ABSTRACTS COLLECTION

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e-Posters

EP01 Reproductive Genetics

EP01.001 Aspirin alleviates hypoxia-induced inhibitory effect of fibronectin on trophoblast invasion in preeclampsia

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Background: Preeclampsia is a severe gestational hypertensive disorder that poses a major threat to maternal and fetal health. The pathoetiology is to have defects of trophoblast invasion and uterine spiral artery remodeling, which leads to a hypoxic placenta. Aspirin is an effective agent for preeclampsia prevention but the drug acting mechanism needs further investigation. Elevated Fibronectin (FN) expression in maternal circulation and placenta has been shown to be associated with preeclampsia, however, the role of FN in human pregnancy and its expression under hypoxia is unclear.

Methods and Results: In this study, FN was upregulated in the trophoblasts with decreasing cell invasiveness under hypoxia. By recombinant FN treatment, FN was shown to inhibit trophoblast invasiveness under hypoxia. Aspirin suppressed FN expression of trophoblasts and the FN-mediated effect on cell motility under hypoxia. FN activated signaling pathway of ERK, Akt and JNK, while aspirin induced Akt, JNK and p38 signaling in trophoblasts. By treating inhibitors of these signal molecules, aspirin was demonstrated to reverse FN-mediated cell motility through Akt and JNK signaling.

Conclusion: FN was upregulated but inhibited trophoblast invasion under hypoxia. Aspirin may exert its prevention effect from preeclampsia through suppressing FN expression and alleviating FN-mediated inhibitory effect on trophoblast invasion in early pregnancy.

Grants: National Science and Technology Council, Taiwan

Conflict of Interest: None declared.

EP01.003 Assessment of the clinical value of exome sequencing in infertile men with cryptorchidism

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Background/Objectives: Cryptorchidism is a congenital malformation with at least one testicle missing from the scrotum. It occurs in 1-2% of newborn boys and is more prevalent in premature infants[1]. Variants in >20 genes have been described to be the causative factors of cryptorchidism, e.g. *INSL3*[2]. If left untreated, undescended testicles can cause spermatogenic failure (SPGF) and/or testicular cancer later in life. In Estonia, about 8% of male infertility cases are caused by cryptorchidism[3]. We have hypothesized that a fraction of unexplained patients presenting

congenital cryptorchidism and SPGF (<39×10^6 sperm/ejaculate) may be explained by unknown monogenic causes.

Methods/Results: ESTonian ANDrology (ESTAND) cohort recruited at the Andrology Clinic, Tartu University Hospital (AC-TUH) includes patients presenting idiopathic uni/bilateral cryptorchidism. To analyze monogenic causes of cryptorchidism, 151 patients were selected for whole-exome sequencing. All subjects have given their informed consent to perform genetic studies. In silico gene panel analysis of hypothesis-based candidate loci has been compiled and data analysis in progression. Pathogenicity will be evaluated based on the ACMG guidelines and patient interviews are performed to gather additional clinical data and identify potential pleiotropic effects.

Conclusions: The current study will contribute to clarify the genetic aetiology of monogenic cryptorchidism. As this condition is a frequent problem among men with reproductive failure, the outcome will have an impact on patient management and counselling.

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Grants: Estonian Research Council: PRG1021.

Conflict of Interest: None declared.

EP01.004 Preimplantation genetic testing in a woman carrier of double reciprocal translocation t(7;11) and t(8;13)

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Background: Cytogenetic analysis is still an invaluable instrument when diagnosing balanced chromosomal abnormalities, as those cannot be detected by microarray methods. Balanced reciprocal translocations are one of the most common structural rearrangements with a frequency of 0.16% to 0.20% (1:615-1:500), but at the same time, two or three independent two-way reciprocal or Robertsonian translocations are extremely rare to co-exist in the same carrier. Those are classified as complex chromosome rearrangements (CCRs). Those structural rearrangements are increasing the risk of producing unbalanced gametes even further than a single translocation and can be associated with infertility, pregnancy loss, or offspring abnormality.

Materials and methods: We present here a woman with repeated reproductive failures, performing cytogenetic analysis as a part of a routine examination. Preimplantation genetic testing for aneuploidy (PGT-A) was done further by next-generation sequencing.

Results: We discovered two separate reciprocal translocations in all cells analyzed in the patient – karyotype 46, XX,t(7;11)(p21;q23),t(8;13)(q24;p12). After assisted reproductive technique 3 embryos were available for trophectoderm biopsy at day 5. Two of them were aneuploid (45, XX,-7 and 48, XX, +7, +13) and one embryo was euploid.

Conclusion: Double reciprocal translocations are very rare and include at least four breakpoints in different chromosomes. Chromosomal segregation is extremely complex, as both translocations produce 14 gametes separately, but the combinations could be 14×14 and only 4 gametes would produce a normal embryo (chance 1 to 51). PGT-A is the only way to choose the euploid embryo and our patient had the luck to have such an embryo for transfer.

Conflict of Interest: None declared.

EP01.005 Developing a candidate gene pipeline for monogenic causes of spermatogenic failure

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Background/Objectives: Worldwide, ~15% of couples face infertility and half of these cases are due to a male factor. In the current clinical pipeline, ~60% of cases with spermatogenic failure (SPGF) remain idiopathic[1]. Routine genetic testing is limited to chromosomal, *CFTR*, and AZF microdeletion analyses. Recently, exome sequencing (ES) has led to the discovery of many novel monogenic causes of male infertility phenotypes, pointing to substantial genetic etiology[2]. The ESTonian ANDrology (ESTAND) cohort recruited at the Andrology Clinic, Tartu University Hospital will be used to address the genetic etiology of male reproductive failure.

Methods/Results: 366 ESTAND participants with idiopathic SPGF were prioritized for ES to analyze potential monogenic causes of their condition. The study includes 184 non-obstructive azoospermia (no sperm in the ejaculate), 70 cryptozoospermia (<1×10^6 sperm/ejaculate), and 112 oligozoospermia (1–39×10^6) cases. A list of 159 candidate genes for SPGF was compiled based on the literature. Data analysis pipeline for variant prioritization was developed using population frequencies, known functional/medical consequences, and in silico predictions of candidate variants. The final pathogenicity assessment is based on the ACMG guidelines incorporated with extended phenotyping data and family health history to be gathered at follow-up interviews.

Conclusion: The developed pipeline will be used to assess potential monogenic causes of SPGF and to evaluate the added value of ES in improving the diagnostics and management of the reproductive and overall health of SPGF patients.

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[2]Laan et al., (2021) Br Med Bull 16;140:5-22

Grants: Estonian Research Council PRG1021.

Conflict of Interest: None declared.

EP01.008 Transfer of mosaic aneuploidy Embryo in a couple with balanced translocation

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Background: The latest advances in genetics has allowed us to select and improve reproduction. These techniques tends to increase the diagnosis of embryos as mosaics and to overestimate or underestimate their true ability to develop a healthy baby.

Case: A woman with chromosomal formula: 46,XX,t(3;17)(p21.3;p13) who opted for PGS.

Methods: Biopsy of embryos at blastocyst stage and extraction of trophectoderm.

		Chromosome endowment			
Embrio	Genomic amplification	X,Y	Autosomes	diagnosis	Transfer recommendation
2	yes	-Y	-15	Abnormal/balanced	NO
5	yes	-Xq13.3q22.1	+3p26.3p21.2-17p13.3p12	Abnormal/unbalanced	NO
7	No	Not amplify		No diagnosis	NO
8	yes	Normal	-4q28.2q35.2 mos (25%)	Possible mosaic/balancing	After counseling
13	yes	Normal	-2p25.3p11.2, -15	Abnormal/balanced	NO
14	yes	Complex aneuploidy		Abnormal/unbalanced	NO

Amplification by Sureplex Amplification System, analysis results with BlueFuse Multi software (Illumina).

Karyotyping of amniocytes by conventional techniques.

Results: The table shows the results of the study embryos:

Karyotype amniocites: female, 46,XX,t(3;17)(p21.3;p13).

Delivery of a healthy baby at 39 weeks gestation.

Conclusion: the primary purpose of PGT-A is to detect aneuploidy, not the presence of mosaicism. Furthermore, a mosaic PGT-A result does not conclude the presence of mosaicism in the ICM.

Transfer of Mosaic embrios should be performed after genetic counseling of the couple and in the absence of normal balanced embryos. A prenatal study of amniotic fluid is indicated to determine possible chromosomal abnormalities. In this analysis we can also determine the presence of the balanced translocation by karyotyping. It is confirmed that the transfer of embryos with low mosaic aneuploidy can give rise to healthy offspring.

Conflict of Interest: None declared.

EP01.009 Genotyping for killer cell immunoglobulin-like receptors genes in women with recurrent implantation failure

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Background: Killer Immunoglobulin-like Receptors (KIRs) family consists of 16 genes, encoding either inhibitory or activating receptors connected to the cytotoxic activity and cytokine secretion of natural killer cells. The great complexity of KIR genetic polymorphism is simplified into two haplotypes - A and B, which fundamentally differ in the presence of activating KIRs. Since activating KIRs are encoded only in haplotype B, an individual with two A haplotype (genotype AA) has only receptors that will transmit a strong inhibitory signal of uterine NK cells. The combination of the maternal KIR AA genotype with the paternal HLA- C2 allotype is responsible for the deleterious reproductive effect. The objective of our study was to determine the KIRs genotype in women with recurrent implantation failure (RIF) after assisted reproduction techniques and to make some correlation with specific clinical symptoms.

Materials and methods: Totally, 56 women with RIF were genotyped by PCR-SSP for activating and inhibitory KIR genes.

Results: The overall frequency of AA genotype was 34%. All inhibitory receptors' genes had >90% frequency. Among the women with autoimmune thyroiditis (AT; $n = 12$), we detected significantly higher frequency for the activating 2DS1 (58.3% vs 25%) and lower frequency for 2DS2 (16.7% vs 52.5%). Clinical pregnancy was achieved in 16.7% of women with AA genotype after single embryo transfer (SET).

Conclusion: KIR genotyping is a valuable tool in decision making for SET and matching in oocyte donation. It is worthy to investigate the role of KIRs in the development of AT.

Conflict of Interest: None declared.

EP01.010 Single gene variants contribute to recurrent pregnancy loss – results from targeted next-generation sequencing

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Background: Recurrent pregnancy loss (RPL) occurs in 0,8-1,3% of couples attempting to be parents. Fetal chromosomal aberrations are the most common causes of miscarriage, identified in 50-70% of conception products. Among them, trisomy, monosomy, and polyploidy are present in 86% of noneuploid pregnancy losses, whereas submicroscopic chromosomal aberrations are detected in 8-16% of noneuploid pregnancy losses. We know a lot about the RPL etiology, but still, our knowledge is insufficient. RPL causes remain unexplained in more than 50% of cases.

Materials and methods: We have designed a custom next-generation sequencing gene panel encompassing 43 genes selected based on a medical literature review. The diagnostics were performed in trios – on the genomic DNA (gDNA) extracted from chorionic villi samples (with aCGH normal result) and gDNA isolated from peripheral blood leukocytes of 10 couples suffering from ≥3 pregnancy losses. We also tested 8 couples with no history of miscarriages and their healthy offspring.

Results: We found pathogenic or likely pathogenic variants within *KIR2DL3*, *NLRP7*, *CYP1A1*, *GP6*, *THBD*, *KDR*, and *NOS3* genes in the experimental group. These variants were absent in the control groups.

Conclusion: Revealing new RPL candidate genes is crucial in understanding its causes. The results of our study may lead to further research aiming at identifying novel RPL molecular background and may result in genetic counselling improvement.

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Conflict of Interest: None declared.

EP01.013 Reporting on familial POI to study monogenic aetiology

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Background/Objectives: Premature ovarian insufficiency (POI) refers to menopause before the age of 40 due to diminished ovarian reserve. Higher prevalence in a patient's family compared to the general population (1%)[1] suggests genetic aetiology of POI. The prevalence of self-reported familial POI is nearly four times higher than medically confirmed reports of familial POI (31 % vs 6.3%)[2,3]. The study aims to combine two strategies to pick up familial cases to assess the monogenic aetiology of idiopathic POI.

Methods: Patients with oligo/amenorrhea >4 months; age <40 years; FSH > 25 IU/l ≥2 times, were recruited at the Women's Clinics of Tartu University Hospital and East-Tallinn Central Hospital, Estonia during August 2022 – January 2023. An in-depth interview was performed, and the familial cases were divided into two groups:

- POI confirmed: proband's family member fulfilled POI criteria confirmed by a managing clinician
- POI suspected: diagnosis based solely on proband reports

Results: Twelve patients of the 39 total (31%) POI probands reported a family history of female infertility. Only three of them (8%) had a confirmed familial POI diagnosis.

Conclusion: Our results correlate with previous proband *vs* database reports of familial POI[2,3]. Heritability pattern is important to study monogenic aetiology. Given that proband-only reporting reveals a substantially greater frequency of familial POI, a more conservative approach should be advocated to prevent potential over-reporting.

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Conflict of Interest: None declared.

EP01.015 The Patient with Azoospermia and Rare Gonosomal Anomaly

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Background: The case report presents the sterile couple due to the partner's azoospermia.

Methods: In the partner a deletion of the long arm of the Y chromosome in the AZFa, AZFb and AZFc regions was detected by QF-PCR method, the finding testified to the absence of the long arm of the Y chromosome. The partner's karyotype from peripheral blood was 46, XX. The FISH examination revealed the Yp11.3 (SRY) locus translocated to one X chromosome (46,XX.ish-der(X)t(X;Y)(p22.3;p11.3)(SRY+).

Results: The finding testifies to Klinefelter syndrome with deletion of the long arms of the Y chromosome and translocation of the short arms of the Y chromosome to one of the X chromosomes.

Conclusion: This finding explains the partner's azoospermia, but his phenotype is completely normal, without any symptoms of Klinefelter syndrome (only the level of FSH and LH is high and the level of testosterone is lower). The family solved the infertility by assisted reproduction with donated sperm.

Grant References: No

Conflict of Interest: Dagmar Rašková: None declared, Jiří Horáček Centre for Medical Genetics and Reproductive Medicine Gennet, Inna Soldátová: None declared, Monika Koudová: None declared, David Stejskal: None declared.

EP01.016 Exploring the clinical spectrum of disorders of sex development in the Moroccan population

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Background/Objectives: Disorders/Differences of Sex development (DSD) are defined as congenital conditions in which there is a discrepancy in the development of chromosomal and gonadal/genital sex. The aim of this retrospective descriptive study was to identify the clinical spectrum of DSD among patients in the Moroccan population over a 4-year period.

Methods: We reviewed the clinical records of 87 patients with DSD referred between January 2019 and December 2022 to pediatric endocrinology, medical genetics, pediatric genetics, and pediatric urology clinics.

Results: Of the 87 cases identified, 35 (40.22%) were classified as 46,XY DSD, 30 (34.48%) as 46,XX DSD, and 22 (25.28%) as sex chromosomal DSD. Positive consanguinity is reported at 40.22% of the cases. The most common clinical diagnoses among 46,XY DSD patients were androgen insensitivity syndrome, 5-alpha reductase deficiency and 3-beta-hydroxysteroid dehydrogenase deficiency, with several affected siblings within families. The most frequent reasons for consultation were hypospadias, amenorrhea, the micropenis and clitoral hypertrophy. Congenital adrenal hyperplasia accounted for 83.33% of the diagnoses retained in the 46,XX DSD patients. The most common presenting sign is acute dehydration followed by hypospadias. Among the 22 (25.28%) patients with sex chromosomal disorders, 14 (63.63%) had Turner syndrome, 6 (27.27%) had Klinefelter syndrome, 1 (4.54%) had 47,XXX syndrome, and 1 (4.54%) had mixed gonadal dysgenesis. Gender had been reassigned in 5 patients.

Conclusion: In summary, this study demonstrates the clinical diversity of DSD cases in Morocco. Further efforts are needed to improve awareness, screening, and management of DSD in the country, especially for consanguineous populations.

Conflict of Interest: None declared.

EP01.017 Evidences of oligogenic impact on the development of DSD features in a patient with a c.34G>C GATA4 mutation

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Background: Differences in sexual development (DSD) are an important class of rare human diseases involving numerous genes. Nevertheless, about half of 46,XY individuals remain genetically

unsolved. To investigate disease-causing gene variants combination and genotype-phenotype correlation we analyzed 46,XY DSD patient and family members carriers of c.34G>C (p.Gly12Arg) in GATA4, which had not been previously described in DSD patients. Moreover, a GATA4 mutation c.34G>C was registered in ClinVar in a 46,XY person without DSD features. The **aim** of our study was to look for potential causative variants previously not implicated in DSD to analyze the oligogenic origin of described DSD phenotype.

Methods: Clinical, hormonal, and histological investigations as well as whole exome sequencing for 46,XY DSD patient were performed.

Results: 46,XY *SRY*+ patient had a female phenotype, with both gonads being dysgenetic and hypoplastic. Heterozygous missense mutation c.34G>C (p.Gly12Arg) in GATA4 gene (MAF = 0.0001752) was not previously identified as DSD-causing. Moreover, two rare hemizygous mutations: c.8212T>C (p.Ser2738Pro) in CFAP47 (MAF unknown) and c.1214G>A (p.Arg405His) in KIAA1210 (MAF = 0.008459) located on the X chromosome and involved in spermatogenesis were identified in our patient, but previously not described for DSD patients. Bioinformatic analysis revealed that all these variants are considered pathogenic.

Conclusion: Based on obtained results we hypothesize, that in GATA4 gene c.34G>C allele together with c.8212T>C in CFAP47 and c.1214G>A in KIAA1210 are resulting in oligogenic DSD features exclusively in 46,XY individuals.

Grant References: Molecular-Genetic Mechanisms of Human Disorders of Sexual Development, National Academy of Sciences of Ukraine [0121U110054].

Conflict of Interest: None declared.

EP01.020 Prevalence of thrombophilic polymorphism in women with recurrent pregnancy loss

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Recurrent pregnancy loss (RPL) occurs in 1–3% of couples aiming at childbirth. Despite numerous scientific studies, the etiology remains uncertain in about 50% of cases. Inherited thrombophilia has been implicated, as a possible cause of RPL but results differ from study to study. Aim of this case-control study was to determine whether hereditary thrombophilia is more prevalent in women with RPL.

57 females of unexplained RPL and 65 age-matched healthy controls were investigated for inherited thrombophilia. Detection of FGB 455G/A, FII 20210 G/A, FV 1691G/A, ITGA2 807C/T, PAI-1 5G/4G, MTHFR 677C/T loci was conducted by using RFLP- PCR method.

The frequency of the FGB 455 AA genotype and A allele was more prevalent in women with RPL (95% CI = 1.12-5.39, OR = 2.45, P = 0.028). It was showed a significant higher frequency of the heterozygous Leiden mutation (FV 1691GA) than that of controls (16% vs 4%, OR = 4.31, 95% CI = 1.37-13.39, P = 0.013). An increase in the ratio of homo- and heterozygous carriers of 4G allele of PAI-1 675 5G/4G loci in the group of women with RPL was revealed (95% CI = 1.13-3.14, OR = 1.9, P = 0.015). Carriers of MTHFR 677TT genotype and T allele were found to exhibit almost three-fold higher RPL risk (95% CI = 1.04-7.90, OR = 2.86, P = 0.05). We did not find significant difference in other thrombophilic polymorphism.

The results showed that the genetic factors of thrombophilia: alleles 455A of FGB gene, 1691A of FV gene, 4G of PAI-1 gene and 677T of MTHFR gene might be involved in the etiology of RPL in west Ukrainian women.

Conflict of Interest: None declared.

EP01.021 Preliminary results of the first carrier screening study in the Romanian population

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Background/Objectives. Historically, carrier screening demonstrated to be efficient for preventing specific recessive diseases, found in general population or in a particular ethnic group. Nowadays it is possible to screen for hundreds of such conditions in one single test. Growing evidence shows the impact of extended carrier screening in the general population. The aim of our study was to investigate the carrier frequency in a Romanian cohort by a panel designed in-house.

Methods. A number of 150 genes were selected and included in the test, based on internationally recommended criteria such as high frequency in population, moderate/severe phenotype, well-defined phenotype and early onset of disease. Samples collected from 140 patients underwent next-generation sequencing (NGS). Sequencing data was analyzed using dedicated bioinformatic pipelines and variants were curated manually. Only likely pathogenic and pathogenic variants were taken into account.

Results. Out of the 140 patients included in the study, 108(77%) were carriers for at least one pathogenic/likely pathogenic variant. On average, an individual was a carrier for 1.34 pathogenic/likely pathogenic variants. Highest carrier rate was for 1 disease(34.2%) and 2 diseases(32.1%). The most frequent variants encountered were low penetrance variants in HFE (31.4%), followed by variants in ABCA4 (8%), GJB2 (7.8%), MEFV (5.7%), BTD (4.2%), CFTR (4.2%), PAH (4.2%), GBA (3.5%). These findings are in accordance to results described in literature.

Conclusion. Carrier screening proves to be an efficient tool to discover patients at risk for transmitting recessive disorders. Our study shows preliminary results of carrier frequency in Romania consistent with international data.

Conflict of Interest: None declared.

EP01.022 Reassessment of genetic imbalance in human blastocysts detected by PGT-A and PGT-SR

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Background/Objectives: We aimed to verify PGT results in genetically imbalanced blastocysts.

Methods: PGT was performed by FISH at 3-4th day ($n = 20$), aCGH ($n = 1$) and NGS ($n = 12$) at 5th day. For verification of PGT

results, blastocysts were separated into trophectoderm (TE) and inner cell mass (ICM). Then, aCGH was performed in TE and FISH was performed in both TE and ICM of each blastocyst.

Results: PGT results were confirmed in 13/33 (39.4%) blastocysts: monosomy 21, double monosomy 4 and 21, trisomies 8, 11, 16, 19, 22 initially revealed by PGT-A and chromosomal rearrangements initially revealed by PGT-SR. In the remaining 20/33 (60.6%) blastocysts, verification showed different results than PGT. Five blastocysts, classified as aneuploid by PGT-A using FISH, were verified as balanced ($n = 2$), as aneuploid but with another aneuploidy ($n = 2$) and as mosaic combining regular aneuploidy with mosaic aneuploidy of different chromosomes ($n = 1$). Seven blastocysts, classified as mosaic by PGT-A using FISH, were verified as balanced ($n = 4$), as regular aneuploid ($n = 2$), as mosaic with aneuploidy of a different chromosome ($n = 1$). Four blastocysts, classified as aneuploid by PGT-A using NGS, were verified as balanced ($n = 2$) and as mosaic aneuploid ($n = 4$). Four blastocysts, classified as mosaic aneuploid by PGT-A using NGS, were verified as balanced. In neither blastocyst discordance between TE and ICM was revealed during reassessment.

Conclusion: PGT results may not reflect genetic status of blastocyst which could be explained by different reasons including aneuploidy correction between 3 and 5th day and/or technical issues of PGT approaches.

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Conflict of Interest: Andrei Tikhonov Part-time, RSF 22-75-00125 (principal investigator), Mikhail Krapivin Part-time, RSF 22-75-00125, Olga Efimova Part-time, Yanina Sagurova Full, Arina Golubeva: None declared, Olga Chiryaeva Part-time, Olga Malysheva Part-time, Irina Mekina: None declared, Evgeniia Komarova: None declared, Dmitrii Staroverov: None declared, Ekaterina Trusova: None declared, Anna Pendina: None declared.

EP01.023 Expanded carrier screening in gamete donors

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Background: Carrier screening for recessive diseases is a standard part of the examination of gamete donors for assisted reproduction. Czech Society of Medical Genetics and Genomics currently recommends examination of 3 genes: *CFTR*, *GJB2* and *SMN1* (group 1), we have added *DHCR7, ATM* and *NBN* (group 2) and genes on X chromosome: *AR*, *COL4A5*, *GLA*, *IL2RG*, *MTM1*, *OTC*, *DMD* (group 3).

Methods: GENNET CarrierTest© uses custom designed NGS panel for detection of mutations in 72 genes associated with autosomal recessive and X-linked disorders.

In total 32,890 samples were analysed. The limited set of 13 genes was examined in 4,618 donors, data from the remaining 59 genes were stored. The full CarrierTest© was performed in 23,654 patients (couples with infertility, recurrent pregnancy loss or family history of inherited disorders).

Results: In the group 1 mutations were detected in 364 (7.9%) donors and 2972 (10.5%) patients, group 2: 192 (4.2%) donors and 1116 (3.9%) patients, group 3: 9 (0.2%) donors and 71 (0.3%) patients.

Of the examined potential donors, a total of 546 (11.8%) had at least one finding and were excluded, this is 3.9% more than with the basic examination. 5215 (18.4%) patients had mutations in at least one of remaining 59 genes, that would require genetic matching if they need a donor.

Conclusion: The expansion of carrier testing in donors makes it possible to further reduce the risks of recessive diseases. New rules should allow the donation of gametes even to carriers, if the recipient's examination and matching is ensured.

Conflict of Interest: Jan Diblik Gennet, Monika Koudová Gennet, Martina Bittoova Gennet, Michala Hrabíková Gennet, David Stejskal Gennet.

EP01.024 Investigating lncRNA expression patterns in human oocytes from patients with polycystic ovaries

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Background/Objectives: Polycystic ovary syndrome (PCOS) is a common premenopausal female disorder characterized by excess androgen and ovarian abnormalities. PCOS may have an effect on the levels of gene expression in different tissues, including ovarian and possibly oocyte samples. Gene expression levels are controlled by multiple mechanisms, including methylation and non-coding RNAs. Thus, in this study, the expression levels of long non-coding RNAs (lncRNAs) in human oocytes obtained from patients with polycystic ovaries and patients without polycystic ovaries were investigated.

Methods: Thirteen metaphase II stage oocytes were obtained from individuals who applied to the Near East Hospital's IVF Clinic. The expression levels of three lncRNAs targeting the *CYP11A1* gene were investigated in oocyte samples. The real-time polymerase chain reaction was used to collect expression data for each oocyte.

Results: Three lncRNAs (RP11-573D15.8, RP11-156E8.1, and lnc-CYP11A1-1) targeting *CYP11A1* gene were shown to be expressed in human metaphase II stage oocytes. There was no statistically significant ($p > 0.05$) change in the expression levels of these lncRNAs in oocyte samples obtained from patients with polycystic ovaries compared to patients without polycystic ovaries.

Conclusion: The expression of *CYP11A1*, which was previously shown to be upregulated in oocytes obtained from patients with polycystic ovaries, is not implied to be regulated by RP11-573D15.8, RP11-156E8.1, or lnc-CYP11A1-1 since there was no difference in the expression levels of these lncRNAs in two groups investigated. Different target lncRNA expression will be investigated to elucidate the regulation mechanism of up-regulated *CYP11A1* expression level in human oocytes obtained from patients with polycystic ovaries.

Conflict of Interest: None declared.

EP01.025 Genome-wide association study meta-analysis supports association between MUC1 and ectopic pregnancy

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Abstract

Ectopic pregnancy is an important cause of maternal morbidity and mortality worldwide. To better understand the genetic risk factors underlying this pregnancy complication, we conduct a GWAS meta-analysis and identify two genome-wide significant loci on chromosomes 1 (rs4971091, $p = 5.32 \times 10^{-9}$) and 10 (rs11598956, $p = 2.41 \times 10^{-8}$). Follow-up analyses propose MUC1, an epithelial glycoprotein with an important role in barrier function, as the most likely candidate for the association on

chromosome 1. We also characterise the phenotypic and genetic correlations with other phenotypes, identifying a genetic correlation with smoking and diseases of the (genito)urinary and gastrointestinal system, and phenotypic correlations with various reproductive health diagnoses, reflecting the previously known epidemiological associations.

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Conflict of Interest: None declared.

EP01.026 FMR4 as a potential blood biomarker for fragile X-associated primary ovarian insufficiency

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Background/Objectives: Female *FMR1* premutation carriers are at risk for developing fragile X-associated primary ovarian insufficiency (FXPOI), a condition characterized by amenorrhea before age 40 years. Not all *FMR1* premutation women suffer from FXPOI and nowadays there are no biomarkers that can predict the occurrence. Long non-coding RNAs (lncRNAs) comprise a group of regulatory transcripts. Previously, we described a significant association between FXPOI and high expression levels of FMR4 (FMR1-derived lncRNA), suggesting a potential role of FMR4 as a possible biomarker for FXPOI (Alvarez-Mora el al., 2022). A limitation in the study design was that it was exploratory. Herein, we further examined the role of FMR4 as biomarker to assess the risk of developing FXPOI, by characterizing young *FMR1* permutation female carriers who have not been diagnosed as FXPOI.

Methods: Serum anti-Müllerian hormone (AMH) level and antral follicle count (AFC) were used to assess woman's ovarian reserve. FMR4 transcript level was evaluated in total RNA extracted from peripheral blood by digital droplet PCR.

Results: A negative association was found between AMH, AFC and FMR4 ($R^2 = 0.2$ for AMH and $R^2 = 0.4$ for AFC), suggesting that FMR4 might help as an additional marker predicting ovarian reserve.

Conclusion: FMR4 might help to better assess the risk of *FMR1* premutation women of developing FXPOI.

Grants: This study was supported by Fundación Merck Salud (19-FE-011) and Instituto de Salud Carlos III (ISCIII), (through the project PI21/01085), co-funded by the European Union. The CIBER de Enfermedades Raras is an initiative of the Instituto de Salud Carlos III.

Conflict of Interest: None declared.

EP01.027 Cases of newborns with CF after IVF in Bulgaria

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Cystic fibrosis (CF) is inherited condition with a progressive chronic clinical course and commonly premature death. The percentage of CF carriage in the general population in Bulgaria is 1 of 33 persons. Screening for CFTR mutations of donors for IVF procedures is not routine practice in Bulgaria. In the last years we have had several cases of newborns with CF after IVF. The purpose of this study was to assess the frequency of occurrence of cystic fibrosis in cases born after IVF.

We report 5 children conceived through IVF genetically and clinically confirmed with CF after birth. Four families had to use donor oocyte. In one case parent's own gametes were used. The methodology for mutation detection includes Sanger sequencing, MLPA analysis and NGS.

In Bulgaria there are currently 248 alive confirmed CF patients. Five of them are born after IVF. All children were diagnosed in the last 5 years. The age of diagnosis was predominantly in the first year of life, since there is no known family history for CF for one child despite classical failure to thrive and nasal polyposis the diagnosis was confirmed late at the age of 9 years.

At the birth incidence of CF in Bulgaria was estimated using epidemiological approaches more than 20 years ago (1 in 3600 live births). Considering the carrier frequency with the many ethical and social issues that can arise we strongly advocate every IVF center to test its donors for CF carriage especially for those diagnosed with CBAVD.

Conflict of Interest: None declared.

EP01.028 In vitro Effects of Platelet-Rich Plasma on Spermatogenesis in NOA cases

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Azoospermia is a condition that affects 1% of the male population.-While approximately 15% of them develop due to hypogonadotropic hypogonadism or obstructive causes and there is a chance for treatment, the fertility of the remaining cases will only be possible by obtaining sperm from the testicles with microTESE.In about half of them, mature spermatozoa are not found.In such cases, different methods have been tried for the maturation of the progenitor germ cells in the testis with different techniques.

Platelet-rich plasma(PRP) is a blood-derived product enriched with platelet density.In experimental studies, the use of PRP in the proliferation and differentiation of sperm cells has been suggested as an effective treatment method. The aim of the study is to determine the effects on spermatogenic cells and their contribution to differentiation with pre-meiotic, meiotic and post-meiotic markers by culturing testicular tissue samples obtained by microTESE from non-obstructive azoospermic(NOA) patients with PRP in appropriate culture medium conditions.

Testicular tissue samples obtained by microTESE in 4 cases with spermatozoa in the testicles and in 10 infertile men diagnosed with NOA were cultured in vitro in PRP supplemented medium.Expression of pre-meiotic(PLZF,VASA),meiotic(SYCP3,CREM),-post-meiotic(PRM2,ACR) genes as biomarkers in spermatogenesis were evaluated by RT-PCR.

The decrease in VASA and CREM expressions was found to be statistically significant when compared with the controls in the group without PRP($p < 0.05$).Our results indicate that patients diagnosed with NOA may benefit more from PRP treatment, and PRP can provide positive contributions to infertility treatment in NOA cases.

This study was supported by Ankara University(BAP)(Project No:TYL-2022-2421)

Conflict of Interest: O. Sena AYDOS This study was supported by Ankara University Scientific Research Projects Coordination Unit (BAP) (Project No: TYL-2022-2421), Sunay Zenginer: None declared, Tulin Ozkan: None declared, nazila farhangzad: None declared, Asuman Sunguroglu: None declared, Kaan Aydos: None declared.

EP01.029 Inferring genetic architecture of preeclampsia using integrative analysis of genome and transcriptome data

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Preeclampsia (PE) is the main cause of maternal and perinatal morbidity and one of the most common pregnancy complication associated with placental pathology. The cause of PE has not yet been fully defined. We used an integrative approach to identify new genetic markers of PE. The genes for study were selected by genome-wide gene expression analysis of the placental tissue in normal and PE pregnancies. The 83 tagging single-nucleotide polymorphisms (SNPs) in placental genes were chose to conduct genotyping based on a case-control study consisting of 551 PE cases and 712 controls from Russian, Buryat and Yakut populations. The joint effects of SNPs on the PE risk were assessed by haplotypes and structure of linkage disequilibrium analysis. Machine learning methods were used to evaluate the association between SNPs and PE. We discovered new candidate genes associated with PE at the genomic and transcriptome levels. We found common and population-specific associations of SNPs with PE. Allelic variations in *CCSAP*, *CORO2A*, *NDRG1*, *PLIN2*, *SIGLEC6* and *ZNF175* genes are associated with PE in all ethnic groups. Moreover, SNPs of the *RAC2*, *GPT2* and *BHLHE40-AS1* genes are associated with PE in Yakut women only. Association with PE was found for rs6818337 of *ANKRD37* gene just for Buryats. Epistatic interactions of the studied genes were revealed in all ethnic groups via MDR analysis. Overall, this study supports the hypothesis that genes identified as differentially expressed in placental tissue in case of PE and normal pregnancy indicate a genetic association with PE at the genome level.

Conflict of Interest: None declared.

EP01.030 The relationship between ovarian insufficiency and MTR and MTRR genetic polymorphisms

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Background: Premature ovarian insufficiency (POI) is the early depletion of the ovarian reserve affecting 1–2% of women < 40 years of age. It has been demonstrated that female reproduction and gene polymorphisms of the enzymes in the one-carbon metabolic pathway are related. Although there is insufficient data for patients with POI, women who carry these associated gene variations. The aim of this study was to investigate whether there was a causal predisposition between MTRR A66G and MTR A2756G polymorphism in women with POI and world controls.

Methods: In this study MTR A2756G and MTRR A66G genotypes, containing SNPs rs1805087 and rs1801394 were determined in all the participants by Real-time polymerase chain reaction.

Results: The prevalence of MTR A2756G and MTRR A66G polymorphisms was determined in 27 POI patients and 2504 healthy women – the World control group. The genotype distribution of MTR A2756G was identical between POI patients and controls. We discovered nearly twice as many MTRR A66G genotypes in the POI group as in the world control group, but without significance (29.63% and 15.73% respectively, $p = 0.3$).

Conclusion: The POI's etiology does not appear to be related to MTR A2756G genotype gene polymorphisms. The trend of higher MTRR A66G genotypes in homozygous frequency in the POI group should be investigated further.

Conflict of Interest: None declared.

EP01.031 Thrombophilia gene mutations, infertility and in vitro fertilization

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Background: Thrombophilia genes are involved not only in coagulation and fibrinolysis, but also in fertilization, fetal development and tissue remodeling, as well as recurrent implantation failure, recurrent pregnancy loss and congenital abnormalities. The aim of our study was to evaluate incidence of inherited thrombophilia in women with unexplained infertility, which underwent IVF, or were preparing for IVF and association of thrombophilia with IVF failure.

Methods: 60 unrelated Georgian women, with unexplained infertility, undergoing IVF cycles, were genotyped by multiplex PCR for simultaneous detection of six genetic risk factors associated with thrombophilia (Factor V (G1691A; H1299R), Prothrombin (G20210A), MTHFR (C677T; A1298C) and PAI-1/SERPINE1 (4G/5G).

Results: Out of 60 Patients with infertility, thrombophilia screening detects no one patient negative for MTHFR gene (C677T; A1298C) mutations. 56.67% ($n = 60$) was homozygote or compound heterozygote, 10% ($n = 60$) was homozygote for

	Allele frequency		Heterozygous frequency		Homozygous frequency		Significance
	POI	World controls	POI	World controls	POI	World controls	
MTR A2756G rs1805087	17%	21.8%	33.3%	32.8%	0%	5.43%	NS
MTRR A66G rs1801394	46.3%*	36.4%*	33.3%*	41.38%*	**29.63%****	**15.73****	****P = 0.3***NS

MTHFR gene (C677T) mutation, 23.33% ($n = 60$) was homozygote for MTHFR gene (A1298C) mutation. 30% was homozygote for PAI-1/SERPINE1 (4G/4G) polymorphism, 53.33% was heterozygote for PAI-1/SERPINE1 (4G/5G) polymorphism. However, mutations of factor V (G1691A; H1299R) and II (G20210A) were less common, 6.67%, 6.67% and 3.33% respectively.

Almost all patients received 2 month antithrombotic prophylactic therapy before fertilization. It's noteworthy that in 4 patients previous several IVF attempts were negative without prophylactic antithrombotic treatment.

Conclusion: Our study showed an increased frequency of MTHFR (C677T; A1298C) mutations and PAI-1/SERPINE1 (4G/5G) polymorphism in women with unexplained infertility, who underwent IVF or are preparing for IVF, However, mutations of factor V and II were less common.

Conflict of Interest: None declared.

EP01.032 Characterization of Epilepsy and Neurodevelopmental disorders in familiar and sporadic cases of Poirier-Bienvenu Neurodevelopmental Syndrome

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CSNK2B gene encodes a regulatory subunit of casein kinase II, highly expressed in the brain. Heterozygous mutations in CSNK2B are associated to Poirier-Bienvenu Neurodevelopmental Syndrome (POBINDS) (OMIM #618732), characterized by facial dysmorphisms, seizures, intellectual disability and behavioural abnormalities, described for the first time in 2017. In this study, we report eight new cases of POBINDS associated with novel heterozygous variants of CSNK2B gene. In three of these patients, a pathogenic variant was inherited from an affected parent. We describe the molecular and clinical features of our patients, focusing in the epileptic and neurodevelopmental phenotype, and comparing them with the previously reported cases. Moreover, while so far all previously reported patients had a de novo CSNK2B mutation, here we report for the first time two familial cases of POBINDS, confirming the high variable expressivity of the disease, and underlying the importance of a thorough family history collection before performing genetic testing in patients with epilepsy and neurodevelopmental disorders.

Conflict of Interest: None declared.

EP01.033 Telomere length in spermatogonia and spermatocytes I as a new predictor of ICSI with testicular sperm efficiency

Arina Golubeva[1], *Yanina Sagurova*[2], *Mikhail Krapivin*[2], *Evgeniia Komarova*[2], *Irina Mekina*[2], *Andrei Tikhonov*[2], *Ekaterina Trusova*[1], *Dmitrii Staroverov*[1], *Olga Efimova*[2], *Anna Pendina*[1;2]

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Background/Objectives: We have checked whether telomere length (TL) in spermatogonia and spermatocytes I from azoospermic patients whose testicular sperm was used for ICSI can be a predictor of the embryo survival in vitro and of a pregnancy chance.

Methods: Testicular samples were obtained by open biopsy from 25 azoospermic patients. TL was measured by quantitative fluorescence in situ hybridization (Q-FISH) on cytogenetic preparations of mitotic spermatogonia ($n = 359$) and meiotic spermatocytes I ($n = 295$). The embryo survival was estimated as a ratio of blastocysts at 5th day ($n = 52$) to embryos at 2nd day ($n = 132$). A total of 38 cycles were analyzed including 29 with embryo transfer and 9 with registered pregnancy.

Results: TL in spermatogonia and spermatocytes I was linked to the embryo survival up to the blastocyst stage and to the pregnancy rate. Telomeres were longer in spermatogonia and spermatocytes I in patients: (1) from couples with high (>50%) embryo survival compared to the couples with low (<50%) embryo survival, including developmental arrest at cleavage stages ($p<0.0001$ and $p<0.0001$, respectively, the Mann-Whitney U-test); (2) from couples with a pregnancy after embryo transfer compared to the couples in which pregnancy was not registered ($p = 0.01$, the Mann-Whitney U-test).

Conclusion: The survival of embryos obtained by ICSI with testicular sperm as well as chance of pregnancy are associated with TL in spermatogonia and spermatocytes I. In ICSI cycles with testicular sperm, TL in spermatogonia and spermatocytes I could predict successful embryonic development in vitro and a chance of pregnancy.

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Conflict of Interest: Arina Golubeva: None declared, Yanina Sagurova full-time, RSF №18-75-10046, Mikhail Krapivin full-time, RSF №18-75-10046, Evgeniia Komarova full-time, RSF №18-75-10046, Irina Mekina: None declared, Andrei Tikhonov full-time, RSF №18-75-10046, Ekaterina Trusova: None declared, Dmitrii Staroverov: None declared, Olga Efimova full-time, RSF №18-75-10046 (principal investigator), Anna Pendina full-time, RSF №18-75-10046.

EP01.034 High incidence of CPLANE1 related Joubert syndrome in early pregnancy loss

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Until now, monogenic causes of early pregnancy losses (EPLs) remain largely unexplored, and only a small number of studies using whole exome sequencing (WES) have been published. Recently, we have reported CPLANE1 double heterozigosity (c.1819delT;7817T > A and c.5820+3_5820+6del) in two EPLs from a couple of Albanian ethnic origin (ESHG 2022; P01.026B). Pathogenic variants in CPLANE1 (C5orf42) are known to cause Joubert syndrome (JS), a primary ciliopathy with multiple system defects. The CPLANE1 c.1819delT;7817T > A allele has been observed with an allele frequency (AF) of 0,64% (7/1090) among our cohort of 545 patients studied by WES. In this study we used allele specific PCR to screen for the presence of CPLANE1 c.1819delT;7817T > A allele among 246 euploid EPLs (<12 gestational age) from families with Macedonian ($n = 155$), Albanian ($n = 80$) and other ethnic origin ($n = 11$). Three c.1819delT;7817T > A homozygous and two heterozygous fetuses were detected, all of Albanian ethnic origin except one heterozygote with Macedonian origin. The subsequent WES analysis showed no second pathogenic mutation in the heterozygous fetuses. Thus, we have detected a high incidence of JS in the total studied group of EPLs (2%; 5/248), and even higher incidence among Albanian families (6.1%; 5/82). In conclusion, to the best of our knowledge this is the highest incidence of monogenic disease reported as a cause of EPLs. Targeted screening of euploid EPLs for the CPLANE1 c.1819delT;7817T > A allele with subsequent NGS analysis in

heterozygotes is justified, especially in couples of Albanian ethnic origin since it would detect 1 JS in ~16 EPLs.

Conflict of Interest: None declared.

EP01.036 Detection of genetic causes of miscarriage in the products of conception: Analysis of fetal tissues from a miscarriage using QF-PCR and Array CGH

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Background: From a mathematical point of view is human reproduction relatively inefficient. Studies have shown that only 30% of children from all fertilized eggs are born alive. Repeated spontaneous abortions affect approximately 1-2% (some sources state up to 5%) of women. Generally, early reproductive losses have various causes, as far as genetic causes are concerned, chromosomal aberrations, are clearly the most common cause of early reproductive losses Chromosome aberrations may cause around half of early reproductive losses, in different studies chromosome abnormalities have been detected in 20-70% of cases. Chromosome analysis using microarray methods detected supposed causal CNV (copy number variation) in ~2% of miscarriages and CNVs of unknown significance (mainly of parental origin) in up to 40% of abortions.

Methods: Retrospective analysis of the causes of miscarriage in products of conception (POC) routinely examined at the Department of Medical Genetics TUH using QF-PCR and Array CGH.

Results: Between 2020 and 2022 were 72 POC examined in TUH. Numerous chromosomal aberrations were found in 8 cases. Array CGH revealed the complex structural aberrations as a cause of miscarriage in another 7 cases (examination of the parents showed that the cause of the abortion was mostly „de novo").

Conclusion: The cause of a large number of miscarriages is still unknown. Whole-exome sequencing has revolutionized the postnatal diagnosis of genetic diseases but is still rarely used to study reproductive disorders. Consequently, we would like to incorporate WES into the routine diagnosis of unexplained miscarriages in our department.

Conflict of Interest: Natalie Friedova Thomayer University Hospital.

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EP01.037 Potential role of FOXL2 gene missense variations in women with history of recurrent miscarriages

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Haploinsufficient mutations in *FOXL2*, a forkhead transcription factor gene, are known to cause blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) types I and II, a rare genetic disorder associated with premature ovarian failure. While isolated cases of premature ovarian failure have been reported in woman with missense variations in *FOXL2* gene.

Here, we report two couples with early recurrent miscarriages, with a normal chromosomal complement confirmed by karyotyping. Whole-exome sequencing based screening analysis revealed two missense variants of uncertain significance in *FOXL2* gene (p.Pro337Ser, p.Gly187Asp) in the female partners, respectively. Based on the available scientific evidence, no other significant variants were detected in these couples. The p.Pro337Ser variant is absent from general population (gnomAD), while p.Gly187Asp variant was reported in only 2 males in the gnomAD population (0.001%; 02/151714 alleles; 0 in females). Both these variations are in the non-forkhead domain of the *FOXL2* gene.

The p.Gly187Asp variant has previously been implicated in a woman with non-syndromic premature ovarian insufficiency but with elevated levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and Low estradiol (E2) levels. Recent studies have shown that the *FOXL2* is strongly expressed in the uterine tissue and controls the expression profile of the endometrial genes. Mutation spectrum analysis of the *FOXL2* gene revealed that the variations in the forkhead domain cause BPES I and II phenotypes. Thus, we hypothesise that a gain-of-function mutation in the non-forkhead domain of the *FOXL2* gene may have significant implication in recurrent miscarriages alongside with their assumed role in the primary ovarian insufficiency.

Conflict of Interest: None declared.

EP01.038 The need of couple carrier genetic screening in absence of an index child: retrospective analysis

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An Egyptian couple had two children who died at an early stage of life (2 and 8 moths respectively) with brain abnormalities and respiratory failure. Clinical features include: clenched hands, club foot, septal defects, oesophageal atresia, seizure, abnormal facies, spasticity, brain atrophy. No molecular testing was performed on the affected children. The couple also have 2 healthy children.

In absence of the index children samples, the couple had come forward for the genetic screening testing. Whole-exome sequencing (WES) based screening was performed. Based on the previous children clinical phenotypes, the couple was found to carry a heterozygous variant of uncertain significance (NM_005787.5:c.1034C>T, p.Ser345Phe) in the *ALG3* gene. Pathogenic *ALG3* variants are associated to a rare autosomal recessive congenital disorder of glycosylation (CDG) type Id. Clinical symptoms typically develop during infancy and affect many body systems throughout life.

During the next pregnancy of the couple, targeted prenatal testing was performed for the *ALG3* variant and the fetus was found to be homozygous. However, due to advanced gestational age, and in view of the variant classification, the pregnancy was continued. The baby was born and developed clinical features consistent with ALG3-CDG. Later, on index-based WES and retrospective TRIO-WES couldn't find any other significant variant than *ALG3* variant. Healthy children were found to be heterozygous for this *ALG3* variant.

This case emphasises the importance of carrier genetic testing in the couple in absence of the index child and retrospective

approach to identify the disease-causing variant in the families suffering with severe genetic conditions.

Conflict of Interest: None declared.

EP01.039 A combined RNA expression analysis and whole genome sequencing approach for the study of lncRNAs' role in teratozoospermia

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Background/Objectives: Male infertility is a major health problem as more than 20 million men are affected worldwide. Genetic background plays an important role in many types of male infertility, such as teratozoospermia which is associated with defects in sperm morphology. However, the genetic causes of male infertility remain still at large unexplored.

Methods: In this study, whole-genome sequencing was used in combination with RNA expression analysis to identify differentially expressed (DE) lncRNAs in teratozoospermia and mutations on these DE lncRNAs that are found only on teratozoospermic men. Several bioinformatics tools were used to explore the effect of the variants on lncRNAs' structure and function and lncRNAs-miRNAs interactions.

Results: 3582 mutations found only in teratozoospermic men were identified on DE lncRNAs between normozoospermic and teratozoospermic men. Of these, 64 variants on 24 lncRNAs have a potential regulatory role according to 3DSNP and RegulomeDB scores. Furthermore, 14 variants affect the structure of 9 lncRNAs according to lncRNASNP v3 and 65 variants on 27 lncRNAs cause loss or gain of miRNA targets. Pathway enrichment and Gene ontology analyses of the genes targeted by these lncRNAs revealed pathways that are deregulated in teratozoospermia.

Conclusions: The present study confirms the contribution of lncRNAs studied in the past to male infertility and sheds light on new molecular mechanisms by providing a list of variants and candidate lncRNAs associated for the first time with teratozoospermia paving the road for future studies aiming to the improvement of diagnosis and therapy.

Grant references: Spermogene (grant number T1EΔK-02787).

Conflict of Interest: None declared.

EP01.040 Can we rescue the developmental competence of 3PN zygotes ?

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Because human embryo research is ethically and numerically restricted, alternatives are needed, such as abnormal 3 pronuclei (3PN) zygotes. Correction of triploidy is performed by removing the extrapronucleus at the zygote stage. We aimed to investigate the in vitro development and the genetic component of these corrected zygotes.

A total of 43 3PN zygotes was submitted to extrapronucleus resection, and their in vitro development subsequently monitored. Parental genetic origin was assessed in 29/43 zygotes by segregation analysis of 10 microsatellite markers located on 8 different chromosomes. In 4 blastocyst embryos screening for aneuploidies was performed on trophectoderm biopsies by low pass WGS.

In total, 6/48 manipulated 3PN zygotes reached the blastocyst stage, suggesting that extrapronuclear removal did not modify in vitro development ($p = 0{,}32$). Parental origin was assessed for 29/48 embryos. Among them, 10/29 had a biparental contribution, 4/29 a uniparental contribution, and 9/29 had at least one trisomy and 1/29 one tetrasomy. In 5 embryos, multiple trisomies with the same parental origin suggested that embryos were still triploid, despite PN resection. Remarkably, none of the embryos with uniparental contribution reached the blastocyst stage. In 3 blastocysts, comprehensive preimplantation genetic testing showed only one euploid embryo.

In conclusion, our study illustrates the limitations of using 3PN zygotes as a research model. Despite numerous cumbersome micromanipulations, the failure to restore diploidy considerably limits the number of developing embryos available for research purpose.

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Conflict of Interest: None declared.

EP01.041 Low mitochondrial content marks high health status of human euploid embryos

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Recently we have shown that aneuploid human embryos are characterised by an increased ploidy of their mitochondrial DNA (mtDNA) (https://doi.org/10.1101/2022.10.14.512116). The potential mechanism behind this finding is based on a cellular attempt to compensate for wide spectrum of energetical problems by hyper-replication of mtDNA. If this compensatory mechanism is universal, i.e. it can be activated by a broad range of deleterious and slightly-deleterious factors, we can expect an increased mtDNA content to be a marker of a poor health status even among euploid embryos. Here, using the dataset of 15'000 annotated human embryos with available low-coverage whole-genome sequences, we tested an association of mtDNA content with several phenotypes of euploid embryos such as morphology, expansion grade and outcome (pregnancy and childbirth). In a subset of 7,000 euploid embryos in the dataset, we showed that embryos with the healthiest morphology states and expansion grades had decreased mtDNA content. In a subset of 500 euploid embryos with known outcomes, we observed a gradual decrease in mtDNA content from the cohort of all transferred embryos to those resulting in pregnancy and finally to those leading to childbirth. These results are in line with the hypothesis that increased mtDNA content may indicate poor health status under all else equal. Further research is needed to elucidate the underlying molecular and biochemical compensatory mechanisms of mtDNA hyper-replication. More data are needed to the research consortium INITIATOR (IN vItro ferTIlizATion fOr Research) to uncover new aspects of human embryogenesis.

Conflict of Interest: None declared.

EP02 Prenatal Genetics

EP02.003 Importance and application of whole exome sequencing in prenatal genetic diagnostics

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Background/Objective: Fetal anomalies are responsible for 20% of perinatal deaths. Prenatal whole exome sequencing (WES) approaches can provide genetic diagnosis when conventional tests are negative. A major challenge in fetal diagnostics is that many diseases may not have a known prenatal phenotype, moreover a prenatal feature may be atypical compared to the postnatally described phenotype. Microcephaly in a pregnancy is a structural abnormality may lead postnatal neurodevelopmental consequences (intellectual disability, autism spectrum disorders, epilepsy) are associated with abnormal brain growth causing morbidity and mortality in infancy or early childhood. Ultrasound examination allows the detection of microcephaly in a fetus. *ASPM* gene mutations are estimated to account for 10-40% of autosomal recessive congenital microcephaly.

Methods: Based on positive family history and ultrasonography findings suggesting primary microcephaly, prenatal WES analysis was indicated. Prenatal WES was performed using Agilent SureSelectXT library kit and Illumina sequencing technology. Sanger sequencing confirmed the presence of the pathogenic variants.

Results: WES analysis revealed a c.8506_8507delCA (p.Gln2836Glufs*35) and a novel c.3134_3135delTC (p.Leu1045Glnfs*17) pathogenic *ASPM* mutations in the fetus in compound heterozygous state. The c.3134_3135delTC has never been reported in the literature.

Conclusion: WES proved to be a valuable method in diagnostics, in case of carefully chosen group of patients with appropriate indication. It would be important to make it more widely available in prenatal clinical practice. This method can provide clinically relevant information to manage a pregnancy. The correct diagnosis offers an opportunity for early intervention and effective treatment in prenatal or in postpartum period.

Conflict of Interest: None declared.

EP02.004 Focal dermal hypoplasia presenting with split hand/foot malformation, lack of skin findings, broad thumb, renal agenesis, and coloboma: a fetal case report

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Background: Focal dermal hypoplasia (FDH) is a rare X-linked dominant multisystem disorder characterized by cutaneous (skin aplasia/hypoplasia, hypo-/hyper-pigmentation, nodular fat herniation) and limb malformations (syndactyly, oligodactyly, camptodactyly, split hand/foot malformation [SHFM], and long bone shortening), and developmental abnormalities of the eyes, face, and teeth. The prenatal phenotype is emerging. We describe a case with SHFM, lack of skin findings, broad thumb, renal agenesis, and coloboma.

Methods and Results: A 32-year-old G3P1 female presented following anatomy scan at 19 + 2 weeks' gestation that identified a female fetus with short long bones; syndactyly and camptodactyly on the hands bilaterally; left hand absent middle ray fingers; right foot syndactyly and absent toes, consistent with SHFM. Left microphthalmia and right renal agenesis were identified. Amniocentesis was performed with rapid aneuploidy detection (RAD), chromosomal microarray (CMA), and trio whole exome sequencing (WES). The pregnancy was interrupted at 21 + 4 weeks' gestation. CMA and RAD were normal. WES identified a de novo likely pathogenic variant in the *PORCN* gene, c.1016T>G, p.(Leu339Arg), which is associated with FDH.

Autopsy additionally identified a left thumb with enlarged distal phalanx and left choroid/sclera coloboma. There were no cutaneous findings.

Conclusion: FDH presents with variable phenotype even among family members, likely due to skewed X-inactivation. Prenatal cases have been described that are clarifying the prenatal presentation and include major congenital anomalies visible on prenatal ultrasound. This case contributes to the prenatal phenotype with evidence of rare associated features. The lack of cutaneous findings may provide insight into the presentation at this gestational age.

Conflict of Interest: Molly Jackson full, Ian Suchet full, Rati Chadha full, Kristopher Langdon full, Mary Ann Thomas full

EP02.005 The number of non-pathogenic CNVs impacts the risk of preterm birth

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Background: Preterm birth (PTB) is the main condition related to perinatal morbimortality. Environmental and genetic factors have been shown to contribute to PTB risk. Here, we aimed to study the potential impact of benign/non-pathogenic copy number variants (CNVs) on PTB, focusing either on individual CNV-harboring regions (CNVRs) or on the total burden of CNVs across the genome.

Methods: Genomic data from prenatal CMA tests performed at Hadassah Medical Center during 2013-2018 were used to define recurrent CNVRs. Additionally, for each CMA sample the length and number of CNVs that represent loss\gain events were determined (referred hereafter as loss score\gain score). Genomic data were linked with pregnancy outcomes in women who subsequently gave birth at Hadassah. Multivariable logistic regressions were used to examine the associations of PTB with each CNVR and with loss\gain scores, adjusted for covariates.

Results: During the study period 10,650 prenatal CMA test were performed. A total of 48,070 autosomal CNVs (size 50Kb-1Mb) were found and used to define 411 CNVRs. We further analyzed

pregnancy outcomes of 3,340 singleton live births with non-pathogenic CMA test results; of these 5.9% were PTB. A positive association was found between loss score and PTB (OR = 1.17, $p = 0.035$), adjusted to loss length and abnormal ultrasonographic findings. No significant associations were observed between any of the CNVRs and PTB.

Conclusions: Our findings suggest that non-pathogenic loss events have a cumulative effect on PTB risk, that is independent of the length of losses and irrespective of their specific genomic position.

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Conflict of Interest: None declared.

EP02.006 Bi-allelic variations in CRB2, encoding the Crumbs Cell Polarity Complex Component 2, lead to non-communicating hydrocephalus due to atresia of the Aqueduct of Sylvius and central canal of the medulla

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Congenital hydrocephalus is a common condition caused by the accumulation of cerebrospinal fluid in the ventricular system. We report 3 cases from 2 families with congenital hydrocephalus due to bi-allelic variations in *CRB2*, a gene previously reported to cause nephrotic syndrome, variably associated with hydrocephalus. Neurohistopathological analysis allowed us to demonstrate that the pathological mechanisms underlying hydrocephalus are due to atresia of Sylvius Aqueduct and central medullar canal. While CRB2 has been largely shown crucial for apico-basal polarity, immunolabelling experiments in our fetal cases showed normal localization and level of PAR complex components as well as of tight and adherens junction molecules indicating a priori normal apicobasal polarity and cell-cell adhesion of the ventricular epithelium suggesting another pathological mechanism. Interestingly, atresia of Sylvius aqueduct was also described in cases with variations in *MPDZ* and *CCDC88C* encoding proteins previously linked functionally to the Crumbs (CRB) polarity complex, and all 3 being more recently involved in apical constriction, a process crucial for the formation of the central medullar canal. Overall, our findings argue for a common mechanism of *CRB2*, *MPDZ* and *CCDC88C* variations that might lead to abnormal apical constriction of the ventricular cells of the neural tube that will form the ependymal cells lining the definitive central canal of the medulla. Our study thus highlights that hydrocephalus related to *CRB2*, *MPDZ* and *CCDC88C* constitutes a separate pathogenic group of congenital non-communicating hydrocephalus with atresia of both Sylvius aqueduct and central canal of the medulla.

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Conflict of Interest: None declared.

EP02.007 Uncertainty in prenatal molecular diagnosis : dual diagnosis in two fetuses as new challenges for further debate on reporting policies

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Background: Prenatal Exome Sequencing (pES) offers parents the opportunity to guide choices regarding their pregnancy. It also allows detection of (likely) pathogenic variants involved in severe disorders but without phenotype-genotype correlation due to an early term of pregnancy or prenatally undetectable phenotype.

Methods: Two pregnancies were referred for trio-pES after ultrasound findings. At 10 + 1 weeks of gestation (WG), fetus 1 presented with persistent hygroma colli. At 24 WG, fetus 2 presented decreased movements, hypoplasic external genitalia, retrognathia, prefrontal edema, dilated tortuous aorta.

Results: In fetus 1, pES identified two homozygous variants, i.e. a ASCC1 nonsense involved in spinal muscular atrophy with congenital bone fractures and a CSPP1 nonsense involved in Joubert syndrome. External fetal examination revealed arthrogryposis but no autopsy was performed. In fetus 2, pES also identified two homozygous variants, i.e. a EFEMP2 missense involved in cutis laxa and a RAG1 nonsense involved in postnatal severe combined immunodeficiencies. Postnatal examination confirmed redundant skin.

Conclusion: pES analysis could be subject to partial or undetected phenotype during pregnancy. Current practice is to return only primary findings, i.e. (probably) pathogenic variants having clear phenotype/genotype correlation. Prenatal reporting of variants of uncertain significance (VUS) remains debated but ultrasound monitoring may reveal new signs to reclassify the variant. Should we use the terms "incidental findings" or VUS when phenotypes are prenatally undetectable? Is it ethical and not harmful to exclude variants whose impact on unborn children is certain and to avoid genetic counselling with consequences for future pregnancies?

Conflict of Interest: None declared.

EP02.008 Fetal genetic factors associated with sonographic abnormalities and pregnancy loss

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Spontaneous pregnancy loss (SPL) is common during the first trimester of pregnancy and can be caused by various factors

including large-scale chromosomal abnormalities and submicroscopic aberrations. However, in most SPLs that occur after the first trimester the aetiology remains undetermined. This study aims to resolve SPL cases of unknown aetiology by investigating the fetal genome and its effect on pregnancy outcome.

Twenty-nine samples were collected from fetuses that were spontaneously aborted, terminated or died neonatally. All fetuses had abnormal ultrasounds and no findings after karyotype and array-CGH. Trio-based whole-exome sequencing (WES) was performed to identify causative fetal variants.

Out of eighteen tested trios, causative/potentially causative variants were uncovered in six cases. A known de novo heterozygous missense variant within *SCN2A* was found in a fetus presenting Developmental and Epileptic Encephalopathy 11 phenotypes. Two inherited novel missense variants in *SCN4A* were found in a compound heterozygous fetus resulting in severe SCN4A-related congenital myopathy. A known homozygous nonsense variant in *KLHL40* was found in a fetus with Nemaline Myopathy 8. Potentially causative heterozygous variants were identified in three cases, in genes *USP18*, *CC2D2A* and *CPLANE1* with autosomal recessive inheritance.

We identified causative variants in 3/18 cases as well the possible involvement of heterozygous variants in genes *USP18*, *CC2D2A* and *CPLANE1* in fetal development. Further investigation is required to assess the clinical significance of the latter findings. Accurate identification of variants in such genes creates new genotype-in utero phenotype associations, leading to the prospect of new additions in preconception and prenatal diagnostic panels.

Conflict of Interest: None declared.

EP02.009 Neonatal death due to F10 gene mutation

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Non-consanguineous couples was referred to genetic counseling for neonatal death. First pregnancy was extrauterine, next pregnancy was terminated due to Turner syndrom. After two years female proband got pregnant spontaneously. Prenatal screening was negative and pregnancy was physiological and full term with vaginal delivery. Five hours after delivery new born presented with gastrointestinal bleeding, cyanosis, development of petechiae, dyspnoea, life-threatening bleeding and decease in 18 hours of life due to hemorrhage. Undelying genetic disease was unknown. Clinical exome sequencing was performed with KAPA HyperExome solution (Roche) on NextSeq (Illumina) as trio exome analysis postmortem. Analysis uncover missense variant c.166G>A (p.Glu56Lys) (maternal origin) and terminal variant c.837C>A (p.Tyr279Ter) (paternal origin) in *F10* gene. None of the variants have been found yet. Neonate *was* compound heterozygous in *F10* gene´s variants. Factor X is a vitamin K dependent, liver produced serine protease which forms the prothrombine complex convering prothrombin to thrombin. Inherited factor X deficiency is rare autosomal recessive bleeding disorder with prevalence 1: 1000000 individuals, reccurence risk for our couple does 25%. Severity of clinical presentation was unexpectedly grave. In time of second genetic counselling female proband was pregnant after spontaneous conception. Invasive prenatal diagnosis was performed, fetus was as well compound heterozygous for both variants in *F10* gene, pregnancy was terminated due to miscarrige. Preimplantation genetic testing (PGT) was proposed, couple went through in vitro fertilization with PGT. Six embryos was retreived but only one was eligible for transfer. Female proband gets pregnant after the embryo transfer, waiting for prenatal screening.

Conflict of Interest: None declared.

EP02.010 Plasma miRNA Profile in High Risk of Preterm Birth during Early and Mid-Pregnancy

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Background/Objectives: In recent years, evidence has been accumulated showing that miRNAs can act as potential biomarkers or targets for therapy of preterm birth (PTB), one of the most important problems in modern obstetrics. We have performed a prospective study of the miRNA profile in the plasma during the first and second trimesters in pregnant women with high risk of preterm birth.

Methods: Total of 48 blood plasma samples were taken from 24 women in the first and second trimesters. Small RNA isolation and library preparation for sequencing was performed using miRNeasy Serum/Plasma Kit (Qiagen) and QIAseq miRNA Library Kit (Qiagen). Libraries were sequenced on a HiSeq 2500 (Illumina). Bioinformatic data analysis was performed using the GeneGlobe Data Analysis Center and DESeq2 R Package.

Results: We detected differences in the levels of 15 miRNAs (3 upregulated—hsa-miR-122-5p, hsa-miR-34a-5p, hsa-miR-34c-5p; 12 downregulated—hsa-miR-487b-3p, hsa-miR-493-3p, hsa-miR-432-5p, hsa-miR-323b-3p, hsa-miR-369-3p, hsa-miR-134-5p, hsa-miR-431-5p, hsa-miR-485-5p, hsa-miR-382-5p, hsa-miR-369-5p, hsa-miR-485-3p, hsa-miR-127-3p) ($\log_2(FC) \geq 1.5$; $FDR \leq 0.05$) during the first trimester compared with the control (non-high-risk of preterm birth pregnant women). All downregulated miRNAs in the first trimester from the placenta-specific C14MC cluster. During the second trimester no differentially expressed miRNAs were found.

Conclusion: Our results suggest that the miRNA profile in plasma during early pregnancy may predict a high risk of preterm birth, which is important in preventing gestational problems as early as possible. Identified miRNAs can be used as PTB biomarkers.

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Conflict of Interest: None declared.

EP02.011 A rare prenatal case of osteogenesis imperfecta

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Background/Objectives: We present a case referred to our Clinic in the second trimester of pregnancy for "second opinion" because the fetal ultrasound identified bilateral, curved, short

femur (below 3rd percentile). Also general shortening of the long bones was noted. Subsequent ultrasound at 29 weeks revealed long bones under 1st percentile, femur bent in a "phone handle". Rib cage was subjectively smaller than gestational age. Some of the possible diagnoses in this case included skeletal dysplasias, such as thanatophoric dysplasia, campomelic dysplasia osteogenesis imperfecta, chondroectodermal dysplasia, Jeune dystrophy. Due to high risk of genetic syndrome, prenatal diagnosis was advised.

Methods: Amniocentesis was performed after the first evaluation in our Hospital. Apart from fetal karyotype with normal result, a gene panel (10 genes) for skeletal dysplasias was performed.

Results: Gene panel analysis identified a missense heterozygous pathogenic variant, c.2596G>A (p.Gly866Ser) in exon 37 out of 51 of *COL1A1* gene, responsible for osteogenesis imperfecta, more commonly associated with types II and III. It should be mentioned that type II has an increased risk of perinatal lethality, and in the case of type III, bone deformities are associated and progressive.

Conclusion: In conclusion, the multidisciplinary approach, involving feto-maternal specialists and geneticist is important for such complex cases. Prenatal testing confirmed the diagnosis, being able to predict the outcome and the familial reccurence risk.

Conflict of Interest: None declared.

EP02.012 Non-invasive prenatal testing (NIPT) in Bizkaia: a 10-year experience

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Objectives: Prenatal diagnostic testing involves all the analysis done before birth (prenatally) to determine whether the fetus has certain abnormalities, as hereditary or spontaneous genetic disorders. Currently, there are different detection strategies that include serological and genetic markers. Among them, the emerging non-invasive prenatal test (NIPT) is changing the practice of prenatal diagnosis worldwide due to its benefits and its demand. The objective of our study is to evaluate the diagnostic capacity of the NIPT in screening for common aneuploidies.

Methods: This descriptive and retrospective study includes the analysis of cell free fetal DNA (cffDNA) in maternal blood using the non-invasive prenatal test TrisoNIM® (all its modalities) of 4.717 pregnant women in Bizkaia between January 2013 and December 2022.

Results: 1.21% of the total analyses have been classified at high-risk for common chromosomopathies. Trisomy 21 and sexual aneuploidies account for the majority of these cases, specifically 80%. Of the total number of cases confirmed by invasive techniques (chorionic biopsy or amniocentesis), the concordance has been almost complete for trisomies 13 and 21, while it is practically reduced by half for trisomy 18 and sexual aneuploidies.

Conclusión: Our results confirm the high sensitivity and specificity of the NIPT, and support its use as an incomparable prenatal screening test for fetal chromosomal abnormalities.

Grant References: European Society of Human Genetics (2015). Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. Summary andrecommendations.

Conflict of Interest: None declared.

EP02.013 Impact of prenatal ultrasound findings and parental SNP microarray testing in pregnancy management

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Background: We present two pregnancies of a phenotypically normal couple with the same inherited maternal 1q21.1 microdeletion. The 1q21.1 isolated microdeletion is associated with a variable phenotype from the absence of symptoms to microcephaly, mild to moderate global developmental delay, facial dysmorphism, seizures, heart malformations, ADHD. Also, this microdeletion in trans with a mutation in the RBM8A gene is associated with TAR (Thrombocytopenia-absent radius) syndrome.

Methods: For both pregnancies prenatal diagnosis was performed using QF-PCR and SNP microarray.

Results: In the first pregnancy in the 22nd week of gestation the ultrasound revealed the bilateral absence of the radius. Amniocenteses and SNP microarray analysis were performed and a 453Kb microdeletion was identified in the 1q21.1 region. TAR syndrome was suspected and this pregnancy was terminated.

In the second pregnancy, a NIPT test was performed with a Turner syndrome high risk result. CVS, QF-PCR and SNP microarray were performed. The QF-PCR result was negative. The SNP microarray analysis identified again a 576kb microdeletion in the 1q21.1 region. The ultrasound revealed no malformations. Following this result, the couple was tested using SNP microarray and the mother was found to be a carrier for the 1q21.1 microdeletion. Genetic counseling was performed and considering that the mother is phenotypically normal and the fetal ultrasound was normal, the couple decided to continue the pregnancy.

Conclusion: In this couple's decision to continue or interrupt the pregnancies, both the fetal ultrasound and the parental SNP microarray testing were decisive.

Conflict of Interest: None declared.

EP02.014 Prenatal diagnosis of mosaic trisomy 15 and maternal uniparental disomy 15

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We describe a case of mosaic trisomy 15 and maternal uniparental disomy 15 [UPD(15)mat] in a fetus with unilateral hydronephrosis.

Cytogenetic analysis on amniotic fluid revealed a mosaic karyotype of 47,XX, +15/46,XX. Array-CGH analysis detected a whole chromosome 15 duplication of approximately 20%. Microsatellite and MLPA analyses confirmed the same mosaicism percentage of chromosome 15 defining its maternal origin. Furthermore, MLPA showed the presence of hypermethylation of the SNRPN locus at 15q11, indicating the presence of UPD(15) mat in approximately 40% of disomic cells.

Given the extreme rarity of the mosaic trisomy 15 condition (fewer than 20 cases liveborns described) and the variability of the clinical features reported in the literature, predicting the expected phenotype is challenging. In our case, the percentage of trisomic cells would seem

comparable with cases with normal outcome, however the presence of UPD(15)mat represents a further reason of uncertainty.

In general, UPD following trisomy rescue mechanism can be found associated with placental or fetal mosaicism. In particular, UPD(15)mat associated with mosaic trisomy seems to be characterized by a distinct phenotype from the classic Prader-Willi Syndrome one, with a higher incidence of congenital heart disease and worse cognitive and behavioral outcome.

The study of the fetal morphology showed a left unilateral hydronephrosis, accentuated echogenicity of the right kidney and absence of heart defects or other abnormalities. A clinical evaluation and genetic investigations are planned at birth to assess the extent of mosaicism and UPD.

Conflict of Interest: None declared.

EP02.015 Maternal translocation t(7;9) resulting in a not previously described unbalanced fetal karyotype with trisomy 9p and partial trisomy 7q

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Background/Objectives: Cytogenetic analysis of the amniotic fluid of a fetus with conspicuous ultrasound results and suspected trisomy 18 revealed a different cause for fetal symptoms

Methods: Prenatal ultrasound examination; rapid prenatal testing by fluorescence in situ hybridisation (FISH); prenatal chromosome analysis combined with additional FISH analysis; prenatal microarray-based comparative genomic hybridization (array CGH); chromosome analysis of both parents

Results: Ultrasound examination of a fetus at 31 + 4 weeks of gestation showed IUGR with polyhydramnia, ventriculomegaly, complex vitium (AVSD, DORV), rocker bottom feet, flat profile with a receeding chin, and a clenched right hand. Trisomy 18 was suspected based on the clinical picture.

Rapid prenatal testing by FISH showed a regular result; no trisomy 18 was detected.

Prenatal chromosome analysis, additional FISH analysis, fetal array CGH analysis, and chromosome analysis of the parents revealed the following chromosome complements:

Fetal: 47,XY,+der(9)t(7;9)(q31;q21)dmat

Maternal: 46,XX,t(7;9)(q31.2;q21.1)

Paternal: 46,XY

Conclusion: By using complementary cytogenetic analyses, the cause for the abnormal prenatal ultrasound result was found and was of great importance in genetic counselling regarding prenatal clinical prognosis. However, in cases with conspicuous ultrasound results, performance of array CGH analysis as the method of choice would save time. Unfortunately, the German health system complicates the conduct of array CGH analysis, especially for time-sensitive prenatal cases. The fetal karyotype with an additional der(9)t(7;9) resulted from the maternal balanced t(7;9) by quadrivalent forming during meiosis I, followed by 3:1 segregation with tertiary trisomy. A 3:1 segregation is rare in comparison with the more common 2:2 segregation.

Conflict of Interest: None declared.

EP02.017 Three genetic polymorphisms of MTRR, MTR and AGT in association with fetal growth restriction susceptibility

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Background/objectives: Fetal Growth Restriction (FGR) is a pregnancy-associated condition with a frequency of more than 10% of pregnancies worldwide. It is known to cause multiple adverse outcomes on fetal, postnatal and adult quality-of-life. Therefore, prenatal prognosis and diagnosis is of high importance. It does not exist yet, but scientists are focusing on finding new genetic candidates as FGR biomarkers. The purpose of the presented research is to study the association of maternal genetic variants: Methionine synthase reductase-*MTRR*(A66G), Methionine synthase-*MTR*(A2756G) and Angiotensinogen-*AGT*(T704C) with FGR susceptibility.

Methods: For FGR-diagnosed ($n = 46$) and healthy ($n = 57$) pregnant women, leukocytes DNA was extracted using NK-sorbent «Base»; (Lytech. Co. Ltd. Russia) kit. Allele-specific PCR was used for genotyping and MDR was used to analyze SNP-SNP interactions.

Results: Our data showed that *MTRR*(66GG) genotype is significantly associated with higher FGR risk (OR = 3.18, 95% CI:1.31-7.72, P = 0.025). On the other hand, *AGT*(704C) allele has a protective effect and is associated with lower FGR susceptibility (OR = 0.58, 95%CI:0.32-1, P = 0.049). These findings were supported by the antagonistic interaction of *MTRR*(A66G) with *AGT*(T704C) shown in MDR-analysis. MDR also figured that *MTRR*(A66G) has the highest predictive potential among the three studied polymorphisms.

Conclusion: This study suggests *MTRR*(A66G) and *AGT*(T704C) as candidate markers of FGR risk. Results also showed that these polymorphisms have opposite roles in FGR pathophysiology with *MTRR*(A66G) as a risk factor and *AGT*(T704C) as protective one. Future trials are recommended to confirm findings.

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Conflict of Interest: None declared.

EP02.018 Multiple prenatally detected huge duplications. How to deal with it?

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Background/Objectives: We report on a 27-year-old woman presented at 11th gestational week because of increased nuchal translucency and generalized hydrops in the fetus.

Methods: Cytogenetic analysis and SNP-array were performed.

Results: Chromosome analysis on chorionic villus sampling was apparently normal after short-term culture, however revealed a conspicuous karyotype with a derivative chromosome 17 (46,XY,dup(17)(q2?3q2?5)) after long term culture.

For further characterization, an SNP-array analysis was performed and confirmed a gain of 12Mb in 17q24.2-17q25.3, encompassing 198 genes, many of them disease-associated. Moreover, three additional gains, 6.2Mb in 2q31, 8.9Mb in 4q12-4q13.1, and 7.3Mb in 8q23.1-8q23.3 were detected, all four de novo.

To prove the possibility, that these CNVs might be restricted to the placenta only, an amniocentesis was performed. Contrary to the expectations, all four abnormalities were recovered in the amniotic fluid cells too.

Follow-up-sonography at 16th gestational week then showed shortening of bones, complex heart defect, brain anomalies, hepatomegaly and polyhydramnios.

The boy was born at 35th week of gestation with weight 2490 g and length 46 cm after labor induction with planned palliative care. Initially the boy was "stable" and discharged home on day two. Because of health deterioration parents then requested for maximum care and heart surgery, but the child died at the age of two weeks.

Conclusion: This case illustrates that multiple large duplications encompassing many disease-associated genes, unexpectedly can be present in the fetus itself (and need not be restricted to the placenta) and that these do not per se preclude a (limited) viability.

Conflict of Interest: None declared.

EP02.019 Reduction of screen positive rate of 10qter deletions in noninvasive prenatal testing by paired-end sequencing

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Objectives: Noninvasive prenatal testing (NIPT) sequences both maternal and fetal cell-free DNA (cfDNA) isolated from the plasma of pregnant women. NIPT results, therefore, may be confounded by maternal factors. One such example involves 10qter deletions where maternal mosaicism has been implicated in false positives. cfDNA size has been shown to be correlated to its origin where maternal cfDNA fragments are on average larger than fetal cfDNA. This study will evaluate NIPT screen positive rates (SPR) of 10qter when cfDNA size information is taken into consideration.

Methods: Retrospective data from a low pass paired-end whole genome sequencing NIPT assay of 112,250 clinical samples were used. The samples were not screened for partial chromosomal deletions/duplication at the time of testing. Exploratory analysis of the sequence data collected under IRB was performed to assess the impact of leveraging cfDNA size information on the SPR of 10qter deletions should patients have opted for a genome-wide screen.

Results: 16 samples were initially found to have a deletion spanning 10q23 to 10q26.3. When cfDNA size information was considered, 15 of 16 deletions would screen negative for 10qter potentially resulting in a SPR of less than 1:110,000.

Conclusions: Methods that can differentiate maternal vs fetal signal are important to improve the accuracy of NIPT. This study aims on improving NIPT by taking into consideration known size differences between maternal and fetal cfDNA. Although further clinical validation is needed, these results suggest cfDNA size information may assist in reducing overall screen positive rates potentially incurred by maternal factors.

Conflict of Interest: None declared.

EP02.020 Costello syndrome in our population: report of three cases

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Background: Costello syndrome (CS) is a RASopathy caused by heterozygous gain-of-function germline variants in HRAS. The majority of cases are described postnatally, however, a series of suggestive prenatal abnormalities such as hypertrophic cardiomyopathy, fetal hydrops or increased nuchal translucency (NT) may occur. Its exact prevalence is unknown, with a range in the literature from 1/1,250,000 to 1/300,000.

Methods: We report 3 cases of CS confirmed by clinical exome sequencing (CES), due to pathogenic or probably pathogenic variants in the HRAS gene (ACMG 2015 criteria).

Results: Case 1. A 25-week gestational premature girl with clinical suspicion of RASopathy. CES identified pathogenic heterozygous variant NM_001130442: c.34G>T(p.Gly12Cys) in HRAS gene. The patient finally died at 15 days of life due to cardiogenic shock secondary to hypertrophic cardiomyopathy.

Case 2. 33-year-old woman, 22 weeks pregnant. The ultrasound examination revealed a fetus with few movements and hands in permanent hyperflexion. CES identified pathogenic heterozygous variant NM_001130442: c.35G>A (p.Gly12Asp) in HRAS gene. The pregnancy was interrupted.

Case 3: 29-year-old woman, 11 weeks pregnant. Ultrasound evidenced cystic hygroma (TN = 15.7 mm). CES identified likely pathogenic heterozygous variant NM_001130442: c.38G>T (p.Gly13Val) in HRAS gene. The pregnancy was interrupted.

Conclusion: Three cases of CS were diagnosed in our center within a period of 3 years, despite a yearly delivery rate of 2,700. These findings suggest a higher prevalence of the disease in our population than that reported in the literature. This fact may be justified because the recent incorporation of CES into prenatal diagnosis reveals a prior underdiagnosis.

Conflict of Interest: None declared.

EP02.021 Prenatal exome sequencing, a powerful tool to describe unreported prenatal features of monogenic disorders

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Background/Objectives: Prenatal exome interpretation may be difficult because of unknown or restricted fetal features available and limited prenatal data in databases. In this study, we report the prenatal ultrasound phenotype of five monogenic disorders.

Methods: We retrospectively analyzed the 56/150 cases harboring a causal diagnosis in the "AnDDI-Prenatome study". For each causal monogenic disorder, we investigated if a prenatal onset was previously reported in a public phenotype-genotype databases or in the literature.

Results: 5/56 fetuses presented a prenatal ultrasound phenotype unreported. Causal variants were identified in two gene previously involved in syndromic or not ID/DD (*LINS1*, *GNB2*), and three genes associated with a monogenic disorder with a pediatric onset (*ZNF148*, *ASXL1*, *PGM1*).

In three cases (*ZNF148*, *ASXL1*, *PGM1* variants), causal variants were identified in genes involved in disorders with highly variable postnatal features. The identification of causal variant was possible because of a partial overlap between prenatal and postnatal phenotypes, in silico prediction tools and suspected inheritance. A causal heterozygous de novo *GNB2* variant was identified in a fetus with hydrops and IUFD. Although prenatal features have also been reported in one family, we reported an extreme phenotype leading to IUFD. Finally, a bi-allelic truncating *LINS1* variant was

identified in a male fetus with increased nuchal translucency, IUGR and asymmetric cerebral ventricles. To date, bi-allelic *LINS1* variants are involved in non-syndromic ID/DD.

Conclusion: pES allows to accurately describe prenatal phenotype of monogenic disorders. It's now time to collect all prenatal onset of monogenic disorder in database to help pES analysis.

Conflict of Interest: None declared.

EP02.022 Rare case of androgenetic-biparental mosacism causing placental mesenchymal dysplasia

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Background/objectives: Placental mesenchymal dysplasia (PMD) is rare placental vascular anomaly characterized by placentomegaly and grapelike vessels without throphoblast proliferation. It can be associated with adverse fetal outcomes such as intrauterine demise, growth restriction or Beckwith Wiedemann syndrome, but its cause mostly remains unknown.

Case report: A 30-year old gravida was referred to our center at 13 gestation weeks due to enlarged placenta with multiple anechoic cyst detected by ultrasound and normal appearing fetus.

Results: Chorionic villi sampling was performed. QF PCR for common aneuplodies (Aneufast kit) markers showed tetraploidy with extra set being of paternal origin (comparison of fetal short tandem repeat (STR) markers with parental STR markers was done), thus implying presence of triandric teraplody or androgenic biparental mosaicism (ABM). Karyotype analysis showed normal female karyotype, and thus confirming ABM in placental tissue. Subsequent QF PCR and karyotype analysis from amniotic fluid showed normal female karyotype of biparental origin. After pregnancy termination, histology examination and immunohistochemisty showed nuclear expression of p57 in cytothrophoblast cells with stem villous edema and loss of nuclear expression in stromal villous cells, pattern characteristic of ABM.

Conclusion: PDM is usually associated with diploid karyotype. Our case confirms ABM as a rare cause of PMD. Diagnosis of PMD is challenging due to its ultrasound resemblance to more common placental pathologic conditions such as molar pregnancies. Genetic studies as well as immunohistochemisty are need in order to establish appropriate diagnosis, thus enabling adequate pregnancy management.

Grants:
None

Conflict of Interest: None declared.

EP02.024 Genetic prenatal diagnosis of Lethal congenital contracture syndrome 11

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Background/Objectives: Arthrogryposis is characterized by congenital joint contractures in two or more body areas resulting from reduced or absent fetal movements. Prenatal ultrasound imaging is crucial in early diagnosis and genetic diagnosis is important for counseling. Our aim was to perform the genetic diagnosis of a fetus with ultrasound alterations exhibiting hydrops, short long bones, fixed limb joints, absent fetal movements, and polyhydramnios at 28 weeks of gestation.

Methods: DNA from uncultured amniocytes was analyzed using the qChipPrenatal microarray (qGenomics). Whole exome sequencing (WES) was further performed using DNA Prep with Exome Enrichment on a NextSeq 500 (Illumina). Variant classification was performed according to the ACMG recommendations.

Results: The microarray results revealed a normal female profile, arr(X, 1 − 22) × 2. WES evidenced a compound heterozygous for the *GLDN* (NM_181789) gene: c.62C>A p.(Ala21Glu) and c.1494G>T p.(Leu498Phe). Lethal congenital contracture syndrome 11 (OMIM # 617194) is an autosomal recessive syndrome caused by GLDN pathogenic variants. The literature was reviewed and 28 additional cases were collected. A distinguishing clinical feature described in the majority of patients was pulmonary hypoplasia and six patients survived beyond the neonatal period.

Conclusion: The present reported case and the literature review confirms the association of biallelic *GLDN* variants with arthrogryposis and other phenotypic spectra such as pulmonary hypoplasia, reaffirming it should be better classified as fetal akinesia deformation sequence (FADS). Prenatal diagnosis of this condition is challenging since and WES should be recommended when a FADS is suspected.

Grant References: Fundación Mutua Madrileña (Grant/Award Number: AP171442019).

Conflict of Interest: None declared.

EP02.025 Non-immune hydrops fetalis is associated with variants in the MYB Binding Protein 1a (MYBBP1A) gene

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Background: Non-immune hydrops fetalis (NIHF) is an extremely infrequent entity usually characterized by an excessive accumulation of fetal fluid within the fetal extravascular compartments and body cavities. Etiology of NIFH is highly variable with a proportion of idiopathic unknown cases, or associated with syndromic disorders.

Here we present a fetus death at 27 + 3 weeks with a NIHF presenting with oligohydramnios, cystic hygroma, pleural effusion, echography with generalized hydrops with predominance of

subcutaneous edema. The fetus also presented with ascites, severe and precocious IUGR and some skeletal anomalies.

Methods and Results: Whole exome sequencing in a trio way was applied in order to screen for a possible genetic pathogenic variant. Variant prioritization according to a custom in-house algorithm allowed to identify two variants in *MYBBP1A*, one nonsense (NM_001105538.2:c.238G>T:NP_001099008.1:p.Gly80-Ter) and one canonical splice-site variant (NM_001105538.2:c.3196-2A > G:NP_848696.1:p.Leu177Argfs*20), each inherited from a healthy parent. A previous report (PMID:28425981) described another case with similar phenotype with a compound heterozygous variant in *MYBBP1A* (one identical to our patient). The two variants are predicted to be damaging by the in silico tools applied. The protein encoded by *MYBBP1A* play a role in many cellular processes including response to nucleolar stress, tumor suppression and synthesis of ribosomal DNA.

Discussion: Therefore, we suspect that *MYBBP1A* can be a strong candidate gene associated with the development of hydrops fetalis. It is necessary to collect more cases and further studies to understand the role of this gene and the mechanism associated with the development of the prenatal malformation.

Grant: FIS020/01053 & 018/01433

Conflict of Interest: None declared.

EP02.026 NIPT high-risk of trisomy 16 as a predictor of adverse pregnancy outcome

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Background/Objectives: Trisomy 16 (T16) is a chromosomal abnormality (CA) with extra chromosome 16 in every cell (full trisomy) or in some cells (mosaic trisomy), that may also predict adverse pregnancy outcomes in 68% of cases. The non-invasive prenatal test (NIPT) has become widely used in prenatal screening for CAs. NIPT allows to determine the risks of rare CAs, including T16. Here we present 4 clinical cases with T16 and adverse pregnancy outcome.

Methods: Whole genome NIPT was performed for 4 pregnant women with low PAPP-A levels (<0.3 MoM): 3 from the first trimester prenatal screening high-risk group for CA and 1 from the low-risk group for CA. Patients with high-risk NIPT results were recommended to undergo invasive prenatal diagnostics with karyotyping or/and chromosomal microarray analysis (CMA).

Results: Amniotic fluid karyotyping results indicated that all fetuses had normal karyotypes. For 2 cases amniotic fluid CMA was performed – in 1 case no CA was detected, in the other case mosaic T16 (27%) was detected. In all cases T16 was detected in placenta (CMA or karyotyping was performed). 3 women continued their pregnancies (including mosaic T16 – the newborn had Tetralogy of Fallot), all newborns had growth retardation. 1 woman had late-term loss.

Conclusion: The high-risk T16 detected by NIPT may predict an adverse pregnancy outcome despite the normal fetal karyotype.

The work was supported by the Moscow Department of Health grant.

Conflict of Interest: None declared.

EP02.027 Non-invasive prenatal testing positive results for 22q11.2 deletion syndrome in two consecutive pregnancies of a woman harboring an atypical 22q11.2 deletion

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Background: The 22q11.2 deletion syndrome, also known as DiGeorge or velocardiofacial syndrome, results from submicroscopic deletion of a defined part of chromosomal band 22q11.2. It is the most common microdeletion syndrome (1 in 3000–6000 live births) and has a heterogeneous clinical presentation that can affect multiple organ systems with widely varying degrees of severity. Advances in non-invasive prenatal testing (NIPT) technology have allowed coverage of a set of microdeletions with high penetrance and severe phenotype, including 22q11.2 deletion syndrome.

Methods: A 38-year-old healthy woman with no significant history of genetic disorders was referred for genetic counseling because of a positive NIPT result for 22q11.2 deletion (estimated at 0.65 Mb, likely of maternal origin) of her second pregnancy. NIPT at her first pregnancy was also positive for 22q11.2 deletion and the subsequently performed prenatal diagnosis using chorion villus sampling with MLPA for microdeletion syndromes showed a normal result. Spontaneous pregnancy loss occurred later.

Results: MLPA (P245, P064) for 22q11 deletion of DNA extracted from patient's blood detected a deletion of 3 close located gene probes SNAP29, MED15, ZNF74 (0,5Mb) and normal amplification of 8 other probes at 22q11.21. After genetic counseling concerning the presence of a small atypical 22q11.2 deletion and its clinical and reproductive implications the couple decided not to undertake an invasive prenatal diagnosis of this pregnancy.

Conclusion: The presence of a submicroscopic chromosomal aberration in the maternal genome causes positive NIPT results precluding the fetus state assessment and creates difficulties in clinical correlation and pregnancy management decisions.

Conflict of Interest: None declared.

EP02.028 Could increased nuchal translucency be an early marker for type 0 spinal muscular atrophy?

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We describe a case of de novo type 0 spinal muscular atrophy (SMA) diagnosed after the evidence of familial high risk of SMA and prenatal echographic aspecific findings.

After the first trimester ultrasound, which showed increased fetal nuchal translucency (NT), early CVS was performed. Karyotype, chromosomal microarray and NGS analysis of a RASopathy gene panel resulted negative. The couple decided to terminate the pregnancy.

Meanwhile, SMA was diagnosed in a relative of the father and carrier testing was performed on the couple: the father resulted a classic carrier, the mother was found to have two copies of *SMN1*.

Considering that some authors correlate cardiac defects and high NT values with SMA, we decided to analyze also the fetus' DNA, which showed no copies of *SMN1* and one copy of *SMN2*.

On their next pregnancy, the couple decided to perform CVS and molecular testing for SMA in the fetus, who had only one copy of *SMN1*, maternally inherited, and no copies on the paternal allele. This evidence brought doubt on the mother's hypothesized genotype so, to better understand the familial genotype and define a correct recurrence risk for the couple, we performed the test on their first daughter, who resulted to have two *SMN1* copies, one on the maternal and one on the paternal allele, excluding the possibility of a 2 + 0 genotype in the mother.

We discuss potential new scenarios for testing *SMN1* in pregnancies with atypical ultrasound findings to identify affected fetuses as early as possible.

Conflict of Interest: None declared.

EP02.029 Shifts and tendencies in Non-Invasive Prenatal Testing (NIPT) - 10 years of experience of the main Polish test provider

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In 2013 Non-Invasive Prenatal Testing started to be routinely offered in Poland as an out-of-pocket screening test. It is characterized by no miscarriage risk, accuracy (decreased number of confirmatory testing) and early timing (10w of pregnancy). Over the years, local recommendations defined it as a second-tier test, only recently (2022) acknowledging the fact that its accuracy has been confirmed in both high and low risk groups.

Altogether 40 003 reports, issued 2014-2022, were analysed (15850 based on BGI NIFTY and 24153 on Illumina VeriSeq V1,V2). Data have been extracted from a test-dedicated database. Demographic, technical and medical parameters were compared between two tests and over time.

In line with recommendations, the main reason for testing was constant - abnormal results of the combined screen (35.2%). However, a general shift towards younger maternal age (<35y: 41.0% in 2016 vs 60.5% in 2022) as well as an earlier test take-up (<12w: 16.0% vs 27.6%, respectively) has been noticed over the years. The switch from the BGI to the Illumina test allowed to reduce turn-around time by half (5.5 vs 2.5 days) and to decrease the percentage of both false negative (from 0.044 to 0.020%) and no-call (0.43% to 0.10%) cases. The introduction of genome-wide NIPT (VeriSeqV2 in 2020) decreased the choice of the basic range (T21, T18, T13, sex chromosomes) from 70% in 2020 to 12.6% in 2022. Test price was reduced by 14%.

Improvements in the NIPT performance, availability and range have been allowing gradual shifting it towards the first-tier screening.

Conflict of Interest: None declared.

EP02.030 Prenatally diagnosed arthrogryposis multiplex congenita is associated with variants in the Cardiac muscle alpha-actin (ACTC1) gene

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Arthrogryposis, also termed arthrogryposis multiplex congenita, is a descriptive term for conditions with multiple congenital contractures (MCC). Prenatal diagnosis is usually based on the detection of diminished fetal movements and joint contractures on ultrasound. The etiology is extremely heterogeneous and is associated with syndromic disorders.

Here we present a fetus with MCC presenting prenatally with polyhydramnios, non-immune hydrops with predominance of subcutaneous edema and ductus venosus agenesis. The fetus also had abnormal limb position with hyperextended legs and flexed arms and placentomegaly.

Trio-based whole exome sequencing was performed to screen for possible pathogenic variants. Ultra-rare, previously unreported variant ACTC1 (NM_005159.4) c.1121G>A; p.(Arg374His) was identified de novo in the fetus. This variant predicts a conservative substitution of a highly conserved residue near the C-terminal end of the protein.

Cardiac muscle alpha-actin is the major component of the sarcomeric thin filament and plays a key role in endomyocardial development and cardiac contraction. Loss of function of this protein has been associated with the development of cardiomyopathy and atrial septal defects. Exceptionally, alteration of some residues as the one reported in this case may result in skeletal muscle disorders, where this actin has a minor expression.

Conflict of Interest: None declared.

EP02.031 Prospective reanalysis of unsolved prenatal exome sequencing for structural defects: feasibility and diagnostic yield

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Background/Objectives: In case of normal karyotype/CMA, prenatal exome sequencing (pES) provides an additional diagnosis in

around 30% of structural defects. Regardless of the pregnancy outcome, reanalysis of unsolved cases is offered using additional postnatal/postmortem data. In this study, we investigate the additive value of postnatal/postmortem features to reanalyze negative pES.

Methods: We conducted a prospective reanalysis of unsolved cases included in the "AnDDI-Prenatome cohort", a French multicenter study offering pES for major structural defects. For 84/140 cases with unconclusive pES, reanalysis was systematically offered in case of parental request. Referent clinicians were requested to collect all relevant clinical, biological, and imaging data after birth (postnatal or postmortem). Phenotypical data were collected as Human Phenotype Ontology (HPO). Two independent biologists analyze raw data from pES thanks to an update bioinformatic in-house pipeline for annotation and databases.

Results: From 84 unsolved cases, reanalysis was required for 51 and declined for 8 couples. 8 couples are under consideration, 11 cases were lost of follow-up, and 6 not being approached by the referring clinicians. At least one year of life, data were available for 24 cases, 12 cases were lost of follow-up and 38 are under consideration. At the time of writing, reanalysis is ongoing.

Conclusion: We report the first cohort of fetuses investigated during pregnancy by trio pES for structural defect. We investigated the additive value of postnatal/postmortem data for systematic reanalysis after negative pES. Our data may provide significant informatic and influence clinical practice in case of negative pES.

Conflict of Interest: None declared.

EP02.032 Cell free fetal DNA testing in twin pregnancies

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Background/Objectives: cell free fetal DNA (cffDNA) study in Catalonia is offered as a screening test for pregnant women with single or multiple pregnancies with high (1/10-1/250) or intermediate risk (1/251-1/1100) in the combined screening. In twin pregnancies, cffDNA is more complex than in singleton pregnancies because: 1) twins could be either monozygotic or dizygotic (DZ), in which only 1 twin is likely to have aneuploidy when present, and 2) in DZ pregnancies each twin can contribute different amounts of cffDNA into the maternal circulation. These two factors may contribute to discordant cffDNA results.

Methods: We have performed cffDNA test in 118 pregnancies, including 89 dichorionic diamniotic (DCDA) twins, 25 monochorionic diamniotic and 4 monochorionic monoamniotic. Four DCDA pregnancies presented a vanishing twin.

Results: cffDNA results detected 3 gestations with a high risk of T21, 1 with a high risk of T18 and 2 with a high risk of T13. Invasive testing was performed in 4 cases and aneuploidies were confirmed in each pregnancy (in one of the twins) except in one case that presented a vanishing twin. In the two gestations that refused an invasive test, 1 twin of each pregnancy was born with T21. In addition, 1 false negative was detected in a pregnancy with a vanishing twin.

Conclusion: Our results reinforce the idea that cffDNA testing in twins is feasible, although special caution is needed in pregnancies with a vanishing twin as they may lead to discordant results for up to 6–8 weeks after fetal demise.

Conflict of Interest: None declared.

EP02.035 The use of whole-genome sequencing in the study of the etiology of complex birth defects in 85 fetuses

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Whole-exome sequencing has become a widely used clinical genetic diagnostic test as a second-tier test after chromosomal microarray analysis in the prenatal evaluation of fetuses with multiple congenital anomalies. The study included 85 fetuses with congenital anomalies. The inclusion criteria were the presence of at least two congenital defects of different organs or numerous defects of one system. It primarily concerned defects resulting in severe organ dysfunction and/or congenital defects with poor prognosis, which may lead to fetus death or baby death after birth. The fetal anomalies were diagnosed in ultrasound scans in fetuses of II and III trimesters of pregnancy. The research materials were amniocytes, fragments of the umbilical cord or other fetal tissues. These were collected appropriately from live fetuses during invasive prenatal diagnosis, after miscarriages or stillbirths. WES was performed in fetuses with normal karyotype and chromosomal microarray analysis results.

Molecular findings were found in 47% of studying fetuses. In 35 % of these, de novo variants were found. Variants in genes with autosomal recessive (*17*) and autosomal dominant (*17*) were detected in most cases.

Pathogenic/likely pathogenic variants explained the congenital anomalies in most of studying fetuses. In a few cases, the variants did not correspond with the observed symptoms, in three cases were found germline variants in the genes, which have not been associated with known human diseases.

WES analysis is essential for improving prenatal diagnosis of fetuses with congenital abnormalities. Although in some cases genetic variants potentially relevant for the specific phenotype require functional testing.

Conflict of Interest: None declared.

EP02.036 Investigation of Genes Responsible for Diaphragmatic Developmental Defects with Next Generation Sequencing Technologies

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Background/Objectives: Congenital Diaphragmatic Hernia (CDH) that occurs as a result of defects in the development of the diaphragm is identified as a severe anomaly with high mortality. CDH is caused by chromosomal anomalies, copy number variations, or sequence variations in increasing number of genes. Besides the retinoic acid signaling pathway which is known to play crucial role in diaphragmatic development, the genes modulating other processes like cell migration, cytoskeleton organization, and myogenesis are also involved in CDH etiology. Therefore, next generation sequencing (NGS) technology based whole-exome sequencing (WES) method is expected to be effective to identify new canditate genes for the genetic etiology of CDH.

Methods: In order to identify new candidate genes, trio-WES analysis was performed in eight fetuses and their parents, and solo-WES analysis was performed in ten fetuses with excluded CNVs by chromosome analysis and a-CGH study.

Results: Pathogenic variants have been identified in the genes (*NR2F2, ZFPM2, ARID1A, CREBBP, PLAT, POGZ, RARB*) with known CDH association, and in candidate genes (*CDKL4, STAB2, NEIL2, SETD5, STAB2, TAF4, ZBTB38, ZNF423, COL11A1, PCSK5, RBM8A*) with function-, database- and literatüre-based association.

Conclusion: The results of this study, support the notion that a single gene or variant is not responsible for the majority of CDH cases. The findings of our study will contribute to the literature on the genes that play a role in the etiology of CDH.

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Conflict of Interest: Somayyeh Heidargholizadeh BAP. PROJECT NO: 30101, Cagri Gulec full-time, consultant- project no: 30101, Gulnihal Bulut BAPSİS, project no:30101, gözde tutku turgut full-time, BAPSİS, project no:30101, Seher Basaran full-time, BAPSİS, project no:30101, Umut Altunoğlu full-time, BAPSİS, project no:30101, Birsen Karaman full-time, principal investigator, BAPSİS, project no:30101.

EP02.037 Prenatal WES diagnostic yield in fetuses with congenital defects detected by antenatal ultrasound

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Background/Objectives: Whole exome sequencing (WES) is a diagnostic tool in postnatal settings for individuals with a suspected genetic condition. In prenatal settings, WES diagnostic yield ranges from 15% to 35%. The aim is to evaluate the usefulness of WES in identifying the genetic etiology of congenital defects.

Methods: WES was performed in 25 fetal samples with ultrasound anomalies and previous prenatal CGH-array with a normal result. Kit Nextera Flex for enrichment, oligos Illumina Exome using NextSeq (Illumina). Variant and segregation studies were confirmed by Sanger sequencing.

Results: WES detected five pathogenic variants (*EFTUD2, TBX5, OFD1, SEC23B, FANCA*), one likely pathogenic (*PTPN11*), and three variants of uncertain significance (VUS). The diagnostic yield for pathogenic and likely pathogenic variants was 24%. In 2 fetuses, the diagnosis followed an autosomal recessive pattern, one homozygous variant (*SEC23B*) in a nonconsanguineous gypsy couple with recurrent hydrops in two pregnancies and one diagnosed with Fanconi anemia. In addition, two pathogenic variants inherited from a healthy parent were identified in *GNB1* and *GLI2* genes previous to an accurate compilation of family history that led to a reassessing of the clinical pathogenicity of the variants.

Conclusion: Despite the small sample size, the results show the clinical utility of prenatal WES for detecting pathogenic/likely pathogenic variants in fetuses with congenital defects. As in postnatal WES, detailed phenotyping is necessary to filter and prioritize variants to obtain a molecular diagnosis.

Conflict of Interest: Neus Baena full, Núria Capdevila full, Juan Pablo Trujillo Quintero full, Carmen Manso full, silvia pina full, cristina lesmes full, Montserrat Comas full, Victor Martinez-Glez full.

EP03 Sensory Disorders (Eye, Ear, Pain)

EP03.001 Recurrent benign paroxysmal positional vertigo in DFNB16 patients with biallelic STRC gene deletions

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Objective: Deletions of *STRC* gene (DFNB16) account for 12% of isolated congenital mild to moderate hearing loss (HL). In mice, the stereocilin protein, encoded by *STRC*, is present in the vestibular kinocilium embedded in the otoconial membrane of the utricular macula. Despite this, effects on vestibular function have not been widely investigated. The aim of this study was to investigate the prevalence of Benign Paroxysmal Positional Vertigo (BPPV) in a cohort of DFNB16 patients.

Study Design: Observational descriptive epidemiological study.

Setting: Single-centre study, in a tertiary referral center.

Patients: Over 5 years of age, with a genetic diagnosis of HL related to biallelic *STRC* gene deletions, diagnosed between 2015 and 2021

Intervention: Patients or their parents were interviewed to determine whether they had experienced vertigo or episodes of BPPV.

Main outcome measure: Criteria were at least five acute episodes of rotatory vertigo, each lasting less than one minute, episodes triggered by changes in specific head position, and an absence of neurological symptoms.

Results: 64 patients were included, having mild (33%) to moderate (66%) HL. Median age was 15 years, (range: 6-48). Prevalence of BPPV was 39% (25/64). Median age of first onset was 13 years, (range: 3-18).

Conclusions: This study showed recurrent BPPV and early age of onset in patients with biallelic *STRC* gene deletions. BPPV may be associated with the HL phenotype in patients with *STRC* gene deletions. It is important to inform patients and families of this potential risk such that appropriate management can be proposed.

Conflict of Interest: None declared.

EP03.002 The identification of biallelic LAMA1 pathogenic variant in a consanguineous Saudi family with presumed stickler syndrome

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Purpose: Poretti– Boltshauser syndrome (OMIM # 615960) is a rare autosomal recessive non-progressive cerebellar dysplasia disorder with wide range of ophthalmic manifestations such as high myopia, strabismus, and retinal dystrophy. We aim to study a consanguineous Saudi family with two affected siblings (female 30 yrs.) and (male 28 yrs.) who were primarily diagnosed with stickler syndrome with a positive history of ataxia, delayed speech and a bilateral prophylactic laser that was done 20 years ago for high myopia.

Methods: Comprehensive clinical and molecular approaches were applied including detailed ophthalmological examination followed by whole-exome sequencing (WES).

Results: Detailed ophthalmological examination revealed visual acuity of 20/200 bilaterally associated with pendular nystagmus and esotropia. Anterior segment examination showed a bilateral posterior subcapsular cataract with no sign of lens subluxation. A myopic fundus was observed with a pale optic disc, flat retina, and prominent laser marks. The additional neurological assessment confirmed the presence of mildly delayed gross motor skills that resolved with age with ongoing MRI requests for the full characterization. Genetically, WES revealed novel homozygous

laminin alpha-1 (*LAMA1*) nonsense mutation; NM_005559.4: c.5801C>G(p.Ser1934*), class 4 according to ACMG. This variant was segregated well within available family members confirming the diagnosis of PTBHS.

Conclusions: Our identified novel nonsense *LAMA1* pathogenic variant supports the pathogenicity of the *LAMA1* gene and expands the phenotypic spectrum of PTBHS. Our study highlights the importance of genetics testing in refining the clinical diagnosis for such heterogeneous cases where ocular features are overlapping with other vitreoretinopathies such as stickler syndrome.

Conflict of Interest: None declared.

EP03.003 Digenic cause for optic atrophy due to heterozygous variants in SPG7 and AFG3L2 genes?

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Background/Objectives: Recently, a case series showed that heterozygous variants in SPG7 and AFG3L2 genes are involved in the regulation of mitochondrial protein homeostasis and maturation by m-AAA proteases to maintain optic nerve physiology (PMID:32548275). Here we present a patient were optic atrophy could be explained by digenic mechanism.

Methods: Clinical evaluation, thorough ophthalmological assessments and extensive genetic testing were performed for the patient and parents.

Results: A 6 years old male presents with decreased visual acuity, stating age 4 years. He had normal neurological development and healthy non-consanguineous parents. Bilateral optic disc pallor, decreased thickness of retinal fiber layer, macular ganglion cell loss (on OCT) and diminished functionality of the rods (on ERG) were documented. WGS solo (including mitochondrial genome) and sanger sequencing in parents showed: *AFG3L2* NM_006796.1:c.1286_1288dup,p.(Asn429dup),heterozygous,VUS, paternal and *SPG7* NM_003119.4:c.1045G>A,p.(Gly349Ser),heterozygous, pathogenic, maternal. An article (PMID:30252181) reports an m-AAA mitochondrial damage-associated phenotype characterized by early-onset optic atrophy with spastic ataxia and L-dopa-responsive parkinsonism. The affected individual had a heterozygous de novo AFG3L2 mutation (p.R468C) together with a heterozygous maternally inherited intragenic deletion of SPG7. Analysis of the patient's fibroblasts showed an abnormal pattern of processing of OPA1, a protein essential for mitochondrial fusion and responsible for hereditary optic atrophy. A similar mechanism could explain the optic atrophy in our patient; however, functional studies were not performed.

Conclusion: Possibly, the patient's optic atrophy is explained by both heterozygous variants in the SPG7 (maternal) and AFG3L2 (paternal), being involved in the same mitochondrial cellular metabolic pathway (mitochondrial m-AAA protease involved in OPA1 processing).

Conflict of Interest: None declared.

EP03.004 Spectrum of congenital and inherited ocular disorders seen in a genetic clinic: experience of a developing ocular genetic service

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Background: Hereditary causes are an important etiological category of childhood blindness. This study reports real world experience of a developing ocular genetic service.

Material and Methods: The study was carried out from Jan 2020 to Dec 2021 jointly by the departments of Pediatrics and Ophthalmology of a tertiary care hospital in North-West India. Children presenting to the genetics clinic with congenital or late onset ocular disorder(s) and any individual (irrespective of age) suffering from an ophthalmic disorder and referred by an ophthalmologist for genetic counseling for himself/herself and/or his/her family member(s) were included. Genetic testing (exome sequencing /panel based sequencing /chromosomal microarray) was outsourced to third party laboratories with the cost of the test been borne by the patient.

Results: 8.6% of the registered patients in the genetics clinic had ocular disorders. Maximum number of patients belonged to category of Anterior Segment Dysgenesis followed by Microphthalmia Anophthalmia Coloboma spectrum, Lens disorders, Inherited retinal disorders in decreasing numbers. The ratio of syndromic ocular to isolated ocular disorders seen was 1.8/1. Genetic testing was accepted by 55.5% of families. The genetic testing was clinically useful for ~ 35% of the tested cohort with the opportunity for prenatal diagnosis being the most useful application of genetic testing.

Conclusion: Syndromic ocular disorders are seen at a higher frequency as compared to isolated ocular disorder in a genetic clinic. Opportunity for prenatal diagnosis is the most useful application of genetic testing in ocular disorders.

Conflict of Interest: Anupriya Kaur Full time, Animesh Sahu Full time, Savleen Kaur Full time, Jaspreet Sukhija Full time, Priyanka Srivastava Full time

EP03.005 3 different iPSC-derived cell models for the study of Stargardt Disease and Retinitis Pigmentosa

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Purpose: Stargardt disease (STGD) and Retinitis Pigmentosa (RP) are two of the most prevalent inherited dystrophies of the retina. The first one is caused by recessive mutations in the *ABCA4* gene, expressed in both RPE cells and photoreceptors. RP is the most common form of rod-cone dystrophy. *RHO* - the gene encoding for the specific opsin of rods – is one of the most prevalent mutated genes in the dominant forms.

Methods: We generated 3 different iPSC-derived cell models: RPE, photoreceptor precursors (PhRPs) and Retinal Organoids (RO) from 3 patients affected by STGD disease or RP (with a dominant *RHO* mutation). Using qPCR, Western Blot, and immunochemistry, along with other cellular biology methods, we analyzed their phenotype.

Results: The effect of patient-specific mutations induces aberrant splicing, reduced mRNA expression and reduced protein expression of ABCA4 while showing early signs of autophagy and reduced function on RPE, PhRPs and RO. The RP line shows accumulation of the rhodopsin protein in rod photoreceptors, and early signs of endoplasmic reticulum (ER) stress and autophagy.

Conclusions: iPSC are a wonderful tool for the investigation of retinal diseases, through differentiation to different cell types. While some models with a faster differentiation time can be useful for detection of mRNA or protein expression, more complicated models such as RO are necessary for a deeper insight into the pathophysiology of each disease.

Conflict of Interest: None declared.

EP03.006 Whole-exome sequencing allows the identification of new causal mutations and candidate genes in unsolved inherited retinal dystrophy patients

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Background/Objectives: Inherited retinal dystrophies (IRD) are a group of rare diseases leading to visual loss. After genetic analysis of prevalent IRD genes, ~40% of patients remained unsolved. Here, we applied whole-exome sequencing (WES) to address unsolved IRD cases.

Methods: A total of 47 families (55 IRD patients and 88 unaffected relatives), initially screened for variants in 99 known IRD genes, were analysed using SOPHiA Whole Exome Solution and a personalized bioinformatic pipeline, initially designed to analyse whole-genome sequencing (WGS) data, that uses a virtual panel containing 297 IRD-genes as the first prioritization step. Additionally, all genes were evaluated in seven families so far. Segregation studies were conducted by Sanger sequencing.

Results: This approach allowed the identification of likely causal variants in 12 families, and variants of uncertain significance in other three families. These variants were identified in IRD-related genes not previously included in the panel, located in challenging regions (*RPGR-Orf15*) or not detected due to variant calling errors (*NR2E3*). Also, a new genotype-phenotype correlation was proposed related to *SLC4A7*. Additionally, we detected variants in new candidate genes in another family, for which additional studies are currently being completed.

Conclusions: These results have increased our diagnostic yield in IRD patients and have improved the efficiency of our population-specific IRD panel. Also, we have proposed a new genotype-phenotype correlation and new IRD-candidate genes. Since our algorithm has been adapted from a WGS-pipeline, we suggest the use of a single pipeline to analyse NGS-derived data.

Grant References: ISCIII-ERDF/ESF(PI21-00244), Andalusian Government(PEER_0501_2019;RH-0049-2021), F.Isabel Gemio/F.Cajasol(2019-01), ISCIII-IMPaCT(IMP/0009).

Conflict of Interest: None declared.

EP03.007 Towards a better understanding of genotype-phenotype spectra in hereditary pain loss disorders

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Background/Objectives: Genetic pain loss disorders include congenital insensitivity to pain (CIP) and hereditary sensory (and autonomic) neuropathy (HSN/HSAN). Patients have no or reduced response to pain stimuli, leading to trauma and mutilation with potentially fatal complications. In addition to congenital cases, milder forms with adult onset exist. Here we report on a multicenter study that aims at improving the knowledge of the clinical features with regard to the genetic basis. The collected data are compiled in a registry to enable genotype-phenotype correlations and natural history studies.

Methods: We conducted whole exome or whole genome sequencing and clinical work-up of a large retrospective cohort of CIP/HSAN patients in a global network of neuromuscular centers. When appropriate, we included segregation analyses, sphingolipid

profiles from patient plasma, or long-range sequencing techniques to further characterize variants.

Results: We describe 70 patients with previously unpublished disease-associated variants in 11 genes. The most frequently affected genes are *NTRK1* and *SCN9A* in children and *SPTLC1* in adult patients. We report mutations in previously rarely described entities such as *ATL3*-, *FLVCR1*- and *NGF*-associated pain loss and combine the molecular results with clinical information.

Conclusion: Our results broaden the knowledge of HSAN and thereby improve patient care. The register established here is the starting point for natural history studies and the identification of biomarkers. In view of the already existing first therapy approaches in clinical trials and expected further therapies, the cohort will allow subtype-specific classifications in the future.

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Conflict of Interest: Katja Eggermann: None declared, Annette Lischka: None declared, Miriam Elbracht: None declared, Florian Kraft: None declared, Matthias Begemann: None declared, Daniela Dey: None declared, Christopher Record: None declared, Mary M Reilly: None declared, Petra Lassuthova: None declared, Arman Cakar: None declared, Yesim Parman: None declared, Jonathan Baets: None declared, Vincent Timmermann: None declared, Jan Senderek: None declared, Angelika Lampert: None declared, David Bennet: None declared, James Cox: None declared, Maike Dohrn NIH (R01NS105755), Stephan Züchner: None declared, Thorsten Hornemann: None declared, Michaela Auer-Grumbach: None declared, Christian Hübner: None declared, Geoffrey Woods: None declared, Enrico Leipold: None declared, Jonathan de Winter: None declared, Danique Beijer: None declared, Atchayaram Nalini: None declared, Wilson Marques Junior: None declared, Ingo Kurth EJP RD COFUND-EJP N° 825575.

EP03.008 PP1 criteria pitfall assessing autosomal dominant TECTA-variant in non-syndromic hearing loss

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Introduction: Autosomal dominant (AD) inherited non-syndromic hearing loss (NSHL) is characterized by genetic heterogeneity. More than 50 genes are associated to AD NSHL, including TECTA. NSHL specific ACMG variant interpretation guidelines have been published. However, caution is advised regarding the PP1 criterion.

Material and methods: We describe the finding of NM_005422.44(TECTA):c.5990T>C using gene panel screening of 165 NSHL genes and the subsequent segregation analysis in a Danish NSHL-family. The variant was previously reported to segregate with NSHL in a large Japanese family. The variant was classified as C4 with the following ACMG criteria: PM2_sup, PP3, PM1 and PP1_strong. The latter criterion was decisive and given based on 11 affected segregations in the Japanese family. However, no additional screening of other NSHL genes was reported for the Japanese family. We initially omitted the PP1 criterion and classified the variant as C3 until further segregation analysis had been performed by Sanger sequencing.

Results: The variant was confirmed in the Danish family in two additional affected family members and not found in one unaffected. Based on a total of 13 affected segregations in two families of different ethnicity we included the criterion PP1_strong and reclassified the TECTA variant as C4.

Conclusion: Caution is advised when using the PP1 criterion, as variants could be in linkage disequilibrium with other causative variants. Therefore, PP1 could falsely elevate a C3 variant to C4 with subsequent clinical significance for entire families. In this case NM_005422.44(TECTA):c.5990T>C co-segregated with AD NSHL in both families, supporting a true causative effect.

Conflict of Interest: None declared.

EP03.009 Molecular genetic investigation of CHM in the Czech patient population reveals seven novel pathogenic/likely pathogenic variants including 3 large deletions and one duplication

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Objectives: The aim of the study was to define the spectrum of pathogenic variants in 18 Czech families with choroideremia (CHM).

Methods: All probands underwent complex ocular examination including fundus autofluorescence. Initial molecular genetic investigation comprised direct sequencing of the *CHM* coding region or next-generation sequencing (ocular gene pane or exome), followed by MLPA analysis and genome sequencing to map the exact positions of breakpoints. Segregation analysis was done by Sanger sequencing.

Results: 13 male probands were suspected to suffer from CHM, while 5 (2 males and 3 females) were referred with retinitis pigmentosa and one female with Stargardt disease. Pathogenic or likely pathogenic variants in *CHM* were identified in 17 probands including four novel mutations predicted to introduce a premature stop codon. In addition, four previously unreported copy number variations were found; specifically 2.8 kb deletion involving exon 3, 692 kb deletion comprising exons 3-15 plus *MIR1321* and part of *POF1*, and a deletion of the entire *CHM* together with *DACH2* and *KLHL4*. The only duplication identified was 110 kb involving *CHM* exons 2-8. In one proband the pathogenic c.525_526del variant occurred de novo which is a rare observation in CHM. In one proband with typical CHM phenotype the c.1511-10_1511-6del variant was evaluated as of unknown significance.

Conclusion: Identification of *CHM* pathogenic/likely pathogenic variants in patients referred with other diagnoses than CHM highlights the utility of molecular genetic testing. Disease-causing structural variants in *CHM* may be more prevalent than currently anticipated.

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Conflict of Interest: Petra Liskova Charles University, Prague, Ministry of Health of the Czech Republic NU20-07-00182, Novartis, Monika Pankievic: None declared, Gabriela Storkanova General University Hospital, Hana Vlaskova General University Hospital, Marie Vajter General University Hospital, Andrea Vergaro General University Hospital, Arpad Boday Agel Laboratories, Lubica Dudakova Charles University.

EP03.010 Autosomal Recessive Leber's Hereditary Optic Neuropathy Caused by a Homozygous Variant in DNAJC30 Gene in Two Unrelated Patients

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Recently, Stenton et al., described a new, autosomal recessive inheritance pattern of Leber's hereditary optic neuropathy (LHON) caused by missense variants in the *DNAJC30* gene. The *DNAJC30* c.152A>G, p.(Tyr51Cys) variant was by far the most common variant reported in patients originating from Eastern Europe, thus it is believed to be a founder variant in these populations. We report the first two cases of *DNAJC30*-related autosomal recessive LHON in a young male and a female originating from Estonia. The patients presented severe loss of central vision and clinical features indistinguishable from mitochondrial LHON. The whole exome sequencing carried out in the male patient, and the next-generation sequencing panel in the young female patient identified the same homozygous missense variant in the *DNAJC30* gene. Our cases further reinforce the pathogenicity of c.152A>G, p.(Tyr51Cys) *DNAJC30* variant causing autosomal recessive LHON. We support the parallel sequencing of the mitochondrial DNA and the *DNAJC30* gene in patients with LHON.

Funding information: Estonian Research Council; Grant/Award Number: PRG471, PSG774.

Conflict of Interest: None declared.

EP03.011 A novel HPS5 acceptor splice-site mutation causing Hermansky Pudlak Syndrome type 5

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Objectives: Three affected siblings of two branches of a Bedouin consanguineous kindred presented with nystagmus, mild cutaneous and ocular hypopigmentation, and a history of easy bruising, recurrent epistaxis, and severe bleeding after uneventful surgery, commensurate with a diagnosis of Hermansky Pudlak Syndrome (HPS). We aimed to identify the molecular basis of HPS in the studied kindred.

Methods: Affected individuals were studied following informed consent and approval of Soroka Medical Center IRB. Senior ophthalmologist and geneticist determined the phenotype. Linkage analysis, testing 13 family members, was done using SNP arrays. Segregation analysis of the putative pathogenic variant was done using Sanger sequencing.

Results: Linkage analysis delineated a 17 Mbp disease-associated homozygosity locus on chromosome 11 (LOD score 2.4), containing *HPS5*. *HPS5* sequencing identified a novel homozygous splice-site mutation, c.285-2, T > C, near the end of intron 4. The mutation is in a highly evolutionary conserved residue predicted by SpliceAI to cause acceptor loss (score 0.78). Sanger sequencing of patients' cDNA blood samples revealed that the mutation led to a frameshift and premature stop codon due to a deletion of five nucleotides.

Conclusions: The novel *HPS5* mutation causing HPS in the Bedouin kindred is in a highly conserved splice acceptor site, resulting in an aberrant transcript. The HPS5 protein is part of the Biogenesis of Lysosome-related Organelle Complex 2 (BLOC-2) containing also proteins HPS3 and HPS6. BLOC-2 is involved in the formation and migration of lysosomal-related endosomal compartments and is crucial in melanosome formation and maturation.

Grant: Morris Kahn Family Foundation

Conflict of Interest: None declared.

EP03.012 Audiological phenotyping evaluation in KBG syndrome: Description of a multicenter review

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KBG syndrome is a rare genetic disorder due to monoallelic pathogenic variations of *ANKRD11*.The typical phenotype includes facial dysmorphism, costal and spinal malformation and developmental delay. Hearing loss in KBG patients has been reported for many years, but no study has evaluated audiological phenotyping from a clinical and an anatomical point of view.

Our objective was to reinforce clinical knowledge of hearing impairment in KBG syndrome.

This French multicenter study included 32 KBG patients with retrospective collection of data on audiological features, ear imaging and genetic investigations.

We identified a typical audiological profil in KBG syndrome: conductive (70%), bilateral (78%), mild to moderate (84%) and stable (70%) hearing loss, with some audiological heterogeneity.

Among patients with an abnormality on CT imaging (55%), ossicular chain impairment (67%), fixation of the stapes footplate (33%) and inner-ear malformations (33%) were the most common abnormalities.

In this innovative study, we have shown that hearing impairment may be the first clinical sign of KBG syndrome and we describe the specific characteristics of hearing loss, underlining the variability of clinical, anatomical and radiological features.

We recommend a complete audiological and radiological evaluation and an ENT-follow up in all patients presenting with KBG. Imaging evaluation is necessary to determine the nature of lesions in the middle and inner ear.

Conflict of Interest: None declared.

EP03.013 PRPH2-related retinal dystrophies: mutational spectrum in Fundación Jiménez Díaz University Hospital cohort

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Background/Objectives: Inherited Retinal Dystrophies (IRD) are a group of rare diseases with a prevalence of 1:3000-4000 people. *PRPH2* is one of the most frequent non-syndromic IRD (NS-IRD) causative genes (3,4-4,6%). It is linked to a wide variety of retinal phenotypes (retinitis pigmentosa, cone-rod dystrophy and different macular dystrophies). Here, we describe the *PRPH2* mutational spectrum in the FJD-University Hospital cohort.

Methods: After genetic screening through classic genotyping or targeted next-generation sequencing (NGS) methods, 108 unrelated IRD families from our database (4800 families) were characterized with *PRPH2* variants. The variants were classified according to their typology and localization in the protein domain. Patient's clinical data were obtained from self-reported ophthalmological history. NS-IRDs were classified according to the reported referral phenotype into cone- (NON-RP) or rod-predominant diseases (RP).

Results: In the FJD-cohort, 4% of solved NS-IRDs were genetically characterized with *PRPH2* variants. Most patients carried missense (33%) or frameshift (12%) variants. Regarding the location in the protein, the majority of mutations are found in the D2-loop domain (75%). Approximately 4/5 of these patients have NON-RP phenotypes with an age onset ranging from adolescence to the 5th-6th decade. Our findings show high phenotypic variability within patients even belonging to the same family.

Conclusion: Given the *PRPH2*-associated phenotypic variability, further studies in the phenotype implication of some of the variants are needed. This could help to improve clinical management and genetic counseling and thus, hopefully, help to bring new therapies to patients.

Grant References: CIBERER, ISCIII (PI19/00321-PI22/00321), IIS-FJD_Biobank, University Chair UAM-IIS-FJD Genomic Medicine, ONCE.

Conflict of Interest: None declared.

EP03.014 Identification of common and rare genetic variants reveals a possible role in early-onset maculopathy

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Background/Objectives: Age-related macular degeneration (AMD) is one of the leading causes of severe visual impairment among elderly worldwide. Although AMD mainly affects the elderly (≥65y), some individuals develop AMD characteristics, including drusen at a much younger age. Early-onset maculopathy (EOM, ≤65y) can lead to advanced macular degeneration later in life. The aim of this study was to determine the contribution of common AMD-associated variants in patients with AMD and EOM, and to identify potential disease-causing rare variants in patients with EOM.

Methods: We performed whole exome sequencing (WES) in 10 ADM and 8 EOM patients. Common genetic variants were analysed in 124 AMD-associated genes. We searched for rare variants causing inherited retinal degenerations (IRD) that resemble AMD to identify potential deleterious variants that might contribute to EOM development.

Results: Eight common genetic risk alleles in 7 AMD-associated genes (*C2, C3, CFH, CFB, TLR3, ARMS2*, *CX3CR1)* were present in the two phenotype groups. Four SNPs (*CFH*-rs1061170, *ARMS2*-rs10490924, *C3*-rs1047286, *TLR3*-rs3775291) showed prevalence in AMD probands. Multiple-filtering pipeline identified 14 rare variants in 11 AMD-genes (*CFHR4, CFB, ABCG1, HMCN1, APOB, CFI, CSMD2, CD36, SLC16A8, ABCA1*, *VLDR*) in EOM probands. Evaluation of IRD genes revealed 7 rare variants (3 of which in *PROM1, SEMA4*, and *COL2A1)*, were predicted to be potentially deleterious based on variant type and in silico tools.

Conclusion: Common AMD-associated variants contribute to AMD and EOM. The early-onset nature of EOM and our results suggest a possible role for genetic rather than environmental factors.

References: de Breuk et al. 2022

Grants:KP-06-N33/12/18.12.2019,D01-285/17.12.2019,D01-395/18.12.2020,D-130/14.06.2022.

Conflict of Interest: None declared.

EP03.015 Analysis of VEGFA SNPs towards susceptibility of Diabetic Retinopathy in North West Indian population

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Background/Objectives: Diabetic retinopathy (DR) is one of the major microvascular complications associated with Type 2 Diabetes (T2D) and is one of the leading causes of blindness worldwide. Vascular endothelial growth factor (VEGF) is a multifunctional cytokine that promotes angiogenesis and vascular permeability. VEGF overexpression promotes vessel endothelial cell proliferation, migration, tube formation, and sprouting, thereby subserving as a contributing factor for DR. *VEGFA*-2549 insertion/deletion (I/D) and *VEGFA* -7 C/T have been implicated in a number of diseases with angiogenic basis, and hence are polymorphisms of particular interest. The present study is an attempt to evaluate the association of selected *VEGFA* polymorphisms with Diabetic Retinopathy.

Methods: In this case-control study 414 DR patients, 422 T2D patients (internal controls) and 401 healthy controls (CN) were analyzed. DNA samples were screened for *VEGFA* -2549I/D and *VEGFA* -7C/T polymorphisms using PCR (polymerase chain reaction) based methods.

Results: The genotypic and allelic frequencies of *VEGFA* -2549I/D polymorphism showed no significant difference between the study groups. The analysis of *VEGFA* -7C/T polymorphism showed significant association in T2D + DR vs. CN ($p = 0.0339$) and DR vs. T2D ($p = 0.002$).

Conclusion: Our results indicated that *VEGFA* -7 T allele showed 2.12-fold risk for T2D over CN and showed protection for DR over T2D.

Grant References: Indian Council of Medical Research- Senior Research Fellowship (ICMR-SRF)

Conflict of Interest: Manroop Singh Buttar ICMR-SRF, Vasudha Sambyal Professor, Kamlesh Guleria Associate Professor.

EP03.016 The Genetic Basis and the Diagnostic Yield of Nonsyndromic Hearing Loss in Qatar

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Background/Objectives: Hearing loss (HL) is the most common sensory defect worldwide, and 50-60% of cases are hereditary. The prevalence of Hereditary Hearing Loss is in Qatar is ~5.2%, and several tests are available including *GJB2* sequencing, gene panel, whole exome sequencing and mitochondrial testing. We aim to investigate the genetic spectrum of Nonsyndromic Hearing Loss (NSHL) in Qatar and to assess the diagnostic yield of these test.

Methods: We performed a retrospective chart review for 127 pediatric patients with NSHL who attended the Department of Adult and Pediatric Medical Genetics at Hamad Medical Corporation (between 2014 and 2019).

Results: 39 of cases were genetically solved resulting in a diagnostic yield of 30.7%. The pathogenesis was attributed to 19 single gene variants detected among 11 genes, and two copy number variants. *GJB2* variants were the most common cause for NSHL (36.8% of solved cases), and the c.35delG variant was the most common (9 cases). We also associated for the first time the variant c.283C>T in *FGF3* with NSHL. 7 case with variants of uncertain significance were solved based on family segregation analysis. The diagnostic yield was significantly associated with the use of *GJB2* sequencing and HL gene panel.

Conclusion: Our work provided new insights into the genetic basis of NSHL in Qatar. In the clinical setting, we recommend performing *GJB2* gene sequencing as a first-tier genetic test for NSHL and HL gene panel as a second-tier genetic test for NSHL, and familial segregation for cases with uncertain variants.

Grant references: NA

Conflict of Interest: None declared.

EP03.017 Clinical and genetic spectrum of coloboma: a proposal for a comprehensive approach to newly diagnosed pediatric patients with coloboma

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Background/Objectives: Coloboma is a defect in the closure of the optic fissure that can affect any part of the eyeball. It has been described in isolation or together with ophthalmological or systemic alterations, forming part of genetic syndromes. We aim to (1) establish the variables with the highest probability of a genetic diagnosis and worse visual outcome (2) develop a comprehensive diagnostic and follow-up protocol.

Methods: Descriptive, retrospective and single-center study. Patients under 18 years of age diagnosed with coloboma under follow-up by a Pediatric Ophthalmology Unit have been selected from January 2012 to December 2020.

Results: We included 214 patients (54% female), 50.9% presented with bilateral coloboma and 62.1% with other ophthalmological alterations (28.5% with microphthalmia). Systemic involvement was observed in 28%: neurological dysfunction (24.8%) and dysmorphic features (18.2%). A molecular diagnosis was reached in 26.2% (CHARGE syndrome was the most prevalent), being the clinical exome the most profitable test (22.2%). The variables associated with the highest probability of a diagnosis were: (1) secondary diagnosis for coloboma ($p < 0.001$), (2) nystagmus ($p < 0.05$), (3) systemic affection ($p < 0.05$), (4) performance of hearing, cardiology, and neuroimaging studies ($p < 0.05$), and (5) Clinical Genetics assessment ($p < 0.001$). Bilaterality, macular chorio-retinal coloboma and neurological, cardiovascular, and growth abnormalities were associated with a worse visual prognosis ($p < 0.05$). A diagnosis and follow-up strategy has been elaborated.

Conclusions: It's important to identify diagnosis and prognosis indicators in patients with coloboma in order to offer genetic counseling, prevent possible complications and improve the quality of life of patients and their families.

Conflict of Interest: None declared.

EP03.018 Asymptomatic retinal dysfunction in alpha-methylacyl-CoA racemase deficiency

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Background/Objectives: Alpha-methylacyl-CoA racemase (AMACR) deficiency is a peroxisomal disorder due to biallelic mutations in *AMACR*. At least 13 genetically confirmed patients have been reported to date. Seven had obvious pigmentary retinopathy; however, for the other six, no retinal phenotype was mentioned. Most of the previously reported patients with clinically obvious pigmentary retinopathy did not have electrophysiology studies, and for the few who did the details were limited. The purpose of this report is to document subtle retinal findings in an additional affected family.

Methods: Retrospective case series (three affected siblings and their unaffected parents).

Results: Three Arab siblings (16, 19, and 22 years old) with prior juvenile cholelithiasis had been diagnosed with AMACR deficiency based on biochemical analysis, whole exome sequencing, and confirmatory segregation analysis (AMACR NM_001167595.1: c.877T>C; p.C293R). For all three, there were no visual complaints, but retinal multimodal imaging and electroretinography suggested subtle retinal dysfunction.

Conclusion: Retinal dysfunction is a parameter that should be measured in patients with known or suspected AMACR deficiency even in the absence of visual symptoms. This may be helpful with clinical diagnosis and monitoring response to dietary interventions.

Conflict of Interest: None declared.

EP03.019 Long-read sequencing for improving the characterization of rare inherited eye diseases

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Background/Objectives: Emerging long-read sequencing (LRS) technologies have great potential to improve the detection of structural variants (SVs) in rare diseases. This work presents our experience with nanopore-based sequencing in studying SVs in a cohort of patients with ophthalmogenetic diseases, mainly inherited retinal dystrophies (IRD).

Methods: Long-read genome sequencing was conducted in 35 patients with inherited eye diseases following short-read sequencing approaches. Libraries were prepared using high-weight molecular DNA following Oxford Nanopore Technologies protocols and sequenced at 30x on a PromethION platform. SVs were validated using Sanger sequencing, CGH, MLPA and/or FISH analysis.

Results: To date, LRS revealed causal SVs in 6 patients, including 3 copy number variants (CNVs) in 3 IRD patients and 3 copy-neutral rearrangements, 2 chromosomal inversions and one translocation, in 3 patients with developmental eye diseases. LRS allowed the detection and breakpoint refinement of micro-deletions affecting one or multiple exons of three IRD-related genes (*EYS, KCNV2* and *MYO7A*). In the latter patient, LRS also revealed a previously overlooked likely pathogenic single nucleotide variant (SNV), thus allowing a complete genetic characterization. Additionally, we identified other variants of unknown significance, such as a chromosome X duplication rearrangement and chromosome 1 retrotransposon insertion.

Conclusion: Our study underscores the utility of this technology for unveiling and characterize SVs involved in ophthalmogenetic diseases and, thus showcasing advantages for the detection and characterization of complex SVs in repetitive regions and other variants in non-coding regions.

Grant References: H2020 EasiGenomics program (PID10291), ISCII (PI19/00321, PI20/00851 and PI22/00321), University Chair UAM-IIS-FJD Genomic Medicine and ONCE.

Conflict of Interest: None declared.

EP03.020 Genetic diagnostic of inherited retinal dystrophies through clinical exome sequencing

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Background/Objectives: Inherited retinal dystrophies (IRD) are a group of diseases that cause visual impairment. There are currently 281 genes related to IDR. However, many patients still remain without a genetic diagnosis. The objective of this study is the genetic diagnosis of IRD patients through clinical exome (CE) sequencing.

Methods: We sequenced the clinical exome "Custom Constitutional Panel 17MB" of 61 IRD patients. The libraries were prepared with the protocol SureSelect XT HS (Agilent) and sequenced in the Illumina platform. The bioinformatic analysis was carried out with the Alissa resource (Agilent) filtering IRD genes. The potential pathogenicity of the variants was evaluated with in silico predictors, databases, and functional assays. Furthermore, we validated the (likely) pathogenic mutations by Sanger sequencing and/or MLPA.

Results: Causative variants were detected in 39/61 IRD patients (64%). The patients were solved with mutations in 25 different genes, being *ABCA4* and *USH2A* the most frequent. Furthermore, one of the patients was solved with variants in *RPE65*, the first IRD gene with a gene therapy approved.

Overall, we identified 93 variants that were classified as (likely) pathogenic using the standards of the American College of Medical Genetics and Genomics. It is worth to mention the novel c.1299A>G;p.(Glu433=) variant in *ABCA4*, with a pathogenic effect confirmed by minigene assay.

Conclusions: CE sequencing is an effective strategy for the genetic diagnosis of genetically heterogeneous diseases, such as IDR. Moreover, the minigenes are a good tool to demonstrate the splicing effect of mutations.

Grant References: PI19/00303, ACIF/2019/252, PI22/00213, FPU20/04736, CP22/00028, ONCE.

Conflict of Interest: Pilar Barberán-Martínez Full, FPU20/04736, Belén García-Bohórquez full, Cinta Navarro-Moreno full, Teresa Jaijo full, Elena Aller full, Gema García-García full, José M. Millán full, IP.

EP03.021 When WGS is key to sort out clinical traits: a case of syndromic hearing loss

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Background: In patients presenting with profound bilateral sensorineural hearing loss, some degree of dysarthria is regularly observed, especially if cochlear implantation is conducted with delay. Vestibular areflexia is regularly associated to hearing loss and can induce delayed walk acquisition and some degree of imbalance.

Case report: A male patient has been investigated in the national reference centre for genetic hearing loss for more than 25

years. Initially, he presented with profound bilateral sensorineural hearing loss, implanted at age 5 years, with persisting dysarthria despite good cochlear implant performance. Fifteen years later, he started complaining of imbalance, leading to the discovery of vestibular areflexia. The association of profound bilateral sensorineural deafness and vestibular areflexia mandated the conduction of several rounds of retinal testing, all showing subnormal results. Lastly, he presents with persisting imbalance with subnormal clinical testing. Imaging revealed mild cerebellar hypoplasia and electrophysiological testing only showed proprioceptive deficit. For years, clinicians taking care of him had trouble delineating which symptoms were consequences of his cochleovestibular disorder and which could be associated symptoms.

At 27 years old, whole genome sequencing revealed a probably pathogenic hemizygous de novo c.212G>C p.(Arg71Pro) *BCAP31* variation. It matches with a mild form of Deafness, dystonia, and cerebral hypomyelination (DDCH) syndrome, due to missense variations in *BCAP31*, as described by Whalen et al., (2021).

Concusion: In this case, reaching a diagnosis through WGS and with the recent published work by Whalen et al., was the only way to understand the patient's clinical presentation.

Conflict of Interest: None declared.

EP03.022 Whole exome sequencing of 20 Spanish families: candidate genes for non-syndromic paediatric cataracts

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Background/Objective: Non-syndromic paediatric cataracts are defined as opacification of the crystalline lens that occurs during the first years of life without affecting other organs. This disease is the most frequent cause of reversible blindness in childhood, so the main objective of this study is to propose new candidate genes responsible for paediatric cataracts that allow a better genetic approach to this pathology.

Methods: We present a whole exome sequencing (WES) study of 20 Spanish families with non-syndromic paediatric cataracts who have obtained a previous inconclusive result in a customised cataract Next Generation Sequencing (NGS) panel. After ophthalmological evaluation and collection of peripheral blood samples of these families, WES was carried out to find genes which could cause the phenotype of the patients.

Results: On the one hand, we found at least one alteration that could cause non-syndromic paediatric cataracts in 45% of the families studied (9/20). Of the 11 variants found in the 9 families, 18.2% (2/11) are classified as pathogenic (P), 72.7% (8/11) as variants of uncertain significance-likely pathogenic (VUS-LP) and 9.1% (1/11) as variants of uncertain significance (VUS). On the other hand, we did not find conclusive results in 55% of the families studied, which suggests that further studies are needed.

Conclusion: The results of this WES study allow us to propose *LONP1, ACACA, TRPM1, CLIC5, HSPE1, ODF1, PIKFYVE, CHMP4A, AQP5* and *loci* 2q37 as possible candidate genes for non-syndromic paediatric cataracts.

Grant References: Instituto de Salud Carlos III (ISCIII) project 507 "18/1234" and ONCE grant number 2020/0197782.

Conflict of Interest: patricia rodríguez-solana: None declared, carmen gonzález-atienza: None declared, eloísa sánchez-cazorla: None declared, natalia arruti: None declared, maría nieves-moreno: None declared, Rocío Mena full, principal investigator, marta guerrero-carretero: None declared, Ángela del Pozo: None declared, victoria e.f.montaño: None declared, lucia de dios-blázquez: None declared, Celia Fernandez Alcalde: None declared, María de los Ángeles Gómez Cano: None declared, Luna Delgado de Mora: None declared, Susana Noval: None declared, Elena Vallespín: None declared.

EP03.023 Aiming at the development of the optimal targeted approach to genetic diagnostics of retinal dystrophies

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Previous experiences concerning the genetic diagnosis of inherited retinal dystrophies (IRD), based on whole exome data and the first targeted panel approach, indicated several aspects worth consideration in the development process. Here we present our insights based on the results for the second group of patients.

NGS analysis based on a Twist Bioscience custom panel targeting coding regions of 343 IRD genes, along with selected deep intronic regions and mtDNA sequence, was applied to identify genetic causes of IRD in a group of Polish patients (N = 272). Bioinformatics involved the GATK(v4) best practice pipeline. The CNV analysis was conducted using CoNVaDING and GATK GermlineCNVCaller. The variant classification was performed according to ACMG/AMP guidelines with the help of the in-house interpretation tool BroVar(v3).

Positive results were provided for 176 patients (64.71%). In several cases, this was possible due to the analysis of deep intronic regions. An increase in mean coverage levels (200-250x) and uniformity (F80 1.2-1.4) enabled noise reduction during the CNV analysis (10 patients with the contribution of CNVs in the diagnosis, in two cases diagnosis was based solely on CNV variants). Coverage in the difficult *RPGR* ORF15 region was improved as a result of additional probe tiling.

Presented changes in the panel construction, as well as the improvement in the QC parameters and the CNV analysis, resulted in a 5% increase in the diagnostic yield compared to previous approaches. Four of the patients could be potentially treated with gene therapy as pathogenic variants were uncovered in the *RPE65* gene.

Conflict of Interest: Ewa Matczyńska Employed in Genomed S.A., Marta Beć-Gajowniczek Employed in Genomed S.A., Larysa Sivitskaya Employed in Genomed S.A., Elżbieta Gregorczyk Employed in Genomed S.A., Ewa Suchecka Employed in Genomed S.A., Przemysław Łyszkiewicz Employed in Genomed S.A., Robert Szymańczak Employed in Genomed S.A., Maria Jędrzejowska Employed in Genomed S.A., Sławomir Teper: None declared,

Maciej Krawczyński Employed in Center of Medical Genetics Genesis, Anna Boguszewska-Chachulska Employed in Genomed S.A., Member of Genomed S.A. advisory board.

EP03.024 Analysis of 40 index patients with non-syndromic and syndromic hearing impairment using whole exome sequencing (WES) discovers variants in rare or recently identified causative genes

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Background: Hearing impairment is a common congenital sensory disorder, which is genetically heterogeneous. Identification of the causative variants underlying hearing impairment is challenging, since >100 different genes for non-syndromic hearing impairment and >450 different genes for syndromic hearing impairment have been reported so far.

Methods: In this study, 40 index patients with hearing impairment and variable additional clinical features underwent whole exome Next-Generation Sequencing (WES). Patients were prescreened for DFNB1 and *SLC26A4*-related hearing impairment by Sanger sequencing and for most patients also by analyzing 90-150 genes in a genepanel for non-syndromic and syndromic hearing impairment using an Illumina platform.

Results: Of the 40 patients sequenced, we were able to detect causative variants in eleven cases. The study emphasizes the genetic heterogeneity of hearing impairment since the variants were found in different rare and relatively recently identified causative hearing loss genes such as: *CDK5RAP2*, *CEP78*, *CEP250*, *COL11A1*, *COL9A3*, *FDXR*, and *TFAP2A*. In total, thirteen different likely pathogenic or pathogenic variants were detected, and nine variants were novel (absent from the literature).

Conclusion: Whole exome sequencing, after negative gene panel analysis, allowed us to detect causative variants in eleven of 40 tested cases. The causative variants were found in rare and/or recently identified causative hearing impairment genes. Regarding the non-solved cases, selected families will be analyzed by whole genome sequencing.

Conflict of Interest: None declared.

EP03.025 Bilateral optic nerve coloboma in a floppy infant as a first presentation of Bosch-Boonstra-Schaaf syndrome

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Background: Bosch-Boonstra-Schaaf optic atrophy syndrome - BBSOAS (OMIM: 615722, ORPHA 401777) is a rare, autosomal dominantly inherited syndrome characterized by moderate developmental delay, dysmorphic features and significant visual impairment resulted from optic nerve atrophy. Patients with BBSOAS carry heterozygous mutations in *NR2F1* gene, encoding a nuclear hormone receptor and transcriptional regulator.

Methods: The material for the study was DNA isolated from the peripheral blood leukocytes of a symptomatic patient using automated Maxwell system (Promega). The whole exome sequencing (WES) was performed using Twist Human Core Exome kit (Twist Bioscience). The result was confirmed by Sanger sequencing.

Results: We report a 7-month-old infant who was tested due to severe neurodevelopmental delay, generalized hypotonia and bilateral optic nerve coloboma. The first symptoms were observed at the age of 2 weeks when anisocoria occurred. WES revealed that the patient carries a de novo, heterozygotes, pathogenic variant located within DNA-binding domain of *NR2F1* gene (NM_005654.6: p.Met151Thr) which causes the phenotype of BBSOAS.

Bioinformatics analysis identified the *NR2F1* variant as pathogenic according to the ACMG recommendation. The variant was not found in database collecting polymorphic gene variants (Gnomad, dbSNP), while it is described as probably pathogenic in the ClinVar database.

Conclusions: In conclusion, we found pathogenic variant of *NR2F1* in patient with severe neurodevelopmental delay and coloboma. The presented data confirms the whole exome sequencing is a highly useful tool to identify genetic background of severe optic nerve abnormality in children with a no family history of visual impairment.

Conflict of Interest: None declared.

EP03.026 Genetic etiology of non-syndromic hearing loss in Hungarian patients with cochlear implant

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Introduction: Hearing loss is the most prevalent sensory disorder, affecting millions of people worldwide with an incidence of 0.1-0.2% in the newborn population. Most congenital nonsyndromic hearing loss (NSHL) cases are caused by hereditary factors. Previously, the majority of studies focused on Connexin 26 (encoded by the *GJB2* gene), however with the improvement of new-generation sequencing (NGS) methods, the identification of novel variants has shown a great improvement in NSHL.

The purpose of this study was to elaborate the most effective screening process in a Central-European population of NSHL.

Methods: 139 non-related NSHL patients were enrolled in our study. All patients underwent cochlear implantation or were candidates for it. A stepwise comprehensive genetic approach, including bidirectional capillary sequencing, MLPA and an NGS panel of 108 HL genes was used in the study.

Results: Genetic diagnosis could be established in 99 patients, resulting in an overall 71% diagnostic yield. Targeted sequencing and MLPA could identify the genetic background of 50% of the cases and the NGS panel added another 21% to this diagnostic yield. The vast majority of the diagnosed cases showed autosomal recessive inheritance (86%). The distribution of GJB2 and non-GJB2-cases was 68% vs. 32%, respectively.

Conclusion: The implementation of this step-wise analysis markedly increased our diagnostic yield and proved to be cost-effective as well. To further improve the diagnostic yield, the implementation of whole exome sequencing (WES) may be taken into consideration.

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Conflict of Interest: None declared.

EP03.027 Exploring the Role of CERKL and RIMS1 in Inherited Retinal Disorders: A Case report

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Background: We present the case of a 16-year-old man from Pakistan who experienced a progressive and severe visual night vision impairment and maculopathy. The patient presented with bilateral scotoma and alopecia areata; a family history of early-onset blindness was referred in a first-degree cousin. Given the personal and familial history of visual impairment a clinical exome was performed.

Method: SureSelect-Agilent Custom-Constitutional-Panel-17Mb encompassing 5219 genes. Virtual panel "Inherited retinal diseases" (235 genes).

Results: The analysis led to the identification of a homozygous likely pathogenic variant in the CERKL gene (NM_201548.5: c.1073+3_1073+6del, rs1553513437) and a nonsense variant in RIMS1 gene [NM_014989.7: c.3391C>T - p.(Arg1131Ter)]. The former is predicted to disrupt the canonical donor site of CERKL exon 7 with the retention within the transcript of the 79 nucleotides from intron 7. The latter, never reported previously, should introduce a premature termination codon within exon 22 of a gene for which the role in inherited retinal disorders is disputed.

Conclusion: RIMS1 has been considered a candidate gene for dominant cone-rod dystrophy 7 (MIM #603649), but recent evidence challenges the causative role in the disease. The identification of a homozygous pathogenic/likely-pathogenic variant in the CERKL gene seems to explain the patient's phenotype and challenges the previously reported causative role of RMS1 in inherited retinal disorders. However, we cannot exclude a possible contributory role of the nonsense RIMS1 variant in the clinical expression of our patient. Additional data and segregation analyses are needed to better understand the role of RIMS1 in retinal disorders.

Conflict of Interest: None declared.

EP03.028 Hypoacusia and GJB2 gene: a short report of the c.-22-2A>C variant

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Biallelic pathogenic variants of GJB2 gene (NM_004004.6) are responsible for autosomal recessive nonsyndromic hypoacusia.

The c.-22-2A > C likely pathogenic variant is a hypomorphic allele, affecting the splicing-acceptor-site of exon2, supposed to trigger a secondary upstream acceptor-site with a longer 5'UTR and a decreased expression of CX26-protein. Expected phenotype is milder.

We report two cases with c.-22-2A > C substitution.

PatientA. 26-year-old female, mild bilateral sensorineural hypoacusia on higher frequencies, with late childhood onset and slow age-related worsening. GJB2 compound heterozygote for c.246C>G and c.-22-2A > C variants: the first inherited from the 64-year-old father with right-ear hypoacusia and tinnitus of unknown origin; the latter variant inherited from the 59-year-old mother, with normal-hearing.

PatientB. 40-year-old female, presently pregnant. Severe bilateral sensorineural deafness with sudden onset at 22 years (autoimmunity and vascular causes were excluded, no benefit with corticosteroid therapy). NGS analysis of 101 deafness-related genes revealed only the heterozygous GJB2 c.-22-2A > C variant. Since B patient's partner is also a healthy carrier of the c.35del GJB2 pathogenic variant, prenatal diagnosis was offered: both variants were found in the fetus.

Our data support the hypothesis that heterozygote carriers of the c.-22-2A > C variant are usually not hearing impaired, while compound heterozygotes develop non-congenital mild to moderate hypoacusia. However, we cannot rule out a possible contributory role of this variant in hearing loss if associated with unknown other genetic or non-genetic causes. Regarding patientB's pregnancy, we can't predict the audiological phenotype of the unborn child but, according to the available data, hypoacusia is expected to occur during their life.

Conflict of Interest: None declared.

EP03.029 Minigenes and long read sequencing for the analysis of splicing variants located in acceptor sites of the PAX6 gene

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Background/Objectives: *PAX6* is a transcription factor involved in ocular development whose alterations are associated with congenital aniridia. Approximately 15% of *PAX6* mutations affect canonical splicing sites. Generally, spliceogenic variants result in exon skipping but also might enhance the use of unexpected cryptic sites. Our work aimed to study the spliceogenic effects of different variants in the canonical acceptors of *PAX6*.

Methods: From our aniridia cohort or mutational databases, we selected variants located in the nucleotides -1 and -9, close to the canonical acceptor of any *PAX6* exon. After in silico analysis, potential spliceogenic variants were in vitro assessed through exon-trapping minigenes. Three multiexonic minigenes were generated with the genomic sequences of *PAX6* exons 5-7, 8-11 and 10-13. Variants were introduced by directed mutagenesis and then, mutated and wild-type minigenes were transfected into HEK293 cells. Subsequently, total RNA was extracted, and splicing patterns were analysed by RT-PCR using long-read sequencing in an Oxford Nanopore MiNION device.

Results: Finally, seven variants on acceptors showing potential splicing effects were analysed in vitro. Of these, half produced exon skipping while the remaining half also participated in the activation of exonic or intronic cryptic acceptors causing partial exon skipping.

Conclusion: Splicing variants located in canonical regions of different exons can affect splicing differently despite being in the same gene. Therefore, in vitro assays with minigenes are a good approach to test their involvement in the splicing process and their pathogenicity in aniridia.

Grants: Spanish Health Institute Carlos III (PI17_01164 and PI20_00851) and ONCE.

Conflict of Interest: None declared.

EP03.030 NGS genetic testing of a Romanian patient and his family identified a novel OPA1 gene mutation associated with hereditary optic atrophy

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Introduction: Hereditary optic neuropathy refers to a group of inherited diseases that cause optic nerve atrophy, a major cause of visual impairment.

Materials and Methods: A 15-year-old male patient was recently diagnosed with optic atrophy. Sequence analysis and deletion/duplication testing of the 772 genes associated with hereditary ophthalmic disease were performed by Next Generation Sequencing (NGS) was used.

Results: A novel variant, likely pathogenic, in the OPA1 gene was identified in this case. Mutations in this gene have been associated with optic atrophy type 1, an autosomal dominant inherited condition. Six more autosomal recessive genes have been identified in heterozygosity. The pathogen variant of the OPA1 gene (3q28-q29, OMIM:165500) that causes the patient's disease is present in both his mother and the probate's sister. Maternal grandparents do not have the mutation in the OPA1 gene, therefore the mutation occurred de novo in the patient's mother she passed it on to both children.

Sequencing analysis of OPA1 gene revealed a splicing mutation (c.1589+1dup), in intron 16, in heterozygous state, that is expected to disrupt RNA splicing. This variant is not present in population databases and has not been reported in the literature in individuals affected with OPA1-related conditions.

Conclusions: The genetic cause of hereditary optic atrophy in the proband has been identified. Extensive family testing has allowed the diagnosis of two other relatives with the same disease, one of them pre-symptomatic. All this information is especially useful in the post-test genetic counseling and for a prenatal diagnosis in the future.

Conflict of Interest: None declared.

EP03.031 Early diagnosis of syndromic deafness by whole exome sequencing

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Background: Hearing loss is the most common sensory disorder worldwide. Up to 30% of hereditary hearing impairments are syndromic and affect other organs including the kidneys, eyes and heart. Currently, genetic diagnosis of congenital deafness is limited to high penetrance non-syndromic gene mutational status, like *GJB2*, *GJB5* and *OTOF*. However, it is a very heterogeneous entity, and these genes only explain around 25% of new congenital deafness cases. The aim of this study was to identify rare mutations associated with congenital deafness using whole exome sequencing.

Methodology: Genomic DNA was extracted from peripheral blood leukocytes in a cohort of 12 patients. WES was performed to identify germline mutations. Selected mutations were confirmed by Sanger sequencing and studied in samples from patients' relatives.

Results: We selected two patients with hearing loss as their only clinical manifestation, where we identified mutations in syndromic congenital deafness genes. One patient carried the c.1772G>T;p.Arg555Leu *KCNQ1* heterozygous pathogenic mutation, associated with long QT syndrome. Manifestations of this syndrome include prolongation of the QT interval, ventricular arrhythmias and high risk of sudden death. The other carried the c.23G>A;pArg8Gln *BSND* heterozygous pathogenic mutation, related to Bartter syndrome, characterized by congenital deafness and renal affection. This early genetic diagnosis allows prevention, a better follow-up and the improvement of the treatment of these patients.

Conclusion: WES technique is a useful tool to identify mutations in genes with low penetrance, achieving an early diagnosis of syndromic deafness before severe manifestations appear.

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Conflict of Interest: None declared.

EP03.032 Revisiting PRDM12 polyalanine tract expansion in chronic mutilating itch of MiTES

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Background: Expansion of PRDM12 alanine repeat can cause two opposite phenotypes: midfacial toddler excoriation syndrome (MiTES) associated with homozygous 18 alanines and congenital insensitivity to pain (CIP) that might be linked to 19 alanines. MiTES is characterised by restricted congenital itch with normal pain sensing whilst in CIP pain sensation is absent but itch behaviour is normal.

Methods: We assessed 4 new cases of MiTES and 3 possible MiTES with atypical features and followed up 16 previous MiTES cases. Using HEK293 expressing normal, MiTES, or CIP alanine repeats (12, 18 and 19 alanines respectively), we examined aggregation and subcellular localisation by confocal microscopy.

Results: Phenotype study shows that chronic mutilating itch in MiTES is specific to the early years of life and entirely separate from PRDM12-CIP phenotype. The genotype-phenotype study shows that 7 – 15 alanines in PRDM12 results in normal phenotype, expansion to 17 – 18 alanines is associated with MiTES, while 19 alanines leads to CIP. The atypical MiTES having unusual clinical features have normal genotype, confirming that they are not MiTES. Both 18- and 19-alanine cell lines demonstrate aggregates that are predominantly nuclear, but no clear distinction between the 18 and 19-alanine aggregation phenotype.

Conclusion: This study validates the diagnostic criteria of MiTES as a congenital itch condition caused by PRDM12 polyalanine tract expansion. The MiTES and CIP exhibit distinct phenotypes despite their genotypes varying by a single amino acid. MiTES could be a protein aggregation disease.

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Conflict of Interest: None declared.

EP03.033 Functional validation of a novel variant in the MYO6 gene identified in a family with postlingual non-syndromic hearing loss from Argentina

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Hereditary hearing loss (HL) is the most common sensory disorder affecting 1 in 500 newborn children. There are more than 100 hundred genes related to non-syndromic hearing impairment, therefore, the Whole Exome Sequencing (WES) technique becomes the best option to study all the genes at once. However, analysis of novel variants, in particular missense changes which can lead to a spectrum of phenotypes, is not always straightforward. In this work, we aimed to functionally validate a novel variant detected in a family case with HL.

After performing WES followed by the variant priorization process and segregation analysis, the novel variant c.2782C>A p.Arg928Ser in *MYO*6 gene was identified in a family with postlingual non-syndromic hearing loss. To further analyse the functional implication of this variant, protein modeling with the AlphaFold2 software was performed, revealing a change in the electrostatic charge on the surface of the single alpha helix domain. Then, in order to functionally validate the novel variant, we carried out a knockdown phenotype rescue assay in zebrafish by inhibiting the expression of the orthologous myo6b gene. Injection with wild type MYO6 mRNA rescued the phenotype whereas the mutant MYO6 (c.2782C>A) mRNA had no effect, demonstrating the deleterious effect of this variant on the auditory system. These results highlight the importance of a combined strategy in order to identify candidate variants, as well as the in silico and in vivo studies we performed to infer their pathogenicity and better understand the mechanisms underlying the physiopathology.

Conflict of Interest: None declared.

EP04 Internal Organs & Endocrinology (Lung, Kidney, Liver, Gastrointestinal)

EP04.001 Simultaneously monitoring liver response and hepatitis B virus infections during pregnancy through plasma cell-free RNA sequencing

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Background: Hepatitis B virus (HBV) can be transmitted vertically to the fetus, and pregnancies with HBV infection should be closely monitored for HBV load and maternal liver function. Plasma cell-free RNA (cfRNA) encompasses a broad spectrum of RNA species that can be derived from both human cells and microbes, therefore providing a non-invasive means to simultaneously monitor liver response and HBV RNA level during pregnancy.

Methods: We profiled cfRNA in 211 plasma samples from pregnant women with HBV infection ($n = 40$) and healthy donors ($n = 171$). We then analyzed the changes of both the human transcriptome and the HBV RNA of plasma of HBV patients.

Results: We demonstrated that cfRNA can be used to identify HBV infections. The expression level of HBV in the patients with HBeAg positive was higher than in patients with HBeAg negative, and show a positive correlation with clinical liver functional indicators. The comparative analysis identified a group of genes that altered their expression level in the HBV patient, with function annotations consistent with apoptosis, biosynthesis and metabolism, transmembrane transport, and complement and coagulation cascades. In addition, cfRNA signatures from the liver increased in the HBV patients and correlated with the HBV RNA level.

Conclusions: Plasma cfRNA demonstrates the potential to simultaneously monitor liver response and HBV infections during pregnancy, which will be a benefit in HBV prevention.

Grants: none

Conflict of Interest: None declared.

EP04.002 Gene expression profiling through whole transcriptome sequencing predicts immunological mechanisms in Hirschsprung associated enterocolitis

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Hirschsprung's disease (HSCR) is a congenital gut malformation caused by a lack of innervation. One of the most serious is HSCR associated enterocolitis (HAEC), a potentially lethal condition with 30% of incidence. However, the causes of HAEC are still unknown and the onset is difficult to predict.

We have carried out a transcriptome analysis on Intraepithelial lymphocytes (IEL) derived from gut biopsies and on Peripheral Blood Monocyte Cells (PBMCs) from HAEC, HSCR-only and pediatric patients affected by neither Hirschsprung, nor inflammatory related diseases.

The analysis on the IELs showed a clear clustering between the three groups of patients. Several transcripts were nominally significantly over- and under-expressed in HAEC vs HSCR-only patients, among which several transcripts also involved in Inflammatory Bowel Disease (IBDs) pathogenesis. Accordingly, we found an enrichment in immune and inflammatory pathways in the HAEC group of the Intraepithelial lymphocytes. The transcriptome analysis on the gut PBMCs from a subsample of the same patients is still on progress and is aimed to define if PBMCs might be a reliable biomarker. Preliminary results show a good concordance between IELs and PBMCs.

The finding of a shared genetic background with other inflammatory disorders affecting the gut, and the evaluation of gene networks and pathways involved in inflammation provides further knowledge on the mechanisms of gut inflammation. Eventually confirming the concordance between IELs and PBMCs would allow the much more feasible use of blood instead of gut biopsies, making the replication of the present findings in other and bigger samples more reliable.

Conflict of Interest: None declared.

EP04.003 Genetics of rare kidney disease focal segmental glomerulosclerosis (FSGS) in adult central European population

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Introduction: Focal segmental glomerularsclerosis (FSGS) is a clinically and genetically heterogeneous entity that affects glomerular function of the kidney and manifests itself by proteinuria. It often leads to loss of kidney function, accounting for about 15 % of patients with renal failure. A portion of the FSGS cases is of a monogenic etiology.

Methods: Multicentric study, cohort: adult patients with FSGS confirmed by kidney biopsy, FSGS family history or a sporadic primary FSGS resistant to steroids. Methods: whole exome sequencing, whole genome sequencing, bioinformatics.

Results: The current cohort consists of 239 patients of average age 48 years. The DNA analysis revealed pathogenic variants in genes associated with FSGS at 13,4 % unrelated patients. Genetic testing yielded diagnosis in 40% of FSGS families and in 10% of sporadic cases. The most frequent mutations were variants in COL4A genes (50%), compound heterozygous mutations in NPHS2 gene (17%) and in FSGS families *INF2* gene variants (10%). 36% of variants are novel. By the statistical enrichment analysis we identified two Czech-specific founder mutations within *NHPS2* (c.G868A) and *COL4A4* (c.G1598A). The latter variant is specific for patients with Roma ancestry. The most significantly enriched gene with rare mutations within our cohort is *SMARCAL1*.

Conclusion: This is the first large-scale adult-onset genetic FSGS study in Czech population. It brings new insights into genetic and clinical features of the FSGS in adults and the results will improve personalized medical care.

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EP04.004 Impact of bread diet on intestinal dysbiosis and irritable bowel syndrome assessed by high-throughput sequencing of the 16S rRNA gene: A pilot study

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Background/Objectives: Gut microbiota may be involved in the presence of Irritable Bowel Syndrome (IBS)-like symptomatology in remission Ulcerative Colitis (UC) patients. High-throughput sequencing of the 16S rRNA gene allows an agnostic characterisation of the gut microbiota. Bread is an important source of dietary fibre, and a potential microbiota modulator. We aimed to assess the effect of a traditionally elaborated and baked bread, in comparison to a modern elaboration bread, in changing the gut

microbiota and relieving IBS-like symptoms in patients with quiescent UC.

Methods: Thirty-one UC patients in remission with IBS-like symptoms were randomly assigned to a dietary intervention with 200 g/d of either traditional or modern bread for 8 weeks. Changes in faecal microbiota composition were assessed by high-throughput sequencing of the 16S rRNA gene. Clinical symptomatology was tested using questionnaires and inflammatory parameters.

Results: A decrease in IBS-like symptomatology was observed after both the treatment (p-value = 0.003) and control bread (p-value < 0.001) interventions. The treatment bread suggestively reduced the Firmicutes/Bacteroidetes ratio (p-value = 0.058). In addition, the Firmicutes/Bacteroidetes ratio seemed to be associated with abdominal pain mitigation (p-value = 0.059).

Conclusions: The intake of a bread baked using traditional elaboration decreased the Firmicutes/Bacteroidetes ratio, which seemed to be associated with improving IBS-like symptoms in quiescent UC patients. These findings suggest that the traditional bread elaboration has a potential prebiotic effect in improving gut health.

Grant reference: Spanish Ministry of Economy, RETOS program (RTC-2017-6467-2).

Conflict of Interest: None declared.

EP04.005 Linking genetic testing and histopathologic evaluation of the kidney as a way tosuccessfully diagnose Alport syndrome

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Background: Alport syndrome (AS) is a genetic collagen IV disease that causes kidney, hearing and vision damage. In the past, it was diagnosed mainly by kidney biopsy, but now more often by genetic testing. This study aims to combine these two techniques for the AS diagnosis and to test the relationship between the presence of pathogenic variants and renal biopsy lesions.

Methods: The study group included 57 patients (F:M; 31:26), whose average age at the time of genetic diagnosis was 24 years. Genetic studies were performed by NGS technique and confirmed by Sanger sequencing. Kidney biopsy material was analyzed by optical and electron microscopy and immunomorphological studies.

Results: In a group of 57 patients with genetically confirmed AS, 13 underwent renal biopsy prior to genetic diagnosis. Among the biopsies performed, seven led to the suspicion of AS. The most common changes observed were thickening and thinning of the basement membrane and inflammatory infiltrates. Among seven patients in whom the correct suspicion was established after renal biopsy, the most common finding was a pathogenic variant in the COL4A3 gene (42.8%). Two patients with the most severe clinical course of AS showed inflammatory infiltrates on histopathological examination without AS-specific lesions.

Conclusion: In the results presented here, there was no clear relation between the pathogenic variant found by genetic testing in patients with Alport syndrome and the microscopic image of the kidney. Despite the increasing progress of histopathological testing, it seems that it cannot be an alternative to genetic testing in this disease.

Conflict of Interest: None declared.

EP04.006 Clinical exome sequencing as a tool for genetic diagnosis of Alport syndrome and thin basement membrane disease

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Background/Objectives: Pathogenic variants in *COL4A3*, *COL4A4* and *COL4A5* are associated with Alport syndrome (AS) and thin basement membrane disease (TBMD). Patients with AS show glomerular nephropathy and end-stage renal disease, frequently associated with sensorineural deafness/ocular anomalies, while in TBMD most patients are asymptomatic and have normal renal function. We describe a cohort of patients with AS/TBMD.

Method: Clinical exome sequencing (CES) was performed on a cohort of 95 patients with clinical features suggestive of AS referred to our center from 2019 to 2022.

Results: We identified causative (C4/C5) variants in 43 (45.3%) patients. We confirmed genetic diagnosis of TBMD in 16 (37.2%) patients, of whom 12 (75.0%) with monoallelic variants in *COL4A4* and 4 (25.0%) in *COL4A3*; 19 (44.2%) patients with AS had variants in *COL4A3* ($n = 5$, 26.3%), *COL4A4* ($n = 1$, 5.3%) and *COL4A5* (6 females, 7 males, 68.4%). In 2 (4.7%) cases we found variants in *COL4A1* and *MYH9*, associated with other renal diseases. Finally, we found digenic AS in 6 (13.9%) patients. Of note, 3 (50.0%) of them with coexisting variants in *COL4A5/COL4A4* and one (16.7%) in *COL4A3/COL4A4* had early renal failure. In contrast, 2 male patients (33.3%), with *COL4A5/COL4A3* missense variants showed a mild phenotype. The remaining 16 (16.8%) patients had variants of uncertain significance (C3) and 36 (37.9%) tested negative.

Conclusion: CES is a powerful tool to define the AS/TBMD molecular diagnosis, to identify digenic AS and to find variants in other renal genes, improving clinical and therapeutic management.

Grants: Ministero dell'Istruzione, Progetto di Eccellenza Dipartimentale #D15D18000410001

Conflict of Interest: None declared.

EP04.007 Update on Alport syndrome in our healthcare area

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BACKGROUND/OBJETIVES: Alport syndrome (AS) is considered a rare disease and is underdiagnosed partly due to its clinical heterogeneity and variable expressivity. It can be transmitted X-linked (80%), autosomal recessive (15%) or dominant (5%).

METHODS: Descriptive observational study of 386 individuals who underwent genetic study using a panel of 44 genes related to hereditary renal diseases (SOPHiA Genetics).

Sequencing of the libraries was performed on a MiSeq (Illumina Inc), bioinformatics analysis of the data and variant annotation was performed using SOPHiA DDM 5.8.0.3 software, and variant review by querying major databases (ClinVar, Exac, HGMD, NCBI, PKD Foundation, LOVD).

RESULTS: 132 informative results were obtained, 20% corresponding to Alport syndrome, of which: 35% developed deafness, whereas only 7% had vision loss. One third required renal replacement therapy, in which 70% had variants in COL4A3 or COL4A4.

The correlation between clinical and genetic diagnosis was only 11%, the reasons for the study being mainly unfiliated chronic kidney disease, segmental and focal glomerulosclerosis and even polycystic kidney disease.

65% of the variants found were in heterozygosis and dominant inheritance, with COL4A3 being the most frequently implicated gene.

CONCLUSION: We observed that in AS there is a low degree of concordance between clinical and genetic diagnosis, making the genetic study a basic tool for its correct diagnosis.

In our health care setting, in contrast to the scientific evidence published to date, autosomal dominant inheritance is the majority.

We found few cases of recessive transmission, which could be associated with the variable expressivity of the syndrome.

Conflict of Interest: None declared.

EP04.008 A novel TRPM6 gene variant (c.531delA) causing hypomagnesemia with secondary hypocalcemia

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Background: Primary hypomagnesemia with secondary hypocalcemia (HSH) is a rare autosomal recessive disorder caused by mutations in *TRPM6* gene encoding TRPM6 (transient receptor potential melastatin 6). This is a channel responsible for epithelial magnesium absorbtion in colon and renal distal convoluted tube.

Objective: To identify and characterize the genetic cause of a marked hypomagnesemia associated with hypocalcemia in a 4 months old Romanian girl.

Methods: The genomic DNA of the proband was extracted for Trusight One Sequencing Panel (Illumina) clinical exome sequencing and analysis of candidate causal variants.

Results: We identified a novel homozygous frameshift variant in *TRPM6* gene, c.531delA (p.Gly178fs) which pre-maturely terminates protein and results into a truncated product of 178 amino acid residues instead of 2022. This variant is subject to degradation by nonsense-mediated decay (NMD Prediction Tool (shinyapps.io)). In silico analysis using MutationTaster predicted this variant as disease-causing and was classified as likely pathogenic according to ACMG guidelines. The variant has not yet been described in the medical literature and has not been identified in population databases (dbSNP, gnomAD, ExAC, 1000 Genomes).

Conclusion: Our results shows that a novel frameshift variant in *TRPM6* could be responsible for HSH in this patient. The finding has expanded the mutation spectrum of *TRPM6* gene.

Conflict of Interest: None declared.

EP04.009 Novel TCF7L2 familial linkage and association with Type 2 diabetes, depression, and their comorbidity

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Objectives: Alterations in the activity of transcription factor 7-like 2 (TCF7L2) generate defects previously associated with neuropsychiatric disorders. We investigated the role of the *TCF7L2* gene with major depressive disorder (MDD), type 2 diabetes (T2D), and MDD-T2D comorbidity. We tested whether *TCF7L2* is linked to and/or in linkage disequilibrium (LD, namely linkage plus association) with MDD, T2D, and MDD-T2D comorbidity.

Patients and Methods: In 212 families with T2D and MDD and from the Italian population, we analyzed 80 microarray-based SNPs using Pseudomarker software for linkage to and LD with T2D and MDD under the recessive model with complete penetrance (R1). In a secondary analysis, we tested the variants under the models dominant with complete penetrance (D1), recessive with incomplete penetrance (R2), and recessive with incomplete penetrance (R2).

Results: We found several novel linkage signals and genetic LD-based associations. In addition, we detected two new transcription-factor (TF) binding sites created by two found risk variants: the MDD-risk variant rs12255179 creates a new TF-binding site for the CCAAT/enhancer-binding protein α (C/EBPα), and the T2D-risk variant rs61872794 creates a new TF-binding site for the organic cation-uptake transporter (OCT1). Both new binding sites are related to insulin metabolism.

Conclusions: These results highlight the cross-interactivity between T2D and MDD. Further replication is needed in diverse ethnic groups.

Conflict of Interest: None declared.

EP04.010 Case report: Tuberous sclerosis/polycystic kidney disease contiguous gene syndrome case originated by atypical non-contiguous PDK1/TSC2 deletions

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Background: Mutations in PKD1 or PKD2 cause autosomal dominant polycystic kidney disease (ADPKD) while mutations in TSC1 or TSC2 cause the tuberous sclerosis complex (TSC). PKD1 lies immediately adjacent to TSC2 and large deletions can result in the PKD1/TSC2 contiguous gene deletion syndrome (CGS) with a distinctive phenotype of early onset PKD plus various manifestations of TSC.

Case presentation and Methods: A 16 month-old girl, first child of healthy non-consanguineous parents, was initially evaluated in the neonatal period due to cystic kidney disease. Her course was complicated with several TSC associated manifestations (West syndrome, Wolf-Parkinson-White syndrome, subependymal nodules and cortical tubers on brain MRI) and a likely diagnosis of PKD1/TSC2 CGS was considered. Molecular investigations to confirm the diagnosis were undertaken.

Results: An initial 60K array-CGH was normal. Whole exome sequencing (WES) detected 2 heterozygous variants of unknown significance (VOUS), a maternally inherited variant in PKD1(NM_001009944.2): c.5245G>A; p.V1749I and a de novo variant in TSC2 (NM_000548.4:): c.4166C>G p.P1389R, not allowing full confirmation of the clinical diagnosis. Subsequent reanalysis of WES data allowed the detection of 2 de novo non-contiguous partial gene deletions of both PKD1 (exons 1 to 10) and TSC2 (exons 27 to 32), confirmed by MLPA.

Conclusions: We present a case with clinical manifestations of PKD1/TSC2 CGS caused by 2 non-contiguous partial gene PKD1 and TSC2 deletions, probably mediated by a complex genomic rearrangement. Further results and discussion on the possible mechanism will be presented.

Conflict of Interest: None declared.

EP04.011 Case study: the key role of whole-genome sequencing (WGS) in the diagnostics of kidney disorders

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We present eleven patients referred for genetic testing based on symptoms of polycystic kidney disease, nephrocalcinosis, glomerulopathies and tubulopathies. For the purpose of the study, whole-genome sequencing and targeted analysis of 906 genes associated with kidney disorders was performed.

Five patients received genetic diagnosis. A three-year old boy was compound heterozygous carrier of one pathogenic and one likely pathogenic variant (c.1397G>A and c.3545G>C) in the *PKHD1* gene, associated with polycystic kidney disease 4. A likely pathogenic variant in *PKD1*:c.11390A>G was found in a 45-year-old male with family history of polycystic kidney disease type 1. Another proband harbored one likely pathogenic variant in *COL4A5*:c.4538del, associated with X-linked Alport syndrome 1. Two unrelated children carried compound heterozygous pathogenic mutations in *CYP24A1* (c.428_430del and c.443T>C) causing infantile hypercalcemia 1.

Two variants of unknown significance (VUS) that could be related to two of our patients' phenotypes were detected: *HOGA1*:c.410C>T, combined with a known pathogenic mutation (HOGA1:c.700+5G > T) causing primary hyperoxaluria 3 and *LRP5*:c.3403C>T, associated with polycystic liver disease 4 with or without kidney cysts.

One incidental finding was detected in a 15-year-old girl, referred for genetic analysis based on clinical evidence of proteinuria, intellectual disability and dysmorphic features with unknown etiology. The patient carried one pathogenic homozygous missense variant in the *TAF6* gene (c.212T>C) and was diagnosed with the ultrarare Alazami-Yuan syndrome.

Clarification of our patients' genetic diagnosis provided precise genetic counseling for their families, allowed doctors to take appropriate measures for treatment and gave options for disease prevention in the future offspring.

Conflict of Interest: None declared.

EP04.012 The role of oxidative stress in chronic pancreatitis and pancreatic cancer risk

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Background/Objectives: Oxidative stress plays an important role in the pathogenesis of pancreatic diseases such as pancreatic cancer (PC) and chronic pancreatitis (CP). This study aims to assess the role of antioxidant enzymes and oxidative stress markers in PC and CP diseases.

Methods: Enzyme activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx), plus concentrations of plasma total

glutathione (GSH) and lipid peroxidation markers such as malondialdehyde (MDA), were assessed in 6 PC patients, 20 CP patients and 20 healthy controls using enzyme-linked immunosorbent assays (ELISA). In addition, genetically predicted gene expression association analyses (TWAS) were performed for genes involved in oxidative stress homeostasis, combining genetic prediction models and genome-wide association results for PC risk from the PanScan and PanC consortia (7,110 cases and 7,264 controls), accessed through dbGaP.

Results: Controls showed higher activity of SOD (20.44 ± 6.76 u/ml) compared to PC patients (11.80 ± 7.42 u/ml, $p = 0.02$), and higher activity of GPx (52.37 ± 27.35 nmol/min*ml) compared to patients with CP (23.56 ± 23.54 nmol/min*ml, $p = 0.002$). In addition, MDA levels were lower in controls (63.60 ± 32.20 nmol/ml) compared to PC (142.00 ± 51.97 nmol/ml, $p = 0.01$) and CP patients (152.52 ± 71.70 nmol/ml, $p = 4.1 \times 10^{-5}$). No statistically significant differences were found in total GSH concentrations among groups. Finally, higher levels of predicted expression of *GPX1* were inversely associated with PC risk (Zscore = -2.98, $p = 0.003$).

Conclusions: Reduced antioxidant capacity and increased levels of oxidative stress markers were found associated to pancreatic diseases.

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Conflict of Interest: None declared.

EP04.013 Molecular characterization of RCCX module in 21-hydroxylase argentine patients

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Background: Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency, accounts for 90–95% of CAH cases. The gene encoding 21-hydroxylase, *CYP21A2* mapped to at 6p21.3, in the RCCX (RP-C4-CYP21-TNX) module with another duplicated copy composed of the pseudogenes *RP2, CYP21A1P, TNXA*. CYP21A1P shares 98% sequence identity with CYP21A2, therefore, unequal crossover may generate structural rearrangements and copy number changes in the RCCX module. Here we present our results on the molecular characterization of the RCCX module in affected patients from Argentina.

Methods: We analyzed a total of 314 patients: 226 (55 classical and 171 nonclassical, NC) consecutively samples by Southern blot (SB, 34 of them also analyzed by Multiplex-ligation probe amplification, MLPA), and 88 selected patients by MLPA (18 classical, 70 NC) representing 611 non-related alleles.

Results: We found 11 different haplotypes. The most frequent haplotypes were the bimodular standard RCCX module (50.8 and 43.3%, for patients studied by SB and MLPA, respectively), and a RCCX module with a duplicated CYP21A1P (34.6 and 44.6%, for patients studied by SB and MLPA, respectively). Chimeric genes account for 4.6 and 6.4 % respectively, more frequent (16.3%) for classical patients than for NC (1.5%). Haplotypes bearing a duplicated *CYP21A2* gene represent 1.2 and 2.3% of the analyzed alleles by SB and MLPA, respectively.

Conclusions: The data provided add to the knowledge of the complexity of the RCCX region and for the characterization of a large cohort of patients from our region.

Grant References: PICT2006-00208; UBACYT 2002011010000 5BA; FOCANLIS: 2010, 2013 and 2015

Conflict of Interest: None declared.

EP04.015 Influence of MUC5B common sequence variant on the development and progression of fibrotic interstitial lung disease

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Background/Objectives: A genetic variant in the promoter region of *MUC5B* gene (NG_031880.1:g.1927G > T,rs35705950) has been associated controversially not only with an increased risk of developing IPF (idiopathic pulmonary fibrosis), the paradigm of fibrotic interstitial lung disease (ILD), but also with improved survival. The aim of this work is to assess the influence of *MUC5B* on the development and progression of ILD.

Methods: This is an observational, retrospective study. *MUC5B* variant was tested to 213 patients: 74 patients diagnosed with ILD and family history (cases), and another group of 139 asymptomatic subjects (controls). Fibrotic progression was defined as a forced vital capacity (FVC) loss ≥5% and/or carbon monoxide diffusing capacity (DLCO) loss ≥10% over 1 year. The influence of *MUC5B* on disease development and progression was tested by chi-square test ($\chi 2$).

Results: There were statistically significant differences (p-value = 2.478e-9) in the prevalence of the *MUC5B* variant between the control group (28%) and the cases group (71,6%).

Main diagnosis were IPF (31%), followed by hypersensitivity pneumonitis (24%). MUC5B variant was also more prevalent within the IPF group than no-IPF (p-value = 0,023). Although only a minority of cases shared consistent usual interstitial pneumonia pattern, functional progression was observed in 45,9% (41% of which were IPF). No statistically significant relationship of disease progression and *MUC5B* genotype was found.

Conclusions: The presence of the *MUC5B* variant is a risk factor for developing fibrotic ILD. However, no relationship with disease progression could be identified, raising doubts about the previously reported protective role of *MUC5B*.

Conflict of Interest: None declared.

EP04.016 Double trouble: an interesting case report with a double diagnosis

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A five week old boy who was admitted at the pediatric ward of the Radboudumc hospital because of a viral respiratory tract infection causing central apnea's.

His medical history showed a premature birth at a gestational age of 35 weeks and 6 days and a weight of 3780 grams (97th percentile). Next to the macrosomia, a hypospadias, a congenital heart defect and hypercalcemia were noted.

In his family a mitral valve insufficiency in his father and type 1 diabetes and a hypothyroidism in his mother were present

During physical examination some mild facial dysmorphisms were noticed such as bitemporal narrowing, short and upslanting palpebral fissures, upturned nasal tip and a relatively small mouth.

By using rapid trio whole exome sequencing, a double diagnosis was established within 2 weeks. First, a de novo likely pathogenic variant in the CASR gene (NM_000388.4:c.1626C>G p.(Cys542Trp)) was found, likely explaining his hypocalciuric hypercalcemia. Furthermore, a paternally inherited central 22q11.21 deletion was found, explaining his congenital heart defect.

In conclusion, this case report shows the added value of combined SNV/CNV analysis by rapid whole exome sequencing in neonates with multiple medical problems, not only by limiting the diagnostic journey but also by providing clarity for parents and healthcare providers.

Grant References: Not applicable.

Conflict of Interest: Özlem Baysal Radboud university medical center, Jolijn Verseput Radboud university medical center, Rolph Pfundt Radboud university medical center, Bert B.A. de Vries Radboud university medical center

EP04.017 Mutation screening using whole exome sequencing and extended gene panels for individuals who first present with advanced kidney disease

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Background/Objectives: For patients who first present with advanced kidney disease determining the nature of the underlying pathology may be difficult. In cases such as these, genetic diagnosis may be used to guide the treatment, prognosis and counselling.

Methods: After obtaining informed consent, we collected samples from 10 patients with chronic kidney disease (CKD) with uncertain etiology, aged between 30 and 70. Whole exome sequencing on NovaSeq 6 000 (Illumina) was performed and a panel of 773 gene, previously associated with kidney pathologies, was analysed for the presence of genetic variants. Relevant variants were confirmed using Sanger sequencing. When needed, segregation analysis was carried out using the same method. The pathogenicity of new variants was evaluated using ACMG criteria.

Results: Pathogenic or potentially pathogenic variants were found in several genes, known to be involved in kidney development and/or function. Among those were PKHD1, FOXC2 and SEC61A1. The genetic findings led to establishing the correct diagnosis for several individuals, allowing the clinical team to make a treatment plan and offer adequate genetic counselling. Notably, a woman was first diagnosed with autosomal recessive polycystic kidney disease at the age of 41.

Conclusion: We tested the applicability of targeted exome sequencing for diagnosis in Bulgarian CKD patients. For individuals with advanced kidney disease, where renal function tests and biopsy may not give conclusive results, mutation screening using extended panel of genes may be of great help in establishing formal diagnosis.

Grant references: MU180/14.06.2022; MES: D01-395/18.12.2020, D01-278-14.12.2022, D01-302/17.12.2021 and D01-165/28.07.2022

Conflict of Interest: None declared.

EP04.018 De novo mutation in NR5A1 (SF1) in a child with gonadal dysgenesis

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Background/Objectives: Steroidogenic factor-1 is a nuclear receptor associated with 46,XY DSD (disorders of sex development). A spectrum of phenotypes has been reported including gonadal dysgenesis. We report a child with gonadal dysgenesis and mutation in *NR5A1 (SF1)*.

Methods: Six-months-old child (assigned as female) presented due to phallus enlargement with size of 2 cm. The investigations revealed 46,XY karyotype, normal adrenal function with normal levels of 17-OH-progesterone and elevated testosterone of 6.34 nmol/l [0.07-0.17]. Blind-ended vagina with length of 29 mm was found - micturition takes place from the urethra below the clitoris. MRI scan identified 2 gonads and laparoscopy was performed subsequently. No uterus was found, the gonads in abdomen were determined histologically as testes with preserved architecture after biopsy. DNA from K2EDTA blood was extracted from the proband and the parents. Sanger sequencing of coding regions and intron-exon boundaries of *NR5A1 (SF1)* gene was performed after PCR amplification.

Results: A heterozygous genetic variant *NR5A1*:c.985C>T (p.Gln329Ter) with de novo origin was identified in the patient.

Conclusion: The diagnosis and management of 46,XY infant with genital ambiguity is always a challenge as precise genetic and clinical diagnosis has implication for gender assignment and further treatment. *NR5A1* mutations in 46,XY DSD patients are often associated with intact adrenal steroid biosynthesis. Weather our patient will develop adrenal failure remains to be pursued. Genotype-phenotype correlation needs to be determined as it will allow a better follow-up of the DSD patients and proper genetic counselling of their families.

Conflict of Interest: None declared.

EP04.019 Exome sequencing identifies oligogenic inheritance in familial Primary Biliary Cholangitis

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Background/Objectives: Primary biliary cholangitis (PBC) is a rare autoimmune disorder with multifactorial etiology leading to a progressive damage of small bile ducts, eventually causing liver failure. Most of the efforts performed till now to explain PBC heritability were based on genome-wide association studies and meta-analyses, which however miss rare and ultra-rare deleterious variants with high impact. Here, we report the detailed genetic characterization of a Spanish family with the aim to identify additional risk loci for PBC.

Methods: A family with multiple affected individuals was subjected to genome-wide linkage analysis and exome sequencing. Genotyping was performed on 9 individuals from the family using the Illumina Infinium HumanCore-24 v1.1 BeadChip whereas exome sequencing was performed on 3 affected subjects using the Agilent Sure Select exome v7 Library prep kit and an Illumina NovaSeq6000 sequencer.

Results: Parametric multi-point data analysis did not evidence any significant linkage signal with LOD score ≥3. Rare variant analysis by exome sequencing identified potentially pathogenic variants in three genes (*SLC30A10*, *SHROOM2*, and *KRT18*). *SLC30A10* is highly expressed in the liver, and rare variants in this gene are associated with elevated levels of liver transaminases and cirrhosis. *SHROOM2* is a X-linked gene that was found differentially methylated in PBC. *KRT18* pathogenic variants are known risk factors for developing liver disease, and knock-out mice develop antimitochondrial autoantibody – a hallmark of PBC.

Conclusion: Our data suggest an oligogenic inheritance for PBC, with multiple rare variants each likely explaining different phenotypic features of the patients.

Grant References: -

Conflict of Interest: None declared.

EP04.020 Application of Clinical Exome in genetic nephropathies: example of Nephronophthisis

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Background: Nephronophthisis is a chronic tubulointerstitial nephropathy with an autosomal recessive inheritance, characterized by renal cystic disease evolving to end-stage renal disease (ESRD). It is the most frequent genetic nephropathy in children (10% of ESRD cases), with an incidence of 1/50000 to 1/900000 births. Three forms of nephronophthisis are described: infantile, juvenile and adult. These forms are distinguished by clinical and radiological signs and the age of development of ESRD. Nephronophthisis can be isolated or associated with extra-renal signs, defining a variety of other more complex syndromes. Several genes have been identified in nephronophthisis. Given this clinical and genetic heterogeneity, molecular diagnosis using Next-Generation Sequencing (NGS) represents the most efficient and rapid means of diagnosis for this group of diseases.

Methods: Three patients were referred for genetic investigation suffering from clinical signs of nephronophthisis with chronic ESRD, and we report here clinical features and molecular findings. Considering the clinical heterogeneity and the diagnosis difficulties, we performed clinical exome screening in the patients. A virtual panel of 12 genes involved in nephronophthisis was applied to filter variants.

Results: Three novel mutations have been detected in three different genes: *NPHP1*, *INVS* and *NPHP4*. These results were confirmed by Sanger sequencing and qPCR.

Conclusion: The present case report illustrates the important role of Clinical Exome Sequencing in the accurate molecular diagnosis of genetic kidney disease. This approach allows for an appropriate management of patients and adequate genetic counseling. Moreover, this molecular analysis would be more cost-benefit for patients and their families.

Conflict of Interest: None declared.

EP04.021 Identification of NR6A1 as a new gene involved in congenital urogenital anomalies

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Background: Retinoic acid and posterior Hox genes are both required for renal and uterine development in mammals, their alteration resulting in renal and uterine malformations. The nuclear receptor NR6A1 is implicated in anteroposterior patterning, possibly by regulating Hox gene expression and/or by modulating retinoic acid gradient.

Results: By WES, we identified a heterozygous missense variant in *NR6A1* in one family with recurrence of renal and uterine malformations. Sequencing of the gene in a cohort of individuals with Mayer-Rokitansky-Küster-Hauser syndrome identified one additional predicted deleterious variant. Generation of Nr6a1 mutant models was performed by knocking out the zebrafish orthologs nr6a1a and nr6a1b thanks to crispr/cas9 technology. Developmental kidney defects were detected using transgenic reporter line and characterized by in situ hybridization. nr6a1a$^{-/-}$ mutants show impaired development of the kidney with anomalies in pronephros segmentation, which translate in hypoplasia of kidney tissues in adults. As nr6a1a$^{-/-}$ females are infertile, analysis of the genitalia was performed. MicroCT scan showed precocious degeneration of oocytes while the oviduct seems to be formed. In addition, we detected various defects in the mutant skeleton such as abnormal number of vertebrae and absence of the anal fin.

Conclusion: Renal anomalies in our zebrafish model provide the first evidence for causality of *NR6A1* mutations in kidney malformations. The skeletal defects observed in our mutant confirm a role for Nr6a1 in anteroposterior patterning of the paraxial mesoderm. Moreover, we show that Nr6a1 is also involved in anteroposterior patterning of the intermediate mesoderm as revealed by abnormal segmentation of the pronephros.

Conflict of Interest: None declared.

EP04.022 Mutational spectrum of MEN1 and MEN2 syndrome in Croatian patients

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Introduction: Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant syndrome caused by mutations in *MEN1* gene. One of its main hallmarks is endocrine and non-endocrine tumor growth (parathyroid tumors, pituitary tumors, gastrinomas, insulinomas, cutaneous tumors, etc.).

Mutations in the *RET* proto-oncogene are the primary cause of the hereditary syndrome called multiple endocrine neoplasia type 2 (MEN2). It is divided into three autosomal dominant subtypes (MEN2A, MEN2B, and FMTC) with a high risk of developing medullary thyroid cancer.

Materials and Methods: For MEN1 and MEN2, respectively, genomic DNA samples from 176 and 115 individuals were examined. Sequencing of *MEN1* included all 10 exons with flanking intronic regions, whereas, for *RET*, only hotspot exons (exon 10, 11, 13, 14, 15 and 16) were analyzed. Applied Biosystems 3130xl Genetic analyzer and BigDye® Terminator v3.1 Cycle Sequencing Kit were used to identify pathogenic variants in the coding region of these genes.

Results: Heterozygous pathogenic variants were detected in 32 individuals with MEN1. The most prevalent variants were p.Arg516Profs*15 (8 patients) and p.Glu191Glyfs*5 (6 patients). There were 16 individuals with MEN2 heterozygous pathogenic variant. The most frequent variant was p.Cys620Arg (4 patients), followed by p.Cys634Tyr and p.Leu790Phe (found in 3 patients each).

Conclusions: The genotype-phenotype correlations that can be used to determine the likelihood of aggressive medullary thyroid cancer make molecular diagnostics of MEN2 significant. Contrarily, despite the fact that MEN1 syndrome does not exhibit genotype-phenotype correlations, molecular diagnostics of MEN1 is important for identifying at-risk family members.

Conflict of Interest: None declared.

EP04.025 A pathogenic variant in THRA as the cause of Congenital non-goitrous Hypothyroidism in a pair of monozygotic twins

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Introduction: Congenital non-goitrous Hypothyroidism, 6 (CHNG6) is an autosomal dominant condition characterised by growth and developmental retardation, low serum T4 and high serum T3 levels and delayed bone development. THRA is one of several nuclear receptors for thyroid hormone that when mutated confers a resistance to thyroid hormone.

Methods: Twin sisters presenting with ataxic gait, poor fine motor coordination, dysarthria, truncal obesity, dysmorphic features and mild intellectual disability. Personal history was significant for severe generalized hypotonia, feeding difficulties, global developmental delay and learning difficulties. They are treated for hypothyroism since early childhood. Previous investigations including karyotype, metabolic screening and molecular karyotype were non- conclusive. Family history was non-contributory. Exome sequencing performed with Agilent's Exome V8 NGS panel and data analysed using Franklin. Proband, parents and the rest of the family were additionally tested by Sanger sequencing.

Results: The THRA c.1187dupT p.(Phe397LeufsTer10) truncating pathogenic variant, was found in heterozygous state in the two probands, and was absent from their parents. The variant was the first reported mutation in THRA and since then has been reported in literature in at least three affected individuals.

Conclusion: To our knowledge about 10 individuals from 8 families were found to harbour a THRA pathogenic variant and this study can be incorporated to the limited reported cases published. In addition, this study highlights the importance of the pathogenicity of truncating mutations even if these are located at the C-terminal of a protein.

Conflict of Interest: None declared.

EP04.026 Nonsense mutation in the novel PERCC1 gene as a genetic cause of Congenital Diarrhea and Enteropathy

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Background/Objectives*:* Congenital diarrheas and enteropathies (CODEs) constitute a heterogeneous group of individually rare disorders manifesting with infantile-onset chronic diarrhea. Genomic deletions in chromosome 16, encompassing a sequence termed the 'intestine-critical region (ICR)', were recently identified as the cause of an autosomal recessive congenital enteropathy. The regulatory sequence within the ICR is flanked by an unannotated open reading frame termed *PERCC1*, which plays a role in enteroendocrine cell (EEC) function. We investigated 2 unrelated children with idiopathic congenital diarrhea requiring home parenteral nutrition attending the Irish Intestinal Failure Programme.

Methods*:* Currently 12- and 19-years old, these Irish male patients presented with watery diarrhea and hypernatremic dehydration in infancy. Probands were phenotyped by comprehensive clinical investigations, including endoscopic biopsies and serum gastrin level measurements. Following negative exome sequencing, PCR and Sanger sequencing of the entire coding region and intron boundaries of *PERCC1* were performed for each proband and their parents.

Results*:* In both patients, serum gastrin levels were low and failed to increase following a meal challenge. While no deletions involving the ICR were detected, targeted sequencing of the *PERCC1* gene revealed a shared homozygous c.390C>G stop gain variant.

Conclusion*:* We report clinical and molecular findings in two unrelated patients harboring a shared homozygous variant in *PERCC1*, comprising the first description of a point mutation in this gene in association with CODE. That the 2 parenteral nutrition dependent children with unexplained diarrhea at our institution harbored a *PERCC1* mutation underscores the importance of its inclusion in exome sequencing interpretation.

Conflict of Interest: None declared.

EP04.027 First proved case of autosomal dominant ATP6V1B1-related renal tubular acidosis: experience with whole genome sequencing data

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Background/Objectives: In the genomic era, it is not uncommon to have unusual mode of inheritance (MOI) for various genes. Here we present unusual dominant-form of ATP6V1B1-related renal tubular acidosis. To our knowledge, this is the first case proved to have dominant inheritance by covering all parts of *ATP6V1B1* gene using whole genome sequencing (WGS).

Case: Five-year-young girl presented in early infancy with H/O poor weight gain and failure to thrive. She was admitted at 6-months of age to the ICU with H/O lethargy and severe dehydration. Initial blood investigation showed hyperchloraemic - normal anion gap - metabolic acidosis with hypokalaemia causing ECG changes. Urine PH was consistently >5.5 in spite of metabolic acidosis. Renal ultrasound showed bilateral medullary nephrocalcinosis. She was diagnosed with Distal renal tubular acidosis.

Result: whole exome sequencing (WES) revealed a heterozygous c.1181G>A (p.Arg394gln) variant in *ATP6V1B1* gene. None of parents carry the variant. To rule-out possible variant in the 2nd allele; whole genome sequencing (WGS) was done to virtually cover all parts of the gene including promoter, deep intronic variants and 3`-end; which revealed the same variant in heterozygous state.

Conclusion: This study shows it is more common than originally thought to have different MOI even within the same gene. This phenomenon of having multiple MOI's is still relatively new and therefore it is the duty to report unusual MOI of a gene in order to have better understanding of function of a gene and thus better understanding of disease mechanism.

No Grants

Conflict of Interest: None declared.

EP04.028 Genetic analysis in patients with androgen insensitivity syndrome – detection of a novel AR gene variant

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Background: Androgen insensitivity syndrome (AIS) is a rare condition with an X-linked pattern of inheritance, in which patients with 46,XY karyotype demonstrate complete (CAIS) or partial (PAIS) impairment of pre- and postnatal virilisation. The differential diagnosis in CAIS is limited, whereas in PAIS, numerous other causes of disorders of sex development (DSD) can also produce the typical phenotype of micropenis, severe hypospadias and bifid scrotum. The majority of the cases is determined by the presence of variants in the androgen receptor (AR). More than 1100 mutations in the *AR* gene have been reported. We aimed to study the genetic basis of androgen insensitivity syndrome in patients with clinical diagnosis of the condition.

Material and Methods: Eight patients were recruited from the cohort of 92 individuals with DSD referred to the Department of Genetics, Polish Mother's Memorial Hospital RI. The diagnosis of CAIS or PAIS was made based on their karyotype (46,XY), phenotype and corresponding hormone alternations. Custom DSD NGS panel was used.

Results: A novel pathogenic *AR* variant c.1344_1345insTA was found in a patient with clinical diagnosis of CAIS. In 5 other cases known *AR* missense variants were identified.

Conclusions: Despite the fact that *AR* gene has been thoroughly studied in several DSD cohorts we identified new, yet undescribed variant within its sequence. We believe, that continually increasing access to genomic sequencing technology worldwide will allow for further characterization of genomic variants associated with AIS translating into a better personalised healthcare service.

Conflict of Interest: None declared.

EP04.029 Uniparental Disomy of chromosome 16 as a cause of Primary Ciliary Dyskinesia

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Background/Objectives: Primary Ciliary Dyskinesia (PCD) is a rare genetic condition affecting cilia structure and function. Patients manifest chronic respiratory infections beginning in early childhood, with or without abnormally positioned internal organs and infertility. About 50 genes have been described, almost all with an autosomal recessive inheritance. We present a patient with neonatal respiratory distress, *situs inversus*, cough and mucus production.

Methods: Ciliary beat pattern and ultrastructure were studied on samples of nasal curettage using a high-speed camera accoupled to an inverted microscope and electronic microscopy (EM) respectively. Clinical Exome (CE) was sequenced from blood samples and segregation analysis of the candidate variant was assessed with Sanger sequencing. Finally, CytoScan HD trio array (Affymetrix) was carried out to seek copy number variants and runs of homology (ROH).

Results: Cilia were immobile and EM showed outer dynein arm absence. CE revealed the p.Gly343Profs*4 pathogenic variant in the autosomal recessive *DNAAF1* gene in homozygous state. Although paternity was genetically confirmed, segregation analysis failed at detecting this variant in the father. Finally, the SNP-array revealed two ROH of 38,73Mb and 6,68Mb at 16p11.2-16q22.1 and 16q23.3-16qter respectively. The *DNAAF1* gene is located at 16q24.1, thus explaining the homozygous state of the detected variant as a result of a segmental maternal uniparental isodisomy (UPiD(16)).

Conclusion: PCD is a genetically heterogeneous disorder related to motile cilia. Here we describe the first PCD patient due to a UPD of chromosome 16. This information is crucial to genetic counselling.

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Conflict of Interest: Lidon Carretero-Vilarroig Full, Alba Berzal-Serrano Full, Ana Reula Part-time, Rosana Blanco-Mañez Full, Noelia Muñoz-Fernandez Full, Gema García-García Full, Elena Aller

Full, José M. Millán Full, Miguel Armengot-Carceller Full, Instituto de Salud Carlos III PI22/01010, Teresa Jaijo Full, IP Instituto de Salud Carlos III PI22/01010.

EP04.030 Bartter syndrome vs Gitelman syndrome: a case report

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Introduction: Bartter syndrome and Gitelman syndrome are autosomal recessive renal disorders characterized by fluid, electrolyte, urinary and hormonal abnormalities, including renal potassium, sodium, chloride, and hydrogen wasting; hypokalemia; hyperreninemia and hyperaldosteronism without hypertension; and metabolic alkalosis. Clinical findings include electrolyte, growth, and sometimes neuromuscular abnormalities.

Case report: we performed clinical exome sequencing (CES) in a 32 year-old female patient presenting chronic hypokalemia, hypomagnesemia and intense asthenia.

Results: Data analysis revealed the c.1783C>T (p.Arg595Ter) pathogenic variant. Copy number variant analysis from CES data identified a heterozygous complete deletion of CLCNKB gene.

The c.1783C>T mutation was previously reported in homozygous state and as compound heterozygous in families with Bartter syndrome. A functional study determined that this variant abolished chloride conductance in vitro (PMID:28381550). This variant is present in population databases (gnomAD allele frequency 0.0003633). The patient presented a heterozygous complete deletion of the CLCNKB gene: (hg19) NC_00001.10(NM_000085.5):g.(?_16370987)_(16383412_?)del. This copy number variant has been reported in homozygous and compound heterozygous state in Bartter syndrome patients.

Conclusion: This patient was refered with clinical diagnosis of Gitelman syndrome but the CES-based genetic study stablished the diagnosis of Bartter syndrome. To our knowledge, this is the first patient described in the literature with this two variants. Gitelman syndrome and Bartter syndrome are two highly overlapping renal conditions. In this case, the genetic study allows a better diagnosis and follow up of patients.

Conflict of Interest: None declared.

EP04.031 Advantages of the MS-MLPA in the diagnosis of Russell-Silver syndrome

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Russell-Silver Syndrome (RSS) is a genetically heterogeneous disease manifested by growth retardation. Most often, the genetic defect is associated with impaired methylation of the H19 and IGF2 genes located in 11p15, or with maternal uniparental disomy of chromosome 7. The use of methylation-dependent multiplex legase probe amplification (MS-MLPA) for genetic disorders in RSS is important in the search. Case from practice. Boy, 6 years old, height 106 cm (-3DS), weight 12.5 kg (-3DS), BMI 11.1 (-3DS), protruding forehead, triangular face, narrow chin, clinodactyly 5 fingers. Anamnesis: signs of intrauterine growth retardation of the III degree were detected at the 33rd week of gestation during ultrasound screening of the fetus. The boy was born at 37-38 weeks of pregnancy. Birth weight 1700 g (-3DS), body length 41 cm (-3DS), head circumference 29 cm (-1DS). At the age of 1 year 3 months, there was a delay in physical development, a deficiency in body weight of the III degree. A diagnosis of RSS has been proposed. However, when studying the IGF2 gene by real-time PCR and the study of the 7q33-34 locus by microsatellite analysis did not reveal any pathology. Karyotype 46,XY.

We proposed to conduct an additional examination using the MS-MLPA method to determine the methylation status and the presence of microdeletions/microduplications in the NSD1, H19, CDKN1C, KCNQ1OT1 (11p15) genes. Hypomethylation of the H19DMR/IC1 domain was found. The use of MS-MLPA made it possible to verify the diagnosis in a 6-year-old boy: Q87.1. Russell-Silver syndrome, abnormal methylation of the H19 gene.

Conflict of Interest: None declared.

EP04.032 Further evidence of a novel PPP1R12A frameshift variant involved in anomalies of sexual development

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Abnormalities of sexual development are caused by genetic defects, ranging from chromosomal aberrations to single nucleotide changes. They may be limited to the genitourinary system or can be associated with impairment of different organs.

Here we present a rare case of sexual development anomaly determined by a pathogenic variant in *PPP1R12A*. The boy, aged 3 years 6 months, was referred to our clinic for genetic consultation and molecular diagnosis.

He is the first child of a nonconsanguineous couple, born at term, after a pregnancy complicated with polyhydramnios in the third trimester. Duodenal atresia, prenatally suspicioned, was confirmed at birth, when bilateral cryptorchidism was noted, as well. Later, an intraabdominal testis, a rudimentary fallopian tube and uterus were detected. The child has a mild facial dysmorphism and low levels of estrogen, testosterone, AMH and DHEA. Classical karyotype, SNP array and WES were normal, except for a paternal-inherited inversion on chromosome 9.

WGS was performed by Illumina technology and data was analyzed using Congenica platform.

A novel, heterozygous frameshift variant c.2182_2185delGAAA in *PPP1R12A*, disrupting exon 16 of 25 was detected, which leads consequently to a truncated protein. The variant was omitted by WES due to low coverage. *PPP1R12A* is a key regulator involved in cell cycle, cell adhesion and migration during embryogenesis and in morphogenesis. It has been recently (2020) linked with genitourinary syndrome with or without cerebral malformations by Hughes et al., who reported 12 patients with truncating variants. Our study brings new evidence for a better characterization of *PPP1R12A*-related anomalies.

Conflict of Interest: None declared.

EP04.033 Altered microRNA expression in asymptomatic and mild COVID-19 cases

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Background/Objectives: Host-encoded microRNA (miRNA) response to SARS-CoV-2 infection has been reported to be altered mostly in relation to severe COVID-19 cases. However, there is scarce information in relation to host miRNAs profiles in asymptomatic or mild symptomatic COVID-19 cases, that might also provide important insights into both viral pathogenesis and patient management.

Methods: Circulating miRNAs from serum and nasopharyngeal samples belonging to eight asymptomatic or mild COVID-19 patients and eight age and gender matched healthy controls were determined by NGS. RNA was isolated and converted into miRNA NGS libraries to subsequent sequence on a NextSeq (Illumina Inc.) instrument.

Results: We observed 18 miRNAs dysregulated in serum from asymptomatic or mild symptomatic COVID-19 cases when compared to healthy controls, being miR-485-3p and miR-143 the most up-regulated (Log2FC = 3.77 and 3.23, FDR = 2.2e-7 and 0.0018, respectively) while miR-12136 and miR-1275 were the most down-regulated ones (Log2FC = -4.70 and -3.05, FDR = 5.2e-8 and 0.000073, respectively). The nasopharyngeal samples from the same individuals presented the miR-3195 as the strongly upregulated miRNA (Log2FC = 5.24, FDR = 0.00027). The principal component analysis (PCA) revealed that the miRNA profile based on 500 dysregulated miRNAs could independently classified serum from nasopharyngeal samples of COVID-19 cases.

Conclusion: This study demonstrates that SARS-CoV-2 infection induces a robust host miRNA response also in asymptomatic or mild COVID-19 cases. miR-485-3p is a promising useful inflammatory biomarker while miR-143 may efficiently combat SARS-CoV-2 by reducing host cell apoptosis via mTOR. Validation of the present findings in a large patient cohort are warranted.

Grant References: CB21/13/00100 (CIBERINFEC), ISCIII, Spain.

Conflict of Interest: None declared.

EP04.034 Genetic modifiers of Cystic Fibrosis-Related Diabetes in Georgian CF Patients with 1677DelTA mutation

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Introduction: CF Genome Wide Association Studies (CF GWAS) identify cystic fibrosis-related diabetes (CFRD) loci. Genome-wide significance was obtained for variants at *PTMA, TCF7L2* and *SLC26A9* loci. *PTMA* and *SLC26A9* variants are CF-specific; *TCF7L2* variants also associated with T2D. The objective of our study was to assess CFRD genetic risk factors in Georgian CF patients.

Materials and Methods: The study was approved by the ethics committee of the Tbilisi State Medical University. Informed contents will be obtained from all patients or their representatives. 18 CF patients (age: 5-15) with 1677DelTA mutations were enrolled in this study. We genotyped patients regarding the *TCF7L2* rs7903146, *PTMA* rs838440 and *SLC26A9* rs4077468 using a TaqMan assay (Applied Biosystems, USA).

Results: The allele distributions of rs7903146, rs838440 and rs4077468 are summarized in Table 1. We observed 2 CF patients double homozygotes regarding the risk alleles of both, *TCF7L2* and *SLC26A9* genes which may suggest an increased susceptibility to the CFRD.

Table 1. Allele distributions of CFRD gene variants

SNP	Locus	Gene	Major allele/Minor allele	MAF	Genotyped	*P* Value
rs7903146	10q25.3	*TCF7L2*	C/T	0.4	98%	<0.05
rs838440	2q37.1	*PTMA*	G/T	-	0%	-
rs4077468	1q32.1	*SLC26A9*	A/G	0.6	98%	<0.05

MAF, Minor allele frequency

Conclusions: Individuals with cystic fibrosis with the additional diagnosis of CFRD have a higher risk of early death than those without CFRD. Early diagnosis and treatment correlate with slower rates of pulmonary decline and improved growth.

This work was supported by the Shota Rustaveli National Science Foundation of Georgia, Fundamental Research Grant #FR-22-2601

Conflict of Interest: None declared.

EP05 Skeletal, Connective Tissue, Ectodermal and Skin Disorders

EP05.001 A novel heterozygote LORICRIN variant in a father and daughter with palmoplantar keratoderma

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Abstract:

Introduction: Loricrin Keratoderma (LK) is a rare early-onset autosomal dominant skin disorder, characterized by honeycomb-like palmoplantar keratoderma (PPK) and diffuse ichthyosiform dermatosis. There might also be symptoms such as pseudoainhum/autoamputations, knuckle pads, secondary infections and hyperhidrosis. Affected children can be born as collodion babies. To the best of our knowledge 10 different disease-causing variants in *LORICRIN* have been published, of these nine have been related to LK.

Material & Methods: A father and daughter presented with PPK with a honey-comb like pattern and fine-scaling ichthyosis since adolescence and childhood respectively. The daughter had associated hyperhidrosis, discrete knuckle pads and constriction bands. Diagnostic analysis was performed by next-generation sequencing, using an exome-based in-silico panel targeting 95 genes related to palmoplantar keratoderma. A novel variant in *LORICRIN* was identified in both father and daughter.

Results: A heterozygote frameshift variant was identified in *LORICRIN* (c.792dupC,p.(Ile265Hisfs*71)). The variant was absent in

gnomAD and ClinVar. The variant is predicted to result in a delayed stop codon and elongation of the protein to 336 amino acids as opposed to the native length of 312 amino acids. Nine of 10 disease-causing variants reported in the literature were also frameshift variants and six of these variants lead to an elongation of the protein to 336 amino acids, supporting our interpretation of the variant as likely pathogenic.

Conclusion: We identified a novel heterozygote variant in *LORICRIN* in a father and daughter with PPK. We classified the variant as likely pathogenic.

Conflict of Interest: None declared.

EP05.003 Novel mutations and atypical presentation of Papillon Lefevre Syndrome

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Background Papillon Lefevre syndrome (PLS) is an autosomal recessive condition that is caused by mutations in cathepsin C (CTSC) enzyme. Typically, it presents with severe periodontitis that leads to teeth loss in early youth and palmoplantar keratosis.

Aim We present two PLS cases with novel mutations and unusual clinical presentations.

Results We present two PLS cases with confirmed novel CTSC mutations; the first case had the frameshift mutation c.285_286delGT (p.L96Efs*2) and clinically had all teeth existing with minimal attachment/ bone loss along with severe enamel structural defect and open root apices, while the second case had the homozygous frameshift variant c.1331delinsAAAAA (p.G444Efs*4) and presented with extensive buphthalmos secondary to congenital glaucoma and loss of vision in her right eye.

Conclusion The spectrum of PLS causing gene have additional two novel mutations with atypical clinical presentations.

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Conflict of Interest: Maha Abouzaid National Research Centre, Science,Technology and Innovation funding Authority (STDF), Nermeen Ahmed National Research Centre, Science, Technology & Innovation Funding Authority (STDF)., Mohamad Abdelhamid National Research Centre, Science, Technology & Innovation Funding Authority (STDF), Mostafa Mostafa National Research Centre, Science, Technology & Innovation Funding Authority (STDF).

EP05.004 the potential of mummy substance to stimulate healing in mesenchymal stem cells cultured with human fibroblasts

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the potential of mummy substance to stimulate healing in mesenchymal stem cells cultured with human fibroblasts

Background: The objective of this research is to determine the effect of mummy substance on the enhancement of cell proliferation and matrix protein synthesis in wound healing.

Methods: The methodology used for this study involves isolating mesenchymal stem cells and human fibroblasts procured at Pastor Institute, Iran. The cells were treated with mummy substance separately and co-cultured between ASCs and WJSCs and fibroblasts. Proliferation was assessed by Ki67 method in monolayer condition. Synthesis of components of extracellular matrix (ECM) such as collagen type I, type III and fibronectin 1 (FN1) were determined by qPCR.

Results: The effective concentration of Mummy substance on fibroblasts, adipose-derived stem cells, and Wharton jelly was determined to be 1000 µm/ml through the MTT assay. The results were analyzed using Graph Pad Prism 6.01 software. The level of Ki67 was found to be higher in adult stem cells compared to neonatal stem cells ($p < 0.0001$). The study concluded that Mummy material enhanced the expression of collagen I, III, and fibronectin in fibroblast-ASCs co-culture but not in fibroblast-WJ-SCs co-culture.

Conclusion: This research presents a successful in vitro method for promoting healing. Therefore, the potential use of Mummy substance and stem cell-based treatments in wound healing as a new therapeutic strategy is promising.

Conflict of Interest: Sepideh Hassanpour Khodaei full time, shahnaz sabetkam full time, Havva Ozgen Eyupoglu: None declared, Şükrü Tüzmen full time

EP05.005 Hereditary mucoepithelial dysplasia: A report of two patients

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Background/Objectives: Ichthyoses are disorders of cornification characterized by dry, scaly skin and a disruption of skin barrier function (1). Some severe forms of ichthyosis, called syndromic ichthyoses, show extracutaneous symptoms (1). One such ichthyosis is hereditary mucoepithelial dysplasia (HMD), which has sometimes been called ichthyosis follicularis, atrichia and photophobia syndrome 2 (IFAP2) (1). To date, four *SREBF1* mutations in 18 families have been linked to HMD.

Patients/Methods: The patients were admitted to Hospital Infantil Niño Jesús in Madrid. Genomic DNA was extracted from peripheral blood by standard phenol/chloroform protocol, mutations were found using whole-exome sequencing and validated by Sanger sequencing.

Results: Both patients showed diffuse non-scarring scalp alopecia, widespread follicular keratosis, gingival and palatal erythema, and fissured tongue. Whole exome sequencing revealed the patients were heterozygous for mutation c.1669C>T, p.Arg557Cys in *SREBF1* (NM_001005291.3) and Sanger sequencing validated it. These results match those found previously by other groups and seem to further point to disease-causing *SREBF1* mutations being localized to the same three clustered amino acids (arginine 557, asparagine 558, and leucine 560)

Conclusions: The present study reports two additional patients with the p.Arg557Cys *SREBF1* mutation.

Grants: This project was funded by FIS-FEDER PI20/01569

(1) Gutiérrez-Cerrajero, C. et al., Ichthyosis. Nat. Rev. Dis. Prim. 9, 2 (2023).

Conflict of Interest: None declared.

EP05.006 Novel likely pathogenic variant c.588_612del(p.Trp196CysfsTer5) in EBP gene in a Cypriot family with Conradi-Hünermann Syndrome

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Background/Objectives: Conradi-Hünermann syndrome is an X-linked disorder caused by mutation in the gene encoding delta(8)-delta(7) sterol isomerase emopamil-binding protein (EBP; 300205) on chromosome Xp11. The clinical findings are highly variable, ranging from fetuses with multiple malformations and severe growth retardation to milder manifestations. Almost all liveborn individuals with CDPX2 are female. Characteristic features are growth deficiency, chondrodysplasia punctata, asymmetric shortening of the limbs, scoliosis, linear or blotchy scaling ichthyosis in the newborn and later follicular atrophoderma, scarring alopecia, and cataracts.

Methods: We report a female newborn referred in our clinic because of antenatally reported short limbs, and postnatal lower limb asymmetry, ichthyosis, and early stippling in the left epiphysis in the hip x-ray. The family history was indicative as the mother showed also short stature, ichthyosis, lower limb asymmetry and severe scoliosis.

Results: Trio exome sequence analysis revealed a novel maternal inherited frameshift likely pathogenic mutation c.588_612del(p.Trp196CysfsTer5) in EBP gene. The mutation has not been reported before, lies at a functional domain and is absent from gnomAD exomes and genomes. Loss-of-function is a known mechanism of disease in EBP gene.

Conclusions: We described a family with X-linked chondrodysplasia punctata caused by a novel mutation in the EBP gene. Our study further extends the mutation spectrum of the EBP gene in CDPX2.

Grant References Chondrodysplasia Punctata 2, X-Linked, genereviews, https://www.ncbi.nlm.nih.gov/books/NBK55062/, Clinical, molecular and biochemical characterization of nine Spanish families with Conradi-Hünermann-Happle syndrome: new insights into X-linked dominant chondrodysplasia punctata with a comprehensive review of the literature,PMID: 22121851, None

Conflict of Interest: Emilia Athanasiou State health services organisation, SHSO Cyprus, irene savvidou Ministry of Health, Cyprus, Sofia Ourani SHSO, CYPRUS, Andri Miltiadous karaiskakio foundation, Petroula Gerasimou karaiskakio foundation, Paul Costeas karaiskakio foundation, Violetta Anastasiadou Karaiskakio Foundation.

EP05.007 Variants in the TB5 domain of FBN1 are responsible for short stature phenotype in Weill-Marchesani syndrome

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- **Background:** Fibrillin, encoded by FBN1, is a key component of microfibrillar network, responsible for mechanical properties of extracellular matrix and a reservoir for numerous cytokines, including TGFß. FBN1 is associated with several connective disorders, characterized by tall or short phenotype. Among them, acromelic dysplasia which include Weill-Marchesani syndrome (WMS), geleophysic dysplasia (GD) and acromicric dysplasia (AD), is defined by short stature, short extremities, thick skin and joint limitations. GD and AD are associated with severe short stature present all patients, whereas height seems to be more variable in patients with WMS. While pathogenic variants in FBN1 responsible for AD and GD are all located in exons 41-42 encoding TB5 domain, variants responsible for WMS are located throughout the gene. The aim of this work was to establish a correlation between FBN1 variant location and height in WMS patients.
- **Methods:** Retrospective multicenter study and review of literature (NCBI PubMed database:"Weill-Marchesani + FBN1"). Inclusion criteria: clinical diagnosis of WMS with identified FBN1 mutations.
- **Results:** 20 WMS patients with FBN1 mutation have been studied, 12 and 8 from literature and cohort, respectively. Half of the patients presented with normal stature. Patients with a variant located in exons 41 or 42 were significantly smaller than other patients (Mann–Whitney test, $p = 0.0040$).
- **Conclusion:** We conclude that variants located in TB5 domain are associated with severe short stature in acromelic dysplasias. This observation supports the key role of the TB5 domain in growth.

Conflict of Interest: None declared.

EP05.008 De novo balanced translocations disrupting the FBN1 gene: an uncommon cause of Marfan syndrome

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Background: Marfan syndrome is a well-characterized rare genetic connective tissue disease. Main features involve the skeletal, ocular, and cardiovascular systems, and are mainly caused by *FBN1* (MIM #134797) variants.

Patients and Methods: We report two patients, from unrelated families, with sporadic clinically early-diagnosed Marfan syndrome. Among other phenotypic features, both patients presented with marfanoid habitus, ectopia lentis, aortic root

dilatation, positive wrist and thumb signs and high-arched palate. Targeted sequencing and multiplex ligation-dependent probe amplification (MLPA) of the *FBN1* gene, gene panel of Marfan-related disorders, and an exome-sequencing for the older patient, were performed but did not provide any molecular basis.

Results: Both patients benefited from genome sequencing, retrieving a balanced translocation involving chromosome 15 and disrupting the *FBN1* gene. Theses translocations were characterized by specific fluorescence in situ hybridization probes and karyotype. The 25-year-old male carries a de novo t(9;15)(p13.3;q21.1) translocation. The breakpoint disrupts the intron 40 of the *FBN1* gene. The 9-year-old female carries a de novo t(15;16)(21.1;13.13) translocation, disrupting the intron 45. The other breakpoints are not clinically relevant in both patients.

Conclusion: In the literature, one patient can be found, leading to three cases of Marfan syndrome caused by de novo balanced translocation. In case of clinical certainty of diagnosis, structural variants should also be screened, along with sequencing of the *FBN1* and Marfan-related genes. Our patients illustrate the great contribution of genome sequencing, even in well-known diseases.

Conflict of Interest: None declared.

EP05.009 COL1A2 multiexon deletion in Osteogenesis Imperfecta type 2 – clinical case

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Context: Osteogenesis Imperfecta (OI) is characterized by liability to bone fractures mainly due to pathogenic variants in *COL1A1* and *COL1A2*. OI type 2 is at the most severe end of the clinical spectrum of OI usually leading to early death and is typically caused by a dominant-negative effect of glycine substitutions in the triple helical domain.

Case report: We report the first pregnancy of a healthy couple with a high risk in Down syndrome screening and ultrasound anomalies detected at 13 weeks – increased nuchal translucency, subcutaneous edema of lower limbs, shortening of the long bones and bilateral clubfeet. Later, detection of bowing of the long bones, low amniotic fluid, reduction of fetal movements and a small abdominal circumference led the couple to opt for termination of pregnancy. Post-mortem radiological studies confirmed the diagnosis of OI type 2. Molecular studies identified a heterozygous multiexonic *COL1A2* deletion (exons 4 to 17). This deletion was also detected in the healthy mother, in mosaic state, who also carries a second multiexonic deletion (exons 12 to 17), in heterozygosity, in the same gene. RNA studies from the mother's fibroblasts confirmed the presence of both deletions and demonstrated that they were both in-frame.

Conclusion: This case expands the current knowledge of collagen type I variants reinforcing the role of multiexonic deletions in OI type 2. This clinical case also illustrates the challenges in prenatal diagnosis of fetuses with OI and highlights the importance of molecular studies, including familiar studies, to provide accurate genetic counselling.

Conflict of Interest: None declared.

EP05.010 New evidence of the effect of parental age on the likelihood of having children with achondroplasia

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Background/Objectives: Achondroplasia (ACH; MIM:100800) occurs with an estimated prevalence between 1/16,000 and 1/26,000 live births, representing the most common genetic form of human dwarfism. Affected patients have short limbs with macrocephaly and characteristic facial features such as frontal bossing and midface hypoplasia. Mutations in *FGFR3* causing skeletal dysplasia are all inherited in an autosomal dominant pattern, but frequently occur de novo on the paternal allele. It is thought that there are factors that affect DNA replication or repair during spermatogenesis, but not during oogenesis, and may predispose to the G380R mutation in the *FGFR3* gene. In European countries there is a trend of increasing the average age of childbearing.

Methods: We analyzed 38 families with a sick child diagnosed with achondroplasia. The control group consisted of 259 families.

Results: The average age of fathers in the control group was 30.3. The average age of the fathers of children with achondroplasia was 36.2 years old. Comparison of the average father's age in the study and control groups showed significant differences between the groups ($U = 2771.5$, $p = 0.000014$). Thus, the age of the father does increase the probability of having a sick child with achondroplasia. Therefore, it is necessary to create a complex of procedures (such as prenatal diagnosis or preimplantation genetic testing) to prevent the birth of a sick child in high-risk couples.

Conflict of Interest: None declared.

EP05.011 Marfan syndrome in case of 14-years old girl with confirmed missense pathogenic variant in FBN1 gene

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Marfan syndrome is an autosomal dominant multisystem disorder caused by a mutation in the FBN1 gene with an incidence of 1:5000. Currently, the revised Ghent nosology is used in clinical practice. Molecular analysis of the FBN1 gene has reduced the uncertainty of diagnosis, especially in young patients (before 20 years of age) who do not meet the clinical criteria for Marfan syndrome.

We report a 14-year-old Bulgarian girl who did not meet the Ghent criteria for Marfan syndrome but in whom a pathogenic FBN1 variant was detected.

The girl was referred by a pediatrician for genetic counseling because she was with tall stature, had pectus excavatus, and arachnodactyly. The patient was born after the first uneventful pregnancy in the family (mother 27 years old, father 33 years old). At the age of 14 years, she was with height 175 cm (over 97 percentile) and weight 46 kg (between 25 and 50 percentile). She was not diagnosed with cardiovascular and ocular symptoms. Both parents were with normal stature (mother - 164 cm, father - 170 cm) and there was no other family member with Marfan-like features.

The girl underwent genetic testing by NGS with a target panel comprising the exons of 6699 genes. Analysis revealed a

pathogenic variant (missense mutation) c.7532G > A in exon 61 of the FBN1 gene in the heterozygous state.

With the clinical case presented, we confirm the need for NGS technology in cases with suspected Marfan syndrome and not fulfilled clinical criteria, especially in young patients.

Conflict of Interest: None declared.

EP05.012 Two novel mutations in the PLEC gene in a subject with non-lethal epidermolysis bullosa simplex with pyloric atresia

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Background: Epidermolysis bullosa simplex (EBS) with pyloric atresia (PA) caused by autosomal recessive mutations in *PLEC*, which encodes plectin, is characterized by severe skin blistering at birth and congenital PA and is usually lethal within the first year of life. Since EBS-PA is most often caused by mutations in *ITGB4*, which encodes integrin-beta-4, it is likely that the disease-causing mutations abolish the interaction of plectin with integrin-beta-4.

Subject and method: We report a two-year-old boy born to healthy non-consanguineous parents with non-lethal EBS with congenital pyloric atresia and rather mild bullous lesions noted shortly after birth on the trunk and later at the lower limbs. Clinical exome sequencing was performed in the subject using Illumina TruSight One Kit.

Results: In the *PLEC* gene, two novel heterozygous mutations, c.613G>A (p.Glu205Lys) in exon 3 (actin binding domain, ABD) and c. 11471_11472del (p.Tyr3824Trpfs) in exon 32, were identified. According ACMG classification c. 11471_11472del is a pathogenic variant. Although c.613G>A is a variant of uncertain significance (ACMG), six Meta scores and CADD score predict a damaging effect.

Conclusion: We believe that the newly reported *PLEC* mutations located in the ABD and C-terminal domain likely contribute to a milder disease course via plectin-integrin-beta-4 interactions already reported for *ITGB4*-related EBS-PA. This report will further contribute to the mutational and phenotypic spectrum of EBS-PA.

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EP05.013 Familiar Craniofrontonasal syndrome - case reports

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Introduction: Craniofrontonasal syndrome (CFND) is a rare X linked dominant condition caused by a mutation in the *EFBN1* gene coding a protein ephrin B1. A deficiency of ephrin B1 blocks the adhesion and communication between cells with clinical picture of various skeletal and soft tissue abnormalities. The reported physical characteristics include curly and frizzy hair, widely spaced eyes, flat and broad nose with a vertical groove on the top, coronal craniosynostosis with brachycephaly, midline defects, scoliosis, facial and body part asymmetry.

Despite mode of inheritance, female patients with CFND exhibit more severe symptoms than men. This genetic paradox can be explained by ephrin-B1 deficit compensation by other ephrin molecules in male patients and mosaic pattern of wild type and mutant ephrin-B1 protein expression in women.

We present the case of a family where 2 sisters and their father were affected. 23 years old female presented with facial asymmetry, growth retardation and typical CFND facial features. Her 3 years younger sister was less affected and the father of both who denied clinical testing was only mildly affected.

Methods: Sanger sequencing analysis of *EFBN1* gene was performed.

Results: In our case a heterozygous pathogenic missense mutation c.161C>T, p.(Pro54Leu) in exon 2 of *EFNB1* gene was found in both sisters.

Conclusions: Correct diagnosis based on characteristic facial features in combination with targeted genetic testing is essential for confirmation of diagnosis with adequate follow up and complex therapeutic care. Identification of a causative mutation in family allows to offer prenatal or preimplantation genetic testing.

Conflict of Interest: Lenka Horáková part time, Monika Koudová full, Věra Kavánová: None declared, Anna Křepelová full.

EP05.014 Ser40Leu of IFITM5 can manifest as prenatal Caffey disease

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Background/Objectives: Skeletal dysplasias (SDs) are a heterogeneous group of heritable bone and cartilage disorders. We report a prenatal-onset SD where whole exome sequencing (WES) provided an unexpected result and raised novel implication.

Case presentation: We report a case of prenatal Caffey disease or prenatal cortical hyperostosis (PCH) due to a monoallelic pathogenic variant of *IFITM5*. The first trimester screening at 12 + 5 weeks of gestation for a 30-year-old mother showed shortening of long bones and increased risk for trisomy 18 and 21. Ultrasonography at 16 weeks showed a clover shaped skull, a narrow chest, and bowing of all long bones. Polyhydramnios was noted at 27 + 1 weeks requiring amnioreductions. A girl was born at 32 + 1 weeks but passed away shortly after birth. Findings of severely bowed long bones with diaphyseal hyperostosis and mildly thick ribs on postnatal X-rays led to the suspicion of PCH. A *COL1A1* variant has previously been associated with PCH, thus an osteogenesis imperfecta (OI) panel consisting of *COL1A1*, *COL1A2, CRTAP, P3H1* genes was done, with negative results. Research WES subsequently identified a heterozygous de novo *IFITM5* pathogenic variant. The c.119C>T(p.Ser40Leu) variant has been reported in patients with atypical OI, presenting with bowing and broadening of long bones at birth, with multiple fractures developing later. Pathogenesis of PCH is largely unknown, and prenatal manifestation of *IFITM5*-associated OI may constitute a subset of PCH.

Conclusion: Accurate diagnosis of prenatal SDs is challenging, yet important in the prognostication of these disorders. WES can improve the diagnostic accuracy of prenatal SDs.

Conflict of Interest: Jiin Ying Lim Full time, Celeste Jia Ying Yap Full time, Anju Bhatia Full time, Gen Nishimura Full time, Nikki Fong Full time, Saumya Shekhar Jamuar Full time.

EP05.016 Functional analysis of splicing variants in COL2A1 gene helps in interpretation of clinical picture in patients with skeletal dysplasia

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Background/Objectives: Pathogenic variants in COL2A1 are known to cause a large spectrum of skeletal dysplasia. Splicing variants are one of the most curious variants in interpretation as they can affect mRNA and protein structure by various molecular events.

Methods: SpliceAI tool used for prediction of splicing events. To experimental splicing study we created 8 minigene vectors, containing 47 COL2A1 exons out of 54. Variants were introduced in corresponding vectors by site-directed mutagenesis. Splicing changes were analyzed with Sanger sequencing and fragment analysis.

Results: We chose splicing variants from cohort of Russian patients with different forms of *COL2A1*-related skeletal dysplasia and previously published variants: 1 variant found in foetus with hypochondrogenesis, 6 variants in patients with Kniest dysplasia, 3 variants in patients spondyloepiphyseal dysplasia congenita (SEDc), 11 variants in patients with Stickler syndrome. It was demonstrated that 10 splicing variants led to in-frame exon skipping, 9 variants to frameshift events, 2 variants had no influence on splicing. We showed that severe form of skeletal dysplasia such as hypochondrogenesis, Kniest dysplasia, SEDc were mainly associated with dominant-negative effect due to in-frame protein shortening caused by exon skipping. Stikler syndrome as most frequent mild phenotype was mainly caused by haploinsuffiency due to splicing events with frameshift.

Conclusion: We successfully analyzed 21 splicing variants in *COL21* gene. 19 of them had different effect on splicing: exon skipping leads to in-frame deletion, out-of-frame changes in exon length leads to frameshift with premature stop-codon formation. These data helps to clarify diagnosis and management in affected patients.

Conflict of Interest: None declared.

EP05.017 TGF- β signalopathies between clinics and laboratory: case series with overlapping features of Marfan, Loeys-Dietz, Ehlers Danlos and Osteogenesis Imperfecta syndrome

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Background/Objectives: Heritable connective tissue disorders (HCTDs) are a complex group of disorders involving various organ systems: heart, blood vessels, skin, joints, bone, eyes, lungs, and exhibit a wide range of clinical symptoms showing marked phenotypic variability that can be difficult to diagnose only through clinical examination. Many genes have been identified as a cause of HCTD and they encode for structural proteins, modifying enzymes or components of the TGFβ-signaling pathways. Other genes still await their discovery.

Four notable connective tissue conditions: Marfan syndrome, Loeys–Dietz syndrome, Ehlers-Danlos syndrome, and Osteogenesis imperfecta, show some clinical overlap regarding cardiovascular, skeletal, craniofacial, ocular, and cutaneous features. These diseases are caused by or result in pathological alterations of the complex relationship between the TGF-β family of signalling mediators and the extracellular matrix in connective tissues.

Methods: In this study, we report 7 unrelated cases who showed various degree of phenotypical overlap of skeletal, cardiovascular and cutaneous manifestations and also we highlight the importance of Next Generation Sequencing as a molecular diagnostic tool.

Results: The clinical suspicion of hereditary connective tissue disorders was confirmed by identifying different classes of variants in *FBN1, TGFBR2, SMAD3, COL5A1, COL5A2, COL1A1* genes. Two cases presented with additional features were explained by coexisting pathology.

Conclusion: These four typical examples of HCTD are clinically and genetically heterogeneous diseases, sharing some clinical features and the risk of cardiovascular morbidity and mortality. A multispecialty approach for these patients should be encouraged to reach a more personalized management for treatment and prevention. Genetic counselling is mandatory.

Conflict of Interest: None declared.

EP05.018 A homozygous pathogenic variant in DYSF gene caused Miyoshi myopathy in an Iranian family

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Background/Objectives: Miyoshi myopathy is a type of muscular dystrophy that is characterized by weakness mainly in the distal parts of the legs. Affected individuals show difficulty in walking, climbing stairs, and tiptoeing standing. This disease is a member of Dysferlinopathies and shows an autosomal recessive inheritance that is caused by pathogenic variants in the DYSF gene. In this study, we reported the molecular analysis of a family affected by muscular myopathy using whole exome sequencing (WES).

Methods: After neurological examination, WES was done on an affected proband, then research was followed by co-segregation analysis on the participating family members.

Results: The proband was a 25-year-old male whose Miyoshi myopathy has been started 5 years ago. The proband has two affected and one non-affected sisters. Their parents have not shown any disease-related symptoms. WES analysis revealed a homozygous c.6001C>T (p.Gln2001Ter) pathogenic nonsense variant in exon 52 of the DYSF. Co-segregation analysis revealed this homozygous variant in affected sisters and a wild variant in a non-affected sister. Also, parents were heterozygous for this variant.

Conclusion: DYSF gene encodes dysferlin protein that is involved in membrane repair and regeneration. c.6001C>T was found as a compound heterozygote with c.706C>T(p.Arg236Ter) in two brothers with dysferlinopathy. Dysferlin was severely reduced in membrane and absent in cytoplasm of their skeletal muscle cells. Also, this variant was reported in a man affected by LGMD2B as a homozygous variant. This variant is predicted to damage normal protein function through nonsense-mediated mRNA decay or protein truncation.

Conflict of Interest: asiyeh jebelli Higher Education Institute of Rab-Rashid, saba baghshomali: None declared, mohammad yazdchi Tabriz University of Medical Sciences, Fahimeh Firyaei Hamadan University of Medical Sciences, Leila Emrahi Govar Iranian Legal Medicine Organization.

EP05.019 Variant c.895_904del (p.Val301SerfsTer8) in a newborn girl with isolated postaxial polydactyly – a case report

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Polydactyly is one of the most common inherited limb malformations with an overall incidence of 0.3-3.6/1000 in live births. It is characterized by one or more extra digits on the hands and feet. The most prevalent type of polydactyly is postaxial and is characterized by the presence of extra fingers on the ulnar side of the hand and foot. The most common mode of inheritance of isolated polydactyly is autosomal dominant, but autosomal recessive inheritance is also possible.

We present a case of a female baby with non-syndromic postaxial polydactyly with an established pathogenic variant in ICQE gene associated with autosomal recessive polydactyly type A7. The newborn was a product of a first, uneventful pregnancy of healthy, unrelated parents (mother - 27 years old, father - 29 years old). Isolated polydactyly of all four limbs of the baby without any other dysmorphic features was found at birth. The newborn and both of the parents underwent genetic testing via NGS with a target panel including 6699 gene exons. Analysis revealed a pathogenic frameshift deletion c.895_904del (p.Val301SerfsTer8) in ICQE gene in homozygous state in the baby and heterozygous - in both parents. The identified ICQE mutation is associated with autosomal recessive postaxial polydactyly, type A7. Based on the result of the test, genetic counseling of the family was conducted.

NGS testing contributes to a better understanding on the genetic etiology of isolated polydactyly and provision of genetic counselling of the affected family.

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Conflict of Interest: None declared.

EP05.020 A mosaic PDGFRB variant in a patient with Kosaki overgrowth syndrome

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Background: PDGFRB mutations are associated with different conditions from overgrowth (Kosaki overgrowth syndrome) and connective tissue abnormalities to myofibromatosis, intellectual disability and cutaneous phenotypes.

Methods: A 5-year old boy with overgrowth, dysmorphic facial features, patchy skin areas with extreme hyperlasticity resembling cutis laxa.Skin biopsy histopathology reported a cutis laxa diagnosis. DNA was extracted from the peripheral blood, buccal swab and skin biopsy from the patient and his parents. Exome sequencing performed with Agilent's Exome V8 NGS panel and data were analysed using Franklin.

Results: A PDGFRB pathogenic c.1685A>G, p.Tyr562Cys variant was detected at an allelic balance of around 22% in the patient's skin biopsy and was absent from his peripheral blood and the buccal swab sample, denoting a mosaic de novo origin. The PDGFRB: c.1685A>G is located at a mutational hot spot, has extremely low frequency in gnomAD population databases and computational prediction tools unanimously support a deleterious effect on the gene. This variant has previously been described as mosaic in several patients presenting with intracranial aneurysms or segmental hemihypertrophy of the one arm and hand or one sided aneurysm with an ipsilateral cutaneous phenotype.

Conclusion: The presence of a de novo PDGFRB pathogenic variant in a patient presenting with overgrowth and skin hyperelasticity might comprise to our knowledge the first mosaic PDGFRB-associated Kosaki overgrowth syndrome. This study can be incorporated to the limited reported cases published

Conflict of Interest: None declared.

EP05.021 Novel homozygous missense variant in GTF2E2 causes non-photosensitive trichothiodystrophy type 6

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Trichothiodystrophy (TTD) is a rare autosomal recessive neurocutaneous disorder, characterized by brittle sulfur deficient hair and multisystem abnormalities. The main core of the phenotype associates brittle hair with a characteristic tiger tail banding under

polarized light microscopy with a variable set of additional features including developmental delay (DD)/intellectual disability (ID), microcephaly, anemia, decreased fertility, and progeroid features. TTD is divided into two forms: photosensitive (PS-TTD) and non-photosensitive (NPS-TTD).

Several genes have been associated with autosomal recessive NPS-TTD, including GTF2E2 in very rare cases. GTF2E2 encodes for TFIIE-β, the beta subunit of the general transcription factor TFIIE. This transcription factor HE (TFIIE) is an essential component for transcription. So far, only two pathogenic variants in GTF2E2 from four unrelated families were reported, both affected the wing helix 2 (WH2) region of TFIIEβ protein.

Here, we report clinical data of a novel homozygous missense variant located in the WH2 domain in two brothers with NPS-TTD. Our two cases and the review of the literature allow us to broaden the genotypic and phenotypic spectrum of the disease, underlining the intra- and interfamilial variability, notably on the neurodevelopmental phenotype.

Conflict of Interest: None declared.

EP05.022 USP7 is a new candidate gene for nonsyndromic polydactyly

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Background: Polydactyly is a rare developmental disorder characterized by growth of additional digits on the preaxial, postaxial and central axes of hands and feet. Nonsyndromic postaxial polydactyly can be inherited in a recessive or dominant manner. Nine genes (*GLI3*, *GLI2*, *GLI1*, *SHH* (ZRS), *ZNF141*, *FAM92A*, *IQCE*, *KIAA0825*, *DACH1*) have previously been reported for postaxial polydactyly. Genetic analysis of families with polydactyly is very useful to understand human limb development. The purpose of this study was to identify the genetic cause of postaxial polydactyly in two individuals from a Pakistani family.

Methods: Whole exome sequencing was performed to search for the underlying genetic cause and Sanger sequencing was used to check variant segregation in the family. Several bioinformatics tools were used to identify the variant and assess its pathogenicity.

Results: A rare missense variant c.1738G>A, p.(Gly580Arg) was identified in *USP7* gene that encodes ubiquitin specific peptidase 7. The variant segregated with the phenotype dominantly with reduced penetrance. In silico analyses revealed damaging effects of the variant.

Conclusion: *USP7* is previously known to cause limb defects such as 5th finger clinodactyly, hallux valgus and small hands in Hao-Fountain syndrome. ClinVar search showed two variants (c.383+1G > C, c.333C>G:p.(His111Gln)) for a syndromic form of preaxial polydactyly. Based on these phenotypic observations and our finding, *USP7* can be considered as a new candidate gene for nonsyndromic polydactyly. This study expands the phenotypic spectrum of *USP7* which will help in clinical diagnosis. Future studies should be performed to define the role of this gene in limb development.

Conflict of Interest: None declared.

EP05.023 Musculocontractural type of Ehler-Danlos syndrome with severe skeletal findings in the novel variant of CHST14 gene

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Background/Objectives: Ehlers-Danlos syndrome (EDS) is a connective tissue disorder characterized by joint hypermobility, hyperextensibility of the skin and generalized connective tissue fragility. Musculocontractural EDS is an autosomal recessive disorder. Here we present this very rare syndrome with severe skeletal and radiological findings.

Methods: We presented a 16 years old girl born at 38 weeks of gestation from 39-year-old mother by spontaneous vaginal delivery. There was parental consanguinity. She walked independently by 3 years. The body weight, height, head circumference were <3th percentile at the time of her most recent assessment. On physical examination; brachycephaly, prominent forehead, micrognathia, facial asymmetry, low-set and rotated ears, prominent ears, hypertelorism, downslanting palpebral fissures, blue sclerae, hypoplastic columella, long philtrum, thin upper lip, small mouth, thorax deformation, severe scoliosis, skin hyperextensibility, atrophic scars, joint hypermobility, skin fragility, scars of operations, multiple contractures, bilateral adducted thumbs, arachnodactyly, distal arthrogryposis, low fat and muscle mass, muscle weakness. Echocardiography showed a mitral valve prolapse.

Results: Whole-exome sequencing analysis displayed a novel homozygous frameshift likely pathogenic variant in the exon 1 of *CHST14* gene (NM_130468.4 c.660_667del p.Ser221ProfsTer17).

Conclusion: The major characteristics of the musculocontractural form of EDS include distinctive craniofacial dysmorphism, congenital contractures of thumbs and fingers, clubfeet, severe kyphoscoliosis, muscular hypotonia, hyperextensible thin skin with easy bruisability and atrophic scarring, wrinkled palms, joint hypermobility, and ocular involvement. Since 1997, less than 100 patients have been described. We aimed to contribute to phenotype diversity with a new case with severe skeletal findings.

References:
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EP05.024 Update on the allelic heterogeneity and phenotypic diversity in CBFB-related cleidocranial dysplasia

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Background: Cleidocranial dysplasia (CLCD) is a rare skeletal dysplasia with significant clinical variability manifested by delayed closure of fontanels and cranial sutures, dental and clavicular anomalies and short stature. *RUNX2* has been the only known disease-causing gene for CLCD until we recently identified pathogenic variants in *CBFB* in eight individuals from five families with a CLCD-like phenotype (CLCD2). *CBFB* encodes the core-binding factor β subunit (CBFβ) that interacts with all RUNX proteins to form heterodimeric transcription factors, which may explain phenotypic differences between *CBFB*- and *RUNX2*-related CLCD.

Methods: We provide an update on the genotypic and phenotypic data of our current series of individuals with *CBFB*-related CLCD.

Results: We had previously reported five pathogenic *CBFB* variants, all located in the RUNX-binding domain of CBFβ. We have now ascertained a sixth family with a sporadic case presenting with clavicula bipartita, pseudo-epiphyses of the 2nd metacarpal, shortening of distal phalanges, delayed carpal ossification and normal stature. The novel heterozygous missense variant (c.314G>A, p.(Gly105Glu)) of *CBFB* is located in the RUNX-binding domain and in silico programs uniformly predicted its high pathogenicity. Functional studies are currently performed to investigate how these different *CBFB* variants affect the function of CBFβ-RUNX complexes, and how this may contribute to the development of CLCD2.

Conclusion: We now added a sixth family with CLCD2 to our cohort, herewith expanding the genotypic spectrum of this novel rare bone disease. By including this variant in our ongoing functional studies, we aim to improve our knowledge on genotype-phenotype correlations in CLCD2.

Conflict of Interest: Ewa Hordyjewska-Kowalczyk: None declared, Tessi Beyltjens: None declared, Eveline Boudin: None declared, Nicole Revencu: None declared, Nele Boeckx: None declared, Miriam Bertrand: None declared, Leon Schütz: None declared, Tobias Haack: None declared, Axel Weber: None declared, Eleni Biliouri: None declared, Mateja Vinkšel: None declared, Anja Zagožen: None declared, Borut Peterlin: None declared, Shashidhar Pai: None declared, Aida Telegrafi GeneDx, Inc, Lindsay Henderson GeneDx, Inc, Courtney Ells: None declared, Lesley Turner: None declared, David Geneviève: None declared, Wim Wuyts: None declared, Wim Van Hul: None declared, Gretl Hendrickx: None declared, Geert Mortier: None declared.

EP05.026 Pathogenetic mechanisms in the Darier disease (DD): a gene expression and protein interactions' study

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Background/Objectives: Darier Disease (DD) (OMIM #124200) is a rare autosomal genodermatosis which affects 1-4:100,000 people, which is characterized by loss of cell-to-cell adhesion (acantholysis), premature and abnormal keratinization (dyskeratosis) and rounded keratinocytes. DD is caused by pathogenic variants in the *ATP2A2* gene (12q23-24.1), which encodes for the ATPase Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase isoform 2 (SERCA2), a ubiquitously expressed cellular pump responsible for the calcium translocation from cytosol to endoplasmic reticulum. SERCA2 alterations impair intracellular calcium homeostasis leading to ER stress response and cell apoptosis and deregulate the NOTCH1 pathway in several disease models. The project aims include the identification of a DD transcriptional signature associated to SERCA2 defects and of the role of SERCA2 mutations on the NOTCH1 signaling pathway.

Methods: we collected patients' skin biopsies: i) to perform transcriptomic RNA-sequencing (RNA-seq) analysis on affected and unaffected subjects; ii) to generate keratinocytes and fibroblasts primary cell lines for expression analysis of the NOTCH1-downstream effector proteins (HES1, HEY1, c-MYC) and of the autophagy-related mediators to investigate this lysosome-dependent regulated mechanism of degradation; iii) to investigate on FFPE bioptic samples, NOTCH1 signaling deregulations through immunohistochemistry.

Results: we defined a DD transcriptomic gene profile by RNA-seq analyses which show reduction on the NOTCH1 signaling pathway mediated by *ATP2A2* patients' variants. NOTCH1 protein deregulations have been confirmed in vivo by IHC of the nuclear intracellular domain of NOTCH1 and of c-MYC, a downstream Notch1 target.

Conclusions: we are defining the relationships between SERCA2 and NOTCH1 proteins and the pathogenetic molecular mechanisms underlying the DD.

Conflict of Interest: None declared.

EP05.028 Genotype-phenotype correlation and effects of bisphosphonates in rare forms of osteogenesis imperfecta : a retrospective study

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Osteogenesis Imperfecta (OI) is a clinically and genetically heterogeneous group of diseases characterized by brittle bones. Though genetic mutations in *COL1A1* and *COL1A2* account for approximately 85-90% of OI cases, there are now more than twenty genes described. Treatment is based on the wide use of bisphosphonates and though it is well established that they increase lumbar spine (LS) bone mineral density (BMD), the clinical impact on fracture reduction is still debated.

In this study, we first investigated the clinical characteristics of 40 patients with variants in non-*COL1A1/COL1A2* genes in order to study genotype-phenotype correlations as the natural history of these rare forms is still little known. We then studied the usefulness of bisphosphonate treatment by evaluating the effects

on LS BMD, annual non-vertebral fracture rate, bone turnover markers, and height.

This study allowed us to further define two phenotypes. Indeed, patients with *CRTAP* variants had an antenatal presentation with a short (<3rd p) and curved femur. They can also present with more moderate forms, further extending the phenotypic spectrum of OI forms linked to *CRTAP*.

In patients with *SERPINF1* variants, we consistently observed progressive deformities and loss of mobility.

Regarding treatment by bisphosphonates, all patients showed a significant increase in LS BMD while treated and this increase was dependent on the dose received. The increase in LS BMD also translated in a reduction of fracture rate during treatment. Finally, our study showed that the earlier bisphosphonates are initiated, the greater the fracture rate is reduced.

Conflict of Interest: None declared.

EP05.029 A targeted next-generation sequencing panel outcomes for the molecular diagnosis of Ectodermal Dysplasia in Spanish population

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Background: Ectodermal Dysplasia (ED) includes several clinically and genetically heterogeneous disorders shown isolated or as part of a genetic syndrome. Due to the vast number of genes implicated, establishing a molecular diagnosis can be challenging. We aim to develop a targeted next-generation-sequencing (NGS) panel to know the most prevalent mutated genes.

Methods: We designed a panel of 125 genes involved in ED (SureSelect_XT-HS®_Agilent). If negative result, MLPA or CGH-SNP array were used for CNVs detection. We screened a cohort of 142 unrelated patients (89 males/53 females) referred two or more impaired ectodermal derivatives or only one if were hypohidrosis or Selective-Tooth-Agenesis (STHAG).

Results: The causative mutation was identified in 95 unrelated patients (66.9%), involving likely pathogenic/pathogenic variants in 25 genes. *EDA* variants associated to Hypohidrotic Ectodermal Dysplasia (XLHED, OMIM#305100) were the majority, 47.4%(45/95) following by variants in *WNT10A*, 15.8%(15/95) involved in STHAG (OMIM#150400), Odonto-Onycho-Dermal dysplasia (OMIM#2579809) or Schöpf-Schulz-Passarge syndrome (OMIM#224750) and *TP63* (OMIM*603273), 6.3%(6/95) cases. Surprisingly, *TSPEAR* is most frequent ED gene identified for autosomal recessive inheritance (ECTD14, OMIM#618180), 6.3%(6/95) cases above *EDAR* (OMIM*604095), 4.2%(4/95). Mosaicisms in *PORCN* (Focal-Dermal Hypoplasia, OMIM#305600) in 2.1%(2/95), mixed variants (CNV/SNV) in *GJB6* (Clouston syndrome, OMIM#129500) and new clinically overlapping entities as *CLDN10* (Helix syndrome, OMIM#617671) or *FOXN1* (OMIM#618806) were also identified.

Conclusions: We have developed a representative NGS panel for molecular diagnosis of a wide variety of ED with high throughput that will help us better understanding of the contribution of usual and uncommon genes in our population.

Grant References: PI14/01259_PI17/00796_PI21/01082. ISCIII, Madrid (Spain).

Conflict of Interest: None declared.

EP05.030 RMRP-related spectrum – clinical and molecular characterization of a patient cohort in the Portuguese population

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Background: Cartilage-Hair Hypoplasia is a *RMRP*-related disorder comprising a continuum phenotypic spectrum (CHH-spectrum) characterized by disproportionate short stature and other findings such as gastrointestinal dysfunction, immunodeficiency, increased risk for malignancy or anemia.

Methods: Clinical and molecular characterization of 8 CHH-spectrum cases from a Portuguese hospital centre, with performed or ongoing *RMRP* molecular study, based on retrospective analysis of medical records.

Results: We describe 6 male and 2 female patients, from 6 unrelated families, with a CHH-spectrum diagnosis, currently between 1 and 49 years old. Prior to molecular study, most had clinical or radiological diagnosis suggested (5/8), from birth to 46 years old. Two families had skeletal dysplasia panel performed in prenatal setting. In one, only one variant was detected and in the other the diagnosis was missed (*RMRP* not included in exome). Most patients had disproportionate short stature (7/8), 3/5 with prenatal onset, normal occipitofrontal circumference (5/5), normal intellect (5/5), impaired lymphocyte proliferation (6/6) and 2/2 with reported recurrent infections. Variable hypotrichosis was identified in 4/7 patients, 4/5 patients had gastrointestinal dysfunction or failure to thrive and one patient had a persistent idiopathic thrombocytopenic purpura. No malignancies were reported. A total of 10 previously described *RMRP* variants were identified.

Conclusion: Our data are generally in accordance with the literature. In 2/8 cases, one without short stature, the diagnosis was only achieved by reverse phenotyping. We aim to include patients from other Portuguese hospital centres. Detailed description of national cohorts of CHH-spectrum patients contributes to awareness and better-informed counselling and management.

Conflict of Interest: None declared.

EP05.031 A new variant in the LTBP4 gene likely leads to splicing failure

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Background/Objectives: The *LTBP4* gene encodes for a protein known as latent transforming growth factor beta-binding protein 4 (LTBP4). This protein is a member of the extracellular matrix (ECM) protein family and plays a critical role in the regulation of the transforming growth factor beta (TGF-β) signaling pathway.

LTBP4 is particularly important in the development of several organs and tissues, including the lungs, heart, and skeletal muscle. Pathogenic variants in the *LTBP4* gene cause several genetic disorders, including autosomal recessive cutis laxa type 1C (OMIM: 613177). Here we present a patient with cutis laxa and two mutations in the *LTB4* gene.

Methods: Bioinformatic analysis of the WES data was performed using a custom-developed bioinformatics pipeline. Polymerase chain reaction (PCR) Sanger sequencing was used for validation of genetic variants detected by exome sequencing. Amplification products of appropriate size were identified using agarose gel electrophoresis.

Results: A 6-year-old male patient underwent whole exome sequencing. As a result of bioinformatics analysis, two variants were identified. The first variant is a known pathogenic genetic variant resulting in the acquisition of a stop codon and premature termination of translation (ENST00000308370.11:c.1453C>T). The second variant is a new variant possibly leading to the destruction of the branch point and disruption of splicing (ENST00000308370.11:c.452-22A > C). We confirmed these variants with Sanger sequencing and showed that they are formed compound heterozygosity.

Conclusion: We classify the new variant as a variant of unknown clinical significance (ACMG classification). A functional analysis will be done to confirm the pathogenicity of the variant.

Conflict of Interest: None declared.

EP05.032 Homozygous variegate porphyria: a novel PPOX variant in two patients from a family

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Background: Variegate porphyria (VP) is one of the acute hepatic porphyrias caused by the deficient activity of protoporphyrinogen oxidase. VP is an autosomal dominant disease, resulting from the pathogenic variants in *protoporphyrinogen oxidase (PPOX)* gene. It is characterized by adult-onset cutaneous photosensitivity, blistering, fragility of sun-exposed skin, thickening, and hyperpigmentation. Homozygous VP (HVP) with biallelic *PPOX* variants is very rare, presenting with severe infantile-onset cutaneous and neurological findings. Here, we present two patients from a family with HVP.

Patients and Methods: The proband was a 10-year-old female who was referred for skin lesions suspected as epidermolysis bullosa. She developed blistering skin lesions on her face and hands when she was seven months old. She also had developmental delay and epilepsy. On examination, microcephaly, bilateral brachydactyly and camptodactyly of the hands, and ataxic gait were noted. Erosions, scarring, hyperpigmentation, and thickening were observed on sun-exposed skin. Her 7-year-old first cousin had a similar phenotype, and hyperactivity in addition.

Whole exome sequencing (WES) followed by an analysis focused on rare homozygous variants was performed on the proband. Sanger sequencing was used for confirmation and segregation analysis of the *PPOX* gene variant.

Results: WES revealed the novel homozygous *PPOX*(NM_000309.5):c.164A>C(p.Glu55Ala) variant in the proband. The affected cousin was also homozygous. Segregation analysis confirmed autosomal recessive inheritance.

Conclusion: Here, we report a novel *PPOX* pathogenic variant expanding the genotypic spectrum of HVP. HVP is an extremely rare disease and should be considered in patients with photosensitive skin lesions, microcephaly, brachydactyly/camptodactyly, and neurodevelopmental manifestations.

Conflict of Interest: None declared.

EP05.033 Report of first Endocrine-cerebro-osteodysplasia patient to reach childhood age

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Endocrine-cerebro-osteodysplasia (OMIM #612651, ECO) is an extremely rare, neonatal lethal, autosomal recessive disorder with multiple anomalies involving the endocrine, cerebral, and skeletal system. To date, seven cases diagnosed with ECO, that all died in utero or in neonatal period, have been described in the literature.

Here we report a 18 month-old female patient who was born at $37 + 6$ weeks to consanguineous parents. Physical examination revealed hydrocephalic appearance, short nose, depressed nasal bridge, broad nasal ridge, long philtrum, thin lips, low-set ears, brachydactyly, bilateral postaxial polydactyly of the hands, sandal gap and short micromelia. In her cranial MRI, hypoplasia of the brainstem and intensity changes in peripheral white matter in both cerebral hemispheres that may be due to delayed myelination, and external hydrocephaly due to cortical atrophy was detected. In her abdominal ultrasonography dilated pelvicalyceal structures of left kidney and increased echogenicity in both kidneys were noted.

Whole exome sequencing analysis revealed a homozygous likely pathogenic *CILK1*(NM_014920.5):c.1664_1665del (p.Tyr555CysfsTer48) variant at patient and segregation analysis showed the healthy parents as carriers. In addition to that the patient had a pathogenic *LMNA*(NM_170707.4):c.808A>C (p.Lys270Gln) variant that was inherited from healthy mother.

This is the first report of ECO diagnosed at the childhood. There might be several underlying mechanisms that lead to prolonged survival for our patient; such as *CILK1* variant being a null variant located at 13th exon which is the penultimate exon therefore leading partial decrease of protein expression and/or it may be caused by the presence of a secondary pathogenic *LMNA* variant.

Conflict of Interest: None declared.

EP05.035 An indel variant in BHLHA9 causes Mesoaxial Synostotic Syndactyly with Phalangeal Reduction

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Background: Mesoaxial synostotic syndactyly with phalangeal reduction (MSSD) presents a characteristic combination of clinical features that includes mesoaxial osseous synostosis at a metacarpal level, absence of one or more phalanges, and hypoplasia of distal phalanges, clinodactyly, and preaxial fusion of toes.

Methods: We evaluated a large consanguineous Pakistani family with autosomal recessive MSSD with polydactyly. Whole Exome sequencing, segregation analysis and in silico predictions were performed.

Results: The family had presented mesoaxial reduction of digits, cutaneous syndactyly, camptodactyly, clinodactyly and postaxial polydactyly. Whole-exome sequencing and Sanger sequencing identified in affected subjects a homozygous frameshift-indel mutation (NM_001164405:exon1:c.251_270del, c.251_254insCGCA, p.Phe85Glnfs*108) in *BHLHA9* that results in a truncated BHLHA9 protein. In silico study revealed major changes in 3-D structure of BHLHA9 protein that affect its interaction with other proteins.

Conclusion: This study reports second family with a previously identified frameshift-indel variation in *BHLHA9* gene and will facilitate genetic counseling in Pakistani families with a MSSD-related phenotype.

Conflict of Interest: Safeer Ahmad EDUFI Fellowship, Muhammad Zeeshan Ali: None declared, Muhammad Muzammal: None declared, Mari Muurinen full, Shabir Hussain full, Muzammil Ahmad Khan full, Outi Mäkitie full.

EP05.036 genotype and phenotype characteristics of osteogenesis imperfecta in Saudi Arabia

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Background/Objectives: Osteogenesis imperfecta (OI) is an inherited skeletal disease characterized by variable and recurrent fractures and extra skeletal manifestations. Up to date, there is 20 types of OI, majority are autosomal recessive disorders. We aim to describe clinical, molecular, and radiological features of OI and to evaluate the impact effectiveness of bisphosphonate therapy in variable genetic types of OI.

Methods: We performed a retrospective cohort analysis for 71 patients with variable ages between 2005 and 2022. Clinical and radiological assessment performed and confirmed by genetic study. In-house bisphosphonate therapy protocol is applied to all types regardless the type or severity.

Results: 71 patients with confirmed OI included. Collagenous related OI represent 51.43% with majority being de novo. Non-collagenous OI spectrum shown in 49.30%. The Most common mutation is the heterozygous mutation c.903+1G > A in *COL1A1* gene followed by the homozygous mutation c.570_571delTG in *P3H1* gene and c.831dupC in *FKBP10* gene. Collagenous OI present with typical blue sclera, dentogeneous imperfecta and variable failure to thrive while non-collagenous OI have more hearing loss, physical deformities, short stature secondary to multiple fracture, walking disabilities and respond effectively to earlier bisphosphonate therapy with variable outcome especially, patients with *TMEM38B*-related OI in whom 50% reach normal bone density.

Conclusion: Almost half of Saudi OI are collagenous and other half belongs to the non-collagenous forms. We observe very dramatic improvement for bisphosphonate therapy even in those a previously lethal OI form.

Conflict of Interest: None declared.

EP05.037 Exome sequencing of nonsyndromic cleft palate trios reveals interesting candidate genes

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Background: Nonsyndromic cleft palate only (nsCPO) is one of the most common forms of orofacial clefting and has a multifactorial etiology with a high heritability of >90%. Genome-wide association studies have identified ten genome-wide significant risk loci for nsCPO. Accordingly, common variants are involved in nsCPO development. However, genetic and epidemiological data suggest that also rare variants with a larger effect size play a strong role. The aim of this project was to perform exome sequencing (ES) in nsCPO trios and multiply affected families to identify rare highly penetrant (de novo) variants localized in potential candidate genes for nsCPO.

Methods: We performed ES in 342 individuals, 124 with nsCPO and 218 healthy relatives (84 trios, 9 quattros and 9 multiplex families ranging from 5 to 8 family members), the majority of European ancestry. Exome capture was performed using Twist Human Core Exome Kit. Libraries were sequenced on Illumina Nova Seq with 2×100 bp read length.

Results: A preliminary analysis of 43 trios identified 461 potential de novo variants. After stringent quality control, filtering and validation, the analysis revealed 46 de novo mutations in potential candidate genes for nsCPO, e.g. in *KDM6B*, a paralog of *KDM6A*, a gene for Kabuki Syndrome. A protein-protein-interaction analysis using STRING (v.11.5) showed an enrichment of interactions among the candidate genes ($p = 0.009$).

Conclusion: Our preliminary analysis shows the high potential of our dataset and adds further evidence for a strong contribution of rare variants to nsCPO.

Grant References: DFG grant MA 2546/6-1.

Conflict of Interest: Nina Ishorst: None declared, Sarah L. Mehrem: None declared, Dmitriy Drichel For the sake of completeness, we declare that Dmitriy Drichel provides compensated consulting services outside of academia as an independent consultant. Although past and current clients might include biotech, life science, and pharma companies, the services are not related to the presented work and the author is unaware of any possible conflict of interest., Sugirthan Sivalingam: None declared, Carine Carels: None declared, Iris van Rooij: None declared, Andreas Buness: None declared, Kerstin Ludwig Speaker at trainee workshops by Hans-Riegel Foundation, Co-founder and stake holder LAMPseq Diagnostics Inc., Elisabeth Mangold: None declared.

EP06 Cardiovascular Disorders

EP06.001 The relationship between the MTNR1B polymorphism and myocardial infarction susceptibility

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Introduction: Melatonin is a circadian hormone with antioxidant properties and protects against myocardial ischemia-reperfusion injury. Genetic variations of the melatonin receptor gene (*MTNR1B*) play an important role in the development of type 2 diabetes, a risk factor for cardiovascular disease. Accordingly, *MTNR1B* polymorphisms are crucial in numerous diseases of the cardiovascular system. Therefore, the aim of the present study was to investigate a possible association between the *MTNR1B* polymorphism rs10830963 and susceptibility to myocardial infarction.

Methods: The study group consisted of 199 myocardial infarction patients (57% men) and 198 control participants (52% men) without a history of cardiovascular disease. Genotyping for *MTNR1B* rs10830963 was performed from peripheral blood samples using the TaqMan SNP genotyping assay on the Applied Biosystems QuantStudio 5 real-time PCR system.

Results: In patients with myocardial infarction, the rs10830963 polymorphism was associated with cardiovascular risk factors such as type 2 diabetes ($p = 0.04$), hypertension ($p = 0.04$) and shift work ($p = 0.05$). However, no significant association, estimated by the chi-square test, was found in the distribution of alleles and genotypes between myocardial infarction patients and controls.

Conclusion: In this study, no significant association was found between rs10830963 polymorphism and myocardial infarction susceptibility. The present negative results do not exclude the role of this polymorphism in the development of myocardial infarction, as some previous studies have shown. Rather, they may indicate that rs10830963 is a minor risk factor for myocardial infarction.

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Conflict of Interest: None declared.

EP06.002 Haploinsufficiency in MYH7 as a possible mechanism of pathogenicity in a Portuguese family with Hypertrophic Cardiomyopathy

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Background/Objectives: Hypertrophic cardiomyopathy (HCM) is one of the most common inherited cardiac conditions, mostly caused by pathogenic variants in sarcomere-encoding genes. Predicted deleterious missense (pDM) variants in *MYH7* have been often described as having a dominant negative effect on sarcomeric function in HCM patients. The ACMG/AMP variant classification framework for *MYH7*-associated inherited cardiomyopathies also states that loss-of-function (LOF) variants should not be considered as an isolated cause of HCM, although it has been admitted that compound heterozygosity of LOF with missense variants could lead to extremely severe presentations. In addition, a recent paper describes a heterozygous frameshift *MYH7* variant segregating in a multiplex family affected by HCM (PMID: 34460321).

Methods: We present a family of six brothers, two of which with previous clinical diagnosis of HCM. One of these brothers' son died suddenly at 16-years-old, and another brother' son was diagnosed with HCM in his early 20s.

Results: The NGS panel requested in one of the affected brothers identified a frameshift variant in *MYH7* which introduces a premature termination codon. Genetic testing in the remaining relatives identified the same *MYH7* variant in a total of six individuals, all with echocardiographic involvement. Based on that, we reclassified this variant as likely pathogenic and offered preimplantation genetic testing to a family carrier.

Conclusion: This case strongly suggests that LOF variants in *MYH7* may be associated, albeit rarely, with HCM. It also illustrates the importance of periodically reviewing *MYH7*-specific pathogenicity criteria.

Grant References: Nothing to declare.

Conflict of Interest: Diogo Fernandes da Rocha Serviço de Genética Médica, Centro Hospitalar Universitário de São João, Márcia Baixia Ipatimup, Ana Grangeia Serviço de Genética Médica, Centro Hospitalar Universitário de São João, EXPL/DTP-EPI/0376/2012, Raquel Silva Ipatimup, Sérgio Castedo Ipatimup, Ana Sofia Correia Serviço de Cardiologia, Unidade Local de Saúde de Matosinhos, Porto, Portugal, pedro louro Serviço de Genética Médica, Centro Hospitalar Universitário de São João.

EP06.003 The MT-ND1 m.3460G>A pathogenic variant as a cause of isolated cardiac disease in a 77 year old man

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Introduction: Pathogenic variants in MT-ND1, encoding the core protein of mitochondrial respiratory Complex I, cause several disorders, including Leber hereditary optic neuropathy (LHON), Leigh syndrome and MELAS. Cardiomyopathy, particularly hypertrophic (HCM) occurs in ~30% of adults with mitochondrial diseases.

Materials and Methods: We evaluated a 77-year-old man with heart failure secondary to apical HCM. Medical history revealed Wolff-Parkinson-White (WPW) diagnosed at age 14, treated with ablation at age 49 and pacemaker implantation ~10 years ago due to atrioventricular block, and lately upgraded to cardiac resynchronization therapy defibrillator due to ventricular arrhythmia. He has no siblings and family history was limited. No ophthalmological or neurological problems were reported.

Results: Cardiomyopathy multi-gene panel testing revealed a known pathogenic variant in MT-ND1 (m.3460G > A (c.154G>A), p.Ala52Thr) with 29% heteroplasmy. This variant is one of three common mtDNA pathogenic variants underlying LHON.

Conclusions: MT-ND1 m.3460G > A, usually associated with LHON, was found to cause a purely cardiac phenotype. Manifestations included cardiac pre-excitation, found in ~10% of LHON patients, and HCM. The very few reports of left ventricle myocardial thickening in LHON m.3460G > A patients, were all in men with symptomatic optic neuropathy. Optic neuropathy is very unlikely to appear after age 75, so to the best of our knowledge this is the first report of MT-ND1 m.3460G > A presenting as an

isolated cardiac disease. The cardiac-limited phenotype may be explained by low heteroplasmy level (29%), compared to >70% leukocyte heteroplasmy in LHON patients with optic pathology. These results underscore the importance of unbiased genetic testing in cardiomyopathy patients.

Conflict of Interest: None declared.

EP06.004 Prevalence of hyperlipidemia causing genetic variants in patients with coronary artery disease

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Background/Objectives: Hypercholesterolemia is the main cause of atherosclerosis which is the leading cause of death in the developed world. Recent studies have shown that certain genetic variants related to hypercholesterolemia are much more prevalent than previously thought. Thus, familial hypercholesterolemia (FH) which is an autosomal dominant genetic disorder, might be underdiagnosed and undertreated.

Methods: During this study 93 patients with coronary artery disease (CAD) who have not been previously diagnosed with FH were included in this study. All patients had high low-density Lipoprotein cholesterol levels in blood plasma (>5 mmol/L). Next generation sequencing was used to sequence the coding regions and exon/intron boundaries of the *LDLR, PCSK9, APOB* and *LDLRAP1* genes.

Results: One patient had a heterozygous pathogenic variant in the *LDLR* gene which has been described previously (c.1013G>A (p.Cys338Tyr)). Another patient was a carrier of a heterozygous pathogenic variant in the *APOB* gene (c.10580G>A (p.Arg3527Gln)). A third patient was a carrier of a previously undescribed variant in the *LDLR* gene (c.734A>G (p.Asp245Gly)). Based on the American College of Medical Genetics criteria, this variant was determined to be likely pathogenic. A further 10 patients were carriers of variants of uncertain significance in the *LDLR* and *APOB* genes.

Conclusion: This study highlights that patients with FH remain underdiagnosed and undertreated. There is a need to expand molecular testing for patients with CAD. More complex studies are needed to determine the pathogenicity of variants of uncertain significance.

Conflict of Interest: Darius Čereškevičius Employed in Lithuanian University of Health Sciences hospital "Kauno Klinikos" department of genetics and molecular medicine., Research was funded by the Lithuanian University of Health Sciences scientific fund., The Lithuanian University of Health Sciences hospital "Kauno Klinikos" department of genetics and molecular medicine allowed the use of their next generation sequencers., Ali Aldujeli Employed in Lithuanian University of Health Sciences hospital "Kauno Klinikos" cardiology clinic., Kristina Zubielienė Employed in Lithuanian University of Health Sciences hospital "Kauno Klinikos" cardiology clinic., Vacis Tatarūnas Employed in Lithuanian University of Health Sciences Institute of Cardiology Laboratory of Molecular Cardiology.

EP06.005 Cardiogenetic examination of non-ischemic cardiac arrest survivors: concealed arrhythmogenic cardiomyopathy is the most frequent cause, while hypertrophic cardiomyopathy is underrepresented in a representative Czech cohort

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Background/Objectives: We aimed to determine the genetics of non-ischemic cardiac arrest (CA) in a representative cohort of CA survivors (CAS) in the Czech Republic.

Methods: A total of 200 CASs (120 M/80 F; mean age at CA: 35.6 ± 16 years), referred from regional centres underwent cardiogenetic counselling and NGS sequencing using a candidate gene panel (150+ genes) utilizing the SOPHiA GENETICS DDM platform. The presence of variants was confirmed by Sanger DNA sequencing and cascade screening. Additional cardiac exams (e.g. TTE, SaECG, exercise ECG) were performed to clinically validate obtained results.

Results: CASs presented mainly as idiopathic ventricular fibrillation (iVF, 124/200). Other diagnoses comprised arrhythmogenic cardiomyopathy (ACM), including forms with involvement of the right ventricle (RVACM, 11/200), left ventricle (LVACM – 13/200), or both ventricles (BiVACM – 13/200), hypertrophic cardiomyopathy (HCM-1/200), hereditary arrhythmic syndromes (LQT – 23/200, BrS-9/200, CPVT-2/200) and arrhythmogenic mitral valve prolapse (MVP -6/200). Molecular genetic testing identified a causative DNA variant (P/LP) in 54/200 (27%) of CASs. The highest detection rate was in RVACM (73%). Genetic testing clarified the diagnosis in 21% of the iVF cohort, mostly as concealed ACM.

Conclusion: Most cases of CA are of unknown aetiology (iVF -62%). Cardiogenetic examination confirmed approximately one third of these cases, primarily concealed ACM (*PKP2* gene; 12/200). Other frequent causes of CA were LQT2 (9/200), LQT3 (7/200) and CPVT (5/200). Conversely, HCM was underrepresented in this cohort (1/200 and 2/200 after genetic testing).

Conflict of Interest: None declared.

EP06.006 Genetic screening in patients with suspected heritable thoracic aortic disease in Japan

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Background/Objectives: Heritable thoracic aortic diseases (HTAD), such as Marfan syndrome, Loeys–Dietz syndrome, and vascular Ehlers–Danlos syndrome, cause life-threatening aortic events at a young age, and require accurate diagnosis for appropriate treatment. This study aimed to investigate the genetic background of patients with suspected HTAD in Japan.

Methods: The study investigated probands who were suspected of having HTAD and underwent genetic testing in our institute from July 2016 to December 2022. Genetic testing was performed by panel sequencing and Sanger sequencing for the following 11 causative genes: *ACTA2*, *COL3A1*, *FBN1*, *MYH11*, *MYLK*, *SLC2A10*, *SMAD3*, *TGFB2*, *TGFB3*, *TGFBR1*, and *TGFBR2*.

Results: A total of 244 probands (male/female 128/116, median age 37 [0–78] years) were included. One hundred forty patients (57.3%) experienced aortic dissection or aortic surgery prior genetic

testing, or had aortic root dilatation without a history of aortic events. Sixty-eight patients (27.9%) had a family history of aortic events. Pathogenic/Likely Pathogenic variants of HTAD were detected in 118 probands (48.3%): 87 *FBN1*, 8 *COL3A1*, 6 *SMAD3*, 6 *TGFBR2*, 4 *ACTA2*, 3 *MYH11*, 3 *TGFBR1*, 1 *TGFB3*, and none in other genes.

Conclusion: This study presented the positive rate of genetic testing and the composition of causative genes in patients with suspected HTAD in Japan.

Grant reference: JSPS KAKENHI Grant Number 18K15909

Conflict of Interest: None declared.

EP06.007 The role of MRAS in atherosclerosis and relevant cardiovascular risk factors

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A study by Erdmann et al., in 2009, revealed a region on 3q22.3, which encompasses the *MRAS* gene as a risk factor for Coronary Artery Disease (CAD). *MRAS* encodes muscle Ras, a member of the Ras family of small membrane associated GTPases associated with TNF-α signaling. According to GTEx and other eQTL datasets, *MRAS* risk variants for CAD increase *MRAS* mRNA levels primarily in the arterial tissue. Moreover, recently it has been indicated that functional *MRAS* variants are Macrophage- and SMC-specific. The role of *MRAS* in atherogenesis is still elusive. Therefore, we aimed to study the function of *MRAS* in vascular smooth muscle cells (SMCs), one of the key cell types in the etiology of atherosclerosis and in plaque stabilization. To assess the impact of *MRAS* deficiency, human primary aortic SMCs transfected with MRAS-specific siRNA and murine aortic SMCs derived from Mras/Apoe knockout (ddKO) mice were subjected to functional assays including proliferation and migration. Mras/Apoe ddKO murine SMCs significantly proliferate more and migrate faster as compared to wild type SMCs when stimulated with TNF-α ($n = 4$, $p < 0.001$), but not without stimulation. Similar results were obtained from human SMCs after TNF-α stimulation where the knockdown of *MRAS* increases migration and proliferation ($n = 6$, $p < 0.01$). Control experiments with PDGF stimulation showed no impact of *MRAS* deficiency on cellular behavior, indicating that *MRAS* is specific to TNF-α signaling. In conclusion, *MRAS* deficiency modulates SMC biology in response to TNF-α. However, further studies are needed to decipher its exact role in plaque stability. Keywords: MRAS, TNF-α

Conflict of Interest: Pashmina Wiqar Shah Full timw, Full, Student, Dr. rer. nat. Tobias Reinberger Full time, Co-supervisor, Jeanette Erdmann Full time, Prinicpal Investigator, Prof. Jeanette Erdmann is the PI of this project. I am doing my Phd under her kind supervision. I am funded by the DAAD (Deutsches Akademischer Austausch Dienst) for a period of four years to complete my PhD.

EP06.008 Isthmic aortic coarctation, a rare complication of Vascular Ehlers-Danlos syndrome?

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Background: Vascular Ehlers-Danlos syndrome (vEDS) (OMIM #130050) is characterized by arterial, intestinal, and uterine fragility, thin and translucent skin, easy bruising, characteristic facial appearance, and an aged appearance to the extremities.

Clinical report: A heart murmur, not present during his previous medical follow-ups, was diagnosed in an 11-year-old child, justifying the performance of a cardiac ultrasound to demonstrate tight isthmic aortic coarctation, an atypical diagnosis given his advanced age. The intraoperative aspect found a friable and very fragile aortic tissue.

Associated symptoms consisting of thin and translucent skin with visible vascularization, easy bruising, postoperative scalp sore, early-onset lumbar scoliosis, and pes planus led to the *COL3A1* gene testing. A heterozygous missense variant c.2806G>A (p.(Gly936Ser)) confirmed the diagnosis of vEDS. This variant with a dominant negative effect is inherited from his mother. She presented with two complete perineal tears intrapartum. An aneurysm of the celiac trunk, aneurysms of the right renal artery, an ostial stenosis of the left renal artery with post-stenosis dilation, and ischemic sequelae on both kidneys, had been found with no lesion on the supra-aortic trunks on imaging following a traffic-induced trauma. Maternal grandparents were tested, proving that the *COL3A1* variant occurred de novo in the mother.

Conclusion: We report the first case of a child with confirmed vEDS and aortic coarctation, more patients may be affected by this rare complication. vEDS can have dramatic consequences during surgery due to vascular fragility. A diagnosis before surgery could therefore allow additional precautions to be taken to prevent these risks.

Conflict of Interest: None declared.

EP06.009 Prevalence of genetic variants associated with thrombophilia in patients with intracoronary stents

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Background/Objectives: Atherosclerosis and intravascular thrombosis play a central role in acute coronary syndromes treated by implantation of an intracoronary stent. In recent years, the role of thrombophilia in the pathogenesis of arterial thrombosis has become increasingly relevant. The aim of this study was to determine the genetic association of coronary artery disease (CAD) with the genotype of variants in the genes coding for prothrombin (F2), coagulation factor V (F5), plasminogen activator inhibitor-1 (PAI-1), methylene-tetrahydrofolate reductase (MTHFR) and coagulation factor XIII (F13A1).

Methods: The genotyping was performed by TaqMan-based RT-PCR on samples from 60 patients with CAD who underwent intracoronary stent implantation and 29 control subjects, followed by statistical analysis.

Results: Multivariate logistic regression was used to constructs a model for predicting the coronary artery disease with investigated variants genotypes. The AA genotype of the F5 1601G > A Leiden mutation (rs6025), the GA genotype of the F2 20210G > A rs1799963 variant, the 4G/5G genotype of the PAI-1 -675 4G/5G rs1799889 polymorphism, and the AC genotype of the MTHFR 1298A > C variant rs1801131 have the effect of increasing the probability of CAD. In contrast, the GT genotype of the F13A1 103G > T (rs5985) variant has an effect of reducing the odds for

CAD. The effects of the CC and CT genotypes of the MTHFR 677C > T rs1801133 variant on the model are statistically marginal.

Conclusion: The investigated gene variants have a potential in clinical prediction of the risk for developing coronary artery disease.

Conflict of Interest: Marina Stratrova Genomika Medical, Skopje, Hristo Pejkov University Clinic of Cardiology, Medical Faculty, Ss. Cyril and Methodius University in Skopje, Macedonia, Zan Zimbakov University Clinic of Cardiology, Medical Faculty, Ss. Cyril and Methodius University in Skopje, Macedonia, Slavica Josifovska Laboratory for Molecular Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University in Skopje, Sasho Panov Laboratory for Molecular Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University in Skopje.

EP06.010 Rapid exome sequencing for children with acute cardiomyopathy in the PICU – a case series

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Background: Rapid exome sequencing (ES) is increasingly being utilized as an efficient diagnostic tool in the critical care setting with high diagnostic yield. Acutely presenting cardiomyopathy (CM) can often require critical care and has numerous possible etiologies including various genetic disorders, particularly in children.

Here we describe a brief series of pediatric patients hospitalized in the intensive care unit (ICU) with acute cardiomyopathy of unknown cause. Rapid ES provided a timely diagnosis and informed clinical decisions including eligibility for extracorporeal membrane oxygenation ECMO or heart transplant.

Methods: ES was performed for 5 unrelated patients ages 8 days to 10 years with acute cardiomyopathy hospitalized in the ICU. Turnaround time was 5 to 60 days

Results: ES was diagnostic in 3/5 cases including a likely pathogenic variant in *ACTC1*,c.664G>A, in a 6 year old boy with left ventricular non-compaction and a homozygous pathogenic variant in *NRAP*,p.Gln1113Hisfs*40, in a 10 year old boy with dilated CM. In both cases, as results confirmed isolated cardiac involvement, the patients were eligible for transplant and received bridging therapies.

A homozygous pathogenic variant in *MYBPC3* :c.3491-2A > C was detected in a neonate confirming the diagnosis of fatal neonatal CM. Results were delivered within 5 days precluding ECMO.

In two cases, a variant of unknown significant was identified in genes, related to broader phenotypes These results prompted further investigation and extended metabolic workup.

Discussion and conclusion: Rapid ES for pediatric patients with acute CM had a high diagnostic yield and a significant impact on patient care.

Conflict of Interest: None declared.

EP06.011 A homozygous rare TTR variant of transthyretin amyloidosis

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Background/Objectives: Hereditary transthyretin amyloidosis (ATTRv) is a rare autosomal dominantly inherited disease caused by mutations in the transthyretin (*TTR*) gene. More than 140 *TTR* gene variants have been reported in ATTRv. Few cases have been described in which the rare *TTR* variant c.302C>T, p.(Ala101Val) was mostly associated with cardiac ATTRv.

Methods: We present a 45-years-old patient with diagnosed sensomotor polyneuropathy of the upper and lower extremities and pronounced tetraparesis. Cardiac involvement (amyloidosis) was suspected only 4 years later. Cardiac MRI was performed showing asymmetric LV hypertrophy with a suspicion of hypertrophic cardiomyopathy. Haematological analysis for AL amyloidosis were negative. 99mTc-PYP bone scintigraphy showed no myocardial uptake (Grade 0). Endomyocardial biopsy was performed for differential diagnosis. Amyloid deposits were found, but immunohistochemistry showed a likely non-specific reaction to transthyretin. Mass spectrometry was not available.

Results: Next-generation sequencing revealed a likely pathogenic homozygous variant of the *TTR* gene NM_000371.3:c.302C>T, NP_000362.1:p.(Ala101Val). As parental testing was not available, real-time PCR analysis was performed and no heterozygous deletion of exon 3 of the *TTR* gene was detected. This variant in the homozygous state was not described in the literature before. The same c.302C>T *TTR* variant in the heterozygous state was also found in 3 other unrelated probands (2 females and 1 male) with predominant cardiac involvement at our centre. Their ages at diagnosis were 57, 74 and 77.

Conclusion: The homozygous c.302C>T *TTR* variant is associated with earlier disease onset and neurological involvement compared to the heterozygote state.

Conflict of Interest: Dovile Zebrauskiene Created a presentation about ATTR cardiomyopathy which was funded by Pfizer., Egle Sadauskiene: None declared, Sigita Aidietiene: None declared, Agnė Šiaudinienė: None declared, Valdas Pečeliūnas: None declared, Jurate Barysiene: None declared, Egle Preiksaitiene: None declared.

EP06.012 Generation of antibodies against α-Klotho protein using canarian camels

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Cardiovascular disease (CVD) is the leading cause of death worldwide. Atherosclerosis is the substrate responsible for the vast majority of cardiovascular events. Reductions in α-Klotho protein levels have been related with the pathophysiology of CVD, particularly in chronic kidney disease patients.

The main objective of our project is to obtain mini- and nanoantibodies (nanobodies) from Canarian camels to be employed in the detection of human α-Klotho protein in different tissues, and serum and urine samples.

Nanobodies are variable domains of heavy chain-only antibodies (HCAbs) that can be isolated from camelids. In spite of their single domain structure, nanobodies display many unique features, such as small size, high stability, and cryptic epitopes accessibility, which make them ideal for sophisticated applications in plants and animals.

We carried out immunizations in camels with the different regions of the protein to detect the different forms in which the protein is presented. We isolated lymphocytes from camel blood in order to clone the DNA regions codifying for antibodies against α-Klotho, that will be further expressed in bacteria. Effectiveness and sensitivity of these new generation antibodies are being tested in clinical samples and cell lines that express α-Klotho through different approaches, e.g. western blot, ELISA and immunohistochemistry assays. These mini- and nanobodies will allow us to use α-Klotho as a biomarker with clinical applicability.

This work is supported by Cabildo de Tenerife, Tenerife 2030, FDCAN, MEDI, in the Agustín de Betancourt programme and PIFIISC21/23 from Fundación Canaria Instituto de Investigación Sanitaria de Canarias (FIISC).

Conflict of Interest: None declared.

EP06.013 Clinical and laboratory characteristics of congenital heart defects, caused by microdeletion of 22q11.2

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Introduction: Isolated congenital heart defects (CHD) are the most frequent among all congenital defects (CD). Their frequency among live births of Kyiv is 8-9:1000 (2019-2021). Microdeletion of 22q11.2 and trisomy of chromosome 21 are the most common causes of CHD.

Materials and methods: Prospective genetic counseling and examination of 77 children, aged 1 day to 12 years from different regions of Ukraine for the last 10 years was undergone at the Center of Medical Genetics of Children's Hospital "OKHMATDYT" (hospital cohort). All cases of CHD were confirmed by pediatric cardiologist. Cytogenetic examination included G-banding karyotyping according to the standard protocol. The FISH using loci-specific microdeletion of 22q11.2 DNA-probes was applied to verify the microdeletion.

Results: Sixty-seven full-term children (87%) were born via physiological delivery, with body weight more than 2,5 kg (81%). Prenatally diagnosed CHD before 22 week of pregnancy were in 16 women (21%), invasive prenatal diagnostics took place in 12% of cases. Phenotype of probands: presence of stigmas was in 51 children (66%), hypo- and aplasia of thymus were in 50 children (65%), other CD - 27%, among them – multiple CD in 8 cases (38%), polydactyly in 4 cases (19%). Four probands (5%) with microdeletion of 22q11.2 did not have any CHD. Conotruncal defects were in 55% of all CHD, critical CHD were in 14%. Microdeletion of 22q11.2 was confirmed in all probands.

Conclusion: With the aim of study the etiology and differential diagnostics of CHD in children, applying of whole range of molecular studies are recommended.

Conflict of Interest: Nikita Pozhar full, Vira Galagan full, Valentyna Kurakova full, Andrii Kurkevych full, Yuliia Dudierina full, Maryna Tsyhankova full, Yurii Hryshuk full, Diana Mykhailova full.

EP06.014 Time to downgrade genetic testing? Review of pathogenic variants in cardiogenetic

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The financial and societal impact of hereditary cardiac diseases (HCD) is widely acknowledged. One of the most prevalent of these conditions is hypertrophic cardiomyopathy – it affects 1:200 to 1:500 people – where is reported that in half of cases it is found a pathogenic mutation. In 2022 it was published international "Expert Consensus Statement on the state of genetic testing for cardiac diseases".

We reviewed the results of genetic testing performed for HCD in our laboratory between January 2019 and January 2023. A total of 675 genetic analysis were carried out for, confirmed or suspected: hypertrophic cardiomyopathy (HCM N = 243), dilated cardiomyopathy (DCM N = 215), arrhythmogenic cardiomyopathy (ACM N = 38), among others.

We found pathogenic or likely pathogenic variants in 130 samples with genetic diagnostic rates for:

HCM 22,2% (N = 54) – with pathogenic (P) or likely pathogenic (LP) variants on *ACTN2, ALPK3, FHOD3, MYBPC3, MYH7, MYL2, PRKAG2, RBM20, RYR2, SLC25A4, TNNI3, TNNT2, TPM1;*
DCM 21.4% (N = 46) *–DSP, FLNC, GLA, LMNA, MYBPC3, MYH7, PKP2, RBM20, RYR2, SCN5A, TNNT2, TPM1, TTN;*
ACM 31,6% (N = 12) – *DSP, GLA, HAMP, KCNJ2, MYBPC3, PKP2, RYR2*.

Currently, the evident benefits of genetic testing in HCD is widely accepted, namely for early disease detection and management. In our cohort it was found P/LP variants on genes with clinical implications for medical management of patients, not included in the 2022 international recommendations. This work aims to contribute to the ongoing discussion of potential benefits of expanded genetic testing in HCD, namely the possibility of reverse phenotyping on cases of clinical uncertainty.

Conflict of Interest: Diana Antunes Genomed Part-time

Chulc/santa marta full time, Ana Coutinho full-time, Yuri Chiodo full-time, Brigida Meireles full-time, Sofia Pérez CHULC/Estefânia Hospital, Maria Carmo-Fonseca IMM full time, Genomed

EP06.015 Prognostic value of microRNA-126-3p for cardiovascular events in a Spaninsh general population

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Background and objectives: Measurement of circulating levels of microRNAs (miRNAs) is emerging as potential biomarkers for cardiovascular disease. Here we estimate the predictive value of a

plasma miRNAs signature associated with albuminuria in the incidence of cardiovascular events.

Methods: Plasma miRNAs quantified in hypertensive patients by next generation sequencing were validated in a cohort of patients and controls by real-time quantitative PCR. The microRNAs associated with albuminuria were tested and their prognostic value for cardiovascular event incidence were analyzed on a retrospective, population-based study (Hortega Study) using Cox proportional hazard models.

Results: A plasma microRNA profile was identified in the discovery cohort associated with albuminuria and three microRNAs (miR-126-3p, miR-1260b and miR-374a-5p) were confirmed in the validation cohort. The microRNA signature discriminates urinary albumin excretion at baseline ($n = 1025$), and predicts the incidence of cardiovascular events and coronary heart disease and stroke in a general population retrospective study within a 14-year follow-up ($n = 926$). Thus, high miR-126-3p levels were associated with a shorter time free of both cardiovascular events (HR = 1.48, (1.36–1.62), $p < 0.0001$), as well as coronary artery disease and stroke combined (HR = 2.49, (2.19–2.83), $p < 0.0001$).

Conclusion: An increased plasma microRNAs profile was identified in hypertensive patients with albuminuria. Increased miR-126-3p emerges as a prognostic marker for cardiovascular events in a long-term general population. Further studies will assess the potential role of miR-126-3p as a guide for the status of endothelial dysfunction.

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Conflict of Interest: None declared.

EP06.016 Differentially expressed genes in peripheral blood mononuclear cells of coronary artery disease patients

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Aim: To reveal the differentially expressed genes (DEGs) of HDL-cluster and atherogen-cluster in peripheral blood mononuclear cells (PBMC) of CAD patients.

Methods: 77 male patients with CAD diagnosed by angiography and 65 control patients were enrolled. Two sets of genes related to HDL metabolism (HDL-cluster with 23 genes) and atherosclerosis-prone (atherogen-cluster with 41 genes) were selected by bioinformatic approaches. Transcript levels of 64 genes in RNA isolated from PBMC were measured by real-time RT-PCR and compared by REST software. KEGG pathway and STRING analyses of protein-protein interaction of DEGs were exploited.

Results: Thirty DEGs in CAD patients were identified. Compared with control, 8 genes in HDL-cluster (*AMN*, *APOE*, *CETP*, *LDLR*, *LPL*, *PLTP*, *PRKACA*, and *ZDHHC8*) were upregulated and 6 genes (*ABCA1*, *ABCA5*, *ALB*, *APOA1*, *LCAT*, and *SCARB1*) were downregulated in CAD. Analogously, 8 genes in atherogen-cluster (*CXCL5, IL1B, ITGB3, NR1H2, NR1H3, TLR5, TLR8*, and *TNFRSF1A*) were upregulated and 8 genes (*CD14, F5, IL1R1, ITGAM, PCTP, S100A12, S100A8*, and *TNFRSF1B*) were downregulated.

Conclusion: The expression of genes involved in cholesterol efflux decreased in CAD while the expression of genes involved in (re)generation of nascent HDL-like particles increased. The concomitant increase of *APOE* and *LDLR* expression may be responsible for the increased cellular uptake of remnants of TG-rich lipoproteins. Higher expression of *ZDHHC8* may evidence the mitochondrial-regulated apoptosis. Expression pattern of atherogen-cluster genes generally relates to inflammatory processes and immune response through MYD88 and TRAF6, leading to NF-kappa-B activation. Opposite changes of *IL1B* and *IL1R1* expression may weaken the net effect.

Conflict of Interest: Elena Nosova full, Alexander Dergunov full, Mikhail Popov full, Alexandra Rozhkova full, Margarita Vinogradina full, Veronika Baserova full, Svetlana A. Limborska full, Liudmila Dergunova full.

EP06.017 Arrhythmiogenic right ventricular cardiomyopathy (ARVC) phenotype investigated by exome sequencing reveals a Naxos disease case

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Background: Arrhythmiogenic right ventricular cardiomyopathy (ARVC) is a disorder with increased risk for arrhythmia including sudden cardiac death. ARVC is usually inherited following an autosomal dominant pattern, with variable penetrance and expressivity. In rare cases, mutations in *JUP* may lead to a rare autosomal recessive condition in the spectrum, namely Naxos disease, presenting additionally with woolly hair and palmoplantar keratoderma. We present a case of Naxos disease diagnosed at infancy.

Methods: A 13.8 m.o. male was referred to our lab for ARVC investigation, following clinical evaluation and no cardiological signs. Woolly hair, patches of rough/dry skin at knees/palms just after the initiation of crawling and both healthy, non-consanguineous parents originating from the island of Naxos, Greece, set the clinical suspicion. Exome sequencing (ES) was applied on DNA extracted from peripheral blood: library preparation was performed with Clinical Exome Solution (Sophia Genetics) and sequenced on Illumina NextSeq-550 (Illumina). Bioinformatics analysis was conducted by SOPHiA DDM® bioinformatics pipelines.

Results: Naxos disease has a prevalence of 1:1000 in the population of the Greek islands and was suspected based on phenotypic traits and origin of the toddler. A virtual panel of 51 genes related to arrhythmiogenic syndromes was analyzed. Tertiary analysis revealed a homozygous pathogenic *JUP* variant; c.2038_2039del or p.(Trp680Glyfs*11), classic for Naxos disease.

Conclusion: ES permitted early genetic diagnosis of Naxos disease. Although principles of evaluation and treatment are based on ARVC and general heart failure guidelines, early diagnosis is essential for patient management, timely interventions and relative cascade screening for risk stratification.

Conflict of Interest: Georgia Christopoulou Full-time employment at Genotypos M.S.A., Full-time employment at Genotypos M.S.A., Stavros Bournazos Full-time employment at Genotypos M.S.A., Aikaterini Oikonomaki Full-time employment at Genotypos M.S.A., Stavroula Samara Full-time employment at Genotypos M.S.A., Antigoni Miliou: None declared, Charalambos Vlachopoulos: None declared, Pantelis Constantoulakis Full-time employment at Genotypos M.S.A.

EP06.018 Correlations of BHMT (rs3733890) and PAI-I (rs1799889) gene variants with blood parameters in pediatric patients with hereditary thrombophilia

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Background/Objectives: Clinical presentation of hereditary thrombophilia in childhood leads to a deterioration in quality of life, and sometimes to disability, recurrence and death. The aim of the study was to analyze a correlation between genes variants and hemostasis parameters to find new targets in prevention and treatment.

Methods: The study involved 116 children (mean age 9.44 ± 5.01 years) with a clinical presentation of hereditary thrombophilia (thrombosis, stroke, etc.). The investigations of *PAI-1* (rs1799889), *FV* (rs6025), *FII* (rs1799963), *ITGA2* (rs1126643), *ITGB3b* (rs5918), *FGB* (rs1800787, rs1800790), *MTHFR* (rs1801133, rs1801131), *MTRR* (rs1801394), *MTR1* (rs1805087), *RFC1* (rs1051266), *BHMT* (rs3733890) genes variants were performed using PCR and PCR-RFLP. All children patients underwent standard hemostasis examination. Parents of the children gave informed consent. There were used nonparametric Spearman test (SPSS v.27).

Results: Power significant correlations were found between *BHMT* gene variant and D-dimer level, as well as variant of the *PAI-I* gene and the activities of plasminogen and plasminogen inhibitor. D-dimer levels were significantly lower in patients with the 742GG genotype of the *BHMT* gene. Patients with the 4G/4G genotype had the highest levels of plasminogen and plasminogen inhibitor activities.

Conclusion: The identified correlation of *BHMT* gene variants with D-dimer levels may be the basis for the development of a new strategy for the prevention and treatment of hereditary thrombophilia through betaine consumption. At the same time, analysis of *PAI-I* gene variants will be important to assess the risk of thrombotic events and compensatory capacity in patients.

References: No

Grants: No

Conflict of Interest: None declared.

EP06.019 Identification of new risk loci for ischemic stroke in genome-wide data with clustering methods

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Background/Objectives: Stroke is the second cause of death worldwide. Despite a few dozens of genomic loci found to be associated with the ischemic stroke (IS), the genetic bases of the disease remain underexplored. We applied clustering methods to genome-wide data of 5580 individuals with IS and controls to search for genetic loci (groups of single nucleotide polymorphisms, SNPs) associated with the risk of IS.

Methods: The genotypes of 883908 SNPs were transformed into autosome based linkage disequilibrium matrices and clustered with DBSCAN and HDBSCAN algorithms. Haplotypes were inferred for each cluster and tested for association with IS using Plink, p-values were adjusted by the total number of clusters revealed. The sets of SNPs associated significantly with IS were annotated. Genes obtained were tested for overrepresentation in sets of human genes given in MSigDB.

Results: There were identified 97 and 122 candidate genes by DBSCAN and HDBSCAN, respectively, and 88 of which were common. Sixteen genes of protocadherin gamma gene cluster were found to be overrepresented in cell adhesion.

Conclusion: To date little is known about the role of protocadherin genes in IS but there are several studies showing their involvement into neurodevelopmental disorders. Our research showed for the first time the association of several protocadherin genes of gamma subfamily with IS. This suggests protocadherins can be a common part of base mechanisms underlying pathological processes in nervous system.

Grant: The study was funded by Russian Foundation for Basic Research (grant No 19-29-01151).

Conflict of Interest: None declared.

EP06.020 Establishment of Sidra Cardiac Registry in Qatar and the dissection of genetic causes in patients with congenital heart disease

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Background: Congenital heart disease (CHD) is one of the most common birth defects worldwide, with a prevalence of 1–1.2% in liveborn infants. Many factors are involved in the causality of CHD including genetic defects like chromosomal abnormalities and single gene mutations.

Methods: In this study, we establish the Sidra Cardiac Registry at Sidra Medicine in Qatar, which includes so far 92 patients with cardiac problems, including CHD, cardiomyopathy and channelopathy, in addition to their relatives. We investigated the genetic causes of 52 registered patients with CHD and reviewed the results of the genetic testing conducted in those patients as part of their clinical evaluation, including chromosomal testing. Furthermore, we performed whole exome sequencing (WES) analysis to identify potential causative variants.

Results: Sixteen CHD patients had chromosomal aberrations, that explained their complex disease phenotype, including six patients with trisomy 21. Moreover, from the WES analysis, we describe 65 potential variants in 56 genes identified in 24 CHD patients. Four out of the 65 variants were pathogenic/likely pathogenic based on the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) classification; these variants were detected in four patients.

Conclusion: Our study presented potential variants that could contribute to the development of CHD in our patients. Thus, further functional assessments are needed to better understand the role of the identified variants in relation to CHD pathogenesis.

Grant Reference: Sidra Internal Research Fund (SDR200038).

Conflict of Interest: None declared.

EP06.022 Complex congenital heart disease in the 8q21.11 deletion

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Congenital heart diseases (CHD) are the most common human birth defects with an incidence of 0.8-1% livebirths and are a major contributor to morbidity and mortality. Most cases are multifactorial; however, known single gene disorders associated with CHD is increasing gradually with the use of next generation sequencing technology. Pathogenic mutations in over 70 genes have been linked to isolated and syndromic CHD, with most mutations causing a loss-of-function. Identification of CHD-causative genes is significant in preimplantation, pre- and postnatal genetic diagnosis, early prophylaxis and personalized treatment of CHD.

A critical region for 8q21.11 deletion was recently defined, including the gene *HEY1*. Patients with deletions of this critical region frequently have CHD, typically septal defects. However, complex CHD has not been reported. We report a 2-year-old patient with hypoplastic right heart, hyploplastic tricuspid valve, pulmonary atresia, total anomalous pulmonary venous return and ventricular septal defect as well as hypotonia, developmental delay, short palpebral fissures, and low set ears. Microarray analysis revealed 4.34 Mb deletion at GRCh37/hg19 8q21.11q21.13 (chr8:78001401-82340000) overlapping the critical region and encompassing *HEY1*. Whole genome sequencing revealed no other candidate variants related to the phenotype.

HEY1 is involved in the embryonic development of the heart, central nervous system, and vascular system and is part of the NOTCH1-HEY1 pathway. Hrt1/Hey1null mice show perinatal lethality due to congenital malformations of the aortic arch and its branch arteries. This case expands the cardiac phenotype of 8q21.11 deletion syndrome and the cardiac defects seen in association with haploinsufficiency of *HEY1*.

Conflict of Interest: None declared.

EP06.023 Reporting a new ACVRL1 gene variant involved in Rendu-Osler-Weber syndrome

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Background/Objectives: Rendu-Osler-Weber syndrome, also known as Hereditary Hemorrhagic Telangiectasia (HHT), is a rare autosomal dominant disorder characterized by arteriovenous malformations due to defects in angiogenesis. The main clinical manifestation is bleeding that can occur in any location, the most frequent being recurrent and spontaneous epistaxis. We performed a genetic study in a family with several members clinically diagnosed with HHT.

Methods: 13 whole blood samples were obtained from members of the three youngest generations in order to perform a targeting exome of the main five genes associated with HHT (ACVRL1, BMPR2, ENG, GDF2, SMAD4). Variants annotation criteria were: changes with a number of readings >20x and a frequency >30%. Variants were double-checked by Sanger sequencing, compared to the reference sequence (HGMD database), and faced to different databases and in silico prediction tools.

Results: Five members were found to be heterozygous for the variant c.752_772+2dup in the ACVRL1 gene, which is a 23 nucleotide duplication in intron 6 splice donor site. Interestingly, it is the first time this variant has been reported and 3/5 of the tested patients have presented with classical symptoms (mainly epistaxis and cutaneous telangiectasia).

Conclusion: Although the variant c.752_772+2dup in the ACVRL1 gene has not been previously described, its location in the splice donor site and the presence of clinical manifestations in a high number of probands suggest a possible pathogenic effect on ARNm processing. In agreement, a similar intronic variant (c.772+3_772+4dupAA) has been previously reported as pathogenic.

Grant References: not applicable.

Conflict of Interest: None declared.

EP06.024 Sequence analysis unveils a "hot-spot" for microhomology-mediated CNVs in FLNC linked to arrhythmogenic/dilated cardiomyopathy

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Background/Objectives: Loss-of-function variants in the *FLNC* gene have been associated with a specific overlapping phenotype of arrhythmogenic/dilated cardiomyopathy. To date, only splicing, frameshift, or nonsense variants have been reported. In this study, we sought to identify the exact breakpoint of three different copy number variations (CNVs) detected in *FLNC* in patients with this phenotype, as well as to review the sequence near the breakpoints to ascertain the putative mechanism underlying their formation.

Methods: Patients' genomic DNA was genotyped using next-generation sequencing (NGS) with a panel of genes that included *FLNC*. The CNVs were detected using a read-depth approach, and Sanger sequencing was used to confirm the breakpoints.

Results: In a cohort of 6,750 patients diagnosed with dilated and/or arrhythmogenic cardiomyopathy sequenced by NGS, we identified three different CNVs affecting *FLNC*. The breakpoints were characterized at the sequence level. The 5' ends of all three CNVs were found in intron 2 of the gene. According to an analysis of the sequence close to the breakpoints, the CNVs were likely generated by breakpoint microhomology.

Conclusion: Breakpoint sequencing of three different CNVs involving the *FLNC* gene revealed the presence of microhomology at nearby sites that promote DNA breaks, highlighting the importance of intron 2 of the *FLNC* gene as a possible hot spot for CNVs.

Conflict of Interest: Laura Cazón Full time, Emilia Maneiro Full time, Luis De la Higuera Romero Full time, Marlene Perez Barbeito Full time, Rosalía Peteiro Full time, Iria Gómez Díaz Full time, Paula Velez Full time, Maria Sanchez Full time, Anahi Sanluis Verdes Full time, Guillermo Smith Ramos Full time, Xusto Fernandez Full time, Diego Cabrera Argaña Full time, Almudena Amor Full time, María Valverde Full time, Soledad García Hernández Full time, Ivonne Cárdenas Reyes Full time, Martin Ortiz Genga Part-time, Juan Pablo Ochoa Full time.

EP06.025 Effect of MYBPC3 c.913_914del variant: a cohort study

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Objective: Hypertrophic cardiomyopathy, the most common genetic heart disease, is frequently caused by the c.913_914del pathogenic variant in sarcomeric gene MYBPC3. This variant was previously reported as highly penetrant and causative for high rate of adverse cardiac events, including sudden cardiac death (SCD), aborted SCD (aSCD), and heart failure deaths. The aim of present study was to determine the impact of the variant on clinical presentation and prognosis in our cohort.

Methods: Our database of genetic testing results was screened for probands with (likely) pathogenic variants in MYBPC3 and their relatives. Clinical characteristics, rate of adverse events and penetrance were collected and compared between individuals with c.913_914del and those with other (likely) pathogenic variants in MYBPC3.

Results: We identified 30 (19 probands/11 relatives) aged 42 ± 16 years having c.913_914del (NM_000256.3) and 48 (31 probands/17 relatives) aged 33 ± 21 years with other pathogenic variants in MYBPC3. No significant difference in clinical characteristics were observed between probands with c.913_914del and other MYBPC3 variants (left ventricular hypertrophy: 21 ± 11mm vs 18 ± 7mm; p = 0,33). The penetrance for c.913_914del was 73,3% and 72,9% for other MYBPC3 variants. During follow up (9 ± 8 years), none of probands in either group died of SCD or heart failure. One patient in each group had heart transplantation or experienced aSCD (1/19 vs 1/31; p = 0,72). The prevalence of non-sustained ventricular tachycardia was similar in both groups (3/19 vs 5/31; p = 0,97).

Conclusion: MYBPC3 c.913_914del does not lead to significantly increased rate of SCD, different clinical presentation and penetrance in comparison with other pathogenic MYBPC3 variants.

Conflict of Interest: None declared.

EP06.027 Frequency of the most common mutations in the LDLR gene in patients with familial hypercholesterolemia from RN Macedonia

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Background: Familial hypercholesterolemia (FH) is a highly prevalent disorder and a risk factor for early coronary artery disease, caused by inherited changes (mutations) in the *LDLR*, *APOB*, and *PCSK9* genes, which affect processes of regulation and elimination of cholesterol from the blood. Literature data have implicated the involvement of defects in the *LDLR* gene as the most common cause of this condition.

Methods: We evaluated the frequency of the most common mutations in the *LDLR* gene from 110 patients (72 males, 38 females) with clinically defined characteristics of FH. The methodology included TaqMan genotyping analysis for four variants in the *LDLR* gene (c.81C>G p.(Cys27Trp), c.858C>A p.(Ser286Arg), c.1646G>A p.(Gly549Asp) and c.1285G>A p.(Val429Met).

Results: A total of 25.3 % of the patients had a mutation in the *LDLR* gene. The most common mutation was c.81C>G detected in 18 (16.3%), followed by c.858C>A in 7 (6.3%) and c.1646G>A in 3 (2.7%) patients. Three patients had two mutations (one homozygote for c.81C>G, and 2 double heterozygotes for c.81C>G c.858C>A and c.81C>G/c.1646G>A, respectively). No significant difference was found in the distribution of these defects between men and women, whereas a higher frequency of defects was detected in patients >50 (73.1%), compared to patients <50 years of age (26.9%) ($p < 0.05$).

Conclusion: We identified distribution of molecular defects for FH similar to the neighboring countries. The identification of the most common mutations in the LDLR gene will improve the genetic diagnosis of FH in our population.

Conflict of Interest: None declared.

EP06.028 Association between hypertension and coffee drinking based on CYP1A2 rs762551 single nucleotide polymorphism in a Romanian population

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Background: Hypertension is of importance mainly as a risk factor for heart, brain and kidney disease. To date, efforts have been made to reduce and treat high blood pressure levels. Cytochrome P450 1A2 (CYP1A2) is known to be the main enzyme, directly responsable for caffeine metabolism. Rs762551, is a SNP encoding the CYP1A2*1F allele of the CYP1A2 gene, and it known mostly for metabolizing caffeine and its protective role against developing high blood pressure. We aimed to determine if habitual coffee intake has a protective role on hypertension onset, related to CYP1A2 polymorphism.

Methods: For this study 358 hypertensive patients have been considered. PCR technique, analyzing rs762551 (assay C_8881221) on LightCycler 480 (Roche) with Gene Scanning software version 1.5.1 (Roche) has been proceeded.

Results: Absence of statistical association could be observed between the following pairs: gender –hypertension, coffee intake – hypertension and genotype – coffee intake. In addition, a lack of a significant association between the genotype and the presence of hypertension has been observed ($p = 0.530$). A statistically significant difference between the three genotypes regarding the average amount of coffee has been identified ($F = 0.38$; $p = 0.684$). Bonferroni test proved that there are differences between the three genotypes, but they are not statistically significant.

Conclusions: Individuals with the CYP1A2 rs762551 CC genotype proved to consume lower quantities of coffee while the AA genotype tend to consume the highest quantities. No association between coffee intake and high blood pressure have been identified, regardless the genotype.

Conflict of Interest: None declared.

EP06.029 Disconcordant genotype-phenotype in a family with arrhythmogenic right ventricular cardiomyopathy

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Background: *PKP2* gene pathogenic variants are the most common cause of arrhythmogenic right ventricular cardiomyopathy (ARVC). ARVC has variable expressivity and penetrance which might complicate managing patients, especially in cases not fulfilling all ARVC criteria.

Methods: We report a family case with PKP2 gene variant and ARVC

Results: 17 yo patient was referred to cardiologist with suspicion of cardiomyopathy because of brother's sudden cardiac death at 20 yo and uncle (mother side) at 19 yo. Episodes of ventricular arrhythmia were noticed, heart MRI showed 4 mm atrial septal defect with shunt to right, dilation of right ventricle. Cardioverter defibrillator was implanted. Hereditary cardiac disease next generation sequencing gene panel was performed – *PKP2* gene 2-13 exon deletion was found which was confirmed with MLPA. Variant was interpreted as likely pathogenic as truncating variants are known to cause ARVC and no reports in databases of such variant. The same variant was found for patient's sister (no cardiac pathology) and other brother (ongoing cardiac investigation), mother (changes in cardiac MRI – hypokinetic right ventricular segments, lower RV ejection fraction and ventricular arrhythmias), mother's sister (no cardiac pathology). Cousin of the patient from mother side had episodes of chest pain, reduced RV ejection fraction and slight apical dyssynchrony in cardiac MRI, but *PKP2* gene variant was not found.

Conclusion: A patient with phenotype suggesting an AVRC and no *PKP2* gene familial variant might have additional genetic cause for the disease. This complicates family screening as genotype PKP2-negative family members could develop disease and vice versa.

Conflict of Interest: None declared.

EP06.030 Analysis of the distribution of the ANRIL gene rs1333049 polymorphism in patients with acute coronary syndrome in the Ukrainian population

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Background/Objectives: To analyze the distribution of the *ANRIL* gene rs1333049-polymorphism (CDKN2B-AS1) in patients with acute coronary syndrome.

Methods: Venous blood of 195 patients with ACS and 234 without heart pathology was used for the study. DNA was isolated from whole venous blood. *ANRIL* gene polymorphism rs1333049 was studied by Real-time PCR.

Results: The distribution of *GG*, *GC* and *CC* genotypes in patients with coronary artery disease was 23.6%, 46.7%, 29.7%, respectively. In the control group, distribution of genotypes was 29.5%, 50.0%, 20.5%, respectively. Comparison of genotype frequencies did not reveal the existence of a reliable difference in their distribution ($P = 0.071$; $X^2 = 5.292$). The distribution of *C*-allele and *G*-allele in patients with ACS was 46.9% and 53.1%, and in the control group – 54.5% and 45.5%, respectively. The significant difference in alleles distribution between ACS patients and the control group was found ($P = 0.027$; $X^2 = 4.871$). Genotypic analysis in the recessive model has shown that *CC*-genotype carriers had more risk of coronary artery disease development compared to patients with *GC*-genotype and *GG*-genotype almost twice as high ($P = 0.028$, $OR = 1.641$, 95% $CI = 1.055–2.551$).

Conclusion: There is statistically significant link between ANRIL rs1333049 polymorphism and coronary artery disease development in Ukrainian population. Carriers with CC-genotype have more risk of coronary artery disease development compared to patients with major G-allele (GG and GC-genotypes).

Grant References: The present study is the part of the project "The study of the role of genetic factors in the pathogenesis of multifactorial diseases", supported by Ministry of Education and Science of Ukraine (no.0120U102166).

Conflict of Interest: None declared.

EP06.031 the power of collaboration: institutional partnership initiatives for data sharing leads to tnxb phenotype expansion

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Background/Objectives: Biallelic alterations in TNXB are associated with Ehlers-Danlos, classic type; however, vascular aneurysms and dissections have not previously been associated.

Methods: Clinical exome sequencing at our academic medical center lab identified two TNXB variants in a patient with aortic aneurysm and vertebral artery dissections. A retrospective review of additional research exome sequencing found 5/350 cases with two or more TNXB variants in patients with vascular aneurysms or dissections. To evaluate the strength of this association, replication analysis on a cohort of 1844 individuals who underwent clinical testing for suspicion of vascular aneurysm and dissection syndromes was carried out using data from a diagnostic laboratory.

Results: Only 3/22 diagnostic laboratories identified offered genetic testing for aneurysms and dissections that included TNXB in their analysis due to its association with connective tissue disease. After incorporating data from one laboratory, there was a statistically significant difference ($p < 0.01$) in the presence of two or more TNXB variants between cases and controls, suggesting an expansion of phenotype associated with TNXB.

Conclusion: This project reinforces the value of transparent data sharing, within the confines of privacy regulations, between researchers, clinicians, and laboratories in the identification of candidate genes. It also illuminates the utility of broad panel testing and the lack of standardization of gene panel content between laboratories. The variability of panel content has implications for test selection, as clinicians sort through seemingly similar test offerings to identify the best one for patients.

Conflict of Interest: ARPITA NEOGI Yale University, meghan towne Ambry Genetics, daniel j dykas Yale, arya mani Yale, NIH grant.

EP06.032 Genetic background of Czech patients with idiopathic ventricular fibrillation

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The sudden cardiac death (SCD) can be a fatal outcome of several cardiac diseases. SCD is generally caused by ventricular arrhythmias, especially ventricular fibrillation (VF). SCD survivors undergo extensive clinical testing to reveal the underlying cause of VF. Nevertheless, in some cases, neither any signs of the structural heart disease are found, nor diagnostic criteria for any electrical disease of the heart are present. The diagnosis of idiopathic ventricular fibrillation (IVF) is made.

We analyzed a group of 26 patients with SCD aborted by cardiopulmonary resuscitation; structural heart disease was excluded and no clinical signs of primary electrical diseases of the heart were present. We use panel sequencing with 130 genes associated with hereditary cardiomyopathies and arrhythmogenic syndromes for variant detection and CNV analysis.

We present genetic findings in 10 patients with idiopathic ventricular fibrillation. Four variants are classified as pathogenic/likely pathogenic (in *RYR2*, *KCNH2*, *FLNC* and *TTN*), the rest as of uncertain significance (in *CACNB2*, *DSP*, *PKP2*, *RYR2*, *SCN4B*). Majority of these genes has been related to arrhythmogenic syndromes.

Although the patients with IVF do not have a clinical presentation of any familial electrical heart disease, they may have rare genetic variants that may predispose them to malignant life-threatening arrhythmias in certain stress situations. Knowledge of presence of these rare variants may contribute to the understanding of the pathogenesis of SCD. The precise mechanism requires further study. Functional analysis of selected variants is undergoing.

Supported by the Ministry of Health of the Czech Republic–grant project NU22-02-00348.

Conflict of Interest: None declared.

EP06.033 Haplotype R157-V378-T681 in TRPV6 possible risk factor for Ventricular Fibrillation in Acute Myocardial Infarction

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Background/Objectives: Ventricular fibrillation (VF) during acute myocardial infarction (AMI) has been proposed as the main cause of sudden cardiac death. The genetic basis underlying the development of this pathology are still unclear, being many possible related clusters of genes not explored yet. Transient receptor potential (TRP) channels are superfamily of 27 genes coding to cation-permeable ion channels, deeply related to the homeostasis of Ca^{+}. Ion homeostasis is fundamental for the proper function of the cardiovascular system, making this set of genes an interesting target.

Methods: By NGS technologies, the genetic variants present in the 27 TRP genes of 78 patients, 39 with VF during AMI vs 39 without VF during AMI, were analyzed. Depending on their frequency reported in the genomic databases, variants were classified as common or rare. Association of common variants was tested using R package: SNPassoc. Statistical analysis was performed using GraphPad Prism.

Results: A total of 174 common variants were found in the 27 genes analyzed. The haplotype R157-V378-T681 in the TRPV6 gene was identified in 13 VF patients whereas it was not found in any control patients (P.value 0.00007).

Conclusions: The presence of R157-V378-T681 haplotype in the TRPV6 is statistically significant in the VF patients, this correlation has never been reported before. Ca^{+} homeostasis is essential for the excitation and contraction coupling in cardiomyocytes and the inefficient function of channels could represent a risk factor for the development of VF.

Grants: Instituto de Salud Carlos III (PI18/01737)-FEDER funds and a nonconditional grant from Abbott Vascular.

Conflict of Interest: None declared.

EP06.034 Somatic mosaicism in myocardial samples of patients with severe hypertrophic cardiomyopathy

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Background/Objectives: Hypertrophic cardiomyopathy (HCM) is a mainly dominant disorder caused by germline mutations in the >40 genes. Apparent phenotypic anticipation is observed in several HCM families but remains unexplained. We hypothesize that myocardial somatic mosaicism might be a factor driving that phenomenon.

Methods: Ten young probands (15-30 y.o.) were selected who underwent septal myectomy and in whom parents had from no to moderate HCM. For 5 of them, we performed WES of DNA extracted from peripheral blood leukocytes (100x coverage) and myocardial samples (200x coverage) taken intra-operatively. Comparison was performed using a domestic bioinformatics pipeline.

Results: On average, we found 185465 variants on the leukocyte WES and 192427 variants on the myocardial WES. Causative variants of IV-V classes of pathogenicity in target genes (*MYBPC3, MYH7, ALPK3*) were detected in 3 out of 5 probands. All causal variants were confirmed in both type of tissues. In 4 of 5 myocardial samples, LOF variants in the *CTBP2* gene were found. In all cases, more variants, as well as a tendency to increase with age, were identified in the myocardium. No new somatic pathogenic variants were found in myocardium that could explain phenotype anticipation in probands.

Conclusion: There is a tendency to an age-dependent increase in the number of somatic mutations. We were unable to detect new pathogenic variants in the genes of sarcomeric proteins. We plan to increase the number of examined patients in the near future.

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Conflict of Interest: Maria Balashova Centre of Genetics and Reproductive Medicine GENETICO.

Sechenov University, Principal investigator, Mariam Sadekova B.V. Petrovsky Russian Research Centre of Surgery (RRCS)

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Centre of Genetics and Reproductive Medicine "GENETICO"

EP06.035 Child with dilated cardiomyopathy and a novel homozygous splice site variant in FLNC gene

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FLNC gene encodes for Filamin-C (FLNC) protein, a sacromeric protein with important structural and signaling functions in the myocyte. Mutations in FLNC gene were linked initially to myofibrillar myopathy. In 2014, dominant mutations in this gene were linked to familial hypertrophic cardiomyopathy and over time, evidence showed association of this gene with other forms of cardiomyopathy including dilated and restrictive forms.

Recently, a single case report of a child with congenital onset of myopathy with no cardiac involvement associated with a homozygous missense variant in FLNC c.1325C>G (p.Pro442Arg) was reported and the early onset suggest that homozygosity is associated with more severe disease, a phenomena observed with some other dominant disorders.

In this report, we described a boy who presented at the age of 10 years with shortness of breath and found to have dilated cardiomyopathy (DCM) with ejection fraction of less than 20%.

He doesn't have myopathy and family history is unremarkable apart from consanguinity. Whole exome sequencing showed a novel homozygous splice site variant (c.2122-1G > C) in FLNC. The patient underwent cardiac transplantation recently. Sanger confirmation in parents as well as their cardiac evaluation is pending. To our knowledge, this is the second case in literature with biallelic variants in FLNC. While the previous case presented with isolated myopathy, our case presented with isolated DCM. This case suggested that the phenotype associated with variants in FLNC is very heterogenous and can be inherited in dominant or recessive forms, with later being more severe and of earlier onset.

Conflict of Interest: Afaf Alsubhi Full time, Manar Aldarwish Full time, Mohammed Almannai Full time.

EP06.036 Evaluation of the variant spectrum of patients diagnosed with hereditary cardiomyopathy

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Background/Objectives: Hereditary cardiomyopathies can be defined as structural and functional disorders of heart muscle with a wide range of clinical and genetic heterogeneity. They are classified into 5 main groups as hypertrophic cardiomyopathy(HCM), dilated cardiomyopathy(DCMP), restrictive cardiomyopathy(RCMP), arrhythmogenic right ventricular cardiomyopathy(ARVD) and left ventricular noncompaction cardiomyopathy(NCCMP). The aim of our study is to define the responsible genetic etiology and explain its relationship with patients' phenotype for non-syndromic cardiomyopathy.

Methods: Patients referred to Medical Genetics Outpatient Clinic with a preliminary diagnosis of cardiomyopathy between 01.03.2019-31.07.2022 were evaluated with anamnesis,physical examination,pedigree analysis and cardiac examination results. A panel covering *MYBPC3,TNNT2,TPM1,MYH6,MYH7,TNNI3 and MYL2* genes was applied to 144 patients who were thought to be clinically non-syndromic with next generation sequencing (NGS) method.

Results: Pathogenic/likely pathogenic variants explaining the clinic was detected at 35/144 patients and the diagnosis rate was determined as 26% in our study. It was found to be 25%(23/90) in HCMP, 20%(7/34) in DCMP, 50%(2/4) in RCMP and 18%(3/16) in NCCMP. The most common pathogenic/likely pathogenic variants were detected in *MYBPC3* and *MYH7* genes. Variants of unknown clinical significance were detected in a total of 17 patients, and segregation analyzes are ongoing for the validation of variants.

Conclusion: Molecular diagnosis of cardiomyopathies is essential for arranging treatment options and genetic counseling. In this study, genetic etiology related to cardiac pathology was shown at 35/144 patients. Detection of molecular etiology in this disease group is very important in terms of treatment strategy for patients, prediction of prognosis and early diagnosis of asymptomatic family members.

Conflict of Interest: None declared.

EP06.037 The prevalence of mutations in ion channels genes in an unselected cohort of young sudden unexplained death cases

Ainur Akilzhanova[1], *Madina Zhalbinova*[1], *Ayaulym Chamoieva*[1], *Zhanel Mirmanova*[1], *Diana Samatkyzy*[1], *Aidana Gabdulkayum*[1], *Gulbanu Akilzhanova*[2], ***Saule Rakhimova***[1], *Ulykbek Kairov*[3], *Anargul Kuanysheva*[4], *Ulan Kozhamkulov*[1], *Kenes Akilzhanov*[2;5], *Makhabbat Bekbossynova*[6], *Dos Sarbassov*[1;7]

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The purpose of the study was to identify the mutational spectrum in Kazakhstani cohort of young sudden cardiac death (SCD) victims revealed by next-generation sequencing (NGS) of 96 genes associated with cardiac diseases and compare the diagnostic yield of postmortem genetic testing in (1) cases with structural findings of uncertain significance at autopsy (sudden unexplained deaths (SUDs), (2) cases with autopsy findings diagnostic of cardiomyopathy.

Methods: We screened 37 unselected SCD cases (<45 years) using the customized HaloPlex Target Enrichment System (Agilent) and NGS for 96 genes associated with inherited cardiac syndromes and cardiomyopathies. 27 cases had non-diagnostic structural cardiac abnormalities and 10 cases, diagnosed with a cardiomyopathy post-mortem. ACMG/AMP guidelines were applied for variant interpretation of clinical significance.

Results: 31 rare variants were identified in 17 (63%) of the deceased individuals with non-diagnostic structural cardiac abnormalities. Among them pathogenic variants in KCNQ1, KCNJ2,

SCN5A, RYR2 genes were identified. The corresponding frequency in deceased individuals with cardiomyopathies was 35%. The most abundant mutations observed in MYBPC3, LAMA2, MYH6 and GAA.

Conclusion: Genetic screening revealed variants with likely functional effects at high rates, in 63% and 35% of the SCD cases with non-diagnostic and diagnostic cardiac abnormalities, respectively. Targeted NGS screening can support the forensic investigation and help the cardiologist's decision to offer counselling and clinical evaluation to relatives of young SCD victims.

Study was supported by grants from the Ministry of Science and Higher Education of the Republic of Kazakhstan (AP14869903, BR10965164).

Conflict of Interest: Ainur Akilzhanova Principal Investigator, Madina Zhalbinova: None declared, Ayaulym Chamoieva: None declared, Zhanel Mirmanova: None declared, Diana Samatkyzy: None declared, Aidana Gabdulkayum: None declared, Gulbanu Akilzhanova: None declared, Saule Rakhimova: None declared, Ulykbek Kairov: None declared, Anargul Kuanysheva: None declared, Ulan Kozhamkulov: None declared, Kenes Akilzhanov: None declared, Makhabbat Bekbossynova: None declared, Dos Sarbassov: None declared.

EP06.038 Lack of expression of the rare KCNQ1 allele containing two missense variants in the same haplotype

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Genetic variations in the Kv7.1 channel alter membrane repolarization duration and cause inherited rhythm disorders.

We identified two heterozygous variants c.1011C>G (p.Ile337-Met) (Class III, VUS) and c.1025T>C (p.Leu342Pro) (Class IV, Likely Pathogenic) in the *KCNQ1* gene in two Russian probands with QTc interval prolongation. Segregation analysis revealed that both variants were presented in same haplotype. Interestingly that lone variant c.1011C>G (p.Ile337Met) was reported by 2 independent groups. We assume that kcnq1-Met337 variant might be raised first and represent more "archaic" allele.

We introduced two variants in three plasmids: kcnq1-Met337, kcnq1-Pro342, and kcnq1-Met337-Pro342, and compared mutant channels with wt. The plasmids were co-transfected into the CHO-K1 cells along with MinK (auxiliary subunit) coding plasmid. The protein expression level was assessed with immunoblot and confocal microscopy. Electrophysiological recordings of integral IKs current were performed using whole-cell patch clamp. The Ile337Met mutation leads to the slowing of K+ current kinetics and right shift of activation curve, which might decrease the extent of IKs current transferred by mutant Kv7.1 channels at the same values of membrane potential in cardiomyocytes.

Cells transfected with plasmids containing either lone p.Leu342-Pro variant or both mutations together, possess no current. Immunofluorescent staining demonstrated that mutant channels contating p.Leu342Pro mutation were not exposed to the cell surface. It testifies that Leu342 variant affects normal Kv7.1 expression. We assume that missense mutation Leu342Pro realizes in vivo through haplo-insufficiency with or without rare variant p.Ile337Met.

We suggest that p.Leu342Pro may eliminate the consequences of the traffic changes Kv7.1-Met337 and influence the phenotype.

Conflict of Interest: None declared.

EP06.039 Copy number variants in familial hypercholesterolemia genes using targeted NGS, validated through optical genome mapping

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Background/Objectives: Familial hypercholesterolemia (FH) is a common genetic disorder which is primarily caused by pathogenic variants in the *LDLR*, *APOB*, and *PCSK9* genes. Approximately 10% of pathogenic variants in *LDLR* may be CNVs. Here, we combine NGS, MLPA, and Optical Genome Mapping (OGM) to investigate CNVs in *LDLR*.

Methods: A NGS panel was designed for whole gene sequencing (8 genes) of 100 FH patients using Twist technology and Illumina platform. CNVs were detected using CNVexpo, and an in-house pipeline for base-resolved normalized coverage. Identified CNVs were validated using MLPA and OGM. Bionano Services Lab performed the OGM procedure. Purified gDNA was labeled using Direct Label and Stain DNA Labeling Kit. Saphyr chip was run aiming for 100X coverage. De novo assembly and Variant Annotation pipelines were executed on Bionano Solve v3.7. Bionano Access v1.7 was used for CNV reporting and visualization.

Results: In five out of 100 samples NGS and MLPA data showed heterozygous deletions in *LDLR*. Three deletions, affecting different exons, was analyzed and confirmed using OGM. In two samples, OGM better defined the breakpoints as well as the size of the event, which expanded far beyond the gene of interest. In one sample, an additional CNV of *SLCO1B1*, a pharmaco-gene, important for transport of statins used for FH treatment was identified.

Conclusion: CNVs in FH genes in FH patients could be detected using targeted NGS, which was further confirmed by MLPA and characterized using OGM.

Grant References: The study received grants from Örebro County Research Committee.

Conflict of Interest: Anna Green: None declared, Consuelo Alonso employed at Bionano Genomics, Jon Jonasson: None declared, Aniruddh Kashyap: None declared, Emma Adolfsson: None declared, Anna Nordenskjöld: None declared.

EP06.040 Mutation spectrum of potassium channel genes in Kazakhstani patients with Primary Electrical Diseases

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Background/Objectives: Primary electrical diseases (PEDs) or channelopathies are group of genetically determined diseases caused by cardiac arrhythmias. It leads to sudden cardiac death (SCD). Potassium channel genes are particularly important in manifestation of primary electrical disorders. Our research aimed to identification of mutations in potassium channel genes by targeted next generation sequencing (NGS) in Kazakhstani patients with PEDs.

Methods: Venous blood samples were recruited from 79 patients (47.5 ± 17.5 years old) with PEDs. All patients were

sequenced for coding regions of 96 cardiac risk genes by Haloplex Target Enrichment System (Agilent Technologies). Genetic profiles of patients were analysed using SureCall Design (Agilent Technologies) software in order to identify mutated variants. Genetic variants were classified using ACMG/AMP guideline.

Results: According to genetic analysis, 52 variants were detected in potassium channel genes. Annotated data demonstrated 3.8% pathogenic, 1.9% likely pathogenic, 15.4% variants of uncertain significance (VUS), 19.2% likely benign and 46.2% benign variants according to ACMG classification. Among them KCNH2, KCNQ1 genes showed pathogenic status.

Conclusion: The study evaluated mutation spectrum of potassium channel genes in our group of patients by using targeted NGS. Genetic screening identified clinically significant variants within 96 genes associated with inherited cardiac diseases. Targeted NGS screening can be useful to clinicians for early diagnosis, risk stratification of complications and SCD development.

Grant References: Study was supported by grants from the Ministry Education and Science, Republic of Kazakhstan (AP14869903, BR10965164).

Conflict of Interest: None declared.

EP06.041 TAF1A variants in siblings with arrhythmogenic cardiomyopathy

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Background/Objectives: Arrhythmogenic cardiomyopathy is a genetic disorder characterized by the risk of life-threatening arrhythmias, myocardial dysfunction and fibrofatty replacement of myocardial tissue. About 35–50% of patients have no identifiable disease-associated variant. We reported here a family with ACM in a sibling pair. The older sister received heart transplant (HT) at 10 years of age. Post explant cardiac examination showed globular right ventricle (RV) with myocardial adipose replacement. Histological analysis confirmed the presence of arrhythmogenic cardiomyopathy (ACM) signs. She died after HT rejection complications. The younger sister started cardiological checkup for family screening. Magnetic Resonance Imaging (MRI) showed mild to moderate dilatation RV and systolic dysfunction, right atrial dilatation and initial LV dilation with systolic dysfunction, extensive epicardial fat at the free wall of the RV. She presented supraventricular tachycardia and paroxysmal atrial fibrillation episodes requiring preventive ICD implantation.

Methods: To identify the genetic basis of ACM in the sibling pair, exome sequencing was performed using a custom panel kit (Twist Bioscience) on NovaSeq6000 platform (Illumina). Exome sequencing data were filtered for rare, deleterious and recessive variants.

Results: Compound heterozygous recessive mutations in TAF1A, encoding an RNA polymerase I complex protein, were identified in the two sister. The identified variants were rare, predicted to be damaging by in silico tools and located in the functional domain of the protein.

Conclusion: Our family confirm the role of TAF1A gene in a wide spectrum of cardiomyopathy. Identification of additional mutations in TAF1A are required to further establish this genotype–phenotype relationship.

Conflict of Interest: None declared.

EP06.042 Association between Exome Sequencing and Metabolomics to investigate coronary heart diseases in a prospective cohort

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Background/Objectives: Coronary artery diseases (CADs) are linked to metabolic alterations and cause most of the cardiovascular deaths in the adult population in Western countries. The clinical manifestations of CADs (e.g. myocardial infarction and heart failure) are usually acute events that require timely diagnosis and intervention. We aimed at investigating how genetics and metabolomics could improve CADs prevention.

Methods: Whole Exome Sequencing (WES) and untargeted metabolomic data were collected and analyzed for 84 prediagnostic individuals, who developed CAD at the follow-up, and 90 healthy controls, selected within the Turin component of the large European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (EPICOR study).

Results: In the WES analysis, we prioritized rare variants (MAX AF < 1%) based on the status according to ACMG annotation and for all individuals we created a score for each gene given by the addition of specific values based on variant severity. The logistic regression model built with anthropometric variables (age, sex and BMI) had an AUC = 0.55, while adding the genetic score to this model the prediction power increased, AUC = 0.68 (p-value = 0.006). From metabolomic analyses, we selected 49 metabolites which, added to the anthropometric variables, showed an AUC = 0.80. Following the integration of covariates, genetic score and metabolites, the model reached an AUC = 0.84.

Conclusion: This integrated approach could be useful to clarify how the genetic background and metabolite profiles could influence CAD risk development.

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Conflict of Interest: None declared.

EP07 Metabolic and Mitochondrial Disorders

EP07.001 Laboratory challenges by the interpretation of slightly decreased branched chain amino acids concentrations: A case of BCKDK deficiency

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Background: Branched-chain keto acid dehydrogenase kinase deficiency (BCKDKD) is a rare disease caused by pathogenic variants in the *BCKDK* gene located on chromosome 16p11.2. Clinically manifests by autism, epilepsy, developmental delay, seizures, microcephaly, and decreased plasma branched chain amino acids (BCAA).

Methods: We report a case of BCKDKD where first amino acid spectra by HPLC-UV showed only slight decrease in two of BCAA – leucine and isoleucine. The result was considered as non-specific secondary changes.

Results: An 18-month-old girl had the first geneticist consultation due to developmental delay and microcephaly. The examination of amino acid spectra showed isoleucine 26 μM/L (reference range 32-92 μM/L), leucine 40 μM/L (53-149 μM/L) and valine 103 μM/L (79-267 μM/L). At the time of second examination reference range changed due to patient's age, only leucine was identified as slightly decreased 59 μM/L (64-164 μM/L). Diagnosis confirmed by WES analysis identified two heterozygous likely pathogenic variants in the *BCKDK* gene. Treatment by BCAA mixture dose 4 g 3 times a day was not effective. In next three examinations all BCAA was decreased almost half of reference range lowest value. Only after increasing treatment frequency (dose 2 g 6 times a day) isoleucine was 39 μM/L, leucine 73 μM/L and valine 171 μM/L, which were within the reference range.

Conclusions: Determination of slightly decreased some or all BCAA in plasma amino acid spectra should be evaluated as specific changes indicative for an BCKDK deficiency. The final diagnosis is based on the results of molecular genetic studies.

Conflict of Interest: None declared.

EP07.002 The phenylalanine hydroxylase genotype and the expected responsiveness to sapropterin dihydrochloride in the adult Irish population

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Background: Phenylketonuria (PKU) is an autosomal recessive inborn error of metabolism resulting from a deficiency of phenylalanine hydroxylase that catalyzes the hydroxylation of phenylalanine to tyrosine with the help of a cofactor (tetrahydrobiopterin; BH4). Ireland has one of the world's highest incidences of PKU; currently, ~ 400 patients attend the National Centre for Inherited Metabolic Disorders (NCIMD), Mater Misericordiae University Hospital (MMUH).

Sapropterin dihydrochloride, a synthetic form of BH4, is a genotype-specific treatment that has recently been added to the precision genomic medicine program at NCIMD, MMUH. To service the plan for the roll-out of this treatment, all patients with PKU were genotyped, including prediction around sapropterin dihydrochloride responsiveness.

Methods: A study, analyzing the PAH genotype of patients (>18 years) with PKU attending the NCIMD, was performed along with sapropterin dihydrochloride responsiveness analysis.

Results: Two hundred eighty-three patients had PAH genotyping performed. One hundred and four different genotypes were identified in the population, with the R408W/R408W (13.78%) being the most common. The most frequent allelic variants were R408W (34.63%), F39L (12%), I65T (10.78%), L348V (5.48%) and IVS12 + 1G > A (4.78%). Sapropterin dihydrochloride responsiveness was predicted as likely in 12%, unlikely in 47% and difficult to predict in 41% of patients accurately.

Conclusion: The up-to-date PAH genotype landscape in the adult Irish PKU population is presented, detailing percentages of patients who will likely respond to sapropterin dihydrochloride. A large number will require a trial period to investigate responsiveness; information important for service planning as NCIMD continues to develop a precision genomic medicine program.

Conflict of Interest: None declared.

EP07.003 a homozygous c.1604G>A p.(Arg535His) variant of GBA gene with severe phenotype and a rare complication of splenectomy

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Background: The c.1604G>A variant in *GBA* has been reported in only compound heterozygous form with mild or asymptomatic non-neurological cases. Here we report a 15-year-old patient with a homozygous form with early severe manifestations and a splenectomy complication.

Case presentation: At the age of 7 years, a girl presented with poor weight gain, easy fatigability, and thrombocytopenia. Splenectomy done 2 years later. She was referred at 13 years of age to our clinic. She had below average weight and height, moderate hepatomegaly, and a machinery murmur on left scapular area.

CBC showed mild anemia, normal platelets. Liver enzymes and synthetic functions were normal. Liver biopsy revealed Gaucher cells but glucocerebrosidase was normal while lyso G1 was increased. Therefore molecular testing was done revealing homozygous c.1604G>A p.(Arg535His) variant in *GBA* gene. Echocardiography and oxygen saturation were normal. CT pulmonary angiography showed left sub-diaphragmatic tortuous convoluted vessels following contrast enhancement of the aorta with no definitive thoracic connection. CT angiography of abdomen and aorta showed splenic arteriovenous fistula. The patient received ERT regularly with satisfactory response and following with the hepatology clinic for portal hypertension.

Conclusions: The homozygous c.1604G>A variant of *GBA* gene could have an early severe Gaucher disease phenotype with normal enzyme level so a biomarker and molecular testing is necessary. Splenic arteriovenous fistula is a rare complication of splenectomy. Possible causes include mass ligation of splenic artery and vein during splenectomy. Awareness for avoiding splenectomy in Gaucher disease is still needed

Conflict of Interest: None declared.

EP07.004 L-2 Hydroxyglutaric aciduria case in Lithuania

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Background/Objectives: L-2 Hydroxyglutaric aciduria (L2HGA) is a rare (about 140 cases have been reported to date), progressive, autosomal recessively inherited metabolic disorder of organic acid metabolism. It is characterized by macrocephaly, developmental delay, seizures, speech difficulties, cerebellum abnormalities,

typical brain MRI findings and the presence of L-2 hydroxyglutaric acid in urine samples.

Methods: 19 years old woman was reffered to geneticist because of suspicion of metabolic disease. From 1 years of age she had reccurent febrile seizures till 17 yo. Early psychomotor development was normal but with hypotonia. Reaching school age she had intellectual deficit (especially verbal). At 17 yo sleep electroencephalogram showed no abnormalities. Brain MRI – simetrical white matter intense signals in subcortical, basal ganglia and nucleus dentatatus. She was tested for organic acids in urine sample (liquid chromatography-mass spectrometry method). Whole exome sequencing was also performed to confirm the molecular diagnosis.

Results: Very high (12 time increased) 2-hydroxyglutaric acid concentration was detected after organic acids test. Other organic acids were not indicative of other metabolic disorders. Using this method we could not differentiate between L and D configurations of 2-hydroxyglutaric acid. Two likely pathogenic variants in *L2HGDH* (NM_024884.3) gene were found after whole exome sequencing: c.853delT (p.Tyr285fs) (PVS1 very strong, PM2 moderate) and c.164G>T (p.Gly55Val) (PP3 strong, PM2 moderate, PM5 supporting). Variants were classified based on the ACMG guidelines.

Conclusion: Increased urinary excretion of 2-hydroxyglutaric acid, typical MRI findings, clinical symptoms (seizures, developmental delay) and sequencing results in this patient established the diagnosis of L2HGA.

Conflict of Interest: None declared.

EP07.005 Uselfulness of biochemical analyses as functional studies to confirm the pathogenicity of VUS in inherited metabolic diseases

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Background/Objectives: With the implementation of the Next Generation Sequencing (NGS), the process of diagnosing inherited metabolic diseases (IMD) has undergone a substantial change. This powerful tool helps to reach the diagnosis faster when clear mutations in a candidate gene are found, but in other cases, the high number of variants of unknown significance (VUS) detected, may be a nightmare to elucidate the real diagnosis. In this study we checked how the biochemical studies help to reach the diagnosis of the patients when VUS were detected in homozygosity or in compound heterozygosity in a candidate gene.

Material and methods: We analyzed 413 patients with suspected IMD using Whole Exome sequencing (WES). Data were analyzed using virtual gene panels. Biochemical analyses were performed using routine techniques in IMD.

Results: A total of 413 patients were analyzed and the genetic diagnosis was reached in 44% of the patients (181/413). In 29/181 (16%) of these patients, at least one VUS in a candidate gene was detected, questioning the diagnosis. In all these patients, the corresponding biochemical analyses carried out revealed an alteration indicative of the suspicion of disease, confirming allegedly the pathogenicity of the variants and establishing the definitive diagnosis.

Conclusions: In the diagnosis of IMD, the biochemical analyses are essential and very useful functional studies to confirm the pathogenicity of VUS when found in a candidate gene. These studies increase the diagnosis rate of patients presenting with VUS, confirming their usefulness and the important role of IMD biochemical diagnostic laboratories.

Conflict of Interest: None declared.

EP07.006 Identification of obesity-related genetic variants in a family with an exclusively breastfed obese infant

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Background/Objectives: Obesity is a multifactorial disease with genetic and environmental components. Obesity in infancy has been correlated with later childhood and adult obesity, which is prone to increased risk for mortality caused by type 2 diabetes, heart disease, or cancer. We aimed to identify genetic risk factors in a family having an exclusively breastfed obese infant with a history of paternal obesity.

Methods: Four members in a three-generation family were recruited. Physical examination, body mass index (BMI) calculation, biochemical analysis, hormonal evaluations, and hepatic ultrasonography were performed on a 7-month-old boy. Whole exome sequencing (WES) was performed on the case utilizing Illumina NextSeq550, followed by bioinformatic analyses on the Genomize SEQ platform. Variant confirmations and family segregation were performed by Sanger sequencing.

Results: Index had severe obesity (+3.8SD-p > 99.98) on admission with excess weight gain at 2 months, which paralleled paternal history. He showed no sign of syndromic obesity and hepatomegaly was detected at 4 months of age. He reached the normal percentile at 2 years old. His father and grandmother were obese (BMIs: 38 and 31, respectively), while the mother was lean. WES analysis revealed deleterious variants in *SH2B1, GHRL, SRA1, ACADVL, PDE11A, and CAPN10* genes, which were previously associated with obesity. Family segregation confirmed paternal inheritance.

Conclusion: Despite reaching a normal percentile, the infant is under periodic follow-up due to shared genetic variants with his obese father that increase his risk for later childhood obesity.

Grant Reference: Supported by the Scientific Research Projects Coordination Unit of Istanbul University (TDP-2020-37103).

Conflict of Interest: Hazal Banu Olgun Celebioglu Istanbul University, Graduate School of Health Sciences, Istanbul, Turkey, Scientific Research Projects Coordination Unit of Istanbul University (TKP-2020-37103), Ayse Pinar Ozturk Istanbul University, Faculty of Medicine, Department of Paediatric Endocrinology, Istanbul, Turkey, Şükran Poyrazoğlu Istanbul University, Faculty of Medicine, Department of Paediatric Endocrinology, Istanbul, Turkey, Feyza Tuncer Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Genetics, Istanbul, Turkey, Scientific Research Projects Coordination Unit of Istanbul University (TKP-2020-37103).

EP07.009 CFTR mutation spectrum in Georgian CF patients

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Background/Objectives: Cystic fibrosis (CF) is a life-threatening autosomal recessive disease caused by mutations in the CFTR gene. F508del is the most frequent mutation in the world. Other mutations are relatively rare and population specific. The aim of the study was to analyze distribution of CFTR mutations in Georgian CF patients.

Materials and Methods: 91 patients with a clinically confirmed CF diagnosis were tested (70.5% of registered CF patients in the country). Patients have been analyzed for CFTR variants by massively parallel sequencing of CFTR coding region and introns combined with the analysis of intra-CFTR rearrangements.

Results: CFTR gene analysis revealed 29 different mutations in Georgian CF patients. The most common mutation is c.1545_1546delTA (1677delTA) (42.7%), the second most common mutation, W1282X (11.2%). All other 27 CFTR mutations have low frequency, including F508del (6.7%). 3 novel mutations were found (c.708dupT; CFTRdele16_17; c.3170C>G) and reported to CFTR2 database.

Conclusions: Distribution of CFTR mutations in the Georgian CF population differs regarding the high frequency of mutation c.1545_1546delTA (1677delTA) and the low frequency of the predominant F508del mutation. Patients with 1677delTA have typical clinical manifestations and complications of the disease. Genotyping the Georgian CF patients was important to register population eligible for CFTR modulator therapy, and to come up with the better care plan for patients who are not eligible for personalized therapy.

Grant References: Supported by grants from Vertex Pharmaceuticals (CG-2015-104643; unrestricted charitable donation).

Conflict of Interest: None declared.

EP07.010 Diagnosis of ethylmalonic encephalopathy in a patient with a preliminary diagnosis of fanconi anemia: a case report

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Introduction: Ethylmalonic encephalopathy (EE) is a rare, autosomal recessive metabolic disease caused by dysfunction of the ETHE1 protein. EE is usually an early onset fatal disease with developmental delay, recurrent petechiae, orthostatic acrocyanosis, and chronic diarrhea. Rarely, cases with mild clinical findings have been reported in the literature. Different clinical findings are seen even in patients with the same pathogenic variant. This illustrates the clinical heterogeneity of EE and the difficulty of its diagnosis.

Methods: Clinical exome and Next-Generation Sequencing (NGS) based copy number variation (CNV) analysis was performed on the patient who applied with a preliminary diagnosis of Fanconi anemia.

Case Presentation: The case presented here is a 6-year-old girl with consanguinity between her parents. The patient had short stature, hyperpigmentation, microcephaly with bone marrow failure, and also persistent diarrhea from birth and acrocyanosis. Genetic analysis has revealed a homozygous, 2 kilobase pathogenic deletion including exons 1-3 of the *FANCA* gene, and a homozygous *ETHE1* c.3G>T pathogenic variant. Interestingly, our case had a milder phenotype than other patients with *ETHE1* pathogenic variants in the literature.

Conclusion: This case shows that patients with EE can have a milder phenotype and can be affected by another autosomal recessive genetic disease due to the high rate of consanguineous marriages. Therefore, it should be kept in mind that different genetic diseases may manifest together, and clinicians should not always be focused on a single diagnosis. It is also aimed to emphasize the contribution of NGS-based CNV analysis in reverse phenotyping.

Conflict of Interest: None declared.

EP07.011 Undiagnosed monogenic diabetes among young-onset type 2 diabetes – searching beyond current clinical guidelines

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Background: Identification of monogenic diabetes is clinically important for personalized treatment. However, monogenic diabetes remains underdiagnosed and may be overlooked, in the absence of typical monogenic diabetes phenotype (young-onset, non-insulin dependent, non-obese, strong family history). We aim to identify monogenic diabetes among young-onset diabetes patients (onset ≤35 years old) clinically assigned as type 2 diabetes (T2D) by extending genetic testing beyond current guidelines (non-lean and/or absence of family history).

Methodology: 271 patients (median BMI 32.0 kg/m^2) were screened for 16 monogenic diabetes-associated genes using massive parallel sequencing method. The m.3243A > G variant (associated with mitochondrial-inherited diabetes) was detected using quantitative PCR. Multiplex ligation-dependent probe amplification was used to detect copy number variation in *HNF1A, HNF4A, GCK* and *HNF1B*. All pathogenic variants (classified based on ACMG guidelines) were validated using Sanger sequencing.

Results: We identified 8 (3%) patients with monogenic diabetes, including 3 patients with clinically actionable variants in *HNF1A* and *GCK* and one patient with m.3243A > G variant. In comparison with patients without monogenic diabetes, patients with monogenic diabetes had lower median BMI (30.0 kg/m^2 vs. 32.2 kg/m^2, $p = 0.133$), lower prevalence of family history of diabetes (37.5% vs. 84.4%, $p = 0.004$), and lower median MODY PPV based on the Exeter MODY calculator (5.5% vs. 15.1%, $p = 0.657$).

Conclusion: Current clinical guidelines including the Exeter MODY calculator, may not be adequately efficient in selecting patients for genetic testing in the clinics particularly among young-onset obese T2D patients (BMI ≥30 kg/m^2).

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Conflict of Interest: Clara Tan Yes, Yes, Kai Xiang Kee Yes, Yes, Wan Ting Lovynn Chan Yes, Yes, Yuzhen Song Yes, Yes, Rashida Farhad Vasanwala Yes, Yes, Fabian Yap Yes, Yes, Ziliang Lim Yes,

Yes, Tavintharan Subramaniam Yes, Yes, Chee Fang Sum Yes, Yes, Su Chi Lim Full time, Principal investigator, collaborator.

EP07.012 Molecular and biochemical characterization of a novel missense variant in COQ5 causing primary coenzyme Q10 deficiency

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Introduction: Coenzyme Q10 Primary Deficiencies (MIM#607426) are caused by mutation in each of the thirteen coenzymes involved in CoQ-Synthome. COQ5 methylase is crucial for maintaining the coenzyme Q10 level in human cells.

Methods: We described a 9-year-old girl born to consanguineous parents who showed ataxia, distal action tremor, pontocerebellar atrophy, intellectual disability, speech delay, neonatal jaundice, hypoglycaemia. CGH array, Angelman Syndrome and *MECP2*- disorders were excluded. Whole Exome Sequencing (WES) was performed and homozygous or compound heterozygous variants were filtered out.

Result: WES revealed the homozygous missense variant in the *COQ5* gene c.352G>A causing the aminoacid substitution p.(Gly118Ser). Glycine118 is highly conserved among species mapped in Methyltransferase Motif I. This variant, absent in gnomAD, affects the last nucleotide of exon 2. In *silico* effector splice tools predict that the substitution will weaken the exon 2 splice donor. RNA was obtained from peripheral blood cells of the proband and her parents. In the patient we demonstrated exon 2 skipping and a residual full-length product in which glycine 118 was replaced by serine. Instead, her parents showed a full-length product with a mixture of glycine and serine (~20%) at same position and low exon 2 skipping level. Moreover, muscle biopsy was processed for histochemical analysis and CoQ10 level.

Conclusion: Our family is the to be second reported with CoQ10 deficiency caused by loss of function of the *COQ5* gene. Ataxia and intellectual disabilities are clinical features of impairment of this coenzyme. This result is crucial for target treatment with CoQ10 supplementation.

Conflict of Interest: None declared.

EP07.015 Urine-derived cells: a non-invasive approach to the analysis of mitochondrial functions and mitochondrial diseases diagnosis

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Purpose: Mitochondrial diseases are common metabolic disease of complex diagnosis, particularly due to the phenomenon of mtDNA heteroplasmy. Historically, molecular diagnosis was always performed on affected tissues (e.g. muscle, liver), but for the past few years the use of urine appeared to be a good alternative to invasive sampling. Coupled with NGS, it has improved the diagnosis of mitochondrial diseases allowing the identification of many variants of uncertain significance (VUS). However, the functional impact of these VUS is often difficult to assess due to the lack of invasive samples enabling the analysis of mitochondrial functions. The aim of this study was to develop mitochondrial functions analysis on urine-derived cells to allow a fully non-invasive diagnosis of mitochondrial diseases.

Methods: Urine-derived cells were obtained from voiding samples for 2 patients carrying one known pathogenic mtDNA variant (m.3243A > G and m.13513G > A) and 6 controls. The activity of the different mitochondrial respiratory chain complexes, COX, SDH and NADH histochemical staining and the OXPHOS analysis through western blot were performed on primary cultures.

Results: In comparison with the controls, complex I defect was observed for the patient carrying the m.13513G > A and a deficit of complexes I and IV for the patient carrying the m.3243A > G. For the latter, histochemistry also revealed the presence of COX-negative urothelial cells.

Conclusion: This study shows that urine-derived cells are a promising alternative to invasive sampling for the analysis of mitochondrial functions and functional validation of VUS, and confirms the value of urine samples for the diagnosis of mitochondrial diseases.

Conflict of Interest: None declared.

EP07.016 Mitochondrial DNA copy number variation in patients with suspected mitochondrial disease

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Mitochondrial diseases (MDs) comprise genetically and phenotypically diverse group of disorders, characterized by dysfunctional mitochondria. Point mutations, large deletions and duplications are observed in MDs. However, mitochondrial function can as well be indirectly evaluated by measuring mitochondrial DNA (mtDNA) copy number (CN) that is usually increased in MD patients carrying point mutations compared with the control individuals (Filograna R., et al., 2020).

In this study, RT-PCR was applied to detect mtDNA CN in peripheral blood in a cohort of 71 affected individuals with suspected MD. Sanger sequencing of whole-length mtDNA and long-range PCR were used for comprehensive analysis of mtDNA sequence variants, deletions, including haplogroup profiling. mtDNA CN was also analysed in a control group of 15 healthy individuals.

mtDNA CNs were statistically significantly higher in the pediatric (under 18 years of age) patients ($n = 50$; median (IQR) CN = 291.5 (95.75) compared to adults (over 18 years of age, $n = 21$, median CN = 174.6 (55.00), $p = 0.00003$). MD was confirmed in 10 patients and the diagnosis of non-MD was confirmed in 9 patients of the study group. The difference of mtDNA CN in patients and control groups were not statistically significant ($p > 0.05$). However, patients with MD had lower mtDNA CN (median CN = 176.25 (55.5)) compared to patients confirmed with non-mitochondrial disease (median CN = 313 (108.0), $p < 0.05$).

This study indicates the increased mtDNA content in the peripheral blood of non-mitochondrial disease patients manifesting with clinical symptoms relevant to MDs that may be a consequence of compensatory response to secondary mitochondria damage.

Conflict of Interest: None declared.

EP07.018 identification of a novel SLC25A4 mutation causing cardiomyopathy and myopathy in a patient from a consanguineous Saudi family

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Background/Objectives: Mitochondrial DNA Depletion Syndrome 12B (MTDPS12B) is an autosomal recessive depletion syndrome caused by various mutations in *SLC25A4*. Mutations in ANT1 are associated with complex human diseases associated with mitochondrial myopathy and cardiomyopathy [1-3].

Methods: Blood samples were obtained from the family for genetic testing. Skin biopsy was taken from the patient. mtDNA sequencing on the patient DNA sample revealed no pathogenetic variants. We performed a comprehensive muscular dystrophy/myopathy gene panel to identify the genetic cause of the disease. We utilized Sanger sequencing for variant confirmation, and RT-PCR, mtDNA depletion, mitochondrial respiration and glycolysis experiments for further characterization and pathobiological understanding of the variant detected during the gene panel screening.

Results: We identified a novel presumably splicing mutation (c.112-1G > C) in *SLC25A4*. The variant was fully segregated with the phenotype in the family and absent among large ethnically matching controls. The variant was predicted to be pathogenic by different classifiers. Sequencing of the RT-PCR products showed 6 basepairs deletion in the exon 2/3 junctions. Quantitative PCR results indicated decreased copy numbers of mitochondria in both cell types. Functional analyses on fibroblasts obtained from this patient revealed a decrease in both mitochondrial respiration and glycolytic functions as compared to healthy controls.

Conclusion: This homozygous splice site mutation in *SLC25A4* is a strong candidate in causing the disease in our patient and our study expands the genotypic spectrum of SLC25A4 deficiency.

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Conflict of Interest: None declared.

EP07.019 A bibliometric and VOSviewer analysis of molecular mechanisms of obesity

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Background/Objectives: Obesity has been a field of intense research in recent years. Our study aimed to identify the current research hotspot, status and trends in molecular mechanisms of obesity by using bibliometric analysis.

Methods: The bibliometric analysis using VOSviewer software (version 1.6.19) was applied to assess global literatures about obesity, scanned from the Web of Science database (February 2023) using relevant keywords “obesity”, “molecular mechanism”, “gene expression”.

Results: Globally, 442,108 publications on the topic of obesity were identified. The mean citation count of the top 100 most cited articles was 8,3 (range 5-28). Most of them were descriptive studies and 1,151 clinical trials. The “obesity” with a total link strength (TLS) of 3,370 appeared as the most frequent keyword which had a strong link to “inflammation” (TLS = 1,207, and associated with “insulin resistance”, “high-fat diet”, “metabolic syndrome”), and “adipose tissue” (TLS = 904, associated with “adipogenesis”, “diabetes”). Keyword “gene expression” had 358 occurrences (TLS = 461, associated with “epigenetics”, “diabetes”). In total, 23,271 publications on gene expression related to obesity were identified from 2000–2023, 80.6% of them was published in the last decade and the number of publications continues to increase gradually every year.

Conclusions: The current growth trends predict a significant increase in the number of global publications on obesity and its molecular mechanisms. USA made the most outstanding contribution within this field. Research hotspots focused on inflammation, lipid metabolism, insulin resistance, diabetes, metabolic syndrome, adipose tissue and adipogenesis. Gene expression, epigenetics, sarcopenic obesity and adipokines may be the frontiers of future research.

Conflict of Interest: None declared.

EP07.020 uAUG-creating variant in the LDLR gene causes mild familial hypercholesterolemia

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Background/Objectives: Variants that create upstream AUG codons at the 5`UTRs are under strong negative selection and could lead to the Mendelian disorders.

Methods: For patient with clinical diagnosis of familial hypercholesterolemia (FH) we performed custom panel sequencing. Experimental analysis of the effect of the variants on translation efficiency was conducted using dual luciferase assay and qPCR.

Results: A family with a 4-year-old girl applied for genetic counseling due to mild clinical features of hypercholesterolemia. Panel sequencing revealed the c.-8C > A variant in the *LDLR* 5`UTR. This variant leads to the formation of the upstream AUG codon and could create an overlapping uORF that suppresses the translation of the downstream *LDLR* coding sequence. Using experimental approaches, we showed that the c.-8C > A variant did not influence on transcription, but reduced main protein translation efficiency by about two times. Such partial reduction is in good agreement with the mild clinical picture of the patient, which is not typical for familial hypercholesterolemia caused by loss-of-function variants of the *LDLR* gene. We also analyzed three previously published variants in the 5’UTR of the *LDLR* gene. These variants were registered as pathogenic in HGMD and as uncertain significance in ClinVar, however they did not lead to the creation of uAUG. Our experimental analysis showed that these variants

did not affect the level of transcription and translation efficiency. Thus, we suggest that these variants are not associated with FH.

Conclusion: Experimental analysis helps to determine the pathogenicity of rare variants and thereby explain the clinical features of patients.

Conflict of Interest: None declared.

EP07.021 Genotype–phenotype correlation of 2q21microdeletions: report of a 2.5 Mb microdeletion associated with metabolic acidosis and literature review

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Background/Objectives: 2q21 microdeletions are rarely reported with an unclear phenotype/genotype correlation because of the variability of clinical manifestations according to the size of deletions and their locations in the limited reported cases. To elucidate this correlation we report here a case of 2q21.2q21.3 microdeletion associated with severe congenital malformation syndrome and metabolic acidosis.

Patient & Methods: The proband is a termed baby boy of cousin Saudi parents died at the age of 6 months because of lactic acidosis, hyperammonemia, cardiac malformation and bilateral hydronephrosis.

Genetic investigation was performed using karyotype and 180K Agilent oligonucleotides array CGH analysis according to the manufacturer's instructions.

Results: CGH Array revealed the presence 2.5 Mb deletion on the long arm of chromosome 2 at q21.2q21.3 including DARS, MCM6, LCT, and RAB3GAP1 genes. Result was reported as: arr[GRCh37] 2q21.2q21.3(134208726_136736954)x1

Conclusion: We describe here a novel interstitial deletion encompassing 2q21.2-q21.3, which was diagnosed by CGH array. The deletion of approximately 2.5Mb includes four genes. Among them, MCM6 and LCT genes are liked respectively to autosomal dominant and recessive lactase deficiency. We suggest that lactic acidosis is most likely caused by the loss of these contiguous genes. Cardiac malformation and hydronephrosis were observed in previous reported cases with largest deletions including 2q21 region. However, reported cases of 2q21.1 deletions were with mild phenotype and associated only with mental retardation. The comparison between our patient and previously reported cases in the literature contributes to a better definition of genotype–phenotype correlation of 2q21microdeletions.

Conflict of Interest: None declared.

EP07.022 Genetic predisposition for several complex diseases

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Background/Objectives: The predisposition for type 2 diabetes mellitus (T2DM), metabolic syndrome (MetS), obesity (Ob) and arterial hypertension (AH) present intricated elements. We aim to investigate the predisposition for these diseases in a case control study.

Methods: A total of 400 patients (213 men/187 women) were equal distributed in these four groups. 100 healthy subjects (HC) were considered as control. The ACE rs4646994 (I/D), eNOS rs1799983 (VNTR 4a/b), OXTR rs53576 (A > G), ATR1 rs5186 (A1166C), CAT rs7943316 (A > T) and SOD1 rs2234694 (+35A/C) polymorphisms were genotyped by PCR based methods for all samples. Fifteen variables related with life style, paraclinical and anthropometrical date were recorded for each subject.

Results: The distribution frequency of genotypes met Hardy-Weinberg equilibrium law. rs2234694AA genotype were protective for T2DM ($p < 0.05$). and was associated with triglyceride levels in the T2DM and Ob lots. The presence of both rs4646994 I and rs53576 A alleles was associated with T2DM. The rs4646994 DD was associated with hypertension in patients from T2DM and AH lot. Patients who reported drinking alcohol had increased frequency of rs4646994 DD and rs53576 G ($p < 0.05$) compared to nondrinkers from T2DM or Ob lots.

Conclusions: The investigated SOD1, ACE, OXTR polymorphisms seems to be associated with T2DM, obesity or arterial hypertension. No significant association was detected for sublot of subjects stratified according to the gender.

Grant References: This research work was carried out with the support of Ministry of Research and Education, under the Core Program, project PN 23 01 03 03.

Conflict of Interest: Lavinia Mariana Berca National Research and Development Institute for Food Bioresources – IBA Bucharest, project PN 23 01 03 03, Robert-Mihail Sionel National Research and Development Institute for Food Bioresources – IBA Bucharest, project PN 23 01 03 03, Catalina Zenoaga-Barbarosie AMS Laborator Genetic, Danut Cimponeriu University of Bucharest, Faculty of Biology, Department of Genetics, project PN 23 01 03 03, Mihai Toma Central Military Emergency Hospital Dr. Carol Davila, Bucharest, Matei Ioan Nica: None declared, Ortansa Csutak University of Bucharest, Faculty of Biology, Department of Genetics.

EP07.023 The genetic spectrum of maturity-onset diabetes of the young in the Lithuanian diabetic population

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Background/Objectives: The most common type of monogenic diabetes is maturity-onset diabetes of the young (MODY) that affects 1–5% of all patients with diabetes mellitus. Identification of the MODY subtype is important due to subtype-related differences in clinical course and progression, response to treatment and for the genetic counseling. The aim of the study was to determine frequency and genetic spectrum of MODY in the Lithuanian diabetic population selected according to the criteria.

Methods: The study included 55 patients from the diabetic population. Patients were referred for genetic testing based on the results of MODY probability calculator developed by Exeter University. For each individual with the diagnostic criteria for MODY, a panel of 14 MODY genes was screened using targeted next generation sequencing assay.

Results: The (likely) pathogenic variants confirming a diagnosis of MODY were identified in 17 (30.9%) individuals. The majority of patients (88.2%) were found to harbour (likely) pathogenic variants in the *GCK* gene, one patient (5.9%) had pathogenic

variant in *ABCC8* gene and one patient (5.9%) carried (likely) pathogenic variants in both *GCK* and *ABCC8* genes. Also, a variant of uncertain significance in *PAX4* gene was detected in one individual.

Conclusion: The most prevalent MODY subtype in the selected Lithuanian diabetic population is GCK-MODY and only few patients were found having mutations in *ABCC8* and *PAX4* genes. Translational biology, integrative genomic research and studies on both monogenic and polygenic forms of diabetes are required, which will broaden our understanding in terms of pathophysiology and treatment of diabetes.

Conflict of Interest: None declared.

EP07.024 Detailed genetic and clinical analysis of a novel de novo variant in HPRT1: Case report of a female patient from Saudi Arabia with Lesch–Nyhan syndrome

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Hypoxanthine-guanine phosphoribosyltransferase (HPRT1) deficiency is an inborn error of purine metabolism responsible for Lesch–Nyhan syndrome (LNS). The disease is inherited in an X-linked recessive manner and predominantly affects male individuals. Female individuals can carry a mutation as heterozygotes, but typically, they are asymptomatic because of the random inactivation of the affected allele. Nevertheless, although rare, heterozygote female individuals may manifest LNS with full characteristics. Herein, we describe a female patient from Saudi Arabia with LNS. The patient (a 4-year-old girl) presented with typical characteristics of the disease, which include global developmental delay, self-mutilation, hyperuricemia, hypotonia, speech delay, spasticity, and seizures. Her general biochemical laboratory results were normal except for high levels of uric acid. The abdominal MRI\MRS, mostly unremarkable, showed bilateral echogenic foci within the renal collecting system. Genetic testing (whole-exome sequencing, iterative variant filtering, segregation analysis, and Sanger sequencing) pointed a novel de novo frameshift variant in HPRT1. X-inactivation assay using Hpall showed the presence of a 100% skewed X chromosome carrying the affected allele. RT-PCR of the cDNA indicated complete loss of the expression of the normal allele. Our study presents a female patient who has a severe case of LNS and found to be the 15th female patient with the disease in the world. The study emphasizethe need for a streamlined protocol that will help an early and accurate diagnosis of female LNS patients to avoid unnecessary interventions that lead to costly patient care.

Conflict of Interest: None declared.

EP07.025 Metabolomic profiling to resolve complicated case with MMADHC gene mutation

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Introduction: Combined methylmalonic aciduria and homocystinuria cblD type

(MAHCD) is a rare disorder of vitamin B12 (cobalamin) metabolism. It is characterized by decreased levels of the coenzymes adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl) and extremely variable clinical manifestation. MAHCD is an autosomal recessive disorder caused by mutation in *MMADHC* gene.

Materials and Methods: We report a 5-year-old boy with an uneventful premorbid history with current onset of subacute encephalopathy including dementia syndrome, increased sleepiness and ataxia. Differential diagnoses as a progressive cavitating leukoencephalopathy, Canavan disease, vanishing white matter, mitochondrial leukoencephalopathy, primary and secondary CNS vasculitis, subacute sclerosing panencephalitis, HIV- encephalopathy were discussed based on different imaging, hematological and biochemical studies. Metabolic profiling was performed by LC-MS/MS and GC/MS methods. Molecular genetic testing was performed by NGS of genes associated with a defect in cobalamin metabolism.

Results: Metabolic profiling of acylcarnitines in blood and organic acids in urine showed significantly increased levels of propionylcarnitine and extremely elevated excretion of methylmalonic acid and methylcitrates. This profile combined with the high total serum homocysteine found, suggests a defect in cobalamin metabolism. The molecular genetic testing showed a homozygous pathogenic variant c.748C>T (p.Arg250Ter) in *MMADHC* gene which confirmed the biochemical findings.

Conclusions: Metabolomics is an important step to clarify the diagnosis in rare genetic diseases with highly variable phenotype even in cases with a slight clinical suspicion of inherited metabolic disease. The patient received adequate therapy with improvement in speech development but with persistent behavioral abnormalities.

Conflict of Interest: None declared.

EP07.026 Improvement of the genetic diagnosis of severe hypertiglyceridemia through new genes associated with this pathology

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Background/Objetive: Severe hypertriglyceridemia is a disorder of lipid metabolism characterized by elevations in triglycerides and high risk of acute pancreatitis. About 2% of patients present familial- chylomicronemia-syndrome caused by biallelic variants of loss of function in *LPL*, *APOA5*, *APOC2*, *GPIHBP1*, *GPD1* and *LMF1*. Some studies have shown that other genes could be associated with a polygenic hypertriglyceridemia.

The aim of this study was to identify variants in these new genes to increase the genetic diagnosis of hypertriglyceridemia.

Methods: Forty-eight patients with severe hypertriglyceridemia (>10-mmol/L) were studied by next-generation sequencing with a customized panel of 500 genes. A total of eighteen genes were studied, primary genes: *LPL*, *APOA5*, *APOC2, GPIHBP1*, *GPD1* and *LMF1* and secondary genes: *ANGPLT3*, *ANGPLT4*, *ANGPLT8*, *APOC3*, *APOE*, *CREB3L3*, *GALNT2*, *GCKR*, *LIPC*, *LIPI*, *MLXIPL* and *TRIB1*. Variants with allele frequency <0.005 were analyzed in silico and classified according to the guidelines of American College of Medical Genetics.

Results: Fifteen variants were found in 18 patients in primary genes, two of these in homozygous. In addition, thirteen variants were found in 10 patients in secondary genes. Overall, 52% of the patients were carriers of one of these variants.

Episodes of acute pancreatitis were found in the 55% of patients who carried at least one variant in primary genes and in 30% of patients with variants in secondary genes.

Conclusions: Secondary genes analysis increased the genetic diagnostic resulting about 20%. However, carriers of primary gene variants showed a greater association with acute pancreatitis.

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Conflict of Interest: Javier Sanguino full, Carmen Rodriguez-Jimenez full, Francisco Arrieta Blanco full, Pedro Luis Martinez-Hernandez part-time, Daniel Martínez-Jimenez part-time, Ana Carazo part-time, Maria Victoria Del Pozo-Gomez part-time, Cristina Ortega-Patrón part-time, Juan Manuel Montejo part-time, Sonia Rodriguez Novoa Full, This work was supported by grant PI21/01239, Instituto Salud-Carlos-III.

EP07.027 Cytosolic PEPCK deficiency caused by a novel homozygous frame-shift variant presenting as hypoglycemia and acute liver failure at birth

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Here we describe a homozygous frameshift mutation in PCK1, manifested in transient hypoglycemia and acute liver failure with extreme hyperferritinemia, presented on the first days of life.

The patient, a child of non-consanguineous parents from a Jewish Yemeni origin, was born following an uneventful pregnancy. At day 3, she presented acute liver failure with extreme hyperferritinemia of >40,000ng/ml, hyperammonemia and coagulopathy that improved gradually. At the age of 7 months, she was admitted to the hospital following an acute illness with severe hypoglycemia. Whole-exome sequencing (WES) revealed a homozygous frameshift mutation in Cytosolic phosphoenolpyruvate carboxykinase (PEPCK, PCK1). This enzyme plays a rate-limiting step in gluconeogenesis occurring mainly in the liver during prolonged fasting. Biallelic deficiency of this enzyme results in a rare inborn error of metabolism disorder (OMIM #261680).

Typical main Manifestations include fasting hypoglycemia and lactic acidosis with urinary excretion of TCA cycle metabolites. The initial presentation varies between individuals in terms of age and clinical manifestations, however clinical information is lacking as it was diagnosed so far in <30 patients with a total of 6 different mutations, all either missense or splice variants.

To the best of our knowledge, here we describe the first homozygous frameshift mutation in PEPCK causing a severe very early-onset liver failure that was not previously described. This case expands our clinical and genetic understanding of this rare metabolic disorder and emphasizes the need to consider PEPCK deficiency in the differential diagnosis of neonatal liver failure.

Conflict of Interest: None declared.

EP07.028 X-Linked Creatine Transporter Deficiency in Two Saudi Brothers with Autism

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Background/Objectives: X-linked creatine transporter deficiency (CTD) was first described in 2001. A previous genotype-phenotype correlation study of CTD revealed that all affected males have an intellectual disability together with delayed speech development of variable severity. Other frequent clinical features include neurobehavioral disorders such as attention-deficit/ hyperactivity disorder (ADHD), autism spectrum disorder (ASD), seizures, hypotonia, spasticity, and dystonia. There are no previous case reports of CTD in Saudi Arabia. Here, we report X-linked creatine transporter deficiency in two Saudi brothers who presented with autism.

Methods: Case A and B are siblings who were born to a consanguineous Saudi couple. Their father had adult-onset focal seizures and visual hallucinations on Depakene. Their mother and sister were healthy at the time of the presentation. There is a family history of febrile seizures, global developmental delay, and intellectual disability in different cousins.

Results: Case A: Brain MRI showed diffuse cerebral atrophic changes in form of sulcal prominence and ventricular distension. MRS study showed decreased creatinine levels with normal choline and NAA levels. Case B: Brain MRI showed nonspecific periventricular foci of bright T2/FLAIR signal intensity. MRS showed an absence of creatinine peak with normal choline and NAA levels

Conclusion: This case proposes the need to consider creatine deficiency disorders in the differential diagnoses of children with autism spectrum disorder and neurological symptoms in multiplex autism families. Clinicians should have a low threshold for investigation of this possibility, employing brain MRS, genetic testing, and biochemical screening tests for creatine disorders where these are available.

Grants:

Conflict of Interest: None declared.

EP07.029 Case reports of Crigler-Najjar syndrome type 1 in Czech patients

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Background: UDP-glucuronosyltransferase 1A1 is an essential enzyme in bilirubin metabolism. Mutations in the *UGT1A1* gene cause hereditary unconjugated hyperbilirubinemias, which include Gilbert syndrome, Crigler-Najjar syndrome type 1 (CN1) and type 2 (CN2). These disorders differ in level of serum unconjugated bilirubin, severity of symptoms and response to treatment. Gilbert syndrome is the mildest phenotype with only slightly raised bilirubin level. Its clinical presentation sometimes overlaps with CN2, in which the UGT1A1 enzyme activity is

moderately reduced and can be restored by phenobarbital administration. CN1 is extremely rare and the most severe phenotype with total or nearly total absence of enzyme activity and not responding to phenobarbital treatment. Here we present case reports of two Czech patients with CN1.

Methods: *UGT1A1* gene was analyzed by Sanger sequencing. Analysis of mutations in parents was performed to confirm compound heterozygosity in both patients.

Results: Patient 1 had shown persistent unconjugated hyperbilirubinemia since day 3 of life. Diagnosis of CN1 was confirmed at the age of 10 weeks. The present age is 3.5 years. Genotype NM_000463.3:[-41_-40dupTA; 840C > A];[1220delA]. Patient 2 had significant hyperbilirubinemia requiring phototherapy from day 8 of life. The diagnosis was confirmed at the age of 2 months. He is currently almost 3 months old. Genotype NM_000463.3:c.[-41_-40dupTA;722_723delAG];[1021C > T]. Both patients require several hours of daily phototherapy.

Conclusion: Identification of causative mutations confirmed clinical diagnosis and enable future prenatal genetic testing in affected families. Patients with untreated CN are at risk of kernicterus and early death, therefore early diagnosis is critical.

Grant references: RVO-VFN 64165.

Conflict of Interest: None declared.

EP07.030 Quantification of genotype-phenotype relationships in Pompe disease by enzyme activity: a case for graded variant classification

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Background: Pompe disease (Pompe) is a rare autosomal disorder caused by pathogenic variants in acid α-glucosidase (GAA). Two different clinical forms have been described, depending on age of onset: infantile (IOPD, <1 year of age), and late (LOPD, juvenile or adult). Early diagnosis of Pompe and initiation of enzyme replacement therapy are critical for symptom improvement and survival. However, the broad clinical spectrum of Pompe and the large number of GAA variants of unknown significance (VUS) can complicate and delay diagnosis, especially for LOPD.

Methods: We generated a Pompe patient database (n > 1,750), and applied a regression model to determine disease severity (phenotype) from GAA variant combinations (genotypes) through enzyme activity. We further characterized 400 rare GAA VUS taken from a healthy population (ExAc) through an in vitro enzymatic assay, the largest such study to date, and applied a clinical evaluation to determine their pathogenicity.

Results: Overall, our study enabled the clinical characterization of over 600 GAA variants, including 300 VUS. Integration with gnomAD allele frequencies, revealed that 99% of individuals carrying the most common pathogenic variant do not develop Pompe in their lifetime. Conversely, 75 GAA VUS from ExAc showed potential disease relevance. Our model provides a graded classification of variant pathogenicity and is highly reliable, with a predicted activity of 15% suggesting a lifespan disease risk of 1:100.

Conclusion: Our results challenge the binary model of pathogenicity found in variant databases, such as ClinVar, allowing for improved diagnostic potential in Pompe and potentially other monogenic diseases.

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Conflict of Interest: Reet Mishra: None declared, Zhiqiang Hu Illumina Inc., U24HG007346, PI Steven E. Brenner, Illumina Inc., Dona Kanavy ClinGen Lysosomal Storage Disorders Variant Curation Expert Panel, Jennifer L. Goldstein ClinGen Lysosomal Storage Disorders Variant Curation Expert Panel, Deeksha S. Bali ClinGen Lysosomal Storage Disorders Variant Curation Expert Panel, Yuanbin Ru Alara Biotherapeutics, Alara Biotherapeutics, G. Karen Yu Global Blood Therapeutics, Global Blood Therapeutics, Jonathan H. LeBowitz BioMarin Inc., Constantina Bakolitsa University of California, Berkeley, U24HG007346, PI Steven E. Brenner, Wyatt T. Clark BioMarin Inc., BioMarin Inc.

EP07.032 Estimating quality of life in adults with Mitochondrial disease and their carers

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Introduction: Mitochondrial disease is a serious and debilitating neurometabolic disorder causing significant disease burden and mortality, with great heterogeneity in presentation of symptoms. Currently, there are no curative therapies, with treatments focused on managing and reducing the impacts of symptoms. There are limited studies on the Quality of Life (QoL) impacts of the disease and to our knowledge, no study using health utilities to quantify the impact. With advances in genetic testing and the hope of breakthroughs in treatments, QoL data using health utilities will be required for use in cost-effectiveness analysis for publicly funded interventions.

Materials and methods: Patients were recruited from Neurogenetic clinics in Sydney, Australia. 96 participants and 24 carers completed the tailored questionnaire, which collected information on QoL and health utilities through the Assessment of Quality of Life (AQoL)-8D, a validated measure of QoL.

Results: AQoL-8D utility values for patients and carers were significantly lower than the general population. We will present scores for each of the AQoL-8D domains and bivariate and multivariate regressions analysing the drivers of patient QoL.

Conclusion: Mitochondrial disease has substantial impacts on QoL. This paper will present the first analysis of QoL in Mitochondrial disease patients using health utilities. This will be crucial to inform cost-effectiveness of new interventions, including genomic testing, for patients with Mitochondrial disease.

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EP08 Immunology and Hematopoietic System

EP08.001 Genomics role at the identification of patients with inborn errors of immunity

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Background: Inborn errors of immunity or primary immunodeficiencies are humoral or cell-mediated immunity disorders with a genetic etiology that in the absence of a precipitating predisposes to recurrent infections, autoimmune and oncological diseases. In Colombia, it is considered a mandatory notification disease (Law 1392 of 2010, Decree 1954 of 2012, Decree 780 of 2016, Resolution 5265 of 2018 and Resolution 946 of 2019) having a prevalence of 1: 2000 to 10000 in the general population.

Case report: 5-year-old female patient with a clinical history of repeated infections and oral thrush, Juvenile Dermatomyositis since lactation, and low height and weight for age; in multidrug management with little clinical improvement so a New Generation Sequencing (NGS) genetic study with a panel of 228 genes for immunodeficiencies was taken. A pathogenic heterozygous variant was found in the TNFRSF13B gene, associated with common variable immunodeficiency with atypical clinical presentation and the need for genomic diagnosis to establish a specific diagnosis.

Conclusion: Current genomic diagnostic methods allow to establish phenotype/genotype correlation, making a specific diagnosis, to establish directed therapeutic options, predict complications, develop curative target therapies, such as gene therapy, follow-up, genetic counseling, bringing closer to preventive, anticipatory and personalized medicine.

Grant Reference: Not applicable.

Conflict of Interest: None declared.

EP08.002 Genetic variant in complement receptor 1 (CR1, CD35) is associated with a cluster of severe fatal coronavirus disease 2019 (COVID-19) in a Family

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Fatal coronavirus disease 2019 (COVID-19) clustering within a family could indicate a genetic predisposition to contract severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). This study investigated whether such predisposition existed in a family that was severely affected by COVID-19 with four deaths and 3 suffering near-fatal disease. We applied whole exome sequencing and segregation analysis to identify unique genetic variants among those affected by SARS-CoV-2. A novel splice mutation in *CR1* (complement receptor 1) gene was identified [c.5302+1G > A in (rs756221326, 1-207755349 -G-A (GRCh37)] (NM_000651.6). We found a high correlation between those severely affected by COVID-19 and heterozygote alteration. RNA expression analysis identified two isoforms expressed for carries: one with full exon 32 inclusion (wild-type allele) and second with exon skipping (without exon 32). A major outcome of exon skipping is abolishing normal reading frame and creating early stop gain, leading to truncated protein (31 exons compared to a wild-type protein that includes 47 exons). Quantitative allele expression (qPCR) targeting the wild-type allele revealed significantly decreased levels of CR1 among carriers compared to wild-type samples. Given its central role in regulation of complement activity acting on all three complement pathways as a membrane-bound receptor of C3b/C4b, C3/C5 convertase decay accelerator, and cofactor for factor I-mediated cleavage of C3b and C4b, it's intriguing to hypothesize that reduced CR1 expression may have had a significant impact on disease severity and outcome among those carrying the genetic variant.

Conflict of Interest: marah khalaila Full time, nada farran Full time, moran avraham-klabert Full time, Naiel Bisharat Full time.

EP08.003 Describing the bone microenvironment of monoclonal gammopathies patients by single cell RNAseq analysis

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Background/Objectives: Multiple myeloma (MM) is a plasma cell dyscrasia, characterized with the occurrence of bone lesions, that may be preceded by pre-malignant condition as monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM). Up to date we are not able to identify exactly the patients that will progress to active MM. The aim is to study the bone microenvironment (BM) in patients with MGUS/SMM to identify the mechanisms involved in the progression to MM.

Methods: From the bone biopsy of 4 MGUS, 4 SMM, 4 MM patients, we isolated and enriched the CD45-CD138-CD235a-CD31- non-hemopoietic cells. Single-cell RNAseq data were generate on 10X Genomics platform. Cell Ranger 6.1.2 have been used to obtain feature and barcode count matrices. In R software version 4.1.0, the predicted cell populations were calculated using singleR and the HumanPrimaryCellAtlasData. Differential transcript analysis was performed using the linear mixed effects model method in the MAST R package. Pathway analysis was performed using the decoupleR package.

Results: A total of 51672 cells were profiled, and 3 different types of mesenchymal stromal cells(MSCs), osteoblasts(OBs), MSCs-derived cells and a small amount of hemopoietic cells were identified. In MM samples, there was a significant reduction of OBs number, and MSCs-derived cells. Moreover, the BM cells of MM showed an alteration in the expression of genes of WNT, hypoxia, NF-kB and PI3K pathways.

Conclusions: The study at single cells level could be a new method to describe the alterations of BM cells in patients with monoclonal gammopathies.

Grant: AIRC MFAG 2020(ID:24932).

Conflict of Interest: None declared.

EP08.004 Exosomal specificity of microRNAs associated with disease activity and renal damage in systemic lupus erythematosus

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Background and objectives: Little is known about the biofluid specificity of microRNAs (miRNAs) in systemic lupus erythematosus (SLE). Our aim was to identify a specific miRNA profile in plasma exosomes and assess biological functions in response to lupus activity and renal damage using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

Methods: Plasma samples were obtained from 96 SLE patients and 25 controls. RNA was extracted from both plasma and plasma-exosomes and miRNAs were identified using SmallRNA sequencing analysis. Results were validated in a higher cohort by qPCR.

Results: From the small RNA-sequencing, the 25 miRNAs with the highest fold-change expression between biofluids were identified. Nine miRNAs were validated in a larger cohort, and were found to be increased in exosomal fraction and patient groups. Further analysis revealed that two panels combining three miRNAs [panel 1: LN (miR-101-3p, miR-144-5p and miR-15a-5p); panel 2: non LN (miR-144-3p, miR-101-3p and miR-19b-3p)] gave an area under the curve that improves the readout of the single miRNAs (0.964 and 0.828, $p < 0.0001$, respectively). Finally, biology system analysis showed that exosomal miRNAs panels target critical pathways regulating immune response (ubiquitination, TFG-β1-SMAD and VEGF signalling)

Conclusion: Biofluid origin influences on plasma miRNA expression and identified specific exosomal miRNA profiles which target critical pathways in SLE-associated activity and renal damage progression. This finding could contribute to advances in SLE diagnosis and as promising therapeutic targets.

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Conflict of Interest: None declared.

EP08.005 TREX1: The varying and overlapping manifestations of disease

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TREX1 variants are associated with Aicardi-Goutières syndrome (AGS), Familial chilblain lupus (FCL) and Retinal vasculopathy with cerebral leukodystrophy (RVCL). Heterozygous variants are also described in Systemic lupus erythematosus (SLE).

In AGS, *TREX1* variants are generally biallelic and result in dysfunctional exonuclease activity leading to an upregulation of type I interferon and interferon stimulated genes (ISG). Elevated ISGs are also noted in FCL and SLE and variants may be in the C terminus. In RVCL causative variants are heterozygous frameshift mutations, occur at the C terminal end of the protein and impede localization of the protein to the endoplasmic reticulum, rather than impact exonuclease function; in reported cases type I interferon levels have been normal and the phenotype distinct.

We describe a family with features of RVCL, including adult onset vasculopathy, cerebral white matter and contrast-enhancing lesions causing neuropsychiatric sequale and progressive neurological decline. We note however autoimmune manifestations in the kindred, including SLE-like symptoms and Polyarteritis nodosa and an elevated ISG in the proband. We identified a previously unpublished *TREX1* frameshift C terminal pathogenic variant in the family. Functional studies demonstrated that the variant did not impact exonuclease activity, but resulted in mislocalisation of the protein, comparable to that seen in RVCL.

We highlight the diverse manifestations of RVCL and the diagnostic difficulties overlap cases can cause. The correct diagnosis here is pertinent given that different clinical trials with different modes of action are available for each of AGS, SLE and RVCL.

Funding: MRF (TAB), Clayco foundation (SM), WT (DG)

Conflict of Interest: None declared.

EP08.006 Using whole exome sequencing for detecting genetic causes of autoinflammatory diseases in families without common MEFV gene. mutation

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Background/Objectives: Autoinflammatory diseases (AIDs), often known recurrent fever syndromes, are a heterogeneous group of rare conditions characterized by innate immune dysregulation. It is thought that mutations in the Mediterranean fever (*MEFV*) gene are the most common cause of AIDs, but other genetic factors may also contribute. Up to now, more than 30 different genes have been identified as casual genes with autosomal or dominant inheritance patterns for AIDs. Therefore, in this study, we aimed to uncover the genetic causes of AIDs in five Iranian families without common *MEFV* gene mutations using whole exome sequencing (WES).

Methods: Peripheral blood was taken from the core family after clinical evaluation and consent forms were signed by all family members. Then, DNA was extracted and analyzed for quality and quantity. We performed WES on DNA samples from the proband of each family. The WES data were analyzed using different bioinformatics tools and compared to reference databases to identify candidate variants that could be associated with AIDs.

Results: Each family was analyzed multiple times to identify possible candidate genes. The candidate genes were either involved in the inflammation process or known genes associated with AIDs. Segregation analysis is performing to detect probable gene and variants as well as their inheritance pattern in each family.

Conclusion: The potential functional impact of the identified variants will be investigated through further analysis. Furthermore identified candidate genes with their probable causative variants would be useful to accelerate diagnostic processes.

Grant references: USWR: 2920.

Conflict of Interest: Niloofar Bazazzadegan: None declared, Shima Salehi In UIMS, Kimia Kahrizi In GRC, USWR, Mojgan Babanejad In GRC, Sussan Banihashemi In GRC, Hossein Najmabadi In GRC, Seyedeh Sedigheh Abedini In GRC, In GRC, Grant from USWR.

EP08.007 Mendelian susceptibility to mycobacterial disease: clinical and molecular findings of a familial case with variants in IL12RB1 gene

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Background: Clinical disease caused by weakly pathogenic mycobacterial species is a rare entity secondary to a defective immune response with IL-12RB1 deficiency being the most common genetic etiology. In recent years a number of mutations in related genes have been described with variable clinical and immunological findings.

Methods: We describe clinical and molecular findings in an 10-year-old patient with multiple salmonellosis infections, multidrug-resistant intestinal tuberculosis and pulmonary aspergillosis who required multidisciplinary medical treatment. Molecular analysis was indicated, and the study was extended to the nuclear family.

Results: Two variants were reported in IL12RB1 gene c.1456C>T (p.Arg486*) and c.1420T>A (p.Cys474Ser), with pathogenic and uncertain significance classification, respectively, in the index patient and one brother.

Conclusion: Two variants are reported in a patient with clinical diagnosis of mendelian susceptibility to mycobacterial disease. The importance of determining the allelic phase of variants and familial clinical features arises in classifying variants and determining recurrence counseling.

Grant references: cero grants.

Conflict of Interest: None declared.

EP08.008 Functional characterization of genetic variants associated with deficiency of adenosine deaminase II

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Background: Deficiency of Adenosine deaminase 2 (DADA2) is a rare, monogenic disorder characterized by reduced or absent activity of extracellular adenosine deaminase 2 (ADA2) enzyme. DADA2 patients suffer from vasculitis as the primary phenotype, as well as other immunological and hematological manifestations including hypogammaglobulinemia, pure red cell aplasia and lymphoproliferation. ADA2 deficiency is associated with several recessive mutations on the *ADA2* gene. However, the biochemical effect of each defect, as well as the pathophysiological role of ADA2 related to various phenotypes of the disease is still largely unknown.

Methods: The expression pattern and catalytic activity of ADA2 were studied in the lysates and culturing media of HEK293 cells following transfection with 9 selected pathogenic variants. Subcellular co-localization of ADA2 with the Golgi marker TGN45 was also determined for these variants by immunofluorescence assay.

Results: We observed reduced or lack of protein expression in HEK293 media for 6 out of 9 examined ADA2 variants, which were associated with significantly lower levels or absence of enzyme activity in both media and cell lysates. Immunostaining results indicated that 8 ADA2 variants co-localized less within the Golgi apparatus than the wild type protein, suggesting an altered protein secretion pathway in cells under the effect of these variants.

Conclusion Our study suggests impaired protein secretion as a contributing factor behind pathogenesis of some DADA2-associated variants. We further aim to explore possible affected pathways of ADA2 secretion, and understand the biochemical significance of these selected variants on the structural properties and stability of the protein itself.

Conflict of Interest: None declared.

EP08.009 Association toll-like receptor TLR 2, TLR 4 and TLR9 gene polymorphisms with COVID-19 severity

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Background/Objectives: Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a pandemic. Toll-like receptors (TLRs) play an important role in the activation of innate immunity, regulation of cytokine expression, indirect activation of the adaptive immune system. TLRs are key sensors that recognize of pathogen-associated molecular patterns of SARS-Cov-2. This study aimed to explore the association between the of *TLR2, TLR 4, TLR9* polymorphisms with COVID-19 severity.

Methods: The polymorphisms *TLR2 (*rs5743708), *TLR 4(*rs4986791) and *TLR9 (*rs5743836) were genotyped in 315 COVID-19 patients: 118 mild disease, 114 non-severe and 83 severe, age and sex-matched Genomic DNA was extracted from blood of participants, genotyping was performed using RT-PCR.

Results: No differences were found between groups regarding the allelic and genotype distribution of the *TLR 4* and *TLR 9*. For the polymorphism of *TLR2*, statistically significant differences were found in the distribution of allele ($\chi2 = 6.322$; $p = 0.043$) and genotypes ($\chi2 = 6.595$; $p = 0.0037$) frequencies. Carriage of dominant homozygous *Arg/Arg TLR2* trait increases the likelihood to protect against severe COVID-19 in participants mild disease (OR = 0.373; 95 % CI 0.14-0.993, $p = 0.042$) and non-severe (OR = 0.329; 95 % CI 0.118-0.916 $p = 0.027$). Participants with *Arg/Gln* genotype of *TLR2* had 2.68 - and 3.049-times higher odds severe, than mild disease and non-severe (95 % CI 1.007-7.131, $p = 0.042$ and 95 % CI 1.092-8.477, $p = 0.027$ respectively).

Conclusion: Our study indicated, that the polymorphisms *TLR2 (*rs5743708), may be possible risk factors for severe COVID-19.

Conflict of Interest: None declared.

EP08.010 PCSK3 overexpression in Sjögren's Syndrome patients may be regulated by rs4932178 SNP in the promoter region and correlates with IFN-γ expression

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Sjögren's Syndrome (SS) is an inflammatory autoimmune disease, characterized by chronic lymphocytic infiltrates in the exocrine glands. SS etiology is still partially unknown and the identification of new genes associated with this disease could therefore help to gain a more complete view of the mechanisms involved in its

development. Several studies have suggested a possible involvement of *PCSK3* gene in the pathogenesis of chronic inflammatory diseases. This gene encodes for the protease enzyme Furin, which promotes proteolytic maturation of important regulators of the immune response and enhance also the secretion of interferon-γ (IFN).

We investigated, by RT-qPCR, the *PCSK3* gene expression level in peripheral blood mononuclear cells isolated from SS patients and healthy controls and we evaluated a possible correlation with *IFN-γ* gene expression. Moreover, we also explored the variability of two *PCSK3* genetic polymorphisms (rs4932178 and rs4702) by direct sequencing, to evaluate a possible association between these polymorphisms and the expression levels of *PCSK3* gene.

We observed that *PCSK3* expression level was significantly higher in SS patients, compared to the controls ($P = 0.028$) and that it showed a positive correlation with *IFN-γ* expression level ($P < 0.001$). Moreover, we reported that the variant homozygous genotype of rs4932178 SNP is associated with a higher expression of *PCSK3* gene ($P = 0.038$). The homozygous variant genotype of this SNP is also associated with a higher risk of SS development ($P = 0.016$).

Our data suggest that Furin could play a role in SS development, also promoting IFN-γ secretion.

Conflict of Interest: None declared.

EP08.011 Clinical characteristics of patients with Alpha-1-antitrypsin deficiency

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Background: Alpha-1-antitrypsin (AAT) deficiency is an underdiagnosed autosomal recessive disease. The burden of underdiagnosis is high since risk factor modification (primarily smoking cessation), because of diagnosis awareness, can profoundly alter the natural course of the disease. The current study aimed to illuminate clinical aspects of the disease by characterising the cohort of patients with AAT deficiency managed at Golnik University Clinic.

Methods: Genetic testing for the two most common pathogenic variants, PI*Z (c.1096G>A, p.Glu366Lys) and PI*S (c.863A>T, p.Glu288Val), in *SERPINA1* gene followed by sequencing was performed, and clinical information of patients was obtained from the hospital's information system: i.) age, ii.) the serum concentration of AAT, iii.) pulmonary function parameters (FEV1 (%), TI, DLCO (%)), iv.) liver function parameters (AST, ALT, yGT), v.) presence of emphysema, and vi.) smoking status.

Results: Out of 700 patients with a genetic test, 210 had one (187 PI*MZ, 23 PI*MS), 39 had two pathogenic variants (1 PI*SS, 6 PI*SZ, 32 PI*ZZ genotype). Serum AAT concentration differed significantly between the groups (single-allele impairment/bi-allele non PI*ZZ/bi-allele PI*ZZ) with the lowest concentration present in PI*ZZ group. Pulmonary function tests, namely FEV1(%), and DLCO(%) were likewise the lowest in the PI*ZZ group. A similar trend was apparent with emphysema. There were no signs of clinically meaningful liver disease in the PI*ZZ group.

Conclusions: Among adult patients with AAT deficiency, lung disease – manifested as a decrease in pulmonary function and emphysema – was substantial. As expected, lung disease was more pronounced among smokers, even in patients with only single-allele impairment.

Conflict of Interest: Matija Rijavec Full-time, ARRS J3-2532, Julij Šelb Part-time, ARRS, Peter Korošec Full-time, ARRS J3-3072, J3-6787, J3-3626, J3-2234., Katarina Osolnik Full-time.

EP08.012 An exome-based, genotype-driven approach improves the genetic characterization of thrombocytopenias with or without predisposition to myeloid malignancies

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Inherited thrombocytopenias (ITs) are extremely diverse diseases with wide genetic heterogeneity.

We applied Exome Sequencing (ES) and targeted analysis of 43 known IT genes to a large cohort of 187 cases (135 families) presenting with non-syndromic IT. Most cases have undergone a validated diagnostic algorithm including phenotypic investigation and sequencing of suspected genes.

ES achieved an overall diagnostic yield of 32.5%, with 44 pathogenic variants occurring more frequently in *ACTN1*, *ETV6, GP1BA and RUNX1*. Moreover, ES-based Copy Number Variant analysis disclosed an unexpected high prevalence of *RUNX1* large deletions (2.2%). Overall, 8% cases carried variants in genes potentially predisposing to hematological malignancies (*ETV6, RUNX1*).

When compared to the validated diagnostic algorithm, ES gained 16% yield. This could be explained by events of atypical inheritance, sex-related effects and phenocopies for which the candidate-gene approach hindered the diagnosis or false negative laboratory results. One of the major challenges of ES application to IT is the high proportion VUS (25% cases in our study). Since IT-associated variants are generally highly penetrant, segregation data even in small pedigrees can be useful to change variant classification, especially downgrading VUS to B/LB status, as occurred in 20/135 of our cases.

In conclusion, we report that an exome-based, genotype-driven approach contributes to the accurate diagnosis of ITs and to the surveillance over hematological malignancies. We propose the application of ES to overcome the limitations of current candidate-gene approaches. Simultaneous NGS of multiple family members is recommended to reduce the impact of VUS and therefore time-to-diagnosis.

Conflict of Interest: None declared.

EP08.013 CD26+ Leukemic Stem Cells identification in Tunisian chronic myeloid leukemia patient : a preliminary study

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Objectives: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by an acquired genetic alteration of the hematopoietic stem cells behaved as leukemic stem cells (LSC). CD26 is a highly specific marker expressed in CML LSC. Assessment of CD26+LSCs may be an optimal biomarker for the monitoring of CML patients.

This study investigated the role of CD26+ LSCs in the follow-up of Tunisian CML patients and its correlation with molecular response in the area of tyrosine kinase inhibitors.

Methods: Flow cytometry and standard qRT-PCR technique were performed to evaluate CD26 + LSC and BCR-ABL1 transcript levels in peripheral blood (PB) in CML patients, respectively.

Results: A total of 48 patients with CML were enrolled in this study. The CD26 + LSC was higher in patients with failure than those with an optimal molecular response.

CD26+LSCs were significantly correlated with the molecular response expressed as a BCR-ABL1 ratio($p < 0.05$).

Conclusions: To the best of my knowledge this is the first study that investigated CD26+ LSCs in Tunisian CML patients. CD26+LSCs identification can be a useful marker for monitoring patients with CML when a BCR-ABL1 quantitative assay is not available.

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Conflict of Interest: None declared.

EP08.014 The results of the pilot project on primary immunodeficiencies in Sverdlovsk region (Russian Federation)

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Background: Newborn screening (NBS) for primary immunodeficiency (PID) is based on the detection of T-cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KREC). They are sensitive biomarkers for T- and B-cell lymphopenia, but not specific and usually can let to secondary findings and false-positive results. That's why a choice for correct cut-off is a very important factor.

Methods: In 2021 we initiated a pilot study on newborn screening for PID in Sverdlovsk region. The analysis of TREC and KREC was performed by real-time polymerase chain reaction in neonatal dry blood spots by test system "Immunobit" (Generium). The DBS samples were collected between July and September 2021.

Results: In total, 5,044 newborns were screened. Three blood DNA samples were used for control: 1 – with X-linked agammaglobulinemia, 2 – with SCID (ADA-SCID and ILR2G deficiency). At first we used the cut off from instruction: 500 copies for TREC and 300 copies for KREC (both in 100000 leukocytes). Retest from the first DBS was provided in 6.1% of patients. New sample test was needed in 158 (3.1%) of newborns, referral to immunologist was used in 57 cases (two abnormal values successively). Then we recalculated cut-off (99.9 percentile) retrospective. In fine the group with risk for primary immunodeficiency reduced to 1.0% and all 57 newborns who was referred to immunologist stayed in it. Wherein a number of false-positive was reduced significant.

Conclusions: Using of own data to form a risk group for PID is more effective in preventing false-positive referrals.

Conflict of Interest: None declared.

EP08.015 Comparison of two methods for TREC, KREC molecules measuring for inborn errors of immunity NBS

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Background/Objectives: The level of T-cell receptor excision rings (TRECs) and kappa-deleting excision rings (KRECs) could be used for newborn screening patients with inborn errors of immunity. The comparison and unification of the data obtained with measurement by two different methods are essential.

Methods: The analysis of TREC and KREC was performed by real-time PCR followed by analysis of melting curves (HRM) and with using the "Biocore® SMA/TKID plus" diagnostic kit. HRM analysis was performed among 9 000 DBS. Measuring of TREC, KREC levels was performed among 18 000 DBS.

Results: The pilot project was launched to determine the levels of TREC, KREC molecules in DBS of children from the Ternopil region of Ukraine were carried out by the method of quantitative PCR, using plasmids with known concentrations of TREC, KREC, and albumin as standards. The reactions were carried out in three separate tubes followed with HRM to determine the specificity of low-copy products. TREC, KREC and RNAsP as internal control levels involve the detection of three products in one reaction with Biocore® SMA/TKID. We compare Ct and ΔCt (TREC, KREC, albumin) and (TREC, KREC, RNASaP) for both methods. According to the results, ΔCt (T-A) = 6,47, ΔCt (K-A) = 6,46 and for second method ΔCt (T-R) = 4,69, ΔCt (K-R) = 7,10.

Conclusion: Both methods were sensitive to detect T- and/or B-lymphopenia. The changing internal control from RNASaP to albumin gene is needed to find out the TREC, KREC molecules absolute number.

Grant References:

Conflict of Interest: None declared.

EP08.017 Down syndrome phenotype associated with TRNT1 gene

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Introduction: Biallelic mutations in TRNT1 gene cause SIFD syndrome-Sideroblastic anemia, B-cell Immunodeficiency, periodic Fevers and Developmental delay.

The aim: s to present two cases of SIFD syndrome, both of them with Down syndrome phenotype, association that wasn't reported.

Case presentation: Patient 1 (P1)-female, patient 2 (P2)-male come from non-consanguineous parents with insignificant disease family history. In P1 disease started at 3 months and in P2 at 3 weeks, with fever every 2-3 weeks, lasting 5-10 days accompanied by diarrhea and vomiting. Both patients have a Down phenotype with psychomotor retardation and microcephay in P1. There were excluded Down syndrome (karyotype, microarray), celiac disease, hypothyroidism, cystic fibrosis, Schwachmann-Diamond syndrome, inflammatory bowel disease. Laboratory tests showed sideroblastic anemia, CRP > 100 mg/L, elevated serum amyloid, low IgA, IgG and IgM, poor response to vaccination at P1, B lymphopenia and decreased switched memory B cells. We performed WES in P1, revealing a heterozygous double missense pathogenic mutation c.608+1G > T/c.1246A > G in the TRNT1 gene. In P2 the diagnosis was much faster, being suggested by the Downian phenotype associated with periodic fever and proven by gene panel sequencing-heterozygous double mutation c.428_431del /c.1246A > G. Corticosteroid therapy and immunoglobulin replacement were administered to both patients.

Conclusions: SIFD is a rare immunodeficiency, with 46 patients reported in the literature. It should be suspected in any case of recurrent fever associated with hypogammaglobulinemia and Downian phenotype. Early diagnosis will allow the early establishment of treatment and even the optimal time for bone marrow transplantation.

Conflict of Interest: None declared.

EP08.018 Identification of a splice variant in PSMB10 gene in a multiply affected family with undiagnosed autoinflammatory syndrome

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Background/Objectives: Monogenic autoinflammatory syndromes are heterogenous group of disorders that do not always follow simple genetic inheritance patterns. Here we present exome sequencing and interferon signature analysis in a family diagnosed with autoinflammatory syndrome who were clinically and genetically not fulfilling FMF, CAPS, TRAPS, HIDS, and MVK diagnoses.

Methods: Whole exome sequencing is performed in a family with 3 affected siblings, father and unaffected mother using Illumina TrueSeq capture kit. Broad GATK germline short variant calling pipeline was used. Variants were filtered based on frequency (MAF < 0.01), tissue expressions, variant effect predictions (CADD, SpliceAI) and family segregation patterns. PBMC's were isolated from all the family members and healthy controls fresh blood samples. Selected interferon signature genes (*IFI27, IFI44L, IFIT1, ISG15, RSAD2, SIGLEC1, IFIT3, OASL, IFI35*) were analyzed by RT-qPCR. Delta Ct fold changes relative to housekeeping gene (HPRT1) were calculated. Independent T-test was used to calculate the significance in expression levels.

Results: A rare splice variant (rs201451622, NM_002801:exon1:c.56+1G > A) in Proteasome 20S Subunit Beta 10 (*PSMB10*) gene (heterozygous) was found in affected father and 3 affected siblings. This variant is predicted to cause a loss at the donor-splice site at exon 1. Among the interferon signature genes *ISG15* was significantly upregulated (2.24-fold, $p = 0.0108$) in patients compared to healthy controls.

Conclusion: c.56+1G > A variant in *PSMB10* gene is a candidate variant in the present family and future work will explore more interferon gene expressions and in vitro cell culture models of splicing.

Grant: This work is supported by ACU, ABAPKO, 2022/07-16

Conflict of Interest: None declared.

EP08.019 Genetic evaluation of inborn errors of immunity

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Background/Objectives: Inborn errors of immunity (IEI) constitute a heterogeneous group of over 450 different disorders, polygenic pathologies with a wide variability of clinical manifestations.

We aim to establish the optimal test to perform for the diagnosis of IEI and to compare diagnostic rate using WES (whole exome sequencing) or WGS (whole genome sequencing) versus gene panels analysis (including different number of genes from 300 to 4813 genes).

Methods: The medical data collected from the patients who fulfilled the IEI criteria were: personal and family history, clinical picture and paraclinical investigations.

In total, 131 unique index patients were studied with genetic tests. We performed 56 gene panels, 22 WES and 53 WGS tests to determine a conclusive diagnosis.

Results: A total number of 176 tests was performed. The majority of patients (104 patients) have done 1 test, 24 patients had 2 tests, 3 patients had 3 tests and 1 patient had 4 tests.

27 patients had a positive primary immunodeficiency NGS Panel, with a 48% rate of positive result identified; 9 patients had a WES positive result (41%), and 41 patients had a positive WGS result (77%).

Conclusion: A larger number of patients is needed to establish which molecular genetic test has optimal diagnostic yield in the identification of disease-causing variants of IEI.

Conflict of Interest: Cristina-Loredana Pantea Emergency Hospital for Children "Louis Turcanu", Maria Puiu Emergency Hospital for Children "Louis Turcanu".

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EP08.020 When a routine blood test can change it all-Hereditary hyperferritinemia cataract syndrome

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Introduction: Hereditary hyperferritinemia cataract syndrome (HHCS) is an autosomal dominant disorder consisting of high levels serum ferritin and juvenile bilateral cataracts. It is often caused by mutations in the ferritin L-subunit (FTL) gene. We report a 17-year-old adolescent who presented with persistently hyperferritinemia.

Methods: The clinical workup was completed for the affected individual. Sequence analysis and deletion/duplication testing of the 27 genes listed in the NGS iron related disorders panel was performed, using Illumina technology; reads were aligned to the reference sequence GRCh37. Moreover, Sanger sequencing was performed to validate the mutation.

Results and discussions: The patient aged was diagnosed with hyperferritinemia (average 1300 ng/ml) in May 2019. For 2 years, she undergone multiple clinical and laboratory investigations to exclude the presence of iron overload and secondary causes of hyperferritinemia and even overloading with vitamin and iron supplements was taken into consideration by the medical team. A detailed personal history revealed the presence of early-onset cataract by the age of 7, when a specific literature search has oriented us towards HHCS. Two pathogenic heterozygous variants were identified in the patient: (c.-161C > T,rs139325959) of the FTL gene and (c.187C>G,rs1799945) of the HFE gene. HHCS was therefore confirmed, after ruling out the other 2 possible diagnoses, and our young patient can receive a proper care by only visiting the ophthalmologist, instead of a lifetime of searching reasons for hyperferritinemia.

Conclusion: HHCS should be considered in the differential diagnosis of hyperferritinemia, especially in the presence of cataracts, normal serum iron concentration and transferrin saturation.

Conflict of Interest: None declared.

EP09 Intellectual Disability

EP09.001 Developmental and epileptic encephalopathy 87, caused by CDK19 mutation, could include two distinct phenotypes

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Background: CDK19 is a paralog of CDK8, which constitutes the CDK/cyclin module of the mediator complex. Recently, CDK19 was identified as a causative gene for developmental and epileptic encephalopathy 87 (MIM618916; DEE87). The main symptoms are generalized developmental delay, hypotonia, and recurrent epilepsies, but the number of reported DEE87 patients is small and most of the details are yet unknown.

Methods: We compared 16 patients with DEE87 reported in previous papers, including one outpatient of our hospital, in terms of the details of symptoms and mutations.

Results: All of the 16 patients' mutations were located within the kinase domain of CDK19. Of these, 11 patients had (1) mutations in the ATP-binding pocket and 5 patients had (2) mutations in the activation segment. Patients of (1) had no short stature greater than -2.5 SD, whereas two of the five patients of (2) had severe growth retardation (-9.7 and -4.6 SD). Patients of (1) commonly showed hypertelorism, blephaophimosis, wide nasal bridge, wide nasal base, thick nasal wings, and wide mouth. Arched eyebrows, prominent nasal bridge, narrow nasal wings, and upturned nostrils were common in patients of (2).

Conclusion: Patients with DEE87 could be divided into two major clinical groups: those with mutations in the ATP-binding pocket and those with mutations in the activation segment. Regarding the pathogenesis of DEE87, it has been shown that both loss-of-function and gain-of-function patterns are possible. Further elucidation of the symptoms and pathomechanisms of the disease is needed by collecting informations of patients with DEE87.

Conflict of Interest: None declared.

EP09.002 Cost of exome analysis in patients with intellectual disability: a micro-costing study in a French setting

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Aim: To determine an adequate tariff for exome sequencing (ES) in individuals with intellectual disability (ID), we assessed the unit cost per ES diagnostic test from the preparation of

the pre-analytical step until the report writing step and identified its main cost drivers.

Methods: A micro-costing approach was conducted for the year 2018 in a French setting as part of the DISSEQ study, a cost-effectiveness study funded by the Ministry of Health and performed in collaboration with the genetic GAD team from the Dijon University Hospital, and a public sequencing platform (CNRGH). All the resources (labor, equipment, disposables and reagents, reusable material) required to analyze samples were collected and valued. Several sensitivity analyses were performed.

Results: The unit cost per ES diagnostic test was estimated to be €2,019.39 Labor represented 51% of the total cost. The analytical step represented 88% of the cost. Sensitivity analyses suggested that a simultaneous price decrease of 20% for the capture kit and 50% for the sequencing support kit led to a cost estimation of €1,769 per ES diagnostic test.

Conclusion: This is the first estimation of ES cost to be done in the French setting of ID diagnosis. The estimation is especially influenced by the price of equipment kits, but more generally by the organization of the centers involved in the ES analysis. This information can now be used to define an adequate tariff and assess the efficiency of ES.

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Conflict of Interest: None declared.

EP09.003 Male patient with a novel likely pathogenic variant in HDAC4 causing neurodevelopmental disorder with central hypotonia and dysmorphic facies

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Background/Objectives: Neurodevelopmental disorder with central hypotonia and dysmorphic facies (NEDCHF; #619797) is a rare autosomal dominant disorder, caused by heterozygous pathogenic variants in *HDAC4*. So far there are 7 patients described. Here we report a 6 year old patient with NEDCHF and a stop gain variant in *HDAC4*.

Methods: We acquired a patient's family history and clinical data. Chromosome banding analysis, array-CGH and exome sequencing were performed using blood samples. The disease's severity of our patient was assessed and we did literature research for similar patients.

Results: The patient presented with mental retardation, behavioral abnormalities and typical facial features of NEDCHF, such as such as a mild ptosis, deeply set eyes, low-set ears, thin upper lip and pointed chin. Furthermore, he displayed with autism spectrum disorder and eyebrows fanned out medially, features so far not associated with NEDCHF. Chromosome banding analysis and array-CGH showed unremarkable findings. Whole-exome sequencing revealed a heterozygous likely pathogenic variant in *HDAC4* (NM_006037.4:c.3208G>T; p.Glu1070*) predicted to lead to a translational stop at position 1070 of in total 1080 amino acids. The variant was not present in the gnomAD database. A segregation analysis is pending.

Conclusion: Here we report an additional patient with NEDCHF and a novel stop gain variant in *HDAC4*. The variant has so far not been described. Our case report emphasizes the genetic heterogeneity and the variability of the phenotype of variants.

Conflict of Interest: None declared.

EP09.004 The elucidation of the intrafamilial phenotypic heterogeneity of neurodevelopmental disorders by trio-based exome sequencing

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The intrafamilial phenotypic heterogeneity of neurodevelopmental disorders represents a challenging issue in the molecular diagnostics of paediatric NDDs and subsequent genetic counselling. Trio-based ES serves as the unique solution to identify multiple genetic hits and explain their contribution to the clinical manifestation of NDDs.

Our study included 89 paediatric patients with NDDs and MCA and their parents. The strategy of trio-based ES utilized the commercial kit Human Core Exome (Twist Biosciences), Illumina NovaSeq 6000 and in-house bioinformatic pipeline.

Trio-based ES has elucidated 49% of paediatric NDDs (44/89), including recurrent and novel causative gene variants affecting the central nervous system development and functioning.

The detailed variant prioritization and analysis uncovered the familial occurrence of causative variants in "OMIM-morbid" genes in fourteen paediatric patients from twelve families. Familial causative variants in "OMIM-morbid" genes or CNVs were detected in seven patients from six families whereas the variable combinations of causative variants in "OMIM-morbid" genes and rare CNVs encompassing "OMIM-morbid" genes were identified in seven patients from six families. The segregation analyses were performed in other familial members, including those with the clinical manifestation of NDDs.

The intrafamilial phenotypic heterogeneity of NDDs may be explained by "two-" or "multiple-hit" model for NDDs or by dual diagnosis.

Our results shed light on the genetic basis of this phenomenon and demonstrate how trio-based ES can facilitate the molecular diagnostics of paediatric NDDs.

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Conflict of Interest: None declared.

EP09.005 Ultra-rare causes of growth and speech development delay – new cases of Alazami syndrome and Rauh-Steindl syndrome

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Background: Alazami syndrome and Rauh-Steindhal syndrome are two ultra-rare monogenic disorders sharing clinical features.

Both syndromes are characterized by intellectual impairment, severe growth restriction, and similar, but subtle, facial phenotype. Alazami syndrome (ALAZS, OMIM #615071) is an autosomal recessive disorder caused by biallelic disruption of *LARP7* gene and Rauh-Steindhal syndrome (RAUST, OMIM# 619695) is an autosomal dominant disorder resulting from heterozygous pathogenic variant in *NSD2* gene.

Material and methods: Here we report two unrelated females of Polish origin (individual 1 and 2), identified by the search for monogenic causes of speech development delay. Both girls presented severe growth restriction, facial dysmorphism and speech development delay. Initial genetic assessment included negative testing for Silver-Russell syndrome, negative *SRCAP* sequencing for Floating-Harbor syndrome, negative testing for uniparental disomy of 7 and 14 chromosomes, and normal aCGH.

Results: Whole exome sequencing detected compound heterozygosity for in trans *LARP7* frameshift variants: Asn252ArgfsTer7 (maternal origin) and Glu286ArgfsTer4 (paternal origin) in individual 1, and heterozygosity for missense *NSD2* variant Phe344Val in individual 2.

Conclusions: To our best knowledge *LARP7* and *NSD2* genes variants identified in our study are novel and were not reported to date, hence this work expands the molecular spectrum of both disorders. Given the shortage of data regarding both conditions, clinical findings described here expand the knowledge of both diseases variability.

Conflict of Interest: None declared.

EP09.006 NSD1 Mutations in Sotos Syndrome Induce Differential Expression of Long Noncoding RNAs, miR646 and Genes Controlling the G2/M Checkpoint

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Background/objectives: NSD1 gene (nuclear-receptor-binding-set-domain-protein) encodes a methyltransferase [1] implicated in the transcription and methylation of histone H3 at lysine 36 (H3-K36), but the molecular mechanisms involved in these processes remain largely unknown. An increasing amount of evidence indicates the critical role of the NSD1 gene in Sotos syndrome (SoS), a rare genetic disease, and in tumors [2].

Methods: In order to assess the impact of NSD1 haploinsufficiency in the pathogenesis of SoS, we analyzed the gene expression profile of fibroblasts isolated from the skin samples of 15 SoS patients and of 5 healthy parents.

Results: We identified seven differentially expressed genes and five differentially expressed noncoding RNAs. The most upregulated mRNA was stratifin (SFN) (fold change, 3.9, $p < 0.05$), and the most downregulated RNA was goosecoid homeobox (GSC) (fold change, 3.9, $p < 0.05$). The most upregulated lncRNA was lnc-C2orf84-1 (fold change, 4.28, $p < 0.001$), and the most downregulated lncRNA was lnc-C15orf57 (fold change, -0.7, $p < 0.05$). A gene set enrichment analysis reported the enrichment of genes involved in the KRAS and E2F signaling pathways, splicing regulation and cell cycle G2/M checkpoints.

Conclusion: Our results suggest that NSD1 is involved in cell cycle regulation and that its mutation can induce the down-expression of genes involved in tumoral and neoplastic differentiation.

References:
1. Douglas J et al., 2003 Am J Hum Genet
2. Mohanty S et al 2020 Cancers

Grants: AssiGulliver Association, Fondazione Sardegna and Banca d'Italia

Conflict of Interest: None declared.

EP09.007 Gonadal mosaicism in FOXP1 related neurodevelopmental syndrome

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Background: Mutations in FOXP1 gene are responsible for a well characterised neurodevelopmental syndrome, the Intellectual developmental disorder with language impairment with or without autistic features (OMIM 613670). FOXP1 gene encodes the fokhead box protein 1, which is a transcription factor important for the early embryonal development. The main features of the condition are intellectual disability, language deficits, autism spectrum disorder, hypotonia, and congenital anomalies, including mild dysmorphic features, and brain, cardiac, urogenital abnormalities.

Methods, results: Here, we present two siblings with de novo heterozygous FOXP1 variant. The childrens were born from a healthy non-consanguineous couple. Both the four years old boy and the 10 months old girl have delayed psychomotor development, hypotonia, inappropriate laughter and very similar facial features. The four years old boy has no expressive speech. We performed whole exome sequencing for the boy, which identified a pathogenic heterozygous c.1541G>A FOXP1 mutation. His sister's targeted mutation analysis also showed the same heterozygous FOXP1 variant. The segregation analysis revealed de novo origin of the mutation, thus it suggests gonadal mosaicism.

Conclusion: De novo variants are common causes of rare intellectual disability syndromes, associated with low recurrence risk. However, when such variants occur in parental germ cells, the recurrence risk is much higher. The prevalence of gonadal mosaicism is usually unknown. To the best of our knowledge this is the first report in medical literature of gonadal mosaicism in case of FOXP1 related neurodevelopmental syndrome.

Conflict of Interest: None declared.

EP09.009 Further phenotypical delineation of DLG3 related Intellectual developmental disorder: description of 9 new cases

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Objectives: *DLG3* encodes a member of the membrane-associated guanylate kinases protein family, SAP102, expressed in brain and involved in synaptic function. Loss-of-function (LoF) variants in *DLG3* are a known cause of non-syndromic X-linked intellectual developmental disorder (ID) and to date, only 13 families have been reported. Limited clinical descriptions do not permit to precise the developmental trajectories of affected individuals. Also, the pathogenicity of missense variants has to be discussed. In this context, we proposed to constitute an international clinical series of novel patients in order to better define the clinical and molecular spectrum of this condition.

Methods: Recruitment of probands was made by using GeneMatcher.

Results: We report 9 new male patients from 2- to 23-year-old presenting ID and developmental delay in association with other clinical features including ocular (strabismus 2/9 and visual impairments 2/9), psychiatric and behavioral disorders (auto- and hetero-aggressiveness, intolerance to changes and anxiety 5/9), but also some other previously unreported features, including cleft palate (1/9), sacrococcygeal cyst (2/9) and pain hypoesthesia (2/9). Variants were generally inherited from the mother (8/9). Four variants were loss-of-function, one is a deletion encompassing the last exon and the stop codon of the gene and one is a delins leading to a predicted splice site effect. Two variants were missense and classified as VUS.

Conclusion: These descriptions broaden the phenotypic spectrum of the X-linked intellectual developmental disorder induced by *DLG3* variants. Additional patients with missense variants are required to help in their reclassification.

Conflict of Interest: None declared.

EP09.010 Neurocognitive and neurobehavioral characterization of two frequent forms of neurodevelopmental disorders: the DYRK1A and the Wiedemann-Steiner syndromes

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DYRK1A and Wiedemann–Steiner syndromes (WSS) are two genetic conditions associated with neurodevelopmental disorders (NDDs). Although their clinical phenotype has been well described, their behavioral phenotype has been little studied and not systematically using standardized assessment tools. To characterize the latter, we conducted a retrospective study, collecting data on developmental history, autism spectrum disorder (ASD) (using the Autism Diagnostic Interview-Revised and the Social Communication Questionnaire), adaptive functioning (using the Vineland Adaptive Behavior Scales II), behavioral assessments (using the Aberrant Behavior Checklist, the Screen for Child Anxiety Related Emotional Disorders and the Conners' Parent Rating Scale) and sensory processing (using the Sensory Profile) of individuals with these syndromes ($n = 14;21$). In addition, we analyzed information collected from families ($n = 20;20$) using the GenIDA database, an international patient-driven data collection aiming to better characterize natural history of genetic forms of NDDs. In the retrospective study, individuals with DYRK1A syndrome showed lower adaptive behavior scores compared to those with WSS, whose scores showed greater heterogeneity. An ASD diagnosis was established for 57% (8/14) of individuals with DYRK1A syndrome and 24% (5/21) of those with WSS. Language and communication were severely impaired in individuals with DYRK1A syndrome, which was also evident from GenIDA data, whereas in WSS patients, exploration of behavioral phenotypes revealed the importance of anxiety symptomatology and attention deficit hyperactivity disorder signs, also flagged in GenIDA. This study, describing the neurobehavioral profiles of individuals with WSS and DYRK1A syndrome, highlighted some specificities important to be considered for patients' management.

Conflict of Interest: None declared.

EP09.011 Identification of a rare homozygous variant in the gene GPT2, causing a neurodevelopmental disorder in a consanguineous Pakistani family

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BACKGROUND: *GPT2* is also known as ALT2 enzyme that catalyzes the reversible addition of an amino group from glutamate to pyruvate, producing alanine and α-ketoglutarate, pathogenic variants in *GPT2* result in clinically heterogeneous neurodevelopmental disorder (OMIM#616281). The clinical phenotype is variable and extremely heterogeneous and includes the symptoms such as intellectual disability, speech impairment, postnatal microcephaly, spastic paraplegia, delayed developmental milestones.

METHODS: A consanguineous Pakistani family consisting of two affected and two normal individuals along with their phenotypically healthy parents were analysed using whole exome sequencing (WES). The data obtained were filtered against control data bases to remove the polymorphisms. The prioritized variant testing was performed by Sanger sequencing.

RESULTS: The patients were presented with delayed developmental milestones, intellectual disability, microcephaly, speech impairment, aggressive behaviour and behavioural abnormalities. WES data analysis revealed a novel homozygous variant, c.601G>A (p.G201R) in *GPT2* (NM_133443.4) gene. The identified variant is highly conserved and was predicted to be "deleterious" by various

bioinformatic prediction tools. The predicted pathogenicity score using CADD was 22.7. The variant was also not found in homozygous form in public variant databases such as gnomAD, 1000G and dbSNP. Sanger sequencing confirmed the segregation of the *GPT2*: c.601G>A (p.G201R) with the phenotype.

CONCLUSIONS: This study does not not only expand the genetic variability associated with this phenotype but also demonstrate the usefulness of WES in clinical heterogeneous disorders.

Conflict of Interest: None declared.

EP09.012 Case report of a hemizygous intragenic deletion of WDR13 - Further evidence for the role of WDR13 in X-linked intellectual disability

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Introduction: X-linked intellectual disability (XLID) accounts for over 10% of all cases of ID in males. Next generation sequencing techniques have enabled the detection of a multitude of novel causative and candidate genes for ID over the last years. To date, more than 150 causative genes for XLID and several candidate genes have been reported.

Case Report: We report on an 8-year-old boy with global developmental delay, significantly delayed speech and language development, intellectual disability (IQ 50), mild postnatal macrocephaly, obesity, behavioural abnormalities and bilateral leg spasticity with pointed foot position. Furthermore, he showed minor facial dysmorphism and brachyphalangy.

Methods and Results: Conventional cytogenetic analysis, microarray CGH analysis and testing for fragile X syndrome gave normal results. Trio exome sequencing revealed a hemizygous deletion of exons 6-10 of the *WDR13* gene (NM_001347217.2: c.523+68_*2393del;p.(Val175Glyfs*21)) in our patient, which was confirmed by quantitative PCR. The patient's healthy mother is a heterozygous carrier of the variant.

Conclusion: *WDR13* (OMIM *300512) is a poorly characterized gene previously proposed as a candidate gene for XLID. Here we present an intragenic deletion of *WDR13* in a boy with intellectual disability and his healthy mother. To our knowledge, there are only four descriptions of *WDR13* variants as a potential cause of learning disability and ID in the literature so far (PMIDs: 20655035, 20662849, 29302074, 34946860). Our case is a further indication that *WDR13* is a causative gene for XLID. A GeneMatcher search to identify additional patients is ongoing.

Conflict of Interest: None declared.

EP09.013 Identification of a novel de novo missense variant in FBXO11 and a known variant in TECTA

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Background: We report on a 7 year old female child with speech delay, intellectual disability, congenital hearing impairment, recurrent infections of the lung with febrile convulsions. Her father as well as her paternal grandmother suffer from progressive hearing impairment.

Methods: Array-CGH (180k Agilent, design 086332) and trio whole exome sequencing (TWES, Twist Human Core Exome with additional customized probes and NGS with NovaSeq Illumina)

Results: Array-CGH of the child gave normal results. By trio analysis the heterozygous novel de novo missense variant c.1715C>T p.(Ala572Val) in *FBXO11* (NM_001190274.2) was identified in the child.

Missense variants in *FBXO11* cause syndromic neurodevelopmental delay with variable degree of intellectual disability. Rarely patients have seizures. Additional common features include short stature and distal mild skeletal malformations. Most patients have behavioral abnormalities and dysmorphic features (Gregor et al., 2018). In comparison to this reported phenotypical spectrum, our patient has a rather mild manifestation without skeletal or behavioral abnormalities. Noteworthy, to our knowledge, susceptibility to recurrent infections was not reported so far.

In the father, the known pathogenic heterozygous variant c.5597C>T p.(Thr1866Met) in *TECTA* (NM_005422.4) was identified, which was present in the daughter as well. This variant is known to cause autosomal dominant hearing impairment with pre- or postlingual manifestation. Therewith, this variant in *TECTA* explains the hearing impairment in father and daughter.

Conclusion: This case report highlights the heterogeneity *FBXO11* related neurodevelopmental delay. Furthermore, this example emphasizes the need of considering multiple genetic diseases as causal for different symptoms in one family.

Conflict of Interest: None declared.

EP09.014 Clinical exome sequencing for diagnosis of patients with intellectual disability, autism and psychomotor delay

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Background/Objectives: Intellectual disability, autism and psychomotor delay are common features seen in several rare inherited syndromes and diseases, which often exhibit a wide range of severity and accompanying symptoms. The aim was to use clinical exome sequencing to identify the underlying genetic cause of these disorders.

Methods: We utilized the Clinical Exome Solutions (CES v3) panel by Sophia Genetics to perform massive parallel sequencing on NextSeq550. CES v3 covers the coding regions (± 5bp of intronic regions) of 4,728 genes, the entire mitochondrial genome and non-coding variants known to be associated with rare and inherited disorders. The SOPHiA DDM™ platform was used to analyze the data and identify variants, including SNVs, Indels, and CNVs.

Results: A 14-year old girl had a personal medical history of atypical autism and suspected Rett syndrome. Using CNV analysis, we have reported a de novo heterozygous deletion in *PHIP* gene affecting exons 10-15. The patient was then diagnosed with the autosomal dominant syndrome Chung-Jansen. Similarly, in a 8-year old girl with psychomotor delay, we identified a de novo frameshift heterozygous variant c.552_553delTA (p.Asp184GlufsTer14) in *WAC* gene, leading to a diagnosis of the autosomal dominant syndrome Desanto-Shiwani. Both syndromes are characterized by varying degrees of developmental delay, impaired intellect, learning disabilities and behavioral abnormalities, such as autistic features, ADHD, anxiety, aggression, impulsive behavior and mood swings.

Conclusion: The use of clinical exome sequencing proved to be beneficial in determining the final diagnosis for patients with

intellectual disability of different clinical manifestations caused by rare inherited disorders.

Conflict of Interest: Helena Paszeková research associate, Kristýna Hanuláková research associate, Tomáš Píš research associate, Věra Hořínová clinical geneticist, Zděnka Vlčková clinical geneticist, Renáta Michalovská head of laboratory.

EP09.016 Patient with characteristic features of Lamb- Shaffer syndrome. Case study

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Background: Lamb-Shaffer syndrome is a neurodevelopmental disorder characterized by global developmental delay, intellectual disability, poor expressive speech, and mild dysmorphic facial features.

Our aim is to discuss the case of a patient with a new heterozygous mutation in *SOX5* gene.

Methods: Molecular analysis showed a new, de novo heterozygous mutation p.Arg426Ter in *SOX5* gene. The genetic analysis was performed by whole-exome sequencing. The library was prepared using Agilent Sure select VI exome Kit and analyzed with a NovaSeq sequencing platform. The presence of the detected variant was confirmed by Sanger sequencing.

Results: Our patient was born on time of healthy, nonconsanguineous parents. Her birth weight was -3250g, length- 55 cm, OFC-34 cm. Array CGH testing showed normal balanced female karyotype. The whole-exome sequencing showed mutation in *SOX5* gene. Among the main health problems of our patient at the age of five years were: speech delay, delayed motor development (she started sitting without support at the age of 9 months, walking at the age of 22 months), dysmorphic facial features, strabismus, wide toes and hypoplasia of cerebellar vermis.

Conclusion: The case of our patient contributes to the studies of the phenotypes of patients with Lamb-Shaffer syndrome which occur as a result of *SOX5* mutation.

Conflict of Interest: Katarzyna Wojciechowska full, Whitley Zie Student, Joanna Rusecka full, Monika Lejman Full.

EP09.017 Co-occurrence of Vissers-Bodmer syndrome and a likely pathogenic CHD8 variant: a case report

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Background/Objectives: In 2014, heterozygous CHD8 variants were found to cause intellectual disability (ID)/developmental delay (DD) with autism and macrocephaly. In 2020, the eponym "Vissers-Bodmer syndrome" was adopted to characterize a neurodevelopmental disorder caused by disease-causing CNOT1 variants. Here, we report a patient with co-occurrence of pathogenic variants in CNOT1 and CHD8 genes.

Case Report: We report a 5-year-old boy with unremarkable family history. The prenatal and the neonatal periods were uneventful. He evolved with severe DD and autism. On physical examination, he has relative microcephaly, mild dysmorphic features, and asymmetry of the calves. Electroencephalogram, head magnetic resonance imaging and extensive metabolic studies were normal. While genetic workout including array-CGH and fragile-X testing was normal, whole-exome sequencing identified two heterozygous likely pathogenic variants: a de novo variant in CNOT1, c.6916C>T, p.(Arg2311Trp), and a variant in CHD8, c.5393G>A, p.(Trp1798*) inherited from his healthy father.

Conclusion: Herein, we report a case of co-occurrence of variants in CNOT1 and CHD8 genes. Both genes codify for proteins that are believed to modify the epigenetic machinery. The patient's father, who harbors the variant in CHD8, is healthy, suggesting that even truncating CHD8 events can be not sufficient to promote disease. In our subject, the contribution of the CHD8 variant on top of the Vissers-Bodmer syndrome phenotype, however, is difficult to ascertain. Multi-omics approaches also including the methylome analysis are required to confirm the occurrence of more than one causal variant in the same patient, complicating the (reverse) phenotyping and making genetic counselling very challenging.

Conflict of Interest: None declared.

EP09.018 Two novel mutations in ANKRD11 gene in KBG syndrome patients and incomplete penetrance of missense mutations

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Background: KBG syndrome is a rare genetic disorder caused by either pathogenic variants in *ANKRD11* gene or the 16q24.3 microdeletion involving this latter. It is characterized by a distinctive facial appearance, typically macrodontia of upper central incisors, intellectual disability, seizures, developmental delay, skeletal abnormalities and short stature. The severity of KBG syndrome vary greatly, even among individuals with the same genetic mutations. In this work, we report two Moroccan children with typical KBG syndrome, and discuss the incomplete penetrance of missense *ANKRD11* mutations by comparing the phenotypes of patients with inherited pathogenic variants and their transmitting parents.

Methods: Both patients have typical KBG syndrome phenotype. Clinical Exome Sequencing was performed on both patients. Segregation analysis was done via conventional Sanger sequencing in both patients' parents. KBG syndrome cases with inherited pathogenic mutations were collected from literature. Our cases were compared to those reported in the literature.

Results: The first patient harbors a monoallelic missense mutation that was inherited from her mother while the second one harbors a monoallelic de novo frameshift mutation. The analysis of collected clinical data shows that most of the transmitting parents tend to have mild features. Our first patient's parent does not have any relevant clinical sign, which may be explained by the location of the mutation on protein level.

Conclusion: By reporting new cases and analysing clinical data of patients with inherited *ANKRD11* pathogenic variants that are increasingly observed in KBG syndrome, we confirm the variable expression and incomplete penetrance of this syndrome.

Conflict of Interest: None declared.

EP09.019 Characterization of blood and urinary levels of markers of elastin metabolism in 7q11.23 imbalances (Williams and 7q11.23 microduplication syndromes)

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Williams syndrome (WS), due to 7q11.23 microdeletion, is characterized by arterial stenoses, congenital abnormalities and a typical neurocognitive profile. The vasculopathy is the main cause of mortality. 7q11.23 duplication syndrome (7qDS) is characterized by aortic dilatation and variable neurodevelopmental involvement. The 7q11.23 critical region encompasses 26-28 genes including *ELN*, whose haploinsufficiency causes the WS vasculopathy; the pathogenesis of aortic dilatation in 7qDS is not known. *ELN* encodes tropoelastin, which undergoes post-translational modifications of specific lysine residues into desmosine and isodesmosine (D&I), which can be considered as specific biomarkers of elastin metabolism. Our study aims to characterize the blood and urinary levels of D&I in WS (1 *ELN* copy), normal controls (2 *ELN* copies) and 7qDS (3 *ELN* copies).

We set up D&I analysis by Liquid Chromatography/tandem Mass Spectrometry, not previously available in France in a routine setting. Statistical analysis was performed by Mann-Witney non-parametric test.

We recruited 23 WS (0.9-32y), 7 7qDS (4-43y), and 39 unaffected individuals (gender/age matched). Urines D&I/creatinine levels varied depending on age and were significantly lower in WS patients aged <2y (7.1[3.9-8.6] vs controls: 29,4[8.4-97.6], $p < 0.05$) and >15y (1.7[0.9-2.4] vs controls: 2,4[1.2-8.3], $p < 0.05$). D&I levels were normal in WBS serum and in 7qDS serum/urine samples. No obvious correlation was noted with vascular involvement severity.

This study suggests that urinary D&I might be considered as potential biomarkers of elastin metabolism for WS patients (<2y and >15y), in the perspective of the development of future therapeutic trials. Further studies are needed to characterize other markers of elastin biosynthesis.

Conflict of Interest: None declared.

EP09.020 A novel pathogenic mutation in ARID1B gene in a child with syndromic diabetes mellitus - presentation of Coffin-Siris syndrome

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Background/Objectives: We present a case of a 6-year-old girl with insulin-dependent diabetes mellitus developed at 2 years of age; intellectual deficit/autism, with a severe delay in speech; muscle hypotonia and difficult eating. Parents are interested in the genetic etiology of the condition.

Methods: Clinical exome sequencing by NGS was performed covering the coding regions of about 5000 genes.

Results: A genetic variant c.2025C>G, p.(Tyr675*) was detected in *ARID1B* gene. This variant is described for the first time and generates a premature stop codon, resulting in a shortened and damaged protein – we defined it as possibly pathogenic. Mutations in *ARID1B* are connected to the development Coffin-Siris syndrome (CSS). Differentiation of cranial neural crest cells is disturbed, thus explaining the expressed intellectual deficit.

Discussion: CSS is a rare autosomal dominant genetic disorder with de novo arising mutations. It is characterized by retardation in mental and physical development, microcephaly, thick lips, flat nasal root, hypoplasia of the distal phalanges (most commonly the 5th finger) and other facial features. The disease is associated with neurological problems (EEG changes, epilepsy, low IQ) and endocrinological changes; visual and hearing problems.

Conclusion: Next-generation sequencing is a powerful tool in making an accurate diagnosis in children suffering from diabetes in combination with symptoms from other organs and systems and dysmorphism. Our case presents syndromic diabetes mellitus due to Coffin-Siris syndrome and supports subsequent clinical behavior in the child.

Grant References: The study was supported by Grant No7400/19.11.2021 of Council of Medical sciences of Medical University Sofia.

Conflict of Interest: None declared.

EP09.021 Noonan-like phenotype with pathogenic variant in FBXW11

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Background/Objectives: *FBXW11* is a member of the ubiquitin ligase complex. It's involved in the Wnt and Hh signaling. Its role in human disorders is poorly known. Only 7 patients were described with pathogenic variants in *FBXW11* which cause autosomal dominant development disorders. One patient had Noonan-like phenotype. Noonan syndrome is associated with the RAS-MAPK pathway. Ubiquitination is involved in Ras metabolism and *FBXW11* may play a role in its signalling.

Methods: Analysis of the Whole exome sequencing (WES). Sanger sequencing for variant confirmation and trio analysis.

Results: The patient is a Caucasian 5-years old boy. He was born to healthy parents. At birth: weight 3300 g, length 50 cm, Apgar score 8/8. His motor development was delayed. He spoke his first words at 2 years, simple sentences appeared at 4.5 years. Self-care skills are almost formed. His current height is 102.5 cm (-1.47SD), weight is 17 kg (-0.72SD), OD is 54.5 cm (+2.44SD). Phenotype features: low-set protruding ears, strabismus, crease of the lower eyelid, flat nose bridge, wide nose tip, long smooth philtrum, talipes valgus. High degree of hyperopia. MRI revealed Arnold's-Chiari malformation type I, EEG was normal, no variant was found by WES. Reanalysis of the WES data revealed a heterozygous missense variant NM_012300.2:c.793T>C(Trp265Arg) in *FBXW11*. De novo origin of the variant was confirmed by trio Sanger sequencing. The variant was classified as likely pathogenic according to ACMG recommendations.

Conclusions: This is the second case of the Noonan-like syndrome caused by a likely pathogenic de novo variant in *FBXW11*.

Conflict of Interest: None declared.

EP09.022 Case report of rare neurodevelopmental Menke–Hennekam Syndrome

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Background: Menke–Hennekam Syndrome (MHS) is rare genetic multiple congenital anomalies/dysmorphic disorder characterized

by variable intellectual disability, developmental delay, autistic behaviour, short stature, and microcephaly. MHS type 1 is caused by missense variants of *CREBBP* gene, located in exons 30 or 31 and it is allelic disorder for Rubinstein–Taybi syndrome.

Methods: The proband is 7-year-old female who was born at 39 weeks of gestation from normal pregnancy, birth weight 2495 g (<3‰), birth hight 48 cm. Her mother received growth hormone during adolescence. Global developmental delay was noticed in the first year of life. At 5 years of age, she was assessed by scale of Diagnostic inventory for screening children, 1984 (DISC). Test value was 38-61%. Later, she had learning disabilities and special school needs. Paediatric endocrinologist assessed her for short stature multiple times and growth hormone deficiency diagnosis was rejected. At 4 years of age sleep EEG showed bilateral frontotemporal epileptic activity and epilepsy diagnosis was made. Dysmorphic facial features were noticed: strabismus, short and upslanted palpebral fissures, telecantus, depressed nasal ridge, short nose, anteverted nares, short columella, and long philtrum.

Result: Whole exome sequencing (WES) showed likely pathogenic missense variant in *CREBBP* (NM_004380.3) gene c.5128T>C (p.Cys1710Arg) (PP3 strong, PM1 moderate, PM2 moderate, PP2 supporting) in the 30th exon. Only the mother was available for genetic testing and she was negative for the variant.

Conclusions: The proband's phenotype and genotype was compatible with diagnosis of rare neurodevelopmental Menke–Hennekam Syndrome. Appropriate genetic counselling was provided.

Conflict of Interest: None declared.

EP09.023 ITGB8 is a candidate disease gene for recessive trait form of neurological disease

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Background/Objectives: Integrins are a family of cell adhesion molecules that facilitate cell-cell and cell-extracellular matrix (ECM) contact. Integrins form heterodimers of an alpha and a beta subunit. There are 26 integrins in the human genome (18 alpha, 8 betas), nine of which have been associated with Mendelian conditions. Integrins have been primarily associated with skin diseases such as epidermolysis bullosa (MIM #226730) and none have been associated with neurological disease to date.

Methods: Whole exome sequencing was performed on proband and parents' genomic DNA, when possible, and sequenced on the Illumina NovaSeq6000 platform. The variants were filtered by in silico analysis considering the frequency in the public databases and the prediction of deleterious non-synonymous SNVs for human diseases. Global minor allele frequency (MAF) was calculated according to gnomAD. The variants were evaluated by VarSome and categorized in accordance with the ACMG recommendations.

Results: We identified two siblings with putatively pathogenic variants in *ITGB8* (NM_002214.3). The probands are compound heterozygous for the start-loss c.1A>C (p.Met1?) and the splicing variant c.1056+1G > A. Functional studies on cDNA of both patients confirmed the impairment of the transcription, with the loss of an allele and the skipping of exon 7. These probands present with neurological phenotypes including developmental delay, intellectual disability, microcephaly, and dysmorphic features, and an altered Interferon-signature.

Conclusions: Together, our data suggest that variants in *ITGB8* could be associated with a recessive neurodevelopmental rare disease trait.

Conflict of Interest: Scott Barish: None declared, Elisa Lorefice: None declared, Chiara Passarelli Biologist in Laboratory of Medical Genetics, Children Hospital Bambino Gesù, Rome, Monia Ginevrino: None declared, Diego Martinelli: None declared, Antonio Novelli: None declared.

EP09.024 Extension of the phenotypic manifestation of the Shukla-Vernon syndrome with renal and cardiac malformations?

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Background/Objectives: Pathogenic variants in the transcriptional corepressor *BCORL1* were recently associated with Shukla-Vernon syndrome (SHUVER), X-linked disorder manifested with intellectual disability, dysmorphic features and behavioural abnormalities. *BCORL1* is highly constrained for the loss-of-function (LoF) mutations (pLI=1, o/e = 0.05), and so far, only hemizygous missense (Z = 2.06, o/e = 0.79) mutations were reported in the affected patients. Here, we present a case with LoF mutation with additional clinical presentation, not reported previously in SHUVER patients.

Patient and Methods: Eleven-month male infant with psychomotor delay, hypotonia, dysmorphic features, polythelia, digital hand contractures, crossed fussed ectopia of the left hypoplastic kidney with hydronephrosis of the right kidney, atrial septal defect and patent ductus arteriosus, was admitted for genetic diagnosis. Conventional karyotyping, aCGH with CytoScan 750K Array, clinical exome sequencing with Illumina TruSight One kit and Sanger sequencing were performed.

Results and Discussion: After normal findings of 46,XY karyotype and aCGH result, clinical exome analysis revealed presence of novel pathogenic hemizygous frameshift mutation BCORL1:c.2706dupT (p.Thr903TyrfsTer14). Sanger sequencing showed that mutation was a de novo event (not present in proband's healthy mother; biological relatedness confirmed). Renal and cardiac malformations were additional symptoms to those previously described in SHUVER. Interestingly, pathogenic mutations in *BCOR*, another transcriptional corepressor and paralog of the *BCORL1*, are associated with similar cardiac malformations and laterality disorders, which implies possible overlap in disease manifestation in patients with LoF mutations in these two genes.

Conclusion: Recognizing and reporting genotype-phenotype correlation in patients with pathogenic *BCORL1* mutations will further improve the SHUVER characterization.

Conflict of Interest: None declared.

EP09.025 Co-occurrence of two rare autosomal recessive disorders due to double heterozygosity in RYR1 and BCS1L genes in a patient from non-consanguineous family

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Pathogenic variants in RYR1 gene are the most common cause of congenital muscle weakness, congenital myopathies and malignant hyperthermia. On the other hand, BCS1L pathogenic variants cause widely different clinical phenotypes, such as mild Björnstad syndrome, characterized with abnormal hair growth and sensorineural hearing loss, severe GRACILE syndrome, characterized by growth restriction, aminoaciduria, iron overload, lactic acidosis and early death, and a heterogenous condition of complex III deficiency, involving hepatic pathologies, generalized hypotonia, hyperreflexia, cerebral atrophy and global developmental delay.

Here we present a 17 years old girl of Macedonian origin, the first child born from non-consanguineous parents, presented at infancy with severe hypotonia/hemiparesis, congenital deafness and no hair. As growing, severe form of intellectual disability arose. Epileptic seizures started at five years of age, tonic-clonic seizures presented mainly during sleeping. The Whole Exome Sequencing analysis revealed pathogenic variants in two genes: c.548G>A; p.Arg183His inherited from the father and c.871C>T, p.Arg291*, inherited from the mother in BCS1L gene; and c.13013_13032del, p.Ala4338fs inherited from the father, and c.3128G>A, p.Arg1043His, inherited from her mother in RYR1 gene. Our patient presented symptoms associated with both BCS1L and RYR1 genes; while deafness, brittle hair, seizures and intellectual disability are most probably due to only BCS1L gene defects, the generalized muscular weakness might be caused by overlapping effect of both genes. To the best of our knowledge, this is a first presentation of a patient with co-occurrence of RYR1 and BCS1L related disorders.

Conflict of Interest: Emilija Shukarova Stefanovska Macedonian Academy of Sciences and Arts, Gjorgji Bozhinovski Macedonian Academy of Sciences and Arts, Gordana Kiteva-Trenchevska University Neurology Clinic, Medical Faculty, Skopje, Dijana Plaseska-Karanfilska Macedonian Academy of Sciences and Arts.

EP09.026 First description of likely pathogenic variants in ACTB causing Baraitser-Winter cerebrofrontofacial syndrome without intellectual disability in two generations

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Background: Baraitser–Winter cerebrofrontofacial syndrome (BWCFF) (BRWS; MIM #243310, 614583) is a rare developmental disorder characterized by distinctive facial features, intellectual disability, brain malformations, seizures, short stature, coloboma and sensorineural deafness. BWCFF is caused by de novo heterozygous missense variants, with a suggested gain-of-function effect, in *ACTB* (MIM 102630) or *ACTG1* (MIM 102560), both genes encoding cytoplasmic actin proteins. BWCFF patients commonly show global developmental delay and/or intellectual disability, of variable severity, related to the severity of brain malformations. There are no report of individuals presenting without intellectual disability associated with *ACTB* variants. In addition, almost all reported cases were sporadic with only three descriptions of family inheritance.

Methods: Gene panel sequencing including *ACTB* and *ACTG1* was performed in three children. Their symptomatic parents benefited from a targeted sequencing. We collected clinical data for all five individuals.

Results: We describe here for the first time the case of five patients, from two different families, presenting with typical craniofacial features and malformations, but no developmental delay nor intellectual disability. Both adult patients had a normal autonomous life. A Noonan syndrome was initially suspected in two individuals. Gene panel sequencing detected two distinct probably pathogenic, heterozygous, missense variants in *ACTB*. Variants were inherited from their symptomatic parents also presenting with a mild phenotype, conferring a diagnosis of BWCFF in these individuals.

Conclusion: Our observation broadens the phenotypic spectrum associated with *ACTB* and underlines the existence of milder forms without disabilities.

Conflict of Interest: None declared.

EP09.027 Two new cases with HMGB1 loss-of-function variants

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Background/Objectives: Heterozygous *HMGB1* loss-of-function variants are assocated with a newly neurodevelopmental disorder characterized mainly by developmental delay and microcephaly To date, only 6 patients have been reported. We report two new cases in order to better characterize the phenotype of patients with pathogenic variants of *HMGB1* gene.

Methods: We present clinical and molecular characterisation of two unrelated patients with de novo variants in *HMGB1*. Clinical data was obtained by retrospective file analysis, clinical exam and formal neuropsychological evaluation

Results: Case 1, a 14-year-old female, had speech delay, short stature, microcephaly and overweight. Neuropsychological assessment showed cognitive score in the lower limit. She had a history of feeding difficulties in neonatal period and aortic coarctation with bicuspid aortic valve.

Her brain MRI showed cerebral atrophy with simplified gyral pattern. Case 2, a 7-year-old boy, had motor and speech delay, and autism spectrum disorder. His growth was normal, except microcephaly. His brain MRI was normal. The two patients exhibited dysmorphic features with thin upper lip.

Conclusion: An extensive revision of the clinical features of these two patients revealed high concordance with the 6 cases previously reported, including developmental delay with speech delay, feeding difficulties, microcephaly, short stature and overweight. Cerebral atrophy with simplified gyral pattern present in one case expand the spectrum of this new condition.

Conflict of Interest: None declared.

EP09.028 Novel truncating variant reinforces the involvement of ZNF148 in autism spectrum disorder with normal neuroimaging

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Background: Heterozygous variants in *ZNF148* gene, a Krüppel-type transcription factor that has transcriptional regulatory functions, cause an intellectual disability syndrome characterized by global developmental delay, abnormalities of corpus callosum and dysmorphic facial features. Such variants have only been described in five patients worldwide, of which only one presented with autism spectrum disorder (ASD).

Case report: We report a patient with a novel, de novo, heterozygous truncating variant c.1160delC resulting in a premature termination signal in the protein sequence (p.P387Hfs*4) in the *ZNF148* gene.

The patient is a 9 year-old female that presents with mild intellectual disability, ASD, short stature, strabismus and mild facial dysmorphic traits, including long palpebral fissures, anteverted nares, thick lips and prominent chin. Brain magnetic resonance imaging showed normal brain development, without abnormalities of the corpus callosum. Abdominal and cardiac ultrasounds were normal.

Conclusions: During the last decade, Next Generation Sequencing techniques have greatly advanced the identification of new genes related with neurodevelopmental disorders. However, in many cases a thorough clinical description is lacking. Case reports help to better characterize the clinical manifestations.

Here, we describe a novel *ZNF148* heterozygous truncating variant in a patient with distinct phenotype of ASD and normal brain imaging which expands the genotype-phenotype spectrum of *ZNF148*, reinforcing its role as a potential target gene for ASD.

Grant references: not applicable.

Conflict of Interest: None declared.

EP09.029 Dual genetic diagnosis in a patient with complex ocular-neurobehavioral phenotype

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Objectives: The advent of genome-wide analyses based on next generation sequencing (NGS) enabled discovery of novel mutations, disease causing genes as well co-existence of two or more Mendelian disorders in one patient known also as dual/ multiple genetic diagnoses (DGD/MGD). MGD are identified in 2-5% patients tested with NGS methods but the data regarding its specifics is scarce. In this report we present a patient diagnosed with *SETBP1*- and *CNGB3*- related disorders.

Methods: A 28-year-old female was referred for a consultation due to moderate intellectual disability (ID), behavioural abnormalities and visual impairment. Upon examination unspecific dysmorphic features were observed. Firstly, array-CGH was performed, giving negative result. Then NGS analysis was ordered, revealing two heterozygous frameshift variants of known pathogenicity in *CNGB3* gene, correlated with achromatopsia. Family testing confirmed carrier status in parents and biallelic nature of detected variants in patient. However the result did not explain the cause of ID observed in patient. Further extensive analysis of NGS data revealed a novel heterozygous frameshift variant in *SETBP1* gene, which was of de novo origin.

Conclusion: MGD should be considered in patients with complex phenotypes, involving many organs or manifestations that are not clearly related. NGS based methods are of preference. Symptoms, which are not precisely explained by detected variants should be treated with caution, especially in the context of phenotype broadening and require more extensive NGS data analysis.

Conflict of Interest: None declared.

EP09.031 A Novel ZBTB20 Variant In A Patient With Primrose syndrome: A rare clinical entity

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Background/Objectives: Primrose syndrome (PS) is a rare syndrome characterized by developmental delay, intellectual disability, behavior abnormalities, macrocephaly, characteristic dysmorphic features (high anterior hairline, downslanting palpebral fissures, frontal bossing, prominent ears), calcification of the external ear cartilage, sparse body hair, and distal muscle wasting. It is an autosomal dominant disorder caused by pathogenic variants in *ZBTB20*. Herein, we present a 10-year-old female patient who was referred to our centre with the findings of intellectual disability, autism spectrum disorder, macrocephaly and dysmorphic facial fetaures.

Methods: Exome sequencing was performed after informed consent was obtained from the parents.

Results: Physical examination revealed macrocephaly (>+2 SD) and facial features including telechantus, long palpebral fissures, and deep set eyes. Mildly limited extension on interphalangeal joints, scoliosis, and pes planus were noted as well. She also had bilateral deafness, hypothyroidism, mitral valve prolapse, and mildly cerebral atrophy. Abdominal ultrasound was normal. Chromosomal microarray analysis was normal. Exome sequencing revealed a novel, de novo heterozygous variant c.1948A>C (p.Asn650His) in *ZBTB20* gene, which is classified as "VUS" according to the ACMG criteria.

Conclusion: Primrose syndrome is a rare genetic syndrome that should be kept in mind in patients presenting with macrocephaly, intellectual disability, and dysmorphic facial features.

Conflict of Interest: Merve Soğukpınar Full, beren karaosmanoglu Full, Gulen Eda Utine Full, Koray Boduroğlu Full, Pelin Simsek-Kiper Full.

EP09.032 Three novel de novo variants in TAOK1 associated with intellectual disability

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Background/Objectives: The relationship between pathogenic variants in the *TAOK1* gene and neurodevelopment disorders, namely development delay with or without intellectual impairment or behavioural abnormalities (MIM #619575), was reported for the first time in 2019 in 28 patients and in more 13 since then. The molecular spectrum of the identified *TAOK1* variants comprises mainly truncanting and nonsense variants, but also

missense variants and deletions. Here we describe three additional cases of de novo *TAOK1* variants and the associated phenotype.

Methods: During the etiological investigation of three unrelated probands with intellectual disability and macrocephaly, we performed whole exome sequencing (WES), followed by parent segregation studies. We identified three de novo *TAOK1* variants, one nonsense, one intronic, and a deletion involving at least the second exon of the gene. All three variants were absent from gnomAD (Genome Aggregation Database) and were classified as probably pathogenic according to the American College of Medical Genetics and Genomics variant classification guidelines.

Results: The patients described here had mild to moderate intellectual disability, with behavioural problems in two of them. They had non-specific dysmorphic features, macrocephaly and additional history of developmental delay, namely in gross motor and language areas. Two of them also had history of hypotonia. Brain MRI performed during childhood did not show relevant abnormalities.

Conclusion: The phenotype of these three additional patients not only supports the clinical features already associated with *TAOK1* (developmental delay/intellectual disability with or without behavioural problems, hypotonia and non-specific dysmorphic features), but also expands the molecular spectrum of this disorder.

Conflict of Interest: None declared.

EP09.033 A patient with intellectual disability and a de novo missense variant identical to that reported in six previous patients helps to define the phenotype of SCAMP5 syndrome

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Mammalian genomes contain five types of SCAMP genes which encode secretory carrier membrane proteins (SCAMPs). SCAMPs play an important role in vesicle cycling, fusion of vesicles and cell membranes, and regulation of exocytosis and endocytosis. Among them, SCAMP5 is brain-specific and highly abundant in synaptic vesicles. We describe a six-year-old boy with severe intellectual disability, seizures, and delayed speech and fine motor skills. His phenotype includes rough facial features, small head circumference, receding forehead, small chin, and reduced visual acuity. His growth is normal and he shows no autistic features. Trio exome sequencing identified a de novo missense SCAMP5 variant NM_001178112.2:c.538G>T, p.(Gly180Trp) which was confirmed using Sanger sequencing. The same de novo variant in the C-terminal domain of SCAMP5 was identified previously in six unrelated patients with severe developmental delay, seizures, problems with gait, variable dysmorphic features, and with or without autism (Hubert 2020, Jiao 2020). Another missense variant located in the E-peptide domain of SCAMP5 was revealed in homozygous state in a consanguineous family where two affected siblings had paediatric epilepsy and juvenile Parkinson's disease, but not intellectual disability or autism (Zhang 2020). The phenotype of our patient is relatively consistent with the six previous cases carrying the same heterozygous de novo SCAMP5 variant. Because this variant is apparently a very rare cause of neurodevelopmental disorders, each additional patient will be of value for the definition of the full phenotypic spectrum and prognosis of the affected individuals. Supported by AZV NU22-07-00165 and IPE 6980364.

Conflict of Interest: None declared.

EP09.034 Chung-Jansen Syndrome – a new overweight and developmental delay syndrome caused by haploinsufficiency of PHIP – a case report

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Background/Objectives: Monogenetic causes of obesity in children are rare. Chung-Jansen syndrome, a newly described phenotypic entity including overweight and developmental delay/intellectual disability, is caused by haploinsufficiency of *PHIP* (MIM#612870). At present, around 60 patients have been described in the literature – most of them with unique variants. Here, we report of a child, who since early childhood had been followed because of developmental delay and intellectual disability. Initially weight was normal, but from around age 4 years he presented hyperphagia and subsequently obesity. At age 6 years, he was referred to the department of clinical genetics. Subtle dysmorphic features included a flat facial profile, large ears with fleshy earlobes and bilateral clinodactyly of the 5th finger. Weight was 44,8 kg (+4,4SD), height 131 cm (+1,7SD), head circumference 55 cm (+2SD). Behavioural issues and attention deficit disorder was reported. Chromosomal microarray and screening for Prader-Willi syndrome were normal.

Methods/Results: Trio whole exome sequencing was performed and revealed a de novo pathogenic variant (C5) in PHIP: NM_017934.6: c.919_923delATAAA, p.(Ile307Profs*22).

Conclusion: This patient share some of the characteristic features previously reported in other patients with pathogenic *PHIP* alterations, and a distinguishable phenotype is emerging. Obesity is present in many, but not all patients, though there seems to be an age-dependent relationship. PHIP interacts with Proopiomelanocortin (POMC) and extending knowledge about the biological effects of *PHIP* may bring novel targets of treatment of obesity. When genetic screening for Prader-Willi syndrome is negative, Chung-Jansen syndrome should be considered in children with developmental delay and obesity.

Conflict of Interest: None declared.

EP09.035 Co-Occurrence of Fragile X Syndrome with a Second Genetic Condition

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Fragile X syndrome (FXS; MIM 300624) is a genetic disorder whose phenotype is, beyond intellectual disability, characterized by a spectrum of psychiatric impairments and pediatric comorbiditie-s.In more than 99% of cases, FXS is due to expansions of a CGG trinucleotide repeat in the 5′- UTR of the *FMR1* gene exceeding

200 units, leading to DNA methylation of the promoter and transcriptional silencing, although FMRP loss of function leading to the FXS phenotype can also result from other FMR1 sequence modifications. We present the results of Whole Exome Sequencing (WES) analysis performed in a 7- year-old girl with clinical symptoms consistent with FXS, associated with distinct dysmorphic features including thick arched eyebrows, broad nasal bridge with bulbous nose, thin upper lip, widened thumbs and first toes. After negative FMR1 CGG-repeat analysis, and CGH array, WES analysis revealed the presence of a *FMR1* de novo canonical splice-site mutation (NM_002024.5:c.1275+1G > A), predicted in silico as pathogenic. Further analysis of WES data, based on phenotypic features inconsistent with FXS, revealed a *KIRREL3* missense variant segregating in the maternal family with limb alterations. Autosomal dominant KIRREL3 mutations are associated with a neurodevelopmental condition and possible limb malformations. FMR1 transcript analysis, family segregation of the *KIRREL3* variant, and clinical evaluations of carrier relatives are in course. Our data provide further evidence of the possibility that the FXS phenotype variability may at least partly depend by combined effects of possible additional genetic factors. We also show a distinct pattern of dysmorphic features associated with KIRREL3 alterations.

Conflict of Interest: Roberta Polli: None declared, Giorgia Beffagna: None declared, Elisa Bettella: None declared, Marilena Cameran: None declared, Valentina Liani: None declared, Simona Agata: None declared, Elisa Di Giorgio: None declared, Giulia Parmeggiani: None declared, Alberta Leon: None declared, Alessandra Murgia SHIONOGI B.V.

EP09.036 Clinical and molecular characterization of FOXP1 variants in subjects with neurodevelopmental disorders

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Background: FOXP1 (forkhead-box protein P1) is a member of the FOX transcription factor family essential for embryonic development. FOXP1, highly expressed in the nervous system, regulates molecular pathways required for proper brain development and function. FOXP1 heterozygous mutations and deletions are associated with "Intellectual developmental disorder with language impairment with or without autistic features" (OMIM#613670), a neurodevelopmental condition characterized by intellectual disability, speech and language delay, motor delay, ASD, and mild dysmorphic features.

Methods: Two different customized targeted gene panels, one including 74 ID/ASD and a second including the FOXP1/FOXP2/CNTNAP2 genes, were used to screen respectively a cohort of 844 pediatric subjects with Neurodevelopmenatl Disorders (NDDs) and a selected group of 31 pediatric individuals with Specific Language Impairments (SLIs).

Results: 10 FOXP1 variants were detected: 4 novel de novo protein truncating and 1 novel frameshift, all predicted to cause haploinsufficiency, 1 novel paternal missense, 1 maternal and previously reported missense, and 3 ultra-rare missense. Segregation analysis is underway for all the missense variants. Even if more than 100 FOXP1 variants with likely clinical significance are reported in literature, for only few of them the actual impact on protein expression and function has been dissected. We are currently performing a functional characterization of the FOXP1 variants we have identified in order to evaluate their effects on protein expression and transcription activity.

Conclusion: Our work promises to provide new data to improve genotype-phenotype correlation and elucidate the pathogenic mechanisms underlying FOXP1-related conditions.

Conflict of Interest: Elisa Bettella: None declared, Denis Drongitis: None declared, Roberta Polli: None declared, Lucia Verrillo: None declared, Emanuela Leonardi: None declared, Marilena Cameran: None declared, Matteo Della Monica: None declared, Roberta Cernetti: None declared, Chiara Spitalieri: None declared, Franco Stanzial: None declared, Demetrio Baldo: None declared, Licia Turolla: None declared, Donatella Milani: None declared, Giuseppe Cossu: None declared, Maria Giuseppina Miano: None declared, Alessandra Murgia Shionogi B.V.

EP09.038 Revealing novel variants associated with chromatinopathies

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Background: Chromatinopathies are a class of neurodevelopmental disorders caused by mutations in chromatin regulators, comprising more than 50 disorders. Patients affected by chromatinopathies share clinical features, including developmental delay, intellectual disability, facial dysmorphism, and behavioral disturbances. Cornelia de Lange syndrome (CdLS), Rubinstein-Taybi syndrome (RSTS), KBG syndrome (KBGS), Coffin-Siris syndrome (CSS), and Wiedemann-Steiner syndrome (WDSTS) are prominent examples of chromatinopathies.

Methods: In this study, we evaluated 231 individuals with neurodevelopmental delay and additional clinical features. We performed whole-exome sequencing to identify the underlying molecular pathology.

Results: 40 of 231 affected individuals carry 38 different variants in chromatinopathy genes. We detected eight *ANKRD11* and four *SETD5* variants which belong to the most frequently mutated genes associated with neurodevelopmental delay. Furthermore, we found eight variants in genes that encode histone lysine methylases and demethylases (*KMT2A, KMT2B, KMT2C, KMT2D, KDM5C*) in nine patients with overlapping phenotypes. Seven more patients have been diagnosed with CSS carrying variants in *ARID2, BICRA, SMARCB1, SMARCD1*, and *SMARCA4*. *CREBBP* and *EP-300* variants have been identified in patients with RSTS syndrome. Five patients displayed variants in

genes encoding chromodomain-helicase-DNA-binding proteins, CHD3, CHD4 und CHD5. We identified two variants in *BRPF1* encoding a multivalent chromatin reader. In addition, two variants have been detected in other chromatinopathy genes such as *DNMT3A* and *SIN3A*.

Conclusion: In the current study, 17.3 % of our patients with neurodevelopmental delay harbor variants in chromatinopathy genes. We identified 33 novel variants in genes encoding chromatin remodelers and transcriptional regulators, and expand the genotypic spectrum of chromatinopathies.

Conflict of Interest: None declared.

EP09.039 KMT2C pathogenic variants result in a neurodevelopmental disorder with distinct clinical and DNA methylation features

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Introduction: *KMT2C* haploinsufficiency initially was shown to result in a syndromic neurodevelopmental disorder overlapping with Kleefstra syndrome. Moreover, KMT2C (histone-3 lysine-4 (H3K4) methyltransferase) has strong biological interactions with EHMT1 (H3K9 methyltransferase). Therefore, *KMT2C*-related NDD was named Kleefstra syndrome type 2. As the number of diagnosed cases has increased considerably, we sought to characterize this disorder and compare it to the molecularly related Kleefstra (*EHMT1*) and Kabuki 1 (*KMT2D*) syndromes.

Methods: We ascertained individuals with rare *KMT2C* variants through international collaboration, systematically collected and analysed clinical features, performed DNA methylation (DNAm) studies, and used PhenoScore to objectively study facial features.

Results: We ascertained 76 individuals with pathogenic, and 22 with variants of uncertain significance (VUS) in *KMT2C*. Using 16 cases with pathogenic *KMT2C* variants and 50 age- and sex-matched controls, we identified a *KMT2C*-specific DNAm signature. We found this signature to be distinct from those of Kleefstra and Kabuki 1 syndromes. Using the DNAm signature, we re-classified 4/22 *KMT2C* VUS as pathogenic, and, thereby, established a cohort of 80 individuals with pathogenic *KMT2C* variants. We found that *KMT2C*-related NDD is characterized by developmental delay/intellectual disability (~85%), behavioral/psychiatric problems (including autism spectrum disorder (77%) and ADHD (62%)), short stature (36%), hypotonia (33%), recurrent infections (30%), and various congenital anomalies. PhenoScore showed that *KMT2C*-related NDD has a typical facial gestalt ($p < 0.001$) which is different from Kleefstra ($p < 0.001$) and Kabuki 1 ($p = 0.04$) syndromes.

Conclusions: *KMT2C* pathogenic variants result in a unique neurodevelopmental disorder which is distinct from Kleefstra and Kabuki 1 syndromes.

Grant references: SFARI;KNAW(KNAWWF/1327/TMB202120)

Conflict of Interest: None declared.

EP09.040 Biallelic intragenic tandem duplication of CPLANE1 in Joubert syndrome: a case report

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Background/objectives: Joubert syndrome (JS) is a genetic disorder with both neurological and extraneurological manifestations. Of the 40 JS-causing genes currently reported, *CPLANE1* is one of the most frequently mutated, with biallelic pathogenic missense and truncating sequence variants explaining up to 14% of JS cases. We present a case of JS diagnosed after the identification of a novel biallelic intragenic duplication in *CPLANE1*.

Methods: The case is a 3-year-old female patient with developmental delay. We used a clinical exome sequencing (CES) and aCGH for the identification, confirmation and segregation of the duplication. A long PCR and a nanopore-based long-read sequencing (LRS) of the PCR product were used to confirm whether the duplication was in tandem and to identify the breakpoints. The presence of a common haplotype was explored with haplotyping analysis and homozygosity mapping of the CES.

Results: CES and aCGH identified a quadruplication of exons 20-46 of *CPLANE1* and confirmed the duplication was inherited from both unrelated parents. LRS allowed the fine mapping of the duplication with proximal and distal junctions located on intron 19 and intron 46 and a size of 75,559bp. Based on the genetic findings, a brain MRI was ordered, evidencing the molar tooth sign, which confirmed the diagnosis of JS in the patient. A region of homozygosity was identified in the CES data of the patient supporting the duplication being in a common haplotype.

Conclusions: This is the first report of an intragenic duplication in *CPLANE1* as the molecular mechanism of JS.

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Conflict of Interest: None declared.

EP09.041 Exome sequencing in patients with intellectual disability in a large Indian cohort

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Background/Aims: Global developmental delay (GDD)/intellectual disability (ID) is etiologically diverse and has an identifiable genetic etiology in approximately 50% of the patients. We aimed to evaluate the clinical spectrum of patients with ID and the diagnostic yield of exome sequencing (ES).

Methods: Data from patients with ID/GDD from 2016 to 2022 were analysed retrospectively. Patients with chromosomal disorders, triplet repeat disorders, and imprinting disorders were excluded. Clinical geneticists evaluated all the enrolled patients. They were categorized into isolated ID, ID plus (ID with/ without dysmorphism and/or other systemic abnormalities), and ID with neuroregression. Either clinical exome sequencing (CES), whole exome sequencing (WES), or whole genome sequencing (WGS) was performed.

Results: We assessed 411 families with 491 affected individuals (ages 3 months to 46 years) and classified them as ID plus (358/411,87%), ID with neuroregression (46/411,11.2%), and isolated ID (7/411,1.7%). Fifteen percent (64/411) were multiplex while 14% (57/411) had consanguinity. Out of 411, proband-ES, trio-ES, and WGS were performed in 403, 7, and 1 respectively. The overall yield for pathogenic/likely pathogenic variants was 47.7% (196/411), with CES yielding more (51%, 140/274) than WES (36.8%, 56/152). There were 111 novel and 6 copy number variants discovered. Autosomal recessive disorders were more common than others.

Conclusion: In the study cohort, ID plus phenotype had a higher diagnostic yield. It broadens the phenotypic and genotypic spectrum of rare neurodevelopmental disorders.

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EP09.042 GNB5-related neurodevelopmental disorder: A novel variant detected in a large consanguineous family

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Background: *GNB5*-related neurodevelopmental disorders occur due to homozygous or compound heterozygous pathogenic variants in the GNB5 gene. They are characterized by a wide range of intellectual disabilities (ID), developmental delays, language delays, seizures, and visual impairment, and the majority present with bradycardia due to sick sinus syndrome.

Method: We did a retrospective chart review of three affected children from two consanguineous families. A positive family history of four other affected children with a similar phenotype has not been confirmed molecularly. All affected children had overlapping features of global developmental delay, moderate to severe ID, absent speech, and visual impairment. Two behavioral problems and conductive hearing loss, and one with a seizure disorder. One with documented bradycardia and long sinus pause. All had an unremarkable brain MRI.

Results: Chromosomal microarray for all affected children were negative. Proband 1 had inconclusive exome sequencing and genome sequencing. Genome reanalysis later revealed a homozygous variant of unknown significance (VOUS) in GNB5. Molecular testing for probands 2 and 3 also detected the same variant in a homozygous state.

Conclusion: Animal studies have shown that GNB5 plays an essential role in neuronal signaling and autonomic function. So far, around 11 pathogenic, five likely pathogenic, and 17 VOUS have been reported in GNB5. To our knowledge, this variant has not been previously reported in any database; however, it segregated well with the disease and fits the phenotypic description of this disorder. Segregation analysis of the remaining affected members and functional studies to be followed.

Conflict of Interest: None declared.

EP09.043 Paralogous annotation of disease-causing variants and somatic mutations in CREBBP/EP300 can improve variant interpretation in Rubinstein-Taybi syndrome

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Background/Objectives: Rubinstein-Taybi syndrome (RTS) is a rare syndrome caused by loss-of-function mutations in two paralogous tumour-suppressor genes: *CREBBP* and *EP300*. The description of milder cases and the paucity of missense variants reported, make the interpretation of novel changes challenging. We hypothesize that paralogous annotation of disease-causing variants and of tumour somatic mutations can improve variant interpretation in RTS.

Methods: We constructed multiple protein sequence alignments for *CREBBP/EP300* using Clustal-W. We mapped each paralogue residue with a known missense or indel pathogenic variant ($n = 87$, from the literature our own data) or a somatic mutation (COSMIC) onto the equivalent residue of the paralogous gene. Chi-square test was used to calculate if pathogenic variants occurred more often in residues with paralogous pathogenic variants or somatic mutations.

Results: Seven of 29 residues with pathogenic variants in *EP300* (24%) affected residues with a mutation in *CREBBP* ($\chi2 = 0.03$, $OR = 7$). Regarding correlation between germinal and somatic mutations, 8 (27%) of the 29 RTS pathogenic variants in *EP300*, affected a recurrently (>4) somatic mutated residue in COSMIC ($\chi2 = 0.02$, $OR = 8$). For *CREBBP*, 16 (27%) of 58 RTS pathogenic variants in *EP300*, affected a recurrently somatic mutated (>4) residue in COSMIC ($\chi2 = 0.02$, $OR = 8$). Using these criteria, we could reclassify 4/185 and 12/266 variants of unknown significance from ClinVar in *EP300* and *CREBBP* respectively.

Conclusions: Our results show that annotation of somatic and paralogous disease-causing variants in *CREBBP* and *EP300* can facilitate variant interpretation. This method is applicable to other paralogous and tumour-suppressor genes.

Conflict of Interest: None declared.

EP09.044 IQSEC2-associated epileptic encephalopathy: case report

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Background: Whole-genome sequencing (WGS) can detect rare and novel genetic variants associated with hereditary neurodegenerative diseases. We report the case of 3 boys (9, 7 and 2 years old) from the same family with similar clinical features: impaired speech and motor development after the first year of life, muscle hypotension. The older brothers show delayed myelination on MRI and generalized epileptiform discharges on the EEG. The phenotypes of the older and younger children included anomalies of the skull and skeleton, delayed physical development. They have a half-sibling girl without pathological symptoms.

Methods: WGS (PE150, enzymatic fragmentation-based and PCR-free protocol, DNBseq-T7 (MGI, China)) with Sanger sequencing (ABI3500, Applied Biosystems) was performed for siblings and their parents.

Results: Genetic analysis revealed novel hemizygous variant c.1118C>G (p.Ser373Ter) in *IQSEC2* gene in each siblings and heterozygous variant in their mother. In silico algorithms predict the likely pathogenic status for this variant.

Conclusion: Detected by WGS variant *IQSEC2*: c.1118C>G (p.Ser373Ter) can be associated with X-linked intellectual developmental disorder (OMIM 309530) and can cause epileptic encephalopathy in the described siblings.

Grant References: The research was supported by non-profit organization Charity Fund for medical and social genetic aid projects «Life Genome».

Conflict of Interest: None declared.

EP09.045 Keloid Lesions in Intellectual Developmental Disorder with Cardiac Defects and Dysmorphic Facies:A Case Report

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Background: Biallelic variants in TMEM94 were recently reported to cause an autosomal recessive intellectual developmental disorder with cardiac defects and dysmorphic facies -IDDCDF-(OMIM:618316). As the name implies, the main phenotype of this disorder is characterized by intellectual disability/developmental delay, CHD, and dysmorphic features. In the initial cohort reported in 2018, one of the subjects was reported to have boggy subcutaneous lesions. In addition, an adult female patient had a cutaneous lesion in the form of dermal type nevi. In this case report, we describe a 20 year old male who presented with intellectual disability, Ventricular septal defect, refractive error and hypospadias. On exam, he has dysmorphic features with an anterior low hairline, large nails, long eyelashes, protruding ears. In addition to the clinical findings described he has multiple keloids, the largest being in the upper back. And the rest are in the chest, lower abdomen and left arm.Whole exome sequencing showed homozygous splice site variants in TMEM94 (c.2729-2A > G).

Methods: case report

Conclusion: This case report shows that variants in TMEM94 could result in cutaneous manifestations and their presence could help in suspecting this rare syndrome and also in classifying variants in TMEM94.

Conflict of Interest: None declared.

EP09.046 Exploring the phenotype and genotype of Menke-Hennekam syndrome 2: case report and literature review

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Introduction: Menke-Hennekam syndrome 2 (MKHK2) is a recently recognized neurodevelopmental disorder that results from heterozygous missense mutations in exons 30/31 of *EP300* gene, mainly known as a cause of Rubinstein-Taybi syndrome 2 (RSTS2). MKHK2 is characterised by developmental delay/intellectual disability, microcephaly, short stature, autism, epilepsy, feeding difficulties, vision and hearing impairment. Dysmorphims are largely different from RSTS2 and include short palpebral fissures, telecanthus, depressed nasal bridge, short nose, anteverted nares, short columella, and long philtrum. Here, we present a case that contribute to an increasing understanding of MKHK2.

Case presentation: A 1-year-old female was first referred to our outpatient genetic department with global developmental delay. At our observation, she showed facial dysmorphisms, syndactyly, short stature and post-natal microcephaly. Microarray was normal. A WES-based gene panel identified a missense variant in the *EP300* gene, NM_001429.4:c.4783T>C p.(Phe1595Leu) in exon 30. The variant was not previously described in literature, nor in gnomAD population. It occurs at a conserved position across species, at the same position as another pathogenic missense change, and in silico analysis support a deleterious effect. Segregation studies revealed a de novo origin. Reverse phenotyping highlighted that our patient did not have typical RSTS2 characteristics, but rather shared the clinical features of MKHK2.

Conclusions: This case contributes to the expansion of the genotypic spectrum of MKHK2. It also emphasizes the wide clinical phenotype associated with *EP300*, and how reverse phenotyping is an important tool in the interpretation of Next-Generation Sequencing data.

Conflict of Interest: None declared.

EP09.047 An integrated approach to the diagnosis of mental retardation linked to a fragile X chromosome

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The relevance of studying X-linked forms of mental retardation is due to their prevalence and the importance of medical genetic counseling for such families. The most frequent disease of this group is FraX syndrome. The diagnosis of fragile X syndrome is based on clinical criteria and paraclinical examination methods. The technology of complex DNA diagnostics includes high-throughput parallel DNA sequencing, multiplex ligated probe amplification, and multiplex methyl-sensitive PCR. Here is our own clinical observation of a 5-year-old boy suffering from epilepsy and mental retardation. The child was born at a gestational age of 38 weeks, weighing 3600 g, body length 53 cm. He held his head from 7 months, sat from 1 year, walked from 1 year 6 months. At

4 months strabismus appeared. At the age of 4, he suffered from acute glomerulonephritis, epilepsy attacks began. Phenotype: macrocephaly, high forehead, protruding ears with soft cartilage, inferior prognathia. Based on clinical data and the result of portrait diagnostics using the Face2Gene computer program, a suspicion of mental retardation linked to a fragile X chromosome was established. Molecular genetic analysis (MLPA) revealed an allele of the FMR1 gene containing a complete mutation (200 CGG repeats), which made it possible to verify the diagnosis of Martin-Bell syndrome. Thus, an integrated approach to the examination of patients with epilepsy and mental retardation makes it possible to identify the cause, which is important in the treatment and socialization of the patient, as well as in the prevention of new similar cases in the family.

Conflict of Interest: None declared.

EP09.048 Triplication of PCDH19 gene as a potential novel disease mechanism associated to epilepsy phenotype resembling loss-of-function mutations

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Introduction: Loss-of-function variants in PCDH19 cause an early-onset seizure, autism and neurocognitive disorder in heterozygous females or in males with somatic mutations, presumably by a cellular interference mechanism. However, the phenotype associated with a gain of dosage have not been described so far.

Results: One female patient with seizures, including focal, generalized atonic and tonic-clonic seizures, with fever as the triggering factor, cluster presentation and drug resistance. Comorbidities include global developmental delay with impaired socialization (autistic traits), language delay, and difficulties in gross motor skills. Dysmorphic features are absent.

Metabolic, MRI and exome sequencing showed no pathological findings. In the array study (Affymetrix CytoScan 750K) a de novo triplication of 12.2 megabases of the chromosomal region Xq21.3-q22.1 was found (chrX:89355579-101615553; Hg19). The triplicated segment included 41 genes, of which only PCDH19 has been associated with a compatible clinical phenotype. The pattern of inactivation of the X chromosome in blood cells showed a preferential, but not complete, inactivation of the maternal X chromosome, showing some functional mosaicism.

Discussion: A similar pathogenicity mechanism has been reported in the only gene with a similar pattern of inheritance (EFNB1), for which the duplication associated familial hypertelorism only in heterozygous carriers, supported by mouse model studies. Further, this is compatible with the "cellular interference" hypothesis proposed as molecular basis of PCDH19-related disease.

Conclusion: The dose gain in the PCDH19 gene may be a potential novel disease mechanism, which causes a clinical phenotype resembling that caused by loss-of-function variants.

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EP09.049 From autism spectrum disorder to monogenic neurodevelopmental disorder

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Introduction: Intellectual developmental disorder, autosomal dominant 41 (MRD41) is disorder with variable phenotype including nonspecific dysmorphia, delayed psihomotor development, intellectual disability with variable severity, speech delay, epileptic seizures and behavioral manifestations (autistic features). It occurs as a consequence of heterozygous mutation in the *TBL1XR1* gene on chromosome 3q26.

The aim: We report a patient with MKRD41 in order to increase the rate of identification of patients with a rare inherited disease of actually unknown prevalence.

Results: We present a child with clinical diagnosis of autism spectrum disorder with nonspecific dysmorphia (low-set ears, prominent nasal bridge, protruding ear, long eyelashes), hyperactivity, short attention span, delayed speech and language development, atria septal defect. After aCGH result of normal molecular karyotype, we performed whole exome sequencing. A "de novo" patogenic mutation c.58G>T in *TBL1XR1* gene was detected, which is a known causative to MRD41. Mutation c.58G>T in *TBL1XR1* has not yet been reported in association with human diseases in the biomedical literature, however, based on the evidence (PVS1, PM2, PM6_SUP), the identified variant is classified as pathogenic. Detected mutation in *TBL1XR1* gene is responsible for loss of function and inability to synthesize the protein which plays an essential role in transcription activation mediated by nuclear receptors.

Conclusion: Taking into account the very rare occurrence of MRD41 with unknown prevalence, we hope that the presentation of the patient can contribute to the easier recognition of children with MRD41 and inclusion of NGS analysis in diagnostic protocols of children with autism spectrum disorder.

Conflict of Interest: None declared.

EP09.050 Ultra-rare neurodevelopmental disorder with degenerative course and progressivemovement disorders associated with biallelic ZBTB11 variants

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Background/Objectives: Zbtb11 is a conserved zinc finger transcriptional regulator. Fifteen individuals with intellectual disability, bilateral cataracts, combined malonic and methylmalonic aciduria (CMMMA), and neuroradiologic abnormalities have previously been reported to have bi-allelic variants in *ZBTB11*. We present 8 additional individuals with *ZBTB11*-related neurodevelopmental disorder to further delineate the spectrum of clinical features and genetic variants contributing to this syndrome.

Methods: Phenotypic and molecular data from 8 unreported individuals with *ZBTB11* variants were gathered through international collaboration and compared to the previously reported individuals.

Results: Six ultra-rare variants (NM_014415.4: c.2009T>C, c.2517G>C, c.2618A>G, c.2708G>A, c.3130G>A and c.999dup) were identified. Frequent phenotypic features include mild to moderate intellectual disability, motor developmental regression, bilateral cataracts, and complex movement disorders. Hypotonia was prevalent in childhood but evolved into hypertonia with limb contractures as individuals aged. Complex movement disorders included orofacial and limb dystonia, myoclonus, stereotypies, and coarse and resting tremor. One patient underwent deep brain stimulation for generalized dystonia with a good response. Six patients showed bilateral cataracts. One patient presented with CMMMA. MRI of the individuals showed cavum septum pellucidum et vergae, megacisterna magna, T2W hyperintensity and atrophy of the basal ganglia, and T2W hyperintensity of the posterior thalami.

Conclusion: Our description establishes bi-allelic *ZBTB11* variants as a cause of disorder characterized by variable combinations of neurodevelopmental delay, intellectual disability, complex movement disorders, cataracts and CMMMA. Our study adds the phenotype and expands the causative genetic variant spectrum of this complex movement disorder seen in adult patients and the response to deep brain stimulation.

Conflict of Interest: None declared.

EP09.051 Koolen-de Vries syndrome: Two patients with 17q21.31 microdeletion and de novo KANSL1 variant

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Koolen-de Vries syndrome (KdVS; MIM#610443) is a clinically heterogeneous disorder characterized by developmental delay, neonatal hypotonia and facial dysmorphisms. Epilepsy, congenital heart defects, urogenital malformations and ectodermal anomalies were also reported. The syndrome either caused by a monoallelic single nucleotide variant, mostly truncating, in *KANSL1* (KAT8 Regulatory NSL Complex Subunit 1, MIM#612452) gene or by haploinsufficiency or disruption of *KANSL1* gene secondary to 17q21.31 microdeletion. This gene is responsible for encoding a nuclear protein called KAT8 regulatory NSL complex Subunit 1, which is involved in chromatin modification.

Two unrelated boys (seven and five years old) were referred to our clinic from pediatric neurology because of global developmental delay and intellectual disability. The seven-year-old one had cerebral palsy, epilepsy and corpus callosum agenesis, additionally. He was followed by pediatric endocrinology and nephrology due to hypothyroidism and bilateral vesicoureteral reflux. A de novo nonsense heterozygous variant c.2470C>T p.(Arg824*) in the *KANSL1* gene was detected using a multigene panel based exon sequencing.

The five-year-old one had a history of neonatal hypotonia, unilateral cryptorchidism and laryngomalacia. He had typical features of the disorder such as bilateral epicanthal folds, hypertelorism, anteverted ears, long and slender fingers, asymmetric thorax and nasal speech. He also had horseshoe kidney and immunodeficiency due to low B-lymphocyte count. Molecular karyotyping revealed a 536.3 Kb deletion on chromosome 17q21.31 encompassing *KANSL1*.

KdVS is a rare autosomal dominantly inherited syndrome associated with global developmental delay. This report provides insight into clinical differences between KdVs patients with microdeletion and *KANSL1* point mutations.

Conflict of Interest: None declared.

EP09.053 Multiple genetic disorders in one family - a new de novo frameshift variant disrupting ZBTB7A, familial hemiplegic migraine related to ATP1A2 and WNT10A linked hypodontia

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Background: We report on a 5-year-old female index patient with global developmental delay, macrocephaly and overgrowth of adenoid tissue. Her 37-year-old mother suffered from epilepsy and migraine with hemiplegic episodes. Both daughter and mother have hypodontia. The father is healthy.

Methods: Chromosome analysis, array-CGH (180k Agilent, design 086332) and trio whole exome sequencing (Twist Human Comprehensive Exome with additional customized probes and NGS with NovaSeq Illumina) was performed. Validation of de novo variants was done by sanger sequencing.

Results: Chromosome analysis and array-CGH of the index gave normal results. Using trio analysis a heterozygous, de novo frameshift variant c.1073del, p.(Gly358Alafs*68) in *ZBTB7A* (NM_015898.4) was identified in the patient. Loss-of-function variants in *ZBTB7A* cause a newly discovered autosomal dominant intellectual disability syndrome with so far only 13 published cases by Oishi et al., in 2020 and von der Lippe et al., in 2022. Macrocephaly and lymphoid hyperplasia seem to be typical signs

of *ZBTB7A* related disorder and hypodontia is also reported in published cases. In the mother known pathogenic heterozygous variants c.1643G>A, p.(Arg548His) in *ATP1A2* (NM_000702.4) and c.682T>A, p.(Phe228Ile) in *WNT10A* (NM_025216.3) were identified, which were not present in the index, and can account for all symptoms of the mother.

Conclusion: This case report highlights the still growing heterogeneity of intellectual disability syndromes and the importance of using trio analysis to easily identify variants in newly discovered genes in diagnostic processes. Furthermore, this example emphasises the need of considering multiple genetic diseases as causal for different symptoms in one family.

Conflict of Interest: Eva Grauer MVZ genetikum GmbH, Victoria Strohhäcker MVZ genetikum GmbH, Dieter Gläser MVZ genetikum GmbH, Gerlinde Funck: None declared.

EP09.054 Health-related quality of life of carers, their spouse and patients affected by familial ID and change after genomic diagnosis

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Background: Intellectual disability (ID) is associated with far reaching lifetime impacts on patients' health and functioning, resulting in significant care needs. For families, genetic testing can inform an etiologic diagnosis, which can assist in access to appropriate health and social care services. Presently, little is known about the quality of life (QoL) of patients with familial ID or how a genetic diagnosis may impact the quality of life of carers and patients.

Methods: Data on quality of life and genetic testing was collected as part of the EPIC-ID study. 111 households (carers and patients) who had a range of genetic testing including whole genome sequencing were recruited and administered the EPIC-ID survey in New South Wales, Australia. QoL data was collected through validated measures of QoL: the Health Utilities Index (HUI) for children and the Assessment of Quality-of-Life 8D (AQoL-8D) for adult carers and their spouse.

Results: We will present results on QoL of carers and their spouses, measured through health utilities from the AQoL-8D. Results for the health-related quality of patients will be reported using health utilities based on the HUI. Change in quality of life before and after diagnosis will also be reported.

Conclusion: Health-related utility data for carers of those with familial ID should be used to inform cost-effectiveness studies for genetic testing and targeted therapies, supporting access to address the health and social care needs of families with ID.

Funding: Supported by the National Health and Medical Research Council (NHMRC) (Grant ID: 113895).

Conflict of Interest: Deborah Schofield PI on NHMRC Grant 1116360, Joshua Kraindler: None declared, Katherine Lim: None declared, Rupendra Shrestha CI APP1113895, Owen Tan: None declared, Sarah West: None declared, Natalie Hart: None declared, Jackie Boyle: None declared, Lucinda Murray: None declared, Tony Roscioli CI on NHMRC 1113895, Mike Field CI on NHMRC 1113895.

EP09.055 A novel candidate gene for neurodevelopmental disorders: JKAMP

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Bakground/objectives: The widespread use of whole exome sequencing (WES) in children with developmental delay (DD) or intellectual disability (ID) allows to elucidate genetic etiology of neurodevelopmental disorders (NDDs). Herein, we presented a case with a homozygous variant in the JKAMP gene and aimed to show JKAMP could be a novel candidate gene for NDDs.

Case Report: A 4-year-old boy presented with ID, epilepsy and autistic features. He was the fourth child of consanguineous parents, one of his brothers had died due to prematurity and the other two brothers were healthy. Developmental delay had been noticed when he was 6 months old, he had diagnosed with epilepsy at the age of 1 year and had never gained the ability to speak and communicate. He had a narrow forehead, synophrysis, long eyelashes, depressed nasal bridge, short nose, broad nasal tip, long philtrum, and thin upper lip in dysmorphologic evaluation. In the WES, a homozygous frameshift variant (c.243dup, p.Lys82GlufsTer16) classified as "likely pathogenic" was detected in the JKAMP gene (ENST00000556985). His parents and both healthy brothers were heterozygous for the variant.

Discussion: The variant detected in our patient has been previously reported in a 9-year-old case with ID, epilepsy and autism by Strauss et al., The expression of JKAMP was shown in wide range of mouse tissues including brain but the gene does not have a confirmed disease association.

Conclusion: Based on these two cases, JKAMP should be considered as a candidate gene for a new NDD and supported by functional analyses.

Conflict of Interest: None declared.

EP09.056 Combined screening for genetic causes of idiopathic intellectual disability by chromosomal microarray analysis and trio whole exome sequencing in a large cohort of Russian patients

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Background/Objectives: Chromosomal microarray analysis (CMA) and whole exome sequencing (WES) are powerful techniques for the search of genetic causes of intellectual disability (ID). The aim of the study was to evaluate the value of combined CMA and WES analysis for patients with ID due to congenital brain anomalies

Methods: CMA was performed by 60K Agilent microarrays. WES was performed with SureSelect Human All Exon V8 on NextSeq 2000 Sequencing system.

Results: Pathogenic or probably pathogenic CNVs were detected in 422 from 1175 patients with ID (36%). For 9 selected families with a one child and 3 families with two affected children (a total of 39 members including parents) with ID due to congenital brain anomalies, like polymicrogyria, pachygyria, lissencephaly, but balanced karyotype, WES was applied. As a

result, 3 pathogenic variants in 3 families, 3 likely pathogenic in three other families, and 10 variants with uncertain clinical significance for 4 families were identified. Novel pathogenic missense variants were identified in the DYNC1H1, MACF1, CDKL5, MID1 genes. The majority of variants are arisen de novo.

Conclusion: Trio-based WES has been shown to be an important step in making a genetic diagnosis for families with balanced karyotype after microarray analysis. The most important step for the correct interpretation of WES results is a deep phenotyping of patient, which allows to establish the exact genetic cause of the disease if several variants with unclear clinical significance were previously identified.

Grant References: This study was supported by the Russian Science Foundation, grant 21-65-00017.

Conflict of Interest: None declared.

EP10 Neurogenetic and Psychiatric Disorders

EP10.001 A novel variant of the POLR3A gene in a patient with hypomyelinating POLR3-related leukodystrophy

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Background: Hypomyelinating POLR3-related leukodystrophy is a group of rare neurological diseases characterized by degeneration of the white matter of the brain with different combinations of major clinical findings. Here we report the first Korean POLR3-related leukodystrophy caused by bi-allelic *POLR3A* c.1771-6C > G and novel c.1650_1661del variants.

Methods: An 18-month-old girl was admitted for evaluation of a seizure-like activity with spasticity that affected her entire body. She showed dental abnormalities, but not suspicious facial dysmorphism. She was in a bed-ridden state with severe cognitive impairments and episodes of dystonic posturing for 1–2 min. Trio exome sequencing (ES) was performed to determine the potential genetic cause of severe developmental delay with leukodystrophy in our proband.

Results: Trio ES revealed that bi-allelic *POLR3A* deleterious variants, c.1650_1661del of the exon 13, and c.1771- 6C > G of the intron 13 were best candidate as causes of hypomyelinating POLR3-related leukodystrophy. Sanger sequencing confirmed the genetic origin of these *POLR3A* deleterious variants as autosomal recessive hereditary transmission.

Conclusion: Our report provides additional evidence for a phenotypic continuum of hypomyelinating POLR3- related leukodystrophy caused by bi-allelic *POLR3A* variants. Further genetic studies are required to understand underlying pleiotropic effects of different *POLR3A* variants.

Grant References: n/a.

Conflict of Interest: Jeemin Kim Daejeon St.Mary's Hospital (Full time), Ji Yoon Han Daejeon St.Mary's Hospital (Full time), Hyein Yeo Daejeon St.Mary's Hospital (Full time).

EP10.002 Mutational spectrum of Greek patients with axonal Charcot-Marie-Tooth disease

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Background: Axonal forms of Charcot-Marie-Tooth disease (CMT) are classified as CMT2, dominant intermediate CMT (DI-CMT), hereditary sensory and autonomic neuropathy (HSAN) and distal hereditary motor neuropathy (dHMN). They are caused by mutations in over 30 genes. The aim of this study was to decipher the genetic landscape of axonal CMT in the Greek population.

Methods: Seventy-four index patients with axonal neuropathy, negative for CMT1A, were initially Sanger sequenced for GJB1 and, if negative, further screened by an NGS custom gene panel covering 24 of the most mutated genes in axonal CMT. The study was carried out in the Neurogenetics Unit of the 1st Department of Neurology, NKUA, Eginition Hospital.

Results: Overall, we identified 17 cases with GJB1 mutations and 12 cases with pathogenic or likely pathogenic variants in a further six genes (MPZ, MFN2, GDAP1, DNM2, BSCL2, LRSAM1), representing 39.2% of the cohort. One of these variants, originally characterized as variant of uncertain significance (VUS), was further evaluated through ACMG criteria and re-classified as likely pathogenic.

Conclusion: The present diagnostic algorithm had a total yield of 39.2%, while the NGS panel had a yield of 21.1%. Given the limited number of genes tested, these results compare favorably with studies in other European populations. Our study delineates the genetic and phenotypic variability of inherited axonal neuropathies in the Greek, while also contributing to the pathogenicity characterization of one VUS.

Grant: This study was partly supported by a grant from Genesis Pharma (Grant/Award Number: 13044, special account for research grants, NKUA).

Conflict of Interest: None declared.

EP10.004 A preliminary report on fragile X syndrome carrier screening in Thai women

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Background/Objectives: Fragile X premutation carriers have considerable public health significance because they are at increased risk of having a child with FXS and at risk of developing health problems. There have been no reported studies examining the prevalence of fragile X premutation carriers in Thai women. This study aimed to estimate the prevalence of premutation carriers and the distribution of the *FMR1* alleles in Thai women.

Methods: Fragile X screening was performed in 369 Thai female blood donors who had no psychiatric or neurological disorders and no family history of psychiatric or neurological disorders for three generations by questionnaire testing. Fluorescent PCR and triplet repeat primed PCR was performed to determine the *FMR1* CGG repeat. Southern blotting was used to confirm repeat numbers and methylation status of expanded allele.

Results: Of the 369 women, one individual carried a normal allele in whom a premutation allele (89 CGG repeats with one AGG

interruption) was identified. We also found four individuals (1.08% or 1/92) with intermediate alleles (46,49,49,49 CGG repeats). The most common CGG repeat allele was 30, followed by 29 and 36.

Conclusion: This study provides the first evidence for study of fragile X carrier screening in Thai women with a prevalence of 0.27% (1/359). This finding can help to improve fragile X screening in reproductive-age and preconception women to prevent unwanted births of FXS-affected children. We also recommend that carrier screening to identify female carriers should be carried out on a large scale in our population.

Conflict of Interest: Areerat Hnoonual Lecturer (Full time) at Prince of Songkla University, Grant from Faculty of Medicine, Prince of Songkla University (REC62-018-5-2), Sunita Kaewfai: None declared, Oradawan Plong-On Medical scientists (Full time) at Faculty of Medicine, Prince of Songkla University, Pornsiri Sangmanee Medical scientists (Full time) at Faculty of Medicine, Prince of Songkla University, Pornprot Limprasert Professor (full time) at Faculty of Medicine, Prince of Songkla University, Grant from The Education&Public Welfare Foundation.

EP10.005 Differential expression of Alzheimer's disease associated genes in rat brain with ischemic stroke

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Background/Objectives: Alzheimer's disease (AD) and ischemic stroke (IS) are among the most significant neuropathologies. The AD- and IS-related neurodegeneration profiles largely coincide. Moreover, IS served as a significant risk factor for AD. Therefore, both diseases are often considered as comorbidities[1]. Here, using CEU-based proxies for AD-related single nucleotide polymorphisms (SNPs), we created an extended list of candidate AD-associated genes. Then, RNA-Seq of penumbra-associated dorsolateral areas of the frontal cortex of rats at 24h after transient middle cerebral artery occlusion (tMCAO) was obtained, and rat orthologous of AD-associated genes were annotated by gene expression data.

Methods: Wistar rats, tMCAO, magnetic resonance imaging, RNA-Seq, bioinformatics.

Results: Human SNPs that had sqr(r)≥0.2 with known AD-related SNPs were found using LDlink tool. They were annotated with 198 genes. Then, 149 rat orthologous genes were found using g:Profiler and RGD tools. Twenty six of them were differentially expressed genes (DEGs) with cut-off>1.5 and padj<0.05 after tMCAO. They were mainly associated with antigen processing and presentation. Namely, 15 DEGs (*e.g. RT1-Da, Psmb8, Tap1*) were up-, and 11 (*e.g. C1qtnf4, Apoe, Ptk2b*) were downregulated in IS conditions.

Conclusion: The genetic relationship between AD and IS was found by exploration of human DNA variations, functional genomics, and bioinformatics. Perhaps, the identified genes are fragile nodes for the development of neurodegeneration. Our results contribute to the improvement of approaches to the analysis and prevention of complex brain comorbidities.

References: Rost et al. Stroke. 2021;52(8):e499-e516

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Conflict of Interest: None declared.

EP10.006 Interest of exome sequencing in non-syndromic specific learning disorders: a French pilot study

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Background/Objectives: Specific learning disorders (SLD) (dyslexia/dysorthography, dysphasia, dyspraxia, dyscalculia and ADHD) affect about 5 to 10% of school-age children. In France, in the absence of a syndromic diagnosis, array-CGH can be proposed, associated in girls with the search for a X-fragile syndrome. Nevertheless, examples of genes described in ID and also described in patients with SLD are multiplying. Some international teams have identified by exome sequencing (ES) a certain percentage of patients, with severe SLD, associated with a monogenic disease.

Material and Methods: We initiated a pilot project in the care setting, aiming to propose ES in patient referred to genetic consultation for well-documented severe SLD.

Results: 72 patients have been included. Among the 64 patients analyzed, likely pathogenic variants were identified in 7 (CDK13, KCNN2, SMARCC2(x2), SET, TRIO and PKD1), 20 variants of uncertain significance (VUS) in epilepsy or ID genes were identified in 16 patients and 8 variants in genes of unknown significance (GUS). Thus, a diagnosis was obtained in 11% (36% with VUS). Families segregations and episignatures are in progress in order to help in the classification of some VUS.

Discussion: All patients in whom a likely pathogenic variant or variant of interest was identified have either multiple SLD or have an association with behavioral disorders. The fact that the selected genes are involved in other NND is consistent with a broadening of the clinical phenotype. The study will be continued in additional 100 patients in order to confirm this first results.

Conflict of Interest: None declared.

EP10.007 Analysis of intronic and exonic variants in patients with DEPDC5-related epilepsy and correction of splicing using modified snRNAs

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Background/Objective: Considering different effects of splicing variants, it is necessary to perform functional assays to establish the exact molecular mechanism and to estimate the pathogenicity of the variant. It helps to make an accurate diagnosis for patients and develop possible strategies to personalized therapy.

Methods: The impact of the variants on splicing was evaluated using SpliceAI and SPiP tools. Functional analysis was carried out using RT-PCR and minigene assay. Correction of splicing was performed using U1 and U7 snRNAs in HEK293T cells.

Results: We carried out a functional assay for four previously unpublished exonic and intronic variants in patients with DEPDC5-related epilepsy using RT-PCR and presented their effect on splicing. Using minigene assay, we investigated seven previously described exonic variants and showed that three of them affect splicing. One exonic variant c.3264G>A was chosen for splicing correction. We created 22 constructions using modified U1 snRNAs and U7 snRNAs with different splicing regulatory elements: exonic silencers, exonic enhancers and intronic enhancers. The most effective and specific construction contained U7 snRNA with intronic enhancer and increased the amount of the wild-type transcript up to 55%.

Conclusion: This work shows the importance of functional assays to evaluate the effect of intronic and exonic variants on splicing. U7 snRNAs can be used as a promising approach for future therapy in patients with DEPDC5-related epilepsy.

Conflict of Interest: None declared.

EP10.009 Investigating the SNPs involved in metabolism in Cypriot ATTR patients

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Background/Objectives: Amyloidosis polyneuropathy (ATTR) is a rare, inherited and progressive disease, Val30Met is the most common mutation. ATTR is caused by an accumulation of amyloid fibrils, which contains the protein transthyretin. The protein deposits accumulate in several tissues such as the nerves, heart, kidneys and eye. The aim of this project was to investigate metabolic involvement in carriers and patients of V30M mutation, with varying symptoms and age of onset. The SNPs chosen have an involvement in the onset and progression of metabolic disorders.

Methods: The SNPs rs1183910 (HNF1A), rs1421085 (FTO), rs17782313 (MC4R) and rs2943634 (IRS1) were investigated. DNA samples from a cohort of 48 patients and 48 gender and age matched controls were used for the TaqMan SNP Genotyping Assay. PCR purification was performed through the MontageTM PCRm96 plate (Millipore). The Pearson's chi-square test was used to determine if there was a statistically significant difference between expected and observed SNP frequencies of cases vs controls for the investigated SNPs.

Results: Regarding the HNF1A (p-value1), FTO (0.6632), and IRS1 (p-value 0.412244) did not show any statistically significant results. However, MC4R (p-value 0.000988) was found to be statistically significant.

Conclusion: To the best of our knowledge, this is the first study investigating the SNPs of NHF1A, FTO, MC4R and IRS1 in Cypriot ATTR patients. Our study identified that MC4R was strongly related to ATTR. In addition, bioinformatics analysis will be applied in future studies to investigate the role of metabolism SNPs and metabolic pathways in ATTR.

Conflict of Interest: Christiana Christodoulou Full time, elena panayiotou-worth Ful time, Eleni Zamba-Papanicolaou Full time.

EP10.010 De novo variant analysis of childhood-onset obsessive-compulsive disorder in the French-Canadian population

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Background: Obsessive-compulsive disorder (OCD) is a neuropsychiatric disorder characterized by cycles of intrusive thoughts or sensations (obsessions) and repetitive behaviours (compulsions). Despite an estimated heritability of 45-65%, known genetic factors are not sufficient to explain risk. De novo variants (DNVs) arise post-zygotically in individuals but are not present in biological parents and may contribute to OCD risk. However, the number of identified DNVs in OCD remains limited and the genetic contribution to OCD suggests more are likely to exist. Here, we identified DNVs in French-Canadian (FC) childhood-onset OCD trios.

Methods: Whole-exome sequencing was performed on 53 FC OCD trios ($n = 159$). Single-nucleotide protein-coding variants were prioritized. Candidate DNVs were selected based on estimated impact on gene function, population minor allele frequency < 0.1%, elevated deleteriousness (CADD > 20), and biological relevance. The candidate gene list was then used to identify enriched biological and molecular pathways underlying childhood-onset OCD. Regression analyses estimated the impact of DNVs on OCD symptom severity.

Results: 89 DNVs across 89 genes were observed in 42 probands (2.12 DNVs/proband, average CADD Score=26.5). 15 of these genes were identified in previous OCD publications (Halvorsen 2021; Cappi 2020). The top enriched Gene Ontology (GO) term was "regulation of neuron projection development" ($p = 9.50E-05$). The number of DNVs carried by a proband did not significantly explain OCD symptom severity.

Conclusion: Findings from this study shed light on the genetic and biological factors underlying childhood-onset OCD risk, further highlighting the ability to leverage the FC population to elucidate the genetics of complex neuropsychiatric disorders.

Conflict of Interest: None declared.

EP10.011 Mutations in H3.3 that cause a neurodevelopmental syndrome disrupt chaperone interactions

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Background/Objectives: Bryant-Li-Bhoj syndrome is a disorder characterized by neurodegeneration and multiple congenital anomalies. This syndrome is caused by mutations in the genes *H3-3A* and *H3-3B*, which encode the replication-independent histone H3.3. Histone H3.3 is deposited on euchromatin by the chaperone complex HIRA and on heterochromatic regions by DAXX. Prior work investigating the pathogenesis of the causative variants underlying Bryant-Li-Bhoj syndrome has identified

changes in post-translational modification profiles, transcript expression, and proliferation rate in cells with these H3.3 mutations compared to control cells. Notably, patient fibroblasts that harbor H3.3 mutations have a hyperproliferative phenotype. Based on these observed alterations and guided by the patient phenotypes, we are now investigating the effects of patient H3.3 mutations on chaperone interactions and telomere maintenance.

Methods: Using nuclear co-immunoprecipitation assays, we assessed changes to HIRA and DAXX interactions with H3.3 in H3.3-overexpressing HeLa cells harboring different patient mutations. To assess telomere length, we used a telomere restriction fragment (TRF) assay with patient-derived fibroblasts.

Results: The H3.3 patient mutations p.G90R and p.P121R both disrupt H3.3-DAXX interaction, suggesting that H3.3 deposition on heterochromatin may be altered in these cells. TRF analysis showed shortened telomeres in patient-derived cells harboring the p.G90R and p.P121R mutations.

Conclusion: Disrupted DAXX-H3.3 interaction is associated with either loss of establishment of telomere length in stem cells or accelerated telomere shortening. While loss of DAXX interaction is not seen with all Bryant-Li-Bhoj patient mutations, these data may indicate a broader molecular phenotype of convergent H3.3 dysregulation across mutations.

Grant References: Hartwell Foundation, Burroughs-Wellcome Fund.

Conflict of Interest: None declared.

EP10.012 The MAPT H1b haplotype as a risk factor for sporadic amyotrophic lateral sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that affects nerve cells in the brain and spinal cord, ultimately leading to death. The majority of ALS cases (90-95%) are sporadic (sALS) and present without a clear genetic cause or a family history. The microtubule-associated protein tau (*MAPT*) gene has been linked to sALS, with studies showing that altered tau protein is part of the overlap between sALS and the frontotemporal dementia (FTD) spectrum.

Methods: A total of 100 sALS patients from Bulgaria were genotyped for the H1 and H1 subtypes tagging SNPs (rs1467967, rs242557, rs1800547, rs3785883, rs2471738, and rs7521). Genotyping data for 2504 individuals from the 1000 Genomes Project Phase 3 was used to create a control group for the haplotype reconstruction. The most frequent haplotypes were reconstructed with Haploview 4.2 and SHEsisPlus; individual SNP association tests and haplotype analysis was performed with the same software.

Results: Haplotype H1b emerged as a risk factor for ALS, with close to a two-fold increased risk of developing sALS compared to other haplotypes (odds ratio 1.973, 95% CI 1.279-3.044). These results are statistically significant (Fisher's $p = 0.004$, Pearson's $p = 0.001$) after test correction methods and 500000 permutations in Haploview.

Conclusion: The *MAPT* H1b subhaplotype was associated with a two-fold increase in sALS risk. Further functional evaluations of the *MAPT* H1 subtypes are required to determine their contribution to an individual's risk of sALS.

Grant References: The financial support of Medical University Sofia, Grant № D-186 /14.06.2022 is gratefully acknowledged.

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EP10.013 The sensory neuropathy caused by pathogenic amplification in the RFC1 gene is not so frequent among the Czech neuropathy patients

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Background/Objectives: Hereditary sensory neuropathies comprise a broad group of patients manifesting with sensory nerve affection. This genetically determined disorder can be caused by pathogenic variants in almost 20 known genes. Recently, the pathogenic pentanucleotide amplification in the intron of the RFC1 gene was reported in the homozygous state as a cause of the CANVAS syndrome. Except the sensory neuropathy, also the cerebellar ataxia and vestibular syndrome are other clinical manifestation of the disease.

Methods: In our laboratory, we focus on diagnostics and research of hereditary neuropathies. We selected 1519 patients without genetic diagnosis. The selection criteria were; neuropathy, preferably sensory type, recessive or sporadic inheritance and age of onset after 20 years of age. Firstly, we performed the short range PCR to amplify the intronic region of the RFC1 gene. Secondly, in 239 samples with no product or atypical length of the product, we performed the triple repeat primed PCR for three different alleles usually presented in this area.

Results: Finally, in six patients with no product from short range PCR we proved the pathogenic amplification of the AAGGG allele and we genetically proved the CANVAS disease.

Conclusion: The genetics correlated with the clinical findings of the patients, where later onset (30 years +) and primary sensory neuropathy dominated.

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Conflict of Interest: Dana Safka Brozkova Principal investigator of grant Exploring causes of the neurogenetic disorders with the newest genomic methods, Anna Uhrova Meszarosova: None declared, Denisa Stanclova: None declared, Petra Lassuthova: None declared.

EP10.014 Potential associations between PER2, PER3, HCRTR2 and APOE genetic variants with neuropsychiatric symptoms in patients with Alzheimer's disease

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Background/Objectives: Alzheimer's disease (AD) presents multifactorial neuropsychiatric symptoms (NPS). NPS have been associated with *APOE_ℰ4* allele which is also the major genetic AD risk factor. Circadian genes dysregulation and orexins-*HCRTR2* genes are two innovative proposals for BPSD in AD. Period genes and orexins have been associated with mood and sleep disturbances. There are no studies considering gene-gene interactions.

Methods: The association of seven variants in *PER2*, *PER3*, *APOE* and *HCRTR2* genes was evaluated in 31 AD patients and 31 paired cognitively healthy controls. All participants signed an informed consent letter (protocol 11/20). Genotyping was performed from blood samples. Allelic-genotypic frequencies of variants were calculated for the sample study. We explored associations between allelic variants with NPS in AD patients based on NPI, PHQ-9 and sleeping disorders questionnaires. Gene-gene interaction analysis by MDR was included.

Results: *APOE_ℰ4* allele was confirmed as an AD risk variant ($p = 0.03$). The remaining variants did not reveal significant differences between patients and controls. The *PER3*_rs228697 variant showed an increased risk for circadian rhythm sleep-wake disorders in Mexican AD patients (OR = 9.736, p = 0.028). Gene-gene interaction analysis included *PER2*_rs2304672, *PER3*_rs228697 and *APOE_ε4* variants in the best model.

Conclusions: *PER3*_rs228697 variant was associated with a nine-fold increased risk for circadian rhythm sleep-wake disorders in patients. We identified a novel *PER2-PER3-APOE_ε4* interaction in AD patients; therefore, patients carrying *APOE_ε4* allele might present a higher risk for circadian rhythm dysregulation due to this novel interaction. However, these findings need to be further confirmed in larger samples.

Grant References: UAMX, #34605034. CONACyT #1004932

Conflict of Interest: None declared.

EP10.015 Novel intragenic deletion within the FXN gene in a patient with Friedreich ataxia: are they more prevalent than we think?

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Background: Friedreich ataxia (FRDA) is mainly caused by biallelic pathogenic expansions of the GAA trinucleotide repeat in intron 1 of the *FXN* gene. Rarely, affected individuals carry either an intragenic point mutation or a deletion of *FXN* in combination with the abnormally expanded GAA repeat. Here, we describe a novel intragenic deletion identified in a 32-year-old patient with classical clinical features of FRDA. Previous routine genetic analysis of the *FXN* intron 1 led to the assumption that the patient carried the common biallelic expansion.

Methods: PCR and TP-PCR fragment analysis of the polymorphic region in intron 1 of the *FXN* gene was carried out on samples from the proband and both parents. Subsequently, duplication/deletion analysis of *FXN* was performed in the mother and proband samples using the SALSA MLPA Probemix P316-B4 Recessive Ataxias kit (MRC Holland) following the manufacturer's instructions.

Results: The maternal sample only showed a peak corresponding to 9 GAA repeats but no expansion was observed while the paternal sample showed an allele of 7 GAA repeats and an expansion, corresponding to a healthy carrier of the disease. Mother's result did not correlate with a biallelic expansion in the proband. MLPA analysis allowed the identification of an intragenic deletion encompassing 5'UTR and exons 1-2 of *FXN* gene in both the proband and his mother.

Conclusion: With this case, we want to highlight that intragenic deletions may be more prevalent than first thought in patients with FRDA. Performing parental testing is crucial in order to provide accurate genetic counselling.

Conflict of Interest: Anna Esteve Garcia Full-time, Ariadna Padró Miquel Full-time, Carlos Casasnovas Full-time, Valentina Velez Full-time, Laura Rausell Full-time, Pablo Gargallo Full-time, Javier Garcia Planells Full-time, Pedro Alía Full-time, Nuria Llecha Full-time, Cinthia Aguilera Full-time.

EP10.016 Whole exome sequencing identifies a heterozygous TRPC3 missense variant in a patient with adult onset spinocerebellar ataxia

Tim Bender[1;2], *Axel Schmidt*[1], *Kirsten Cremer*[1], *Claudia Perne*[1], *Hartmut Engels*[1], *Stefanie Heilmann-Heimbach*[1], *Pietro Incardona*[3], *Sophia Peters*[1]

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Background/Objectives: Spinocerebellar ataxias (SCA) are a group of genetically heterogeneous neurological disorders with the main symptom gait ataxia. Other symptoms, age of onset, as well as mode of inheritance differ between the respective types of SCA.

Case report: We present the case of a sixty years old woman with slowly progressive gait ataxia, dysarthria, slurred speech and poor fine motor coordination. First symptoms appeared at the age of 38 years. Brain MRIs show a slowly progressive atrophy of the cerebellum. Genetic testing for the most common spinocerebellar ataxias (SCA 1, 2, 3, 6, 7, 11, 13, 14 and 17) gave negative results.

Methods and Results: Whole exome sequencing revealed a heterozygous missense variant (NM_001130698.2:c.2216T>C; p.(Leu739Pro)) in TRPC3 in the patient; no other (likely) pathogenic variant was identified. All applied in silico tools predict a damaging effect of this variant. The Z-score for TRPC3 is 3.84, indicating a high intolerance for missense variants of this gene.

Conclusion: To our knowledge, there is only one published case in which a pathogenic heterozygous missense variant in the TRPC3 gene was reported to cause adult onset SCA (Fogel et al., 2015). In vitro functional expression studies of the variant p.(Arg762His) indicated a toxic gain of function effect. A gain of function etiological mechanism is indirectly supported by the relatively frequent observation of loss of function variants in the general population (pLI=0).

Our findings support the hypothesis that heterozygous pathogenic missense variants in the TRPC3 gene are a possible rare cause of adult onset spinocerebellar ataxia.

Conflict of Interest: Tim Bender University Hospital Bonn, Axel Schmidt University Hospital Bonn, Kirsten Cremer University

Hospital Bonn, Claudia Perne University Hospital Bonn, Hartmut Engels University Hospital Bonn, Stefanie Heilmann-Heimbach University Hospital Bonn, Pietro Incardona University of Bonn, Sophia Peters University Hospital Bonn.

EP10.017 Biallelic variants in HEATR5B associated with severe neurologic phenotypes in two additional families: further evidence for an autosomal recessive syndrome

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Background/Objectives: Biallelic splice site variants in *HEATR5B* have previously been reported in four children from two families with a neurological syndrome including pontocerebellar hypoplasia. Reduced HEATR5B protein expression was observed, and in mouse models, biallelic knockout variants were associated with embryonic lethality. In this case series, we report three additional patients from two families with severe neurologic phenotypes.

Methods: We performed a chart review of the clinical and genetic data of two families with biallelic variants in *HEATR5B*, and compared them to the cases reported in the literature.

Results: Family 1 consisted of two siblings with compound heterozygous frameshift variants in *HEATR5B*: c.3184_3185del; p.(Val1062Glnfs*4) and c.6163del; p.(Ala2055Profs*12). As the latter variant is quite distal we suspect it escapes nonsense mediated decay. Patient 1-A presented in the neonatal period with intractable seizures and died at 6 months of life. His sibling, patient 1-B, is currently 1.5 years old with severe global developmental delay and abnormal neurologic exam, and has evidence of pontocerebellar hypoplasia on MRI. Family 2 consisted of a pregnancy with arthrogryposis, hydrops, cystic hygroma, micrognathia and cleft palate; termination of pregnancy was pursued due to the severity of the findings. The fetus was found to have a more proximal homozygous variant, c.327dup (p.Pro110Thrfs*), expected to cause complete loss of function of *HEATR5B*.

Conclusion: This case series provides further evidence that partial loss of function of HEATR5B is associated with severe neurologic disorders (Family 1), whereas complete loss of function may be lethal in utero (Family 2).

Conflict of Interest: None declared.

EP10.018 Expanding the genotypic spectrum of TUBGCP2 related neurodevelopmental disease: The sixth patient in the literature

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Background/Objectives: TUBGCP2 gene (tubulin-gamma-complex-associated protein 2 (MIM*617817) encodes the γ-tubulin complex 2 (GCP2) protein which is an essential part of the γ-tubulin ring complex at microtubule-organizing-center. Bi-allelic mutations on TUBGCP2 have been associated with neurodevelopmental disease spectrum consisting of microcephaly, hypotonia, epilepsy, and abnormal brain magnetic resonance imaging (MRI) (lissencephaly, pachygyria, cerebral and cerebellar atrophy, heterotopia)[1,2]. We aim to expand the genotypic spectrum of TUBGCP2- delated neurodevelopmental-disease by presenting a patient with a novel homozygous TUBGCP2 variant.

Methods: 6 years-old male with neuromotor developmental-delay, hypotonia, epilepsy, microcephaly, and dysmorphic facial findings has been evaluated with whole-exome sequencing(WES) after normal results of conventional karyotyping and high-resolution microarray analysis. The patient had cortical malformations including thin corpus callosum, pachygyria,and polymicrogyria.

Results: A novel homozygous c.2257dupC(p.Gln753ProfsTer62) variant was detected in the patient. The variant were prioritized in the light of ACMG variant classification guidelines and segragation analysis with sanger sequencing, we evaluated this variant as the underlying molecular cause of the patients' phenotype.

Conclusion: To the best of our knowledge, we present the sixth patient with TUBGCP2- related neurodevelopmental disease in the literature. WES is an effective method for exploring the molecular etiology of undiagnosed neurodevelopmental-disorders.

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Conflict of Interest: None declared.

EP10.019 Neurodevelopmental disorder caused by homozygous variant in NTNG2

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Background: In the Bedouin population in Israel consanguineous marriages are common, contributing to high rates of congenital malformations and genetic diseases. Psychomotor retardation with convulsive disorder was identified in three children of a consanguineous Bedouin family. The aim of this study was to identify the underlying genetic cause of the disease in the studied family.

Methods: Genome-wide linkage analysis was performed using Illumina Omni Express Beadchip (750K SNP loci per sample) and analyzed by HomozigosityMapper and SuperLink software's. Whole exome sequencing was performed and the exome data were narrowed down using the Ingenuity Variant Analysis™ software and our in-house data base of 700 ethnicity-matched controls. Sanger sequencing was conducted to study variant segregation within the affected family.

Results: Two homozygous loci were identified on chromosome 9 (between rs2245389 and rs11516128) and chromosome 19 (between rs11669207 and rs12971399), with maximal LOD score of ~2. Based on our filtering analysis pipeline, analysis of gene expression databases and previous human and mouse studies, a homozygous missense variant in *NTNG2* (Chr9:135073428 c.289G>A; NM_ 032536; p.D97N) was identified as the most probable disease-causing variant in the studied family.

Conclusion: *NTNG2* encodes netrin-G2, a membrane-anchored protein implicated in the molecular organization of neuronal circuitry and synaptic organization and diversification in vertebrates. Our findings complement three studies[1,2,3] of recent years highlighting *NTNG2* homozygous mutations as a cause of autosomal recessive neurodevelopmental disorders. The findings will allow carrier testing and early pre-implantation genetic diagnosis within the studied family and the larger Bedouin kindred.

Grants: Morris Kahn Family Foundation.

Conflict of Interest: Amit Safran The Morris Kahn Family Foundation, Regina Proskorovski-Ohayon Bio-Technology General (Israel) Ltd, The Morris Kahn Family Foundation, Ohad Shmuel Birk Soroka Medical Center, The Morris Kahn Family Foundation.

EP10.021 Altered gene expression by rare variants in gene promoters confers risk to autism spectrum disorder

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Background/Objectives: Autism spectrum disorders (ASDs) are a group of heterogeneous neurodevelopmental disorders characterized by deficits in social communication and restricted, repetitive patterns of behavior or interests. Our preliminary analyses suggests that, beyond novel genes, a portion of the missing genetic etiology lies within promoter elements.

In this study, we assessed the relative contributions of de novo, recessive, and rare inherited noncoding variants in cohorts of WGS from families with ASD.

Methods: We analyzed WGS datasets from 9648 individuals (2560 affected) included in the Homozygosity Mapping Collaborative of Autism (HMCA) and the Simons Simplex Collection (SSC). Variants in cases and controls were identified and annotated using our custom pipeline consisting of >20 mutational impact predictions, and integrating TF motifs, epigenomic, and promoter element predictions data. The potential functional impacts of damaging and benign annotated variants were assessed through a sequence-based prediction of variant gene expression effects with ExPecto.

Results: We identified 3136 unique putative single nucleotide disease-associated variants (SNV) within brain-specific promoters in individuals with ASD. Out of 681 predicted damaging noncoding SNVs, 75 were found to have a greater predicted expression effect in cases compared to variants in controls (95% confidence interval) in at least one of 37 brain-related tissues or cell lines. Interestingly, these variants were located within genes found to be enriched in Intellectual Disability ($p = 1.9 \times 10^{-8}$), Neurodevelopmental Disorders ($p = 1.1 \times 10^{-4}$), and ASD ($p = 1.7 \times 10^{-4}$).

Conclusions: Our study enabled the identification of the likely damaging promoter variants contributing the risk in families within the HMCA and SSC cohorts.

Conflict of Interest: None declared.

EP10.022 Exome sequencing in adult patients with developmental and epileptic encephalopathy or severe epilepsy

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Introduction: Early onset epilepsies, especially developmental and epileptic encephalopathies, are severe epilepsies of childhood with early onset of usually drug-resistant seizures and developmental delay or regression. However, some patients reach adulthood undiagnosed from various reasons such as according-to-age modified phenotype.

Patients and Methods: Here we present results of targeted gene panel and/or exome sequencing in 151 adults with severe epilepsy and majority of them with intellectual disability (72%). From the examined cohort, 97 patients were adult at the time of examination (18 – 48 years of age) and the rest were adolescents ($n = 54$) examined between 12 - 17 years of their age. These patients were selected from the cohort of patients unsolved using standard diagnostic methods.

Results: We found causal or likely causal variant in 42 patients (28%). The most frequent variants were found in genes *DEPDC5* (2x), *KCNQ2* (3x), *MECP2* (2x), *SCN1A* (3x), *SCN2A* (2x) *and STXBP1* (2x), gathering 33% of the reported variants (14 out of 42). The vast majority of causal variants were SNVs confirmed to occur de novo with autosomal ($n = 36$; 86%) or X-linked ($n = 6$; 14%) dominant inheritance.

Conclusion: Although NGS methods represent robust diagnostic tool, the genetic diagnosis in adults may not always be established due to the lack of experience. As well as in pediatric patients, finding the genetic cause is essential for comprehensive care of patients as it enables to understand the comorbidities, knowledge of prognosis, genetic counseling, therapeutic choices and quality of life.

Grant Reference: AZV NU20-04-00279.

Conflict of Interest: None declared.

EP10.023 Interest of chromosomal (array-CGH) and genic (HTS) analyzes in Autism Spectrum Disorder: study of two cohorts of 323 and 64 patients, and proposal of a diagnostic strategy

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder (NDD) affecting about 1% of the population. The phenotype of patients is heterogeneous and many comorbidities may be associated. The underlying genetic etiologies are also heterogeneous and the identification of a causal genetic anomaly is very variable according to the studies.

We studied the clinical phenotype and the results of genetic analyzes performed in two cohorts of patients with ASD: one that benefited from chromosomal analyzes (array-CGH) ($n = 323$); another that benefited from high-throughput sequencing of a panel of genes involved in neurodevelopment ($n = 64$). We then divided the patients into 4 groups according to the isolated or associated character of the ASD.

CGH-array identified 18 chromosomal abnormalities (5.6%) including 10 anomalies with incomplete penetrance and/or variable expressivity and 8 pathogenic variations. Gene panel analysis found 9 pathogenic or probably pathogenic variants and 2 variants with incomplete penetrance and/or variable expressivity (17.2%). In both cases, the diagnosis rate was higher if the ASD was associated with a neurodevelopmental abnormality, in particular ID, and even higher when the ASD was associated with a neurological abnormality and other clinical signs such as facial morphological characteristics or malformations.

These results show the importance of accurately characterizing the clinical signs that may be associated with ASD by a neuropediatric evaluation and a complete malformation assessment before prescribing a genetic analyze. According to these

results, we propose to discuss the strategy of the genetic analyzes to be carried out in a patient with ASD.

Conflict of Interest: None declared.

EP10.024 Biallelic NDC1 variants that interfere with ALADIN binding in neuropathy and Triple-A-like syndrome

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Nuclear pore complexes (NPC) are multi-protein assemblies that regulate nucleocytoplasmic transport. NDC1 is a transmembrane nucleoporin required for post-mitotic NPC assembly and the recruitment of the nucleoporin ALADIN to the nuclear envelope (NE). Biallelic variants in *AAAS*, encoding ALADIN, cause Triple-A syndrome, which is characterized by alacrima, achalasia and adrenal insufficiency. To date, no human disease has been associated with *NDC1* variants. By diagnostically validated RNA-sequencing abnormal *NDC1* splicing was identified in two siblings with a homozygous intronic *NDC1* variant. Through international collaborations five additional individuals from three unrelated families with homozygous variants in *NDC1* were identified by whole exome sequencing. The affected individuals presented with alacrima, achalasia, intellectual disability, hypotonia and severe demyelinating and axonal peripheral neuropathy, reminiscent of Triple-A syndrome. However, none of the individuals had adrenal insufficiency. The identified homozygous intronic variant NM_018087.5(*NDC1*):c.892-21G > A induced skipping of exon 9, resulting in an in-frame deletion p.(Ala298_Lys328del), that impacts a known ALADIN binding site. The two identified homozygous missense variants c.1706C>T,p.(Ser569Leu) and c.1720G>A,p.(Ala574Thr) (GenBank: NM_018087.5 [*NDC1*]) were also located in an annotated ALADIN binding site domain and is predicted to negatively affect ALADIN binding. Functional studies on fibroblasts derived from affected individuals showed decreased recruitment of ALADIN to the NE and reduced post-mitotic NPC insertion. Altogether, our results implicate biallelic *NDC1* variants in the pathogenesis of a Triple-A-like disorder, by interfering with physiological NDC1 functions, such as the recruitment of ALADIN to the NPC.

Conflict of Interest: None declared.

EP10.025 HTT and APOE genes as phenotype modifiers in Parkinson's disease

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Background/Objectives: Previous studies have shown that there is a link between CAG repeat number and the risk of developing neurological disorders, such as Parkinson's disease (PD). Additionally, APOE-Ɛ4+ isoform has been found to increase risk of developing PD. The aim of this study is to asses how genetic and non genetic factors act as phenotype modifiers of PD.

Methods: This study involved genotyping the *HTT* gene CAG repeat size and APOE-Ɛ4+ isoforms in a case-control series that included patients diagnosed with both idiopathic and genetic PD. Statistical analysis were replicated in a Catalonian cohort.

Results: There is a significant increase in the number of CAG repeats found in our PD and replicative cohorts, as well as in the complete meta-analysis, in contrast with controls. Moreover, there is a higher presence of intermediate alleles (5.0%) compared to controls (3.9%). Delayed onset age was found in those patients with intermediate alleles as opposed to those with normal range (idiopathic/genetic PD). Regarding the APOE-Ɛ4+ isoform, it has shown to cause an earlier onset of the disease. Lastly, the presence of cancer manifests a belated age of onset, whereas smoking shows the opposite effect.

Conclusions: In conclusion, this study has demonstrated that both genetic and non-genetic factors can act as phenotype modifiers of PD. The presence of intermediate alleles in the *HTT* genes and cancer are linked to a delayed onset of the disease; meanwhile APOE4 isoform and smoking are associated with an earlier onset of the disease.

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Conflict of Interest: None declared.

EP10.026 Rare variations in NDD genes further elucidate the comorbidity of ASD with other developmental disorders

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Background: Whole exome sequencing (WES) has become predominant in molecular diagnostics for neurodevelopmental disorders (NDDs) including autism spectrum disorders (ASD) by considerably increasing the diagnostic yield. These analyses mostly focus on phenotype driven analysis, where analysis is modified according to the phenotypic features of the examinee. Therefore, the key feature is accurate clinical description of the patient. However, the proportion of undiagnosed patients still remains high. Thus, reverse phenotyping, where we first identify a possible genetic cause, and then determine its clinical relevance has been shown as a more efficient approach.

Methods: WES was performed in 148 Slovenian paediatric patients with suspected ASD. Data analysis pipeline was focused towards analysis of very rare variants firstly in ASD-associated genes than expended to NDD-associated genes and further enriched with protein function and gene prioritization analysis.

Results: By reverse phenotyping pathogenic/likely pathogenic variant in ASD-associated candidate genes was determined in 21% of patients, of which subsequent segregation analysis showed 13 were de novo variants. Diagnostic yield was further increased by 2.7%, through analysis of rare variants in NDD-associated genes.

Conclusion: Our study thus demonstrates that analysis of very rare variants in ADS-genes and other which are functionally similar to them, and by performing reverse phenotyping, we reached higher diagnostic yield despite limited clinical data. Present study also demonstrates that most of causative genes in our cohort were

involved in syndromic form of ASD, and confirms their comorbidity with other developmental disorders.

Conflict of Interest: None declared.

EP10.027 Molecular detection of neuropsychiatric disorders facilitates accurate diagnosis, therapeutic discovery, and effective genetic counseling

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Background: Neuropsychiatric symptoms (NPS) are widespread in clinical practice and are associated with several unfavorable clinical outcomes, such as a detrimental effect on the quality of life, caregiver burden, and hastened disease progression. Currently, NPS is underrecognized and undertreated from a genetic perspective.

Objectives: To elucidate the clinical and molecular characterization of patients presenting with NPS.

Methods: A total of ten cases of NPS were registered in our clinical genetics service during 2020-2022. We include all patients presented with one of these psychiatric conditions, i.e., psychosis, bipolar disorder, mood disorder, and pervasive developmental disorder, and were followed or co-existed with any neurological symptoms. Clinical manifestations, brain imaging findings, and genetic investigations were retrospectively reviewed.

Results: Five of the ten cases were subsequently characterized as chromosomal disorders, including 22q11.2 deletion in three cases, 6q26q27 deletion, and 8p23.2 deletion. In the meantime, the other five cases were characterized by monogenic conditions, i.e., Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts with Leukoencephalopathy, DLG3-related Intellectual Developmental Disorder, Ataxia with Vitamin E Deficiency, Tatton-Brown-Rahman syndrome, and White-Sutton syndrome. The remarkable clinical symptoms among these cases were a history of childhood developmental delay (9/10) and clinical pictures of either neurodevelopmental regression or progressive cognitive impairment (10/10). Regarding the attitude of parents or caregivers, eight of the ten families admitted that the correct genetic diagnosis helped reduce the family's stress and conflicts.

Conclusions: In conclusion, genetic investigation in people affected by NPS is promising in facilitating accurate diagnosis, therapeutic discovery, and effective genetic counseling.

Grant references: none

Conflict of Interest: Sasinipa Trithiphen Full time as affiliation, Takol Chareonsirisuthigul Full time as affiliation, Nareenart Iemwimangsa Full time as affiliation, Bhakbhoom Panthan Full time as affiliation, Sommon Klumsathian Full time as affiliation, Komsan Kiatrungrit Full time as affiliation, Piyawat Dendumrongkul Full time as affiliation, Ajchara Trachoo Full time as affiliation, Chanikhan Sattaporn Full time as affiliation, Kullasate Sakpichaisakul Full time as affiliation, Pirada Witoonpanich Full time as affiliation, Apisit Boongird Full time as affiliation, Charungthai Dejthevaporn Full time as affiliation, Pichet Termsarasab Full time as affiliation, Objoon Trachoo Full time as affiliation.

EP10.028 Identification of novel variants in KCNQ2 gene related to epileptic phenotypic spectrum variability

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Background/Objectives: *KCNQ2* is a gene that encodes the voltage-gated potassium channel subunit Kv7.2, and it is highly expressed in the brain, in particular at the axonal initial segment. Pathogenic variants in *KCNQ2* are associated with neonatal epilepsies, which could range from a milder phenotype of Benign Familial Neonatal Epilepsy (BFNE) to a severe phenotype of Developmental and Epileptic Encephalopathy (DEE). This difference is usually associated with the inheritance or de novo status of the variant. In this study, we performed targeted next-generation-sequencing, using a gene panel including 38 genes, on five young epileptic patients with a wide variability of the epileptic phenotypic spectrum.

Methods: The analysis was conducted by applying Ion Torrent Proton Sequencer. Potential pathogenic variants were selected and Sanger Sequencing was performed to validate the candidate variants.

Results: In each of the five patients we identified a different variant: c.1631+1 G > A; c.2251T>G; c.2593G>A; c.1378G>A; c.2341_2342insC. The first three variants are inherited by affected individuals of the families. To verify the segregation of the last two variants, DNA was not available.

Conclusion: Our findings provides new probably pathogenic variants in *KCNQ2* related to epileptic phenotypes. Further studies are needed to increase knowledge of the pathogenic mechanism underlying these epileptic disorders.

Grants: PRIN Project "Genetic Epileptic channelopathies as disease models for drug discovery toward personalized treatment: an integrated bench-to-bedside and backward approach" - CUP B64I19001030001 – funded by MIUR identification code 2017ALCR7C - Sector LS5 line C – in the following topic: "Epilepsy Genetics"

Conflict of Interest: None declared.

EP10.029 rare copy number variants in CACNA1H implicated in essential tremor

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Background/Objectives: Essential tremor (ET) is the most common movement disorder globally, affecting approximately 5% of the population by the age of 65. ET leads to significant

impairment and diminished quality of life due to tremors occurring in the hands during voluntary motion. Despite ET having an overall heritability of between 45-90%, only a limited number of genetic risk factors have been identified. Here, we leveraged a large ET cohort of 2,133 cases and 10,336 controls to identify copy-number variants (CNVs) associated with ET.

Methods: Samples were limited to those of European ancestry inferred through PCA. CNVs were called from SNP array data using the program PennCNV and annotated for functional consequence and population frequency using gnomAD. Only rare CNVs intersecting protein coding regions of the genome were tested. The enrichment of CNVs per gene between cases and controls was investigated using Fisher's exact test. Only the candidate genes that passed Bonferroni correction were considered.

Results: Following QC, 3,516 CNVs across 1,638 genes were tested across 1,365 cases and 9,585 controls. Gene duplications in the Calcium Voltage-Gated Channel Subunit Alpha1H gene (*CACNA1H*) were significantly more common in ET cases (18 CNVs, $p = 7.1 \times 10^{-9}$) and were absent in any controls examined. Approximately 1.3% of studied cases carried this rare structural variant.

Conclusion: The observation of duplications of the *CACNA1H* gene implicates this gene as a risk factor for ET and adds to the potential involvement of *CACNA* genes in ET pathology.

Grant References: Foundation grants - Canadian Institutes of Health Research

Conflict of Interest: None declared.

EP10.030 Integrative multidisciplinary approach to Frontotemporal Dementia (FTD) spectrum: genetic diagnostic yield in a single-Italian center

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Neurodegenerative diseases (ND) are a heterogeneous set of conditions affecting the Central Nervous System. Neuronal degeneration can lead to memory defects with or without behavioral and movement disorders. Only in a limited percentage of cases it is possible to identify a genetic etiology. Alzheimer's disease represents the leading diagnosis, followed by frontotemporal dementia (FTD), an atypical mixed spectrum that encompasses complex and overlapping clinical entities.

Here we report the experience of a single Italian center. To date, a total of 71 patients have been admitted to our ambulatory (29 men, 42 women: mean age at diagnosis 64.5 ± 8.3, mean age at onset 59.4 ± 8.0). All patients were characterized clinically, using imaging (brain MRI and 18-FDG-PET), neuropsychological testing, and cerebrospinal fluid (CSF) biomarkers ($Beta_{1-42}$ 963.4 ± 426.6, tau 418.7 ± 274.8, p-tau 65.2 ± 47.4). Forty-five patients underwent *C9orf72* testing and a targeted NGS panel analysis, detecting in 3 a pathogenic expansion in *C9orf72* and in 7 pathogenic variants in *GRN* (2 patients), *PSEN1* (2 patients), *MAPT* (1 patient), *NOTCH3* (1 patient), and *HNRNPA1* (1 patient). In 9 patients variants of uncertain significance (VUS) in *NOTCH3, VCP, FUS, GFAP, MAPT, PSEN1 and PLA2G6* were disclosed. Genetic tests did not detect variants in 26 patients. The diagnostic yield of genetic analyses was about 22%. Remaining investigations are still ongoing.

FTD diagnostic criteria have not undergone major updates in recent years to accommodate growing clinical, etiopathological, neuroimaging, and molecular understanding. With this integrative approach, we aim to achieve a better definition and diagnostic stratification of patients belonging to the FTD spectrum.

Conflict of Interest: None declared.

EP10.032 Combining patient phenotypes and transcriptomic data to rationally develop patient models of neurodevelopmental "histonopathies"

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Background/Objectives: Histone mutations are an emerging cause of pediatric Mendelian syndromes. After somatic oncohistone mutations in H3.3 were identified in patients with pediatric gliomas, germline mutations in multiple genes encoding distinct histones were found to cause neurodevelopmental disorders (NDD). We suspected that, while mutations in unpublished histone genes also caused NDDs, WES/WGS analyses and pathogenic variant calling was hindered by unique histone properties, like high sequence homology. To overcome these limitations, we intersected gnomAD constraint-metric data with GTEx tissue expression data to identify novel histonopathy candidate genes (*H2AZ2/H2AFV, MACROH2A1/H2AFY, MACROH2A2/H2AFY2, H2AZ1/H2AFZ, H1-0/H1F)*. Using GeneMatcher, we built cohorts of unreported patients with mutations in a subset of those genes (*H2AZ2/H2AFV, MACROH2A1/H2AFY, MACROH2A2/H2AFY2)*. To delineate the pathogeneses of histonopathies in disease-relevant cell-types, we are developing hiPSC models of patient mutations with lineage differentiation decisions rationally informed by patient phenotypes and publicly-available transcriptomic data.

Methods: We are leveraging bulk RNA-seq datasets, including BrainSpan and GTEx, and single-cell RNA-seq datasets of fetal human brain to identify the developmental time points and the neural and glial populations where histones are expressed.

Results: The same alignment issues that rendered many histone mutations refractory to genetic diagnosis plague many extensively utilized transcriptomic references. We found that only a small subset employing updated alignment pipelines overcome this major limitation.

Conclusions: Our patients continue to motivate which cell lineages and developmental stages we prioritize in hiPSC models of histonopathies. We must carefully select which publicly-available transcriptomic resources we utilize to verify lineage determinations.

Grant References: Hartwell Foundation, Burroughs-Wellcome Fund.

Conflict of Interest: None declared.

EP10.033 Enrichment of melanopsin genetic variants in a delayed sleep-wake phase disorder (DSWPD) patient – whole genome sequencing analysis

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Background/Objectives: Melanopsin (*OPN4*) is a blue light-sensitive opsin-type G-protein coupled receptor. It is highly expressed in photosensitive retinal ganglion cells which mediate regulation of sleep, circadian photoentrainment, and pupillary light response. *OPN4* mutations were shown to affect circadian rhythm and sleep regulation. Previously, we described a male carrier of an *OPN4* missense variant diagnosed with Delayed Sleep-Wake Phase Disorder (DSWPD), with a consistent recurrent pattern of delayed sleep onset. The rs143641898 [NM_033282.4:c.502C>T p.(Arg168Cys)] variant in the *OPN4* gene was shown in a functional study to render the *OPN4* protein non-functional.

Methods: We have conducted a rigorous observational study in 117 suspected DSWPD patients with the aim of detecting variants that may be associated with the delayed sleep phenotype. DSWPD samples were compared to 315 healthy controls.

Results: We report an enrichment of *OPN4* rare coding variants (MAF < 1) in DSWPD samples compared to controls. This significant enrichment implies that other rare predicted loss-of-function (pLOF) variants can similarly contribute to the delayed sleep phenotype. A significant association with rs1079610, a common variant in *OPN4* with delayed bedtime in DSWPD patients, was found, though not seen in a large set of controls without DSWPD diagnoses. This variant has been previously reported in association with altered pupillary responses. These rare *OPN4* variants likely increase the risk of DSWPD via its direct effect on pathophysiology along the melanopsin axis.

Conclusion: This study offers useful insights for the differential diagnosis and ultimately treatment of DSWPD in patients that carry pathogenic variants in the *OPN4* gene.

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EP10.034 Genetic analysis of epileptogenic brain lesions

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Introduction: Malformations of cortical development, such as focal cortical dysplasia (FCD) and hemimegalencephaly (HME), are common causes of intractable pediatric epilepsy. Epileptic surgery has been conducted on carefully selected patients suffering from these disorders, and approximately 60% of patients were seizure-free one year after the surgeries.

Materials and Methods: Genetic analysis of epileptogenic brain lesions was performed for 64 patients with FCD, HME, brain tumors, or hippocampal sclerosis. Targeted sequencing, whole-exome sequencing, and single nucleotide polymorphism microarray were conducted for detecting single nucleotide variants (SNVs) /indel and copy number variants (CNVs).

Results: We identified four germline and 35 somatic variants in 37 of the 64 individuals, consisting of 36 SNVs /indels and three CNVs. Somatic variants in the PI3K-AKT3-mTOR pathway genes were identified in FCD type II and HME, while somatic variants in the PI3K-AKT3-mTOR pathway and RAS/MAPK pathway genes and *SLC35A2* were identified in other types of epileptogenic lesions. One of the *MTOR* somatic variants in FCD type IIB was an in-frame deletion, and somatic variants of *MAP2K1* and *PTPN11* involved in the RAS/MAPK pathway were detected in FCD type I. The in-frame deletions of *MTOR* and *MAP2K1* increased the mTOR pathway activity in transiently transfected cells. In addition, the *PTPN11* missense variant tended to sustain activation of the mTOR or RAS/MAPK pathway, depending on culture conditions.

Conclusions: Our study showed that epileptogenic brain lesions, with the exception of FCD type II, arose from somatic variants in diverse genes but were eventually linked to the mTOR pathway.

Conflict of Interest: None declared.

EP10.035 Prospective evaluation of NGS based sequencing in epilepsy patients: results of 7 clinical genetic laboratories

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Background/Objectives: Epilepsy is a disabling neurological disorder, highly prevalent in children with neurodevelopmental delay. The exact molecular diagnosis is essential to determine prognosis, comorbidity and probability of recurrence and to inform therapeutic decisions.

Methods: Prospective cohort of 317 patients with seizure related Human Phenotype Ontology (HPO) evaluated in 7 diagnostic centers in Germany, duration 2 month. Evaluated data include phenotype, sequencing method and results.

Results: Whole Exome Sequencing (WES) was applied in 50% of patients, followed by panel testing (35%) and Trio WES (T-WES) (15%). Median turnaround time (TAT) for patients was three weeks (median: 22d). Overall, a pathogenic variant (PV) (ACMG cl. 4 /5) was identified in 21%; furthermore, a significant number of patients (15%) carried a clinically suggestive variant of unknown significance (VUS) that was reported. The highest diagnostic yield was achieved by T-WES within the age group 0-12y in syndromic patients (ACMG cl. 5 /4: 39%). The average diagnostic yield in patients 0-12y vs patients >12y was similar (PV (PV + VUS): patients 0-12y: 22% (37%), patients >12y: 21% (33%). Finally, diagnostic findings in syndromic vs non-syndromic patients revealed a significant overlap of frequent causes of monogenic epilepsies including SCN1A, CACNA1A, SETD1B, confirming the heterogeneity of the associated conditions.

Conclusions: In patients with seizures a monogenic cause can be frequently identified; this justifies early and broad application of genetic testing. Our data supports early exome or trio-exome based testing, with possibly profound implications for genetic counselling and therapy.

Conflict of Interest: Maximilian Witzel MGZ Medizinisch Genetisches Zentrum, München, Sören Wenzel GEMEINSCHAFTSPRAXIS FÜR HUMANGENETIK & GENETISCHE LABORE, HAMBURG, Birgit Eichhorn MVZ Mitteldeutscher Praxisverbund Humangenetik GmbH, Dresden, Peter Lorenz MVZ Mitteldeutscher Praxisverbund Humangenetik GmbH, Dresden, Marius Kuhn MVZ Genetikum GmbH, Neu-Ulm, Manuel Lüdeke MVZ Genetikum GmbH, Neu-Ulm, Björn Schulte Zentrum für Humangenetik, Tübingen, Konstanze Hörtnagel Zentrum für Humangenetik und Laboratoriumsdiagnostik (MVZ), Martinsried, Ralf Glaubitz amedes genetics, Hannover, Sarah Knippenberg amedes genetics, Hannover, Anna Teubert amedes genetics, Hannover, Angela Abicht MGZ Medizinisch Genetisches Zentrum, München, Teresa Neuhann MGZ Medizinisch Genetisches Zentrum, München.

EP10.036 GWAS of Clinically Predicted Suicide Liability

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Background: Suicide accounts for more than 700,000 deaths annually. Heritability estimates of suicidal behaviour range from 17-55%, suggesting a significant genetic component to suicide risk. An insufficient number of genotyped cases has caused a lack of adequately powered genetic studies of suicide.

We will address this limitation by leveraging electronic health care records to perform heritability analyses, and a genome wide association study (GWAS) of clinically predicted suicide liability.

Methods: Electronic health registers of the Danish population will be used to clinically predict death by suicide in an age and sex matched case-control subsample of the Danish population.

A LASSO regression will be applied to clinical and social predictive variables using a five-fold cross validation approach. The resulting coefficients will be used to predict suicide liability in a genotyped sample of 146,000 individuals, followed by a GWAS of the clinically predicted suicide liability. We will investigate genetic overlap with the most recent case-control GWAS of suicide, and heritability estimates and genetic correlations between clinically predicted suicide liability and major psychiatric disorders.

Results: Results from this study will shed light on the contribution of common genetic variants to the clinically predicted risk of death by suicide, and demonstrate the level of genetic similarity between clinically predicted death by suicide and observed death by suicide. Finally, this method of leveraging electronic health records for clinical prediction to increase power in genetic studies could pave the way for genetic studies of phenotypes otherwise suffering from low number of genotyped cases.

Conflict of Interest: None declared.

EP10.037 Disease similarity network analysis of Autism Spectrum Disorder and comorbid brain disorders

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Background/Objectives: Autism Spectrum Disorder (ASD) is a clinically heterogeneous neurodevelopmental disorder, with variable severity and multiple comorbidities. Given the previous evidence of genetic overlap between ASD and several comorbid brain disorders, we sought to explore the genetic similarity with ASD across a range of brain disorders, using a genetic similarity disease network approach.

Methods: We developed a genetic similarity disease network between ASD and Intellectual Disability, Attention-Deficit/Hyperactivity Disorder, Epilepsy, Schizophrenia and Bipolar Disease spectrum. Using gene-disease associations from the DisGeNET database, genetic similarities were estimated from the Jaccard coefficient between disease pairs. The Leiden algorithm identified network disease communities and shared biological pathways. Loss-of-function (LoF) rare de novo variants within shared genes underlying the disease communities were identified using the MSSNG whole-genome sequencing dataset.

Results: We identified three disease communities. ASD is included in a heterogeneous community with Epilepsy, Bipolar Disorder, Attention-Deficit/Hyperactivity Disorder combined type, and some disorders in the Schizophrenia Spectrum. ASD and Intellectual Disability are in separate communities. The genes *SHANK3, ASH1L, SCN2A*, and *CHD2*, which are candidate genes for diseases in all communities, have a higher number of de novo rare LoF Single Nucleotide Variants in ASD subjects.

Conclusion: This approach enabled further clarification of genetic sharing between ASD and comorbid brain disorders, as we took advantage from a finer granularity in disease classification and multi-level evidence from DisGeNET, with important implications for disease nosology, pathophysiology, and personalized treatment.

Grant references: *Fundação para a Ciência e a Tecnologia*: MEDPERSYST:PAC-POCI-01-0145-FEDER-016428;DeePer:(EXPL/CCI-BIO/0126/2021);National Institute of Health Doutor Ricardo Jorge.

Conflict of Interest: None declared.

EP10.038 Clinical, radiological, and genetic characterization of a patient with a novel homoallelic loss-of-function variant in DNM1

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Background: Heterozygous pathogenic variants in DNM1 are linked to an autosomal dominant form of epileptic encephalopathy. Recently, homozygous loss-of-function variants in DNM1 were reported to cause an autosomal recessive form of developmental and epileptic encephalopathy in unrelated patients.

Methods: We investigated a singleton from a first-degree cousin marriage who presented with facial dysmorphism, global developmental delay, seizure disorder, and nystagmus. To identify the involvement of any likely genetic cause, diagnostic clinical exome sequencing was performed. Sanger sequencing of the trio, the patient, and her parents was performed to confirm the segregation of the variant. Structural modeling of the variant was produced.

Result: Comprehensive filtering revealed a single plausible candidate variant in DNM1. Sanger sequencing confirmed the full segregation of the variant in the family. The variant is a deletion leading to a premature stop codon and is predicted to cause a protein truncation. Structural modeling implicated a complete loss of function of the Dynamin 1 (DNM1). Such mutation is predicted to impair the nucleotide binding, dimer formation, and GTPase activity of DNM1.

Conclusion: Our work expands the phenotypic spectrum of pathogenic homozygous loss-of-function variants in DNM1.

Grant References: This research was funded by the King Salman Center for Disability Research (KSCDR), Award No. KFSHRC-RAC: 2180004 (Namik Kaya), and the King Abdullah University of Science and Technology (KAUST), Award No. FCC/1/1976-25 and REI/1/4446-01 from the Office of Sponsored Research (OSR) (Stefan T. Arold).

Conflict of Interest: None declared.

EP10.039 Klefstra syndrome in a young woman with unexplained brain dysfunction

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Background: We present a case of a 26-year-old woman referred by a psychiatrist due to psychotic symptoms, aphasia, impaired cognitive functions. She had throat discomfort, difficulty swallowing, feels her tongue swollen; left mouth corner stands lower, tongue deviation slightly to the right; left limbs stand lower; slightly impaired coordination. MRT showed a pineal cyst.

Materials and methods: Array CGH was performed after DNA isolation from peripheral venous blood.

Results: A heterozygous deletion was found - arr[hg19] 9q34.3(140,606,677-140,883,121)x1 (0.276 Mbp). It affects 22 exons of the *EHMT1* gene and the first 15 exons of the *CACNA1B* gene.

Discussion: *EHMT1* gene encodes euchromatin histone-lysine N-methyltransferase 1, which modifies histone functions and is essential for normal development. Heterozygous loss-of-function mutations in this gene are associated with Klefstra syndrome. The CACNA1B gene encodes an N-type voltage-gated calcium channel controlling the neurotransmission of neurotransmitters. Klefstra syndrome is characterized by facial dysmorphism, moderate to severe intellectual disability, speech disturbances, heart defects, kidney defects, severe respiratory infections, seizures, epilepsy, behavioral abnormalities - apathy, lack of attention, sleep disorders.

Conclusion: Heterozygous deletions of all or part of the *EHMT1* gene have been described as a cause of Klefstra syndrome. The establishment of such a deletion allowed the patient's diagnosis to be refined, as the characteristic facial dysmorphisms were observed. DNA microarray analysis is an important method in the diagnosis of psychiatric patients with unclear brain dysfunction.

Conflict of Interest: None declared.

EP10.040 Genes underlying recovery from DSM-5 nicotine use disorder in a US nationally representative sample

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Background/Objectives: Pharmacogenetic studies of nicotine use disorder (NUD) have largely focused on the impact of genetic variation on response to treatment. The contribution of genetic factors to overall recovery from NUD remains poorly understood. We conducted an exome-wide association study to identify genes associated with recovery from DSM-5 NUD.

Methods: We collected saliva samples from National Epidemiologic Survey on Alcohol and Related Conditions–III (NESARC-III) and genotyped them using an Affymetrix Exome Array (363,496 SNPs). We conducted an exome-wide association study among European ancestry samples with prior to past year (PPY) NUD ($n = 3{,}377$). We compared the recovery group (individuals without NUD in the past year, $n = 1{,}339$, treatment in PPY: 16.4%) with the consistent past-year NUD group ($n = 2{,}038$, treatment in PPY: 26.4%). Logistic regression analysis was adjusted for treatment history, severity of PPY NUD, age and sex. Individual-level genetic data (22,848 samples) and phenotypic variables ($n = 4{,}320$) from NESARC-III are available in dbGaP (accession: phs001590.v2.p1).

Results: We identified two SNPs associated with recovery from NUD at 17q24.2: rs11079691 ($P = 6.5 \times 10^{-6}$) in the *PSMD12* gene and an intergenic SNP (rs6504501, $P = 5.4 \times 10^{-6}$). Additionally, cholinergic receptors nicotinic subunits (*CHRNA2*, *CHRNA3*, *CHRNB4* and *CHRNA10*) were implicated in NUD recovery ($P < 0.05$).

Conclusion: Our study is the first exome-wide association study of recovery from DSM-5 nicotine use disorder, providing new insights into the genetic basis of NUD recovery. Our findings warrant further investigation and may have important implications for personalized treatments for NUD.

Grant References: NESARC-III is funded by National Institute on Alcohol Abuse and Alcoholism.

Conflict of Interest: Haitao Zhang Epidemiologist (full time) National Institute on Alcohol Abuse and Alcoholism (NIAAA), USA, The project is funded by National Institute on Alcohol Abuse and Alcoholism and with supplemental support from the National Institute on Drug Abuse.

EP10.041 6 GHz Radiofrequency Electromagnetic Field (RF-EMF) Can Decrease Cell Viability of N9 Microglia Cells in Time-Dependent Manner

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Introduction: Microglial cells which are responsible for innate immunity can be activated in response to various conditions such as brain injury, infection and neurodegeneration. Electromagnetic radiation is emitted by many natural and man-made sources. Several studies have suggested that EMF exposure results in detrimental effects on central nervous system. In the present study, we aimed to investigate viability of microglial cells after 6 GHz RF-EMF exposure at different times.

Materials And Methods: N9 microglia cells were exposed to 6 GHz EMF or sham-exposed for 20 min, 1 hour and 3 hours. After exposure, MTT solution was added to cells in culture. Then, the cells and formazan product were solubilized with DMSO and absorbance at 570 nm was measured. The percentage of viable cells is calculated.

Results: We found that the mean cell viability was %98 at 20 min exposure, %93.7 at 1 hour exposure and %88.1 at 3 hours exposure. There is no significant difference between exposure for 20 min and exposure for 1 hour to 6 GHz RF-EMF on cell viability of N9 microglia cells. However, there is a statistically significant difference between exposure for 20 min and exposure for 3 hour and between exposure for 1 and exposure for 3 hour according to One-Way ANOVA test.

Conclusion: According with our result, N9 microglia cell viability decreases as the exposure time to 6 GHz RF-EMF increses. Our result may suggests that 6 GHz RF-EMF can reduce N9 microglia cell viability in time-dependent manner.

Conflict of Interest: Ayfer Pazarbasi full, Gulsevinc Aksoy full, Mustafa Emre full, Yasin Karamazi full.

EP10.042 A syndrome can hide another: a case of Tay-Sachs disease associated with Prader-Willi syndrome secondary to maternal uniparental disomy of chromosome 15

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We report here the case of a 3-month-old boy born at term, presenting with global neonatal hypotonia, and feeding difficulties. Regarding these clinical manifestations, genetic investigations were performed and showed an absence of expression of the paternally contributed 15q11q13 region consistent with a Prader-Willi syndrome (PWS). This syndrome is characterized by neonatal hypotonia, followed by hyperphagia, development delay, behaviour disorder and hypogonadism. In the context of hypotonia, a brain MRI was also performed revealing a delay in myelination, which was not expected in PWS. The hexosaminidase A assay performed in the context of a suspected leukodystrophy found a low level, in two different samples, in accordance with a Tay-Sachs disease (TSD). TSD is a rare autosomal recessive disease leading to progressive weakness and cognitive decline as a consequence of biallelic variations in the *HEXA* gene on chromosome 15.

The combination of these two disorders in our patient resulted from maternal uniparental disomy of chromosome 15 with a mother carrying a heterozygous pathogenic *HEXA* variant transmitted at homozygous state to her son. To date only one other case combining both PWS and TSD due to maternal uniparental disomy has been reported in a young girl with severe neurological features who died at the age of 25 months.

This observation reminds us that clinicians should always be warren in cases with atypical features that may uncover associated diseases and could modify the clinical care and worsen the vital prognosis.

Conflict of Interest: None declared.

EP10.044 A novel pathogenic variant in VARS1 associated with a syndromic neurodevelopmental disorder with

craniofacial, and neuroradiological abnormalities in a Turkish family

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Control Number: 2538

Topic: ESHG

Presentation Preference: Digital Only Poster

Applied for Early Career Award and/or Fellowship:

Bacground Objectives: The aminoacyl-tRNA synthetases are a vital and universally distributed family of enzymes that plays a critical role in protein synthesis. VARS1 belongs to class-I aminoacyl-tRNA synthetase family and is located in the class III region of the major histocompatibility complex. To date, a total of 31 out of 37 ARS enzymes have been implicated in genetic diseases. Recently 10 patients with biallelic VARS1 variants have been reported in literature.

Methods: We evaluated one individual from a Turkish family. The study was approved by the institutional ethics committees of University College London (UCL) and additional local ethics committees of the participating centres. Exome sequencing (ES) and Sanger segragation anaysis were performed.

Results: In the present study, we identified a novel homozygous VARS1 missense variant by using exome sequencing, in a consanguineus Turkish Family. The affected individual exhibited failure to thrive, increased frequency of seizures, microcephaly, inguinal hernia, jitteriness, proteinuria, otitis. CT value soft tissue density were present in both mastid regions. EEG showed low amplified sharp waves in the left hemisphere.

Conclusion: In this study, we identified of this new ultra-rare variant is intended to further elucidate the clinical features associated with the molecular and phenotypic spectrum of the neurodevelopmental syndrome.

References:

Grants:

Conflict of Interest: None declared.

EP10.045 The functional effect of variants in SCN2A and SCN8A genes drives therapeutic implications: a case series

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Background/Objectives: Dysfunction of sodium ion channels encoded by SCN2A and SCN8A genes leads to epileptic disorders. The functional effect of mutations (gain-of-function/GOF or loss-of-function/LOF) associates specific additional phenotypic features and is essential in choosing the appropriate anti-seizure medication for the patients. The aim of this study is to determine the functional effect of the mutations found in our patients for treatment management.

Methods: Six pediatric patients, four boys and two girls, including one pair of siblings, were referred to our clinic for epileptic seizures. Three of them also presented intellectual disability. One patient associated sensory deficits, osteoporotic recurrent fractures and multiple brain MRI abnormalities. Genetic investigations were performed using an epilepsy gene panel for four patients and WES for the other two.

Results: Four patients, including the pair of siblings, had a SCN2A variant: one pathogenic missense variant, one likely pathogenic frameshift variant and one VUS missense variant. Two SCN8A missense variants were found in the other two patients: a VUS and a likely pathogenic variant. Analyzing patients' phenotypes, literature data and international databases we identified: a LOF, a probable LOF and a probable GOF variant for SCN2A patients, and a GOF together with a probable LOF variant for SCN8A patients. Three of these variants have never been reported before.

Conclusion: Patients displaying genetic driven epilepsies can receive personalized mutation-based treatment. Identifying the functional effect of SCN2A and SCN8A variants is decisive for the pharmacologic management of these patients. Our patients having GOF mutations responded well to sodium channel blockers.

Conflict of Interest: None declared.

EP10.046 Gene Variants Involved in Nonsense-Mediated mRNA Decay Suggest a Role in Autism Spectrum Disorder

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Background/Objectives: Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by impaired social/communication skills and repetitive behaviors. Genetics contribute to 50-80% of ASD risk, but the exact genetic causes are not yet fully understood. In this study, we explored the contribution to ASD etiology of genes involved in an important post-transcriptional regulatory mechanism, the Nonsense-Mediated mRNA Decay (NMD).

Methods: We compiled a group of 46 genes encoding NMD factors and regulators and, in these genes, searched for Single Nucleotide Variants (SNVs) and Copy Number Variants (CNVs) in two datasets of ASD patients ($N = 1828$ and $N = 3570$, respectively). We estimated the frequency of these variants in 60146 controls from gnomAD v2.1.1 and 10355 controls from the Database of Genomic Variants. We then investigated whether the rare variants (MAF < 1% in controls) in these genes that are predicted in silico to be pathogenic affect protein domains required for NMD.

Results: In the selected 46 genes, we identified 270 SNVs and 38 CNVs in 524 and 38 ASD patients, respectively. Of these, 122 SNVs and 11 CNVs were located within protein domains important for NMD function, which contribute to changes in the expression of NMD targets. As many targets encode brain-expressed proteins, NMD impairment due to these genetic variants may contribute to ASD risk.

Conclusion: Functional studies are needed to firmly establish the impact of the identified genetic variants on NMD function. Overall, this study provides novel suggestive evidence for a contribution of the NMD pathway to ASD.

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Conflict of Interest: None declared.

EP10.047 Genetic heterogeneity of epileptic syndromes found by WES: characterization in a cross-section of Colombian patients

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Purpose: Epilepsy is one of the most prevalent neurological disorders. About 35% correspond to epileptic syndromes, characterized by early-onset seizures, severe presentation, refractory to treatment and associated to comorbidities. More than 4,000 causal genes have been described, but more than 50% of cases remain undiagnosed. This study aims to describe the diagnostic yield of Whole Exome Sequencing (WES) in cases with a suspicion of an epileptic syndrome, and to describe the potential causal genes.

Methods: Observational study of Colombian patients with a clinical diagnosis of epileptic encephalopathy. WES was performed to find a possible genetic cause within 2 years.

Results: We included 176 individuals (median age of 6 years). We identified predominant generalized seizures in 72.73%, neurodevelopmental delay in 56.82%, and a positive family history of epilepsy in 29.55% of cases. WES diagnostic yield was 21.02%. We found a pathogenic (P) or likely pathogenic (LP) variant in 40 genes, but diagnosis was confirmed in 22. Seven-teen genes had only heterozygous variants but were associated to diseases with an autosomal recessive pattern, without other genetic finding. One case was considered undetermined because it was not confirmed whether the two variants found were in trans. The most frequently affected gene was *SCN1A* (20.0%) associated to Dravet syndrome, followed by *DEPDC5* (7.5%) related to familial focal epilepsy.

Conclusions: Colombian patients with epileptic syndromes can be efficiently diagnosed by WES. Our results are consistent with Dravet syndrome as the most prevalent epileptic syndrome, which can be relevant for access to future therapies.

Conflict of Interest: None declared.

EP10.048 Improving the genetic characterization in a cohort of HSP patients recruited to the 100'000 Genomes Project by combing Whole genome sequencing and novel bioinformatic tools

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Background/Objectives: Hereditary spastic paraplegia (HSP) describes a spectrum of neurodegenerative disorders presenting with progressive lower limb spasticity. So far, diagnosis of complex disorders has been hampered by the need to perform several tests to assess different genetic alterations. We aim to pass over the conventional diagnostic workflow and to improve genetic diagnosis in HSP by combing whole genome sequencing (WGS) approaches with novel bioinformatic tools to detect different variant types.

Methods: WGS data from patients with HSP recruited to the 100'000 Genomes Project were analyzed to detect single nucleotide variants (SNVs), repeats and copy number variations (CNVs), using ad hoc panels. After SNV calling, CNVs in genes from HSP panel were processed by SVRare algorithm. Short tandem repeats (STRs) genotyping was performed using ExpansionHunter. We initially assessed known disease-causing repeats and then naturally occurring repeats in HSP-related genes.

Results: In a cohort of 606 HSP patients, from 507 families, we identified 125 pathogenic or likely pathogenic SNVs in 107 participants (21.1% of the families). CNV analysis on 78 genes from the HSP panel identified 13 CNVs (12 deletions and 1 duplication), while STR analysis identified pathogenic expansions in *ATXN1, C9orf72, HTT* and *TBP*, increasing the diagnostic yield to 24.6%. Finally, we reported on the ongoing STR analysis on HSP-related genes.

Conclusion: The integration of WGS approaches with novel bioinformatic tools considerably increased the diagnostic yield in genetic neurodegenerative disorders like HSP and sped up the ordinary multistep diagnostic assessment. Further analysis is ongoing to identify genetic variants in HSP genes.

Conflict of Interest: None declared.

EP10.049 Further evidence that biallelic nonsense variants in ATL1 cause complicated hereditary spastic paraplegia

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Background: Spastic paraplegia 3A (SPG3A, OMIM# 182600) is the most common type of early onset hereditary spastic paraplegia (HSP) and is mainly caused by monoallelic missense variants in small GTPase *ATL1* (*606439). Autosomal recessive inheritance due to a homozygous nonsense variant has been reported only once[1].

Case presentation: The female patient presented with tetraspasticity, intellectual disability, absent expressive speech, generalized epilepsy and short stature due to growth hormone deficiency at the age of 12 years at our department. There has been a history of early developmental delay, febrile seizures, muscular hypotonia, pes equinus, and steppage gait. Increased muscle tone of hip adductors and calf muscles was noticed at the age of five years. The patient is the first of three daughters of consanguineous parents from Turkey. Neither the parents nor the siblings showed any neurological symptoms. However, short stature was also present in the mother. Trio genome sequencing was performed in the patient and her parents and showed a homozygous nonsense variant (NM_015915: c.656G>A, p.(Trp219*)) in *ATL1*. The variant is close to a previously reported homozygous variant (c.649C>T, p.(Arg217*)) in a family with three siblings affected by early onset HSP and learning disabilities[1].

Conclusion: This is the second family with a biallelic nonsense variant in *ATL1* causing an autosomal recessive complicated SPG3A and adds epilepsy as a further possible phenotypic feature.

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Conflict of Interest: None declared.

EP10.050 Detection of frontotemporal dementia-associated variants in Bulgarian patients using whole genome sequencing

Dragomira Nikolova[1], *Sena Karachanak-Yankova*[1,2], *Dimitar Serbezov*[1], *Marta Mihaylova*[1], *Mikaela Stancheva*[2], *Diana Belejanska*[3], *Mariya Petrova*[3], *Shima Mehrabian*[3], *Latchezar Traykov*[3], *Savina Hadjidekova*[1], *Draga Toncheva*[4]

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Background/Objectives: Frontotemporal dementia (FTD) is a heterogeneous disorder with a challenging diagnosis showing clinical and genetic overlap with other neurodegenerative, as well as psychiatric diseases. Mutations in three genes-*MAPT*, *GRN*, and *C9orf72*-have been proven in FTD etiology. Recent studies have tried to discover other disease loci by genome analyses. We present our findings of variants discovered by whole-genome sequencing (WGS) of Bulgarian FTD patients.

Methods: Equimolar amounts of DNA samples of 50 Bulgarian patients (18 males and 32 females) with confirmed FTD diagnosis have been pooled and sequenced using BGISEQ-500 system with 100x coverage. Our SNP data were screened for 215 FTD-associated variants from DiscGeNet database.

Results: Altogether, 34 out of 215 variants have been found in our WGS data. All detected variants are polymorphic in Europeans. One variant is coding (*TMEM106B* rs3173615), seven are intergenic and the remaining 26 are non-coding. Three SNP variants are reported as pathogenic in FTD in ClinVar database (*GRN* rs9897526, *C9orf72* rs3849942, rs2814707). Genes containing multiple FTD-associated variants are as follows: *CEP131* gene (rs2659030, rs2725391, rs906175, rs9319617, rs969413), *TMEM106B* (rs7791726, rs1990622, rs3173615), *DAPK1* (rs4877365, rs4878104) and *ENTHD2* (rs1048775, rs2255166).

Conclusion: We didn't detect rare DisGeNet FTD-associated variants in our data, neither variants in *MAPT* gene. We confirm the role of *GRN* rs9897526 and *C9orf72* rs3849942, rs2814707 in FTD in Bulgarian patients. Additionally, we add WGS evidence for the role of the other 31 variants in FTD pathogenesis.

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Conflict of Interest: None declared.

EP10.051 The utility of exome sequencing in the seemingly obvious neurological diagnoses

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Background/objectives: Spasticity, ataxia, gait disorders could be part of several different neurological diagnoses with initially similar clinical manifestation. Patients can be led for a long time (even many years) under a different diagnosis that mimics the correct one. Examination by exome sequencing is a crucial tool in such clinically overlapping diagnoses.

Methods: During 2014 - 2022, we examined more than 200 patients with gait disorders and clinical suspicion of hereditary spastic paraparesis or ataxia using exome sequencing.

Results: Four patients were diagnosed with a completely different disease than was the originally suspected. Spasticity, ataxia, gait disturbances were only the primary clinical manifestations of the disease. We diagnosed Wilson's disease (a disorder of copper metabolism), Galloway-Mowat syndrome (a neuro-renal disorder with a risk of kidney failure), Wieacker-Wolff syndrome (X-linked arthrogryposis, manifesting with spasticity in women) and Niemann-Pick disease (neurodegenerative disease). The interval between the first symptoms of the disease to the correct diagnosis was even 14 or 17 years in two cases.

Conclusion: In seemingly clear neurological diagnoses, clinical symptoms may be only the first manifestations of a completely different disease. In the case of such diseases, exome sequencing is an irreplaceable diagnostic method. Moreover, it may concern not only the rare diseases, but also the more common ones - e.g. Wilson disease.

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EP10.052 Unexpected complexity in the molecular diagnosis of spastic paraplegia 11

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Background: Spastic paraplegia 11 is the most common form of autosomal recessive hereditary spastic paraplegia, and is caused by biallelic pathogenic variants in the *SPG11* gene.

Methods: A 37 y.o. female patient with intellectual disability and spastic paraplegia was studied by array CGH, next-generation and Sanger sequencing, transcriptomics and qPCR.

Results: Array CGH revealed a deletion of 179.4 Kb close to the 5′ end of *SPG11*. Gene sequencing revealed the variants c.3143C>T [p.(Thr1048Ile)] (exon 17, initially classified as of uncertain clinical significance) and c.6409C>T [p.(Arg2137*)] (exon 34, classified as pathogenic). Family segregation studies revealed that the deletion and the variant in exon 17 were found in cis in the patient, while the nonsense variant was in trans. Sanger sequencing of cDNA revealed the nonsense variant in heterozygous state, and the variant in exon 17 was not detected, suggesting exon skipping. This hypothesis was subsequently confirmed by transcriptome analysis. qPCR of the patient revealed that expression of both alleles was significantly reduced compared to controls, which is compatible with both alleles being subject to non-sense mediated decay. Considering all results, the variant in exon 17 was reclassified as pathogenic.

Conclusion: In the patient, a molecular diagnosis of spastic paraplegia 11 was reached after the use of several genetic approaches. The nonsense variant and the variant leading to exon skipping in the *SPG11* gene are causative for the observed phenotype, and the contribution of the upstream gene deletion to the patient's phenotype remains uncertain.

Conflict of Interest: None declared.

EP10.053 Application of New generation sequencing technique in identifying genetic factors involved in gender dysphoria

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Background/Objectives: Transgender refers to people whose gender identity differs from the gender assigned at birth. 0.6-1.1% of the general population belongs to transgender men and women. The cause of transgender can include endocrine, neurobiological and genetic factors. There is a connection between sex hormone signaling genes and gender dysphoria (GD), which later causes abnormal brain differentiation. Mutation and methylation changes are different in trans people than in cisgender people. Multiple genetic variants in different genes including long CAG repeat in androgen receptor, a lower number of TA repeats in SRD5A2CA, CA repeats in estrogen receptors (ER) β, and XbaI A/G SNP in ERα have been reported in transgender women or men.

Methods: New developments in the next generation sequencing (NGS) are expected to be effective in identifying genetic factors of GD.

Results: Mutations in the RYR3 gene were identified using Whole Exome Sequencing (WES), a type of NGS, in the Chinese population. Besides, another WES study revealed 21 variants in 19 estrogen receptor-related genes that were involved in bisexual brain development in 30 transgender. These results suggest that estrogen signaling pathway genes are related to bisexual brain development and GD.

Conclusion: These findings show that NGS methods can help to identify genetic factors related to GD. However, the main limitation of genetic studies is the small number of samples participating in the study and the need to investigate the novel variants in a larger number of samples in order to confirm the important genes as the cause of these disorders.

Conflict of Interest: samin ordubadi: None declared, Mahdiyeh asghari azhiri: None declared, shahriyar tolou: None declared, Leila Emrahi Govar Iranian Legal Medicine Organization, asiyeh jebelli Higher Education Institute of Rab-Rashid.

EP10.054 Identification of 16p11.2 microdeletions and microduplications in a cohort of individuals: a 2014-2021 retrospective analysis

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The occurrence of repetitive and unstable chromosomic regions and specific point mutations during meiosis/mitosis can lead to variations in the number of copies (CNVs) in DNA. These may be microdeletions and/or microduplications, which often cause neurodevelopmental disorders (NDD), such as autism spectrum disorder (ASD), language and speech disorders and schizophrenia. To establish the translation between 16p11.2 genomic imbalances and their clinical indications, a statistical study was done.

A total of 439 samples were evaluated by MLPA (SALSA® MLPA® Probemix P343 and P297), between 2014-2021, with suspicion of 16p11.2 CNV imbalance, for several clinical reasons, such as ASD, intellectual disability, NDD and obesity, either for familial or de novo suspicion.

Concerning the total samples (439 cases), 32 (7,3%) were 16p11.2 microdeletions, 15 (47%) of which were de novo, 8 (25%) were inherited and 9 (28%) were of unknown origin; and 12 (2,7%) were 16p11.2 microduplications, of which 11 (92%) were inherited and 1 (8%) was de novo.

The results of our retrospective analysis are in accordance with the literature which refers 16p11.2 microdeletions are more frequent than the reciprocal microduplication and that the majority of microdeletions occur de novo, while microduplications are more frequently inherited. The authors enhance the importance of retrospective studies done by multidisciplinary teams to achieve the best phenotype-genotype possible correlation.

This article was a collaboration with researchers from UMIBIC-BAS-UP/ITR (Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal and ITR-Laboratory for Integrative and Translational Research in Population Health, Porto, Portugal).

Conflict of Interest: None declared.

EP10.055 Neuropsychiatric symptoms in a child affected by White-Sutton syndrome were rescued by anti-epileptic medical therapy and comprehensive developmental-behavioral intervention

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Background/Objectives: White-Sutton syndrome is a neurodevelopmental disorder caused by a pathogenic variant in POGZ. This disorder is characterized by a broad spectrum of cognitive impairment, developmental delay, hypotonia, autism spectrum disorder, and behavioral problems.

Methods: A retrospective medical record review and a longitudinal clinical course were analyzed.

Results: The patient was born to a schizophrenic mother and adopted at three years old without previous antenatal history. At that time, he had significant motor and speech delays. His adoptive parents provided comprehensive developmental intervention, and the patient could subsequently catch up and perform well in his homeschool program. He presented with neurodevelopmental regression at seven, especially in reading and speaking. Meanwhile, his neurological and autistic-like symptoms commenced, i.e., frequent eye staring without consciousness, gait instability, decreased eye contact, declined social interaction, and expressing fear more than usual. The brain MRI revealed a mild hippocampal abnormality and an inferior vermian hypoplasia. The electroencephalogram exhibited a frequent spike wave over the right temporal region. His WISC-V scores were very to extremely low in all components. Whole-exome sequencing revealed a heterozygous nonsense pathogenic variant in POGZ. One year after treating with Levetiracetam and comprehensive developmental-behavioral intervention, his neuropsychiatric symptoms reversed to his baseline status. His full-scale IQ was then measured at the borderline. Finally, the patient could comprehend four languages and develop excellent social engagement.

Conclusion: We report neuropsychiatric findings in a patient with White-Sutton syndrome that can be rescued by anti-epileptic medical therapy and comprehensive developmental-behavioral intervention.

Grant References: None.

Conflict of Interest: Chanikhan Sattaporn: None declared, Ajchara Trachoo Part-time consultant child and adolescent psychiatrist at Samitivej Srinakarin Hospital, Share holder at Leader Medical Genetics and Genomics, Co., Ltd., and Genome Star, Co.Ltd., Objoon Trachoo Part-time consultant clinical geneticist at Samitivej Sukhumvit, Samitivej Srinakarin, Bumrungrad, BNH, Phyathai 2, Jetanin, and Thonburi Bumrungmuang Hospitals, Shareholder of Leader Medical Genetics and Genomics, Co., Ltd., Genome Star, Co., Ltd., Gene Option, Co., Ltd., and Sofiva Bangkok, Co., Ltd., Consultant at N-Health and Biomolecular Laboratories.

EP10.057 Novel pathogenic variants identified in TRAPP genes in families with Neurodevelopmental Disorders

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Novel pathogenic variants identified in TRAPP genes in families with Neurodevelopmental Disorders

Background/Objectives: Transport Protein Particle (TRAPP) complex plays a pivotal role in ER-to-Golgi transport and contributes to the regulation of multiple membrane trafficking pathways. The precise interaction of different proteins between cellular compartments is fundamental to conducting specialized functions. In humans, TRAPP proteins fall under two related complexes: TRAPP II and TRAPP III. While TRAPP proteins are all related, it is evident that some proteins function independently of the complex. As a result, variants in the genes that encode these proteins are associated with a spectrum of human diseases, including an emerging set of disorders known as TRAPPopathies, which comprise of Neurodevelopmental Disorders (NDDs).

Methods: This study used a large-scale, genotype-first approach where 33 causal candidate variants were found in 41 patients with undiagnosed rare NDDs, some of which have been collected as part of the SYNAPS project. Exome sequencing data was screened in 14 known TRAPP genes linked to NDDs.

Results: Seven novel homozygous variants were identified in TRAPPC6B c.267+3A > G, c.445+5G > T, TRAPPC9 c.676T>G (p.Tyr226Asp), c.1351G>A (p.Gly451Ser), c.2279-3C > G and TRAPPC11 c.560+3A > T, c.373A>G (p.Arg125Gly) that show evidence of pathogenicity with some of them confirmed by Sanger sequencing.

Conclusion: This study provides valuable genomic data for future functional studies to better understand the role of the TRAPP complex in membrane trafficking pathways and how pathogenic variants in TRAPP genes can lead to NDDs. By developing our understanding of the pathogenic mechanisms involved, this will aid in development of potential therapeutic targets.

Conflict of Interest: Rahema Mohammad Full-time Employment, Reza Maroofian Full-Time Employment, Henry Houlden Yes, PI

EP10.058 De novo heterozygous variant in KIF13B gene in a patient with bilateral periventricular nodular heterotopia

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Objective: Gray matter heterotopia (GMH) are rare cortical malformations with a broad spectrum genetic anomalies, including sequence variants in kinesin genes. Kinesin proteins (KIFs) play essential roles in cell migration and primary cilia function. We report on a 4-year-old girl with bilateral periventricular nodular heterotopia (PVNH) and epilepsy with a deleterious variant in *KIF13B*.

Material and methods: The patient belongs to a larger group (32 patients) with various types of GMH. General clinical and MRI investigations and chromosomal microarray were performed for all children. WES testing was done using NextSeq550 equipment for selected patients and their parents.

Results and discussion: Bilateral PVNH was detected by fetal ultrasound and confirmed postnatally by brain MRI. At the age of 23-month-old she presented with mild developmental delay and generalized tonic-clonic seizures, with good therapeutic response. WES trio analysis revealed a de novo variant with deleterious in silico predictions in *KIF13B* gene (NM_015254.3: c.4072C>A; p.Arg1358Ser). No relevant genomic imbalances were detected. There are few reports of deleterious variants in *KIF13B* associated with PVNH. Recent studies on animal models showed that KIF13B interacts with Myosin X, being involved in cell adhesion and migration.

Conclusions: We propose *KIF13B* as a candidate gene for the PVNH phenotype identified in our patient.

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Acknowledgment: Regional Center of Medical Genetics Dolj, Emergency-County-Hospital Craiova for Illumina NextSeq550 access and technical support.

Conflict of Interest: None declared.

EP10.059 Homozygous Frameshift Mutation In Ceruloplasmin Gene That Causing Neurodegeneration, Dementia With Diabetes In A Turkish Man: A Case Report

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Objective: The ceruloplasmin gene encodes the copper transport protein that involved in angiogenesis and neovascularization. Here we report a 60-year-old male with clinical findings of neurodegeneration, demantia and diabetes that caused by frameshift point mutation in ceruloplasmin gene.

Case: The current proband case has some neurodegenerative clinical charactheristics of advanced dementia and progressive cerebellar ataxia for the past three years. Clinical examination was also revealed anemia, dysarthria, torticollis, choreic involuntary movements in the respiratory muscles and hyperreflexia of his extremities. Blood analysis revealed microcytic hypochromic anemia, diabetes mellitus, and decreased copper content in serum and urine. The serum ferritin concentration was high. In the MRI study, there was low intensity on images of the cerebellum, thalamus, dentate nucleus of the putamen and caudate nucleus, and liver. There was no first degree familial history for the similar symptoms in the pedigree diagram.

Method: Blood-EDTA sample were used for exome profilling after pedigree analysis for the proband case.

Results: Homozygous pathogenic frameshift point mutation of pNM-000096.3:c.1173del p.(Asp391Glufs*8) was detected in CP gene with whole exome sequencing.

Discussion: De novo frameshift point mutation in the ceruplasmin gene in our case; early-onset diabetes has led to a dramatic neurodegenerative process, plus sleepiness and behavioral disturbances. The patient's first clinical finding was diabetes, which started at the age of 40. His neurodegenerative symptoms then progressively began, along with lethargy and behavioral problems. We emphasize the importance of careful consideration of detailed clinical examination and questioning of consanguineous marriage in the diagnosis of late-onset disorders.

Conflict of Interest: None declared.

EP10.060 Genetic evaluation of dystonia

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Background: Dystonia is a clinically and genetically heterogenous movement disorder which occurs in isolation, in combination with other neurological symptoms (combined) or in association with other systemic signs (complex). The current diagnostic approach proposed by the expert group of the The European Reference Network for Rare Neurological Diseases (ERN-RND) focuses on syndromic evaluation and classification based on two axes, clinical features and aetiology. The aim of this study was to evaluate the clinical characteristics and diagnostic process of patients with dystonia.

Methods: A retrospective observational cohort study of patients with dystonia referred to our institution. Clinical characteristics were obtained from relevant medical records.

Results: Between 2008 and 2022, 152 patients with dystonia were referred to our institution. Majority were women (57 %) and there was a slight predominance of patients with an early onset (53 %). The distribution of dystonia was most commonly generalized (40 %). Additional neurological features most commonly included ataxia, pyramidal signs, mioclonus and parkinsonism. Next-generation sequencing was performed in 127 patients and identified pathogenic or likely pathogenic variants in 12.6 % of these patients, involving 16 different genes *UFM1, NPC1, SPEN, THAP1, ADNP, MPZ, PANK2, TSEN54, TBK1, CHD8, FGF14, SLC2A1, YY1, GNB1, KMT2B* and *GNAO1*, as well as two chromosomic microduplications. Molecular-genetic tests for a three base-pairs deletion in *TOR1A* (c.907_909delGAG, DYT1) were performed in 24 % of patients.

Conclusion: According to the current recommendations for the diagnostic evaluation of dystonia, we identified a likely genetic aetiology in 12.6% of patients.

Conflict of Interest: None declared.

EP10.061 Rare copy number variation in autism spectrum disorders

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Background: Rare copy number variants (CNVs) have been associated with a wide range of human disorders. Among neurodevelopmental conditions, autism spectrum disorders (ASDs) have an important genetic background, with high impact, rare CNVs being detected in ~ 5-10% of individuals. We report the results of array-based genomic comparative hybridization (array-CGH) screening for rare CNVs in a group of 303 children with ASDs.

Methods: A diagnostic of ASD was established for all individuals included in the patient group. The clinical workup included neurological, psychiatric, and psychological evaluation. Array-CGH was performed for all ASD individuals and a similar number of neurotypical children, using 4 × 180K SurePrint G3 Human CGH microarray (Agilent Technologies).

Results: Rare variants, defined as <1% from the total sample were detected in 241 ASD individuals. Out of 487 rare CNVs, 349 overlapped at least one exon of a coding gene, duplications exceeding the deletions (187 vs 162). Twenty-seven clinically relevant CNVs were identified in 26 children, encompassing pathogenic and likely pathogenic variants. Most of these patients had complex phenotypes which included global developmental delay/moderate or severe intellectual disability, dysmorphic features, epileptic seizures, and congenital heart malformations.

Conclusions: Deep phenotyping and molecular characterization of new groups of ASD individuals add to the existing body of knowledge regarding the contribution of rare CNVs to ASD pathomechanisms.

Funding: The research leading to these results has received funding from the EEA Grant 2014-2021, under the project contract No 6/2019.

Conflict of Interest: None declared.

EP10.062 Natural History of NARS1 Associated Neurodevelopmental Disorder

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Background: Aminoacyl tRNA synthetase (ARS) is a group consisting of essential enzymes for protein translation that attach amino acids to tRNA. De novo and bi-allelic mutations in NARS1, the gene encoding asparaginyl-tRNA synthetase, have recently been associated with a neurodevelopmental disorder. The phenotypic variability of NARS1-associated disease is vast, yet not thoroughly explored. We aim to expand the initially described cohort with new cases and describe the natural history of the disease via a systematic literature review.

Methods: Patients are recruited through the SYNAPS study group at UCL as well as the Rory Belle Foundation and natural history data is collected through Rare-X platform. This study was approved by the institutional ethics committees of University College London and participating centres. The clinical and radiological phenotype, along with the genotype, are presented.

Results: We identified 10 novel affected individuals with bi-allelic or de novo variants in NARS1 who exhibited global developmental delay, microcephaly, gait abnormalities and seizures. The majority of individuals presented with generalised tonic-clonic seizures and the onset was in infancy. Early-onset epilepsy due to variants in the gene shared phenotypic features of epileptic encephalopathy and was characterized by a more severe disease course and poorer survival compared to the other NARS1-associated phenotypes. We also found that demyelinating neuropathy is a cardinal feature of early-onset NARS1-associated disease, either isolated or in combination with central nervous system disease.

Conclusion: We aim to delineate further the clinical features and the course of the disease associated with NARS1 variants.

References:

Grants: Wellcome Trust.

Conflict of Interest: None declared.

EP10.063 Predictors of low efficiency of WES in patients with epilepsy

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Background/Objectives: To determine the predictors of WES inefficiency in epilepsy diagnostic.

Methods: Analysis of clinical data and WES results of 324 patients with epilepsy.

Results: The general group consisted of 51 adults (15.8%) and 272 (84.2%) children, mean age 9.8 ± 10.0 (0.1 ÷ 55.2), sex ratio M(1.0) : W(1,1). The genetic cause of the disease was not detected in 119 (36.7%) cases, and was identified in 68 (21.0%) cases, of which: 9 (13.5%) - CNV, 59 (86.8%) - SNV (28 of them has not been previously described). In 71.2% - variants with a dominant inheritance in 28 genes (in 23.8% in the SCN1A gene); 17.2% - recessive (ADAM22, ARSA, DOCK7, DPYS, KCTD7, KIAA1109, MTHFR, PAH, PIGO, TPP1), 9.4% - X-linked (CDKL5, FLNA, MECP2, PCDH19). In 137 cases (42.3%) – VUS, of which 16 (4.9%) - in genes with incomplete penetrance, inherited from parents. Among patients with an established genetic cause of epilepsy, the proportion of cases with the onset of seizures before 3 years (89.1%) and neuropsychiatric disorders (57.4%) is higher compared to the group with a negative result of DNA diagnostics: 51.2% ($p < 0.001$, $OR = 7.795$; 95%CI 0.483 ÷ 3.023) and 25.2% ($p < 0.001$, $OR = 3.990$; 95%CI 2.116 ÷ 7.522), respectively. The proportion of adolescents and adults is higher in the group with an unknown cause of epilepsy - 48.7% than among patients with a DNA-verified diagnosis - 8.8% ($p < 0.001$).

Conclusion: We can expect a low WES efficiency in adolescents and adults with epilepsy and normal cognitive abilities and the onset of epileptic seizures after the age of 3 years.

Conflict of Interest: None declared.

EP10.064 Dynamic Whole-Exome Sequencing (WES) analysis in a large pediatric cohort of patients with neurodevelopmental disorders and epilepsy

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Background: Over the past decade, our understanding of genetic of epilepsy is rapidly increasing thanks to the availability of high-throughput technologies. Here we report our experience in an Italian Maternal-Children Hospital.

Methods: After a multidisciplinary clinical evaluation (including electroclinical data, neuroimaging, cognitive and behavioural test, dysmorphological data acquisition), patients were recruited for WES.

Results: Since 2019, 70 patients with neurodevelopmental disorders and epilepsy were analysed by WES. In about 50% of the cases a definitive molecular diagnosis was achieved; additionally, in 15% of patients a possibly causative variant in a candidate gene was identified.

Here, we report three cases of particular interest:

1. In a five-year-old boy with autistic spectrum disorder and epilepsy, a pathognomonic EEG drove the molecular analysis. Indeed, we identified a novel, de novo, pathogenic variant (c.3156del, p.(Ser1053Alafs*24)), within *SYNGAP1* gene.
2. In a three-year-old boy with epilepsy, Duane anomaly, vesicoureteral reflux and regression WES highlighted the novel hemizygous likely pathogenic variant c.490C>T, p.(Ser164Pro), in the *PIGA* gene. Regression has been reported only once, therefore, our findings could confirm the expansion of *PIGA*-associated phenotype.
3. In a two-year-old boy with early onset drug-resistant epilepsy, two de novo variants in *SCN1A* (c.695-1G > A) and *CSNK2B* (c.384_394delAGGTGAAGCCA) genes were identified, providing a dual molecular diagnosis and thus highlighting the complexity of molecular data interpretation.

Conclusions: Overall, the high diagnostic yield reached highlights the relevance of a multidisciplinary approach and emphasize the importance of a molecular diagnosis in the comprehensive management of patients, towards a precision follow-up, therapy and family counselling.

Conflict of Interest: None declared.

EP10.066 Next generation sequencing gene panels in children with epilepsy: diagnostic yield and clinical utility

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Background/Objectives: Epilepsy is a frequent neurological condition among pediatric population, having a broad phenotypic spectrum. The advancement in genomic technologies led to the identification of an increasing number of genes involved in epilepsy. We assessed the diagnostic yield and impact of next generation sequencing (NGS) gene panel testing for children with epilepsy.

Methods: We performed a single-center retrospective observational study on a cohort of Romanian children with variable types of seizures, with or without other neurological conditions, between the years 2019-2022. Patients were tested using different NGS multigene panels with a number of genes ranging from 146 to 894.

Results: In total, 89 patients aged 0-19 years (56% males, 44% females) were included in the analysis. Pathogenic variants or likely pathogenic variants were identified in 15 of them, corresponding to a diagnostic yield of 16.85%. The positive results were reported in 14 different genes, SCN1A being the most frequent. Variants of uncertain significance (VUS) were reported in 93% (83/89) of patients. Familial screening of VUS offers additional diagnostic yield.

Conclusion: The information gathered in this study shows high genetic heterogeneity of epilepsy and highlights the importance of genetic testing. NGS-based gene panels contribute to the diagnosis of epilepsy, lead to a better understanding of the pathogenicity of the disease and opens the possibility of a precise and personalized treatment.

Conflict of Interest: None declared.

EP10.067 Association of the APOE gene's rs429358 variant with Alzheimer's disease and Unspecified Dementia but not with Frontotemporal Dementia in Bulgarian patients

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Introduction: Apolipoprotein E (*APOE*) rs429358 variant is a landmark pro "risk" factor for Alzheimer's disease (AD). The less common forms of dementia such as frontotemporal dementia (FTD), and in particularly unspecified dementia (UD), have not yet been ascertained sufficient number of genetic risk variants.

Materials & Methods: Whole-genome sequencing was performed on three DNA pools, set up with DNA isolated from 49 AD patients, 50 FTD patients and 50 UD patients, and whole-exome sequencing was performed on a DNA pool set up with DNA from 61 Bulgarian healthy control individuals. The allele frequency ratios of rs429358 between the AD, FTD and UD patient pools, and the control pool were analyzed.

Results: Our analysis establishes significant difference in the frequency of the rs429358 allele between the AD patients and controls ($OR = 0.25$, $p = 0.02$), and UD patients and controls ($OR = 0.24$, $p = 0.01$), but not between FTD patients and controls ($OR = 0.36$, $p = 0.13$).

Conclusions: In addition to its well established cause for the development of AD, our results indicate that the rs429358 variant of the *APOE* gene might also play role in the etiology of UD in Bulgarian patients. These findings should be further studied in individual DNA samples of patients with unspecified dementia.

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Conflict of Interest: None declared.

EP10.068 A novel mutation in CDK5RAP2 gene causes primary microcephaly with mild intellectual disability, short stature and neurosensorial signs

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Intellectual disability (ID) often co-occurs with other neurologic phenotypes making molecular diagnosis more challenging particularly in consanguineous populations. In this study, we rapport the phenotype of a patient, born to Tunisian consanguineous family, addressed for ID, microcephaly and short stature. A clinical exome sequencing was performed for the proband. We identified a homozygous variant (p.Lys189ArgfsTer15) in the *CDK5RAP2* gene associated with a primary hereditary microcephaly (MCPH), mixed with hearing loss, short stature, hypothalamic, retinal and cochlear developmental defects. Our research reveals the wide phenotype expressivity of the *CDK5RAP2* mutants and expand the spectrum of its neurosensorial defects. The complexity of the genetic causes of microcephaly/ID is highlighted in our study, as well as the effectiveness of exome sequencing in making a precise diagnosis and enhancing patients' care and follow-up.

Conflict of Interest: None declared.

EP10.070 Convergence of bipolar disorder treatments and gene knockdown on the transcriptome

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Background/Objectives: Antipsychotics are a mainstay of bipolar disorder (BD) treatment, though how the drug mechanisms relate to BD disease etiology is poorly understood. We have conducted an analysis into the convergent transcriptomic effects of four antipsychotics used to treat BD (chlorpromazine, clozapine, risperidone, and ziprasidone) with publicly available data from the LINCS L1000 Perturbagen database. Furthermore, we created knockdown iPSC-derived neuronal cell lines for three genes with a downregulatory association with BD (*FURIN*, *MMD*, *VPS45*) to investigate their effects on pathways affected by antipsychotics.

Methods: Convergent gene expression across antipsychotics was identified using Stouffer's Z-score, and pathway enrichment was performed using gProfiler2. Knockdown iPSC lines for genes of interest were created using CRISPRi and differentiated into NPCs followed by glutamatergic neurons. Differentiation was validated by neuronal and pluripotent marker staining (Nestin, Tuj1, Sox2). Knockdown was confirmed by qPCR. Transcriptomic effects of gene knockdown will be analyzed via bulk RNA-sequencing.

Results: Antipsychotic drug treatment resulted in upregulated genes enriched in extra-cellular exosomes (q = 9.21E-5), extra-cellular vesicles (q = 1.14E-4), and extracellular organelles (q = 1.15E-4) pathways in neurons. Knockdown of the genes of interest has been observed (*FURIN* RQ = 0.526, *MMD* RQ = 0.266, *VPS45* RQ = 0.003), and marker staining has demonstrated successful differentiation to NPC and neuronal stages.

Conclusion: Treatments for BD are not generally targeted towards BD biology. These results help elucidate potential transcriptomic mechanisms, such as extracellular pathways upregulated by current antipsychotic treatments. Better understanding of underlying cellular dysfunction could lead to new treatment avenues in BD.

Grant References: Foundation grants - Canadian Institutes of Health Research.

Conflict of Interest: None declared.

EP10.073 SLC9B1 germline mutation in a patient with septo-optic dysplasia

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Background: Septo-optic dysplasia is a clinically heterogeneous disorder traditionally defined by three characteristic features: hypoplasia of the optic nerves, abnormal formation of structures along the midline of the brain and pituitary hypoplasia. From the genetic point of view, it is also a very heterogeneous entity. The aim of this study was to identify the genetic cause of one case of septo-optic dysplasia by whole exome sequencing (WES).

Methodology: Genomic DNA was extracted from peripheral blood leukocytes in a patient with septo-optic dysplasia clinical diagnosis. Her mother is also affected and her son has been diagnosed with a focus of cortical dysplasia. WES was performed to identify germline mutations. 140 genes previously associated with septo-optic dysplasia were analyzed, including *HESX1*, *OTX2*, *SOX2* and *SOX3*. After that, we studied the rest of the variants using bioinformatic tools, in silico analysis and a bibliographical revision.

Results: We did not find any variants in 140 genes previously associated with septo-optic dysplasia. However, we identified two heterozygous mutations in *SLC9B1* gene: c.1234C>T;p.Arg414Ter and c.1318A>T;p.Lys440Ter, both classified as variants of uncertain significance in Varsome and with low population frequency. This gene codifies a transmembrane protein associated with Wolfram Syndrome-2, a neurodegenerative disorder. Familiar study is ongoing. Since the inheritance pattern of this disorder may be autosomal dominant or recessive the familiar study is especially interesting.

Conclusion: *SLC9B1* mutation has been associated with septo-optic dysplasia for the first time. WES is a useful tool to determine the genetic cause of rare diseases and identify new genes involved.

Conflict of Interest: None declared.

EP10.075 Multimorbidity between somatic diseases and neuropsychiatric disorders reflected in their underlying genetics

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Background/objectives: Psychiatric disorders and insulin-related somatic conditions frequently co-occur. Genome-wide genetic correlation analyses indicate shared genetic etiologies between them. Here we aimed to investigate which shared genetic factors underly the observed multimorbidity and if a genetic link exists with brain morphometry.

Methods: We used genomic structural equation modelling to investigate the genetic factor structure underlying genetically correlated psychiatric disorders (ADHD, anorexia nervosa, OCD, major depressive disorder, schizophrenia) and insulin-related somatic conditions (metabolic syndrome, obesity, diabetes mellitus type 2) using the largest GWAS summary-statistics available (N = 10K–898K). We performed a multivariate GWAS on latent factors with loadings from both psychiatric and somatic phenotypes and performed genetic correlation analyses with brain morphometry (n = 29K-38K) using LD score regression.

Results: Factor analyses revealed a two-factor solution with excellent fit (X2(15) = 53.4; AIC = 95.4; CFI = 0.987; SRMR = 0.050). Factor F1 was loaded by psychiatric disorders (all except schizophrenia) and all insulin-related conditions. Factor F2 was loaded only by all psychiatric phenotypes. The multivariate GWAS of factor F1 showed 150 genome-wide risk loci and significant rg with total brain (rg = -.151;P = 1.43E-08), inferior-temporal (rg = -.188;P = 1.09E-07), middle-temporal (rg = -.112;P = 4.77E-04), banks of the superior-temporal sulcus (rg = -.150;P = 4.99E-05), isthmus cingulate (rg = .146;P = 1.64E-04) and lateral occipital (rg = .177;P = 5.81E-05) surface areas.

Conclusion: In this study, we explore the genetic architecture of genetically correlated psychiatric and insulin-related somatic conditions. We identified a latent factor reflecting a shared psychiatric-somatic liability that genetically correlates with specific brain areas. These findings advance our understanding of the shared genetic etiology underlying psychiatric–insulin-related somatic multimorbidity.

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EP10.076 Phenotypic and genomic insights into disorders with deficient myelination in the Indian population

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Background: Several genetic disorders are associated with either a permanent deficit or a delay in central nervous system (CNS) myelin deposition. This ongoing study aims to investigate the clinical and genomic spectrum as well as underlying pathomechanisms of deficient CNS myelination.

Methods: Clinical and radiological evaluation was followed by either targeted and/or genomic testing. Validation of the copy number variants was done using qPCR.

Results: Our cohort includes 34 unrelated families (38 individuals) recruited from October 2019 till date. Overall, twenty-seven disorders were identified in 33 families (97%) who received a genetic diagnosis. Variants were found in *PLP1*, *GJC2*, *GRIN2B*, *HEXA*, *DEGS1*, *FUCA1*, *ATP7A*, *SOX10*, *TUBB4A*, *EPRS1*, *PEX13*, *PEX16*, *PEX26*, *POLR1C*, *NKX6-2*, *RNASEH2C*, *VARS*, *TREX1*, *SETBP1*, *TRAPPC4*, *TRAPPC12*, *MTHFR*, *ZBTB18*, *TPRKB*, *PIGV* and *OCNL*. Significant findings of the current study include (i) Twenty (58%) of 36 disease-causing variants are novel (ii) Deficient myelination was observed as a significant and novel finding in *GRIN2B*-related early infantile epileptic encephalopathy, Menkes disease, pseudo-TORCH syndrome 1, *ZBTB18*-associated intellectual developmental disorder and in an individual harboring a pathogenic 1.412 Mb triplication (iii) Report of extremely rare disorders with variants in *TPRKB, TRAPPC12* and *EPRS1*.

Conclusion: The present study represents the first systematic cohort of disorders of hypomyelination from the Indian population. Molecular diagnosis could be achieved in most individuals in our cohort and is comparable to previously reported yield.

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Conflict of Interest: None declared.

EP11 Neuromuscular Disorders

EP11.001 A novel variant in kinesin family member 5A (KIF5A) causing late-onset hereditary spastic paraplegia type 10 (SPG10)

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Background/Objectives: The kinesin family member 5A (*KIF5A*) gene encodes a neuronal kinesin heavy chain that serves as a molecular motor of microtubule dynamics. It is known that mutations in this gene have been associated with several neurodegenerative disease, including autosomal dominant spastic paraplegia 10 (SPG10). We have identified new heterozygous missense mutation in *KIF5A* from the patient who was diagnosed with spastic paraplegia.

Methods: Genomic DNA was isolated from peripheral blood of the patient and the library was prepared using the Ion Ampliseq Library kit. Raw data from Torrent Suite sequencing runs were processed using Ion Reporter. Direct sequencing was performed and the sample was loaded on the ABI Genetic Analyzer. Three databases were used to predict potentially damaging effects due to amino acid changes.

Results: Through whole-exome sequencing, we found a heterozygous missense mutation in which the guanine base at c.691 of *KIF5A* (NM_004984) was replaced with an adenine, which causes the 231st amino acid valine of the protein to be replaced by methionine. Both parents are normal for this mutation. The 231Val resides within the motor domain of *KIF5A* and is conserved as valine in various species. This variation is predicted to be 'pathogenic' by the three prediction programs.

Conclusion: We have described a novel Val231Met mutation in *KIF5A* in a patient expressing the phenotypical characteristics of SPG10. Our data suggest that the *KIF5A* missense variant of SPG10 loses its ability to activate ATPase processes and thus impairs microtubule motility.

Conflict of Interest: Kyung Min Kang full time, Minyeon Go: None declared, Hyun Min Park: None declared, Ji Eun Park full time, Hyunjin Kim full time, Hee Yeon Jang: None declared, So Hyun Yang full time, Jong Chul Kim full time, Sung Han Shim: None declared.

EP11.003 Antisense oligonucleotide induced pseudoexon skipping and restoration of functional protein for Fukuyama muscular dystrophy caused by a deep-intronic variant

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Fukuyama congenital muscular dystrophy (FCMD) is an autosomal recessive disorder caused by *Fukutin* (*FKTN)* gene mutations. FCMD is the second most common form of childhood muscular dystrophy in Japan, and the most patients possess a homozygous retrotransposal SINE-VNTR-*Alu* (SVA) insertion in the 3′-untranslated region of *FKTN*. A deep-intronic variant (DIV) was previously identified as the second most prevalent loss-of-function mutation in Japanese patients with FCMD. The DIV creates a new splicing donor site in intron 5 that causes aberrant splicing and the formation of a 64-base pair pseudoexon in the mature mRNA, resulting in a truncated nonfunctional protein. Currently, there is no radical therapy for FCMD patients carrying the DIV, and they present more severe phenotype. In the present study, we describe in vitro evaluation of antisense oligonucleotide mediated skipping of pseudoexon inclusion and restoration of functional FKTN protein. In total sixteen 19-26mer antisense oligonucleotide sequences were designed in 2′-O-methyl backbone and screened in patient-derived fibroblasts, lymphoblastoid cells and minigene splice assay. Our results showed one antisense oligonucleotide targeting the exonic splice enhancer region displayed significantly induced the pseudoexon skipping and increased the expression of normal mRNA. It also rescued FKTN protein production in lymphoblastoid cells and restored functional *O*-mannosyl glycosylation of alpha-dystroglycan in patient-derived myotubes. These outcomes thus suggest that antisense oligonucleotide based pseudoexon skipping can be a potential treatment for FCMD patients carrying the DIV. This research was supported by grants from the Japan Agency for Medical Research and Development (18ek0109318h0001, 20ek0109405h0002, 18ek0109249h0002), Grants-in-Aid for Scientific Research (18K07790).

Conflict of Interest: None declared.

EP11.004 Multiomics is needed to increase the detection rate of myopathy patients

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Inherited muscle diseases have an estimated prevalence of nearly 65/100,000. As for many other Mendelian diseases, targeted gene panels are a routine diagnostic test for patients with inherited muscle diseases. However, many tested patients remain unsolved, and a periodical reanalysis of existing data is suggested to increase the detection rate.

This study aimed to find elusive causative variants in patients with neuromuscular disorders who are still missing a molecular diagnosis, focusing on pathogenic/likely pathogenic variants (P/LP) in Varsome and variants predicted to affect the splicing by SpliceAI.

We re-analyzed data collected between 2014 and 2016 of 1084 patients who had been analyzed using a targeted gene panel, MYOcap, covering over 300 genes that are known or suspected to cause skeletal muscle diseases. We used an in-house updated bioinformatic pipeline, including splicing predictor tools and a variant classification based on the ACMG/AMP guidelines, to re-annotate the previously identified variants. We focused on P/LP variants (as predicted by Varsome) and on variants affecting the splicing (SpliceAI threshold >= 0.50).

We re-classified eight variants, and the detection rate of solved cases increased by 0.83%. We also identified many variants predicted to alter the splicing.

The observed modest increase in the detection rate and the high number of variants needing further evidence for a proper classification suggests that additional data, such as exome or genome, transcriptomic and proteomic data, but also segregation and functional data, should be considered to increase the detection rate further.

Grant References: Academy of Finland, Samfundet Folkhälsan, University of Helsinki.

Conflict of Interest: None declared.

EP11.005 Genetic testing algorithm in females with elevated creatinkinase

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Background/Objectives: Establishing the cause of elevated CK values is difficult, especially in young females. In rhabdomyolysis, CK values are found persistent increased along with transaminases, without significant liver injury.

Methods: We evaluated a group of 7 females, with 2 with positive family history for limb girdle muscular dystrophy(LGMD) and age ranging from 1-16 years, referred to the genetics department with persistent elevated AST, ALT and CK values. Sequencing was performed using TSOne panel (Illumina).

Results: Transaminases ranged from 1.5–7 times the upper value of normal range and CK ranging 11–40 times the upper value of normal range. Mean (standard deviation) values for AST/ALT, CK/AST and CK/ALT ratios were 1.1(0.3), 36.1(9.5), respective 37.9(6.2). All female patients were suspected of muscular dystrophy. Sequencing results confirmed LGMD for 4 patients, of whom 2 had pathogenic homozygous variants CAPN3_NM_000070.3:c.1342C>T and CAPN3_NM_173087.1:c.550-delA confirming LGMD type 1; one with likely pathogenic compound heterozygote in FKRP_NM_024301.4:c.148A>G and NM_024301.4:c.985G>A confirming LGMD type 5; one compound heterozygote in LAMA2 with one likely pathogenic NM_000426.4:c.7440-2A > G and one variant of uncertain significance NM_000426.4:c.2448_2450del, that might be associated with LGMD type 23. The remining 3 were found to be carriers of 3 different hemizygous pathogenic variants in DMD gene: NM_004006.2:c.5209C>T, NM_004006.3:c.9661C>T and NM_004006.2:Deletion(Exon 54).

Conclusion: Following persistent transaminase and CK values in young females, genetic investigations confirmed carrier status for disease causing variants in DMD genes in patients without family history, but with implications in medical follow-up and family planning and homozygote status or compound heterozygote for several genes involved in LGMD.

Conflict of Interest: Costela Lacrimioara Serban "Victor Babes" University of Medicine and Pharmacy, Adela Chirita-Emandi "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania, Maria Puiu "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania.

EP11.006 Structural variations affecting the DMD gene detected by whole genome sequencing cause Duchenne muscular dystrophy in two girls

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Dystrophinopathies, comprising Duchenne and Becker muscular dystrophies (DMD and BMD), are the most common neuromuscular diseases in children, with an incidence of 1 in 5000 male births. In women, the manifestation of Duchenne muscular dystrophy is rare due to X-chromosomal inheritance. Here, we present two young girls with muscular dystrophies, CK values > 10000 U/l, severe muscle weakness and mosaicism of dystrophin staining reaction in the skeletal muscle tissues. The discovery of an almost completely skewed X-inactivation in both cases strengthened the suspicion of a dystrophinopathy. However, standard molecular diagnostics (including MLPA and NGS gene panel sequencing) have so far failed to identify the genetic cause of the girls' diseases. Using whole genome sequencing, two reciprocal translocations between their X chromosomes and different autosomes could be detected. In both cases, the breakpoints on the X chromosomes are directly located in the *DMD* gene (in introns 54 and 7, respectively) and are therefore causative for the phenotypes of these patients. These results could be confirmed by additional methods. These cases show, on the one hand, that detailed clinical data, especially confirmed by histopathological analysis, are important to inform about the putative underlying genetic disease and, on the other hand, how time-saving and efficient it can be to find genetic causes of complex genetic constellations in DMD, e.g. by whole genome sequencing.

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Conflict of Interest: None declared.

EP11.007 Circulating microRNAs in extracellular vesicles as skeletal muscle biomarkers in Fukuyama muscular dystrophy

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Fukuyama congenital muscular dystrophy (FCMD) is a severe, childhood-onset muscular dystrophy especially seen in Asian countries. Our group has discovered a potential radical treatment using antisense oligonucleotides and clinical trial has just started. Therefore, reliable serum biomarkers are essential to monitor the progression of the disease or the efficacy of the treatment. Many types of circulating miRNA, such as miRNA-1, -133a, and -206, known as myomiRs have been reported as candidate serum markers for monitoring muscular dystrophies. However, there have been few reports on biomarkers that are specific to FCMD. In this study, we aimed to identify FCMD-specific miRNA markers by collecting extracellular vesicles (EVs) from serum samples of FCMD patients and healthy controls. We generated more specific, reliable methods to extract microRNAs from extracellular vesicle (EV), by using immunoprecipitation methods from patients' serum samples. High throughput miRNA sequencing detected several candidates for FCMD specific miRs, including miR-206 and miR-1. The expression of level miR-26 was upregulated in FCMD patients compared to healthy controls (2.28-fold, 2-ddCt method), and the difference was statistically significant. Our study demonstrates that miRNA analysis on EVs collected by immunoprecipitation can identify miRNAs whose expression levels are specifically increased in FCMD patients. This result suggests that the immunoprecipitation method is effective for miRNA analysis and may pave the way for the development of a novel FCMD diagnosis. This work was supported by the Japan Agency for Medical Research and Development (ek0109249h and ek0109456h).

Conflict of Interest: None declared.

EP11.008 CHN1, a new candidate gene causing Moebius syndrome

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Background/Objectives: Moebius syndrome (MBS) is a rare congenital neurological disease characterized by non-progressive facial palsy and impairment of ocular abduction, due to uni or bilateral paralysis or weakness of the facial and abducens cranial nerves. Other abnormalities may include paralysis of other cranial nerves, lingual hypoplasia, sensorineural hearing loss, craniofacial malformations and abnormalities of the extremities. Both intrauterine environmental factors and genetic causes have been proposed to cause MBS. Until now only two genes, *PLXND1* and *REV3L*, have been identified to cause MBS.

Methods: Whole-exome sequence was performed on a 9-year-old male, clinically and radiologically diagnosed with MBS, presenting facial palsy, bilateral Duane retraction syndrome (DRS), microglossia and congenital torticollis.

Results: A de novo missense variant c.643G>A; p.Gly215Arg in *CHN1*, encoding α2-chimaerin protein, was identified. The variant is not present in population databases and in silico predictors consider the variant as damaging. The p.Gly215Arg variant is located in the C1 domain of *CHN1* where other pathogenic variants have been shown to enhance α2-chimaerin Rac-GAP activity.

Conclusion: Gain of function variants in *CHN1* have been previously shown to cause DRS, which is characterised by the absence or hypoplasia of the 6th cranial nerve. Here, we identified a novel de novo heterozygous mutation in *CHN1* in a patient diagnosed with MBS. We propose to include *CHN1* in the genetic diagnoses of MBS. Further analysis of additional MBS patients may confirm *CHN1* as a new MBS gene.

Conflict of Interest: None declared.

EP11.009 Two years of newborn screening for spinal muscular atrophy in Poland

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Objectives: Spinal muscular atrophy (SMA) fulfills all criteria to be included in newborn screening testing (NBS). Herein, we present our two-year experience with NBS-SMA.

Methods: After a short pilot study since January 2021, the SMA was added to the National Newborn Screening Program in April 2021 and subsequently covered all districts in Poland. Since 03.2022, all newborns with opt-in signed, are tested for SMA (>99% of individuals). Standard dried blood spots are used for DNA extraction. We use SALSA MC002 SMA Newborn Screen test (based on PCR-HRM technique, MRC-Holland) a first-tier test to detect newborns with the homozygous deletion of exon 7 of the SMN1 gene. The second-tier test is based on the MLPA technique (P021 kit).

Results: Since January 2021, 438411 newborns were screened and SMA has been confirmed in 61 children. The results of the first-tier test and MLPA verification were available on the 8th (mean: $9.1 +/-4$) and 14th (mean 15.7 ± 6.0) days of life, respectively. Most of the children had 2 or 3 SMN2 copies (respectively, 18 and 25 individuals). Two children with 1 SMN2 copy were symptomatic at birth and had a heart defect. Four and 5 SMN2 copies, were identified in 13 and 3 children, respectively.

Conclusions: The PCR-HRM method was successfully used in NBS-SMA and allowed us to quickly identify positive patients that can be treated with all available therapies. The calculated prevalence of SMA is 1/7187 in the Polish population.

Conflict of Interest: Monika Gos Biogen, Novartis (lectures), Mariusz Ołtarzewski: None declared, Magdalena Frączyk: None declared, Joanna Wasiluk: None declared, Aleksandra Landowska: None declared, Mariola Jurzyk: None declared, Katarzyna Durda: None declared, Wioletta Wawer: None declared, Paulina Kubiszyn: None declared, Jessica Wieczorek: None declared, Liliia Nosarieva: None declared, Maria Jędrzejowska Biogen, Novartis, Roche (lectures, expert), Novartis.

EP11.010 Early diagnosis in an LGMDR18 case carrying heterozygous TRAPPC11 mutations

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Abstract

Limb-girdle muscular dystrophy (LGMD) is a heterogeneous group of neuromuscular diseases characterized primarily by proximal myopathy. Autosomal recessive LGMDR18 caused by *TRAPPC11* gene abnormalities is associated with childhood-onset of proximal muscle weakness resulting in gait abnormalities and scapular winging. Additional variable features include hyperkinetic movement disorder with chorea, ataxia, or dystonia, global developmental delay, alacrimia, achalasia, cataracts, or hepatic steatosis. Due to wide clinical and genetic heterogeneity with phenotype overlaps, it is often challenging to diagnose subtypes of LGMD. Digenic inheritance of a *TRAPPC11* variant has also been associated with an LGMD subtype.

We report a case of a boy who presented at 17-months of age with hypotonia, mild dysarthria and motor developmental delay. Subsequently at 5 years of age, he showed some features of Duchenne muscular dystrophy such as proximal and progressive muscle weakness, positive Gower sign with calf pseudo-hypertrophy and elevated serum creatine kinase level. However, screening for abnormalities in the *DMD* gene was negative. Muscle biopsy revealed mild loss of dystrophin and patchy loss of α-dystroglycan. Exome sequencing revealed two likely pathogenic compound heterozygous variants inherited in trans, namely *TRAPPC11* (NM_021942.6):c.2938G>A:(p.Gly980Arg) and c.142C>T:(p.Arg48Ter). Subsequent follow-up of the proband at 11 years of age showed additional symptoms of scoliosis, scapular winging, and cataracts consistent with LGMDR18. This case illustrates the utility of exome sequencing in deriving an early and definite diagnosis especially for rare neuromuscular disorders with clinical and genetic heterogeneity.

Grant Reference: NMRC/EDG/1056.

Conflict of Interest: None declared.

EP11.011 Epigenetic signatures in a TARDBP-Amyotrophic Lateral Sclerosis family

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Background: TARDBP promotes the biogenesis of miRNAs and is fundamentally involved in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). To identify potential non-invasive biomarkers of preclinical and clinical progression in the TARDBP-ALS family, we assessed the expression levels of circulating microRNAs in affected patients and asymptomatic mutation carriers.

Methods: we selected 15 tissue- and disease-specific plasma miRNAs involved in targeting TARDBP or binding TDP-43 during their biogenesis/mature form (miR-9-5p\3p,-132-5p\3p,-143-5p \3p, -574-5p\3p, let-7b-5p, -124-3p,-133a-3p, -133b, -142-3p, -146a-3p, -558-3p). Applying qRT-PCR in 7 symptomatic patients (P), 8 mutation carriers (C) and 13 healthy controls (HC), we investigate 10 miRNAs that bind TDP-43 in vitro during their biogenesis or in their mature form and the other 9 are known to be tissue-specific and disease-deregulated.

Results: Five out of 15 miRNAs were significantly dysregulated between HC and P. Furthermore, 13 out of 15 miRNAs were significantly dysregulated in C; eight miRNAs are deregulated exclusively in this group. We highlighted the potential of miR-132-5p, miR-132-3p, miR-124-3p and miR-133a-3p expression levels in serum as biomarkers of preclinical progression for G376D-*TARDBP*-associated ALS.

Conclusion: This is the first study evaluating plasma miRNAs expression in a large TARDBP family of HC, C and P samples. The results showed a specific miRNA differential expression in the symptomatic and asymptomatic mutation carriers compared to healthy controls, underlining a probable peripheral signature capable of stratifying the disease progression.

Conflict of Interest: Paola Ruffo Full, Ines Barone Full, Stefania Catalano Full, Francesca Luisa Conforti Full.

EP11.013 The potential connection between ALS molecular changes and the development and regeneration of CNS

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Background/Objectives: Neurodegenerative disorders are one of the most significant medical burdens of the modern era. Amyotrophic Lateral Sclerosis (ALS) is fatal neurodegenerative disease defined by the progressive loss of motoneurons. While diagnosis is based on clinical assessment and the exclusion of other conditions, there aren't many therapeutic options for neuroprotection. Our understanding of the pathophysiology of ALS has been increased by the discovery of molecular pathways and gene mutations connected to the disease. This has also opened up possibilities for new therapy strategies and diagnostic methods.

Results: Through common and rare variant association analyses, 15 risk loci with distinctive genomic architectures and neuron-specific biology have recently been found to be associated with ALS. Intriguingly, it has been discovered that in mammalian spinal cord tissue (opossum *Monodelphis domestica*), during early postnatal development.at the precise period when neuroregeneration ceases to be possible, the quantity of associated proteins to genes associated with ALS changes.

Discusion and Conclusion: Here, we discuss the possibility that the ALS-related genes/proteins could be connected to neuroregeneration and development. Additionally, since non-coding RNAs frequently regulate the expression regulation of gene in developmental checkpoints, we propose that examining the variations in the composition and quantity of non-coding RNA molecules, both in ALS patients and in the developing central nervous (CNS) system of the opossum at the point when neuroregeneration ceases, may offer potential insight into the development and progression of ALS.

Grant References: Slovenian research agency (Nos. P3-0427, P1-0170), Croatian Science Foundation (IP-2016-06-7060).

Conflict of Interest: None declared.

EP11.014 Correlation between molecular changes and therapeutic efficacy in patients with spinal muscular atrophy treated with gene therapy

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Background: Spinal muscular atrophy (SMA) is a severe neurodegenerative disease, characterized by anterior motor neuron degeneration, which results in increasing muscular weakening and paralysis. SMA is caused by a loss-of-function mutation in the *SMN1* gene, leading to decreased expression of the full-length SMN protein. The presence of the paralogous *SMN2* gene, which partially offsets the *SMN1*, is limited due to alternative splicing that produces a shortened, unstable protein. Approved gene-targeted therapies, designed to increase SMN production, include nusinersen (Spinraza; Biogen), and risdiplam (Evrysdi; Roche). However, because treatment response for later-onset SMA types varies, there is an increasing need to identify new non-invasive biomarkers for the prediction of SMA therapeutic efficacy.

Methods: Our study will assess the clinical response to the nusinersen and/or risdiplam treatment with regard to the selected genetic polymorphisms, and the *SMN2* copy number. A retrospective study with longitudinal follow-up will be conducted, i.e., peripheral blood samples of SMA patients will be collected and coding and non-coding RNA expression profiles will be determined at baseline and approximately 2 years after the treatment initiation. SMA clinical manifestations will be measured by RULM and RHS scales and analyzed with the combined effects of genetic data.

Implications and conclusions: We assume the differences in genetic polymorphisms, numbers of *SMN2* copies, and coding and non-coding RNA expression profiles between treatment responders and non-responders will help clinicians to predict the efficacy of the SMA treatment, which would improve the disease outcome.

Grant References: Slovenian research agency (No. P1-0170 and M.B. PhD thesis grant).

Conflict of Interest: None declared.

EP11.015 Identification of a novel variant of SGCB gene in Moroccan Patient with Limb-girdle Muscular Dystrophy R4

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Background: Limb Girdle Muscular Dystrophy (LGMD) is a heterogeneous group of muscular dystrophies affecting mainly the pelvic and/or scapular girdles but may also affect other muscles including the respiratory muscles and the heart. The mode of inheritance can be either autosomal dominant (LGMD1) or most frequently autosomal recessive (LGMD2).

βeta-sarcoglycanopathy (LGMDR4) is a form of limb-girdle muscular dystrophy caused by a mutation in the *SGCB* gene (4q12).

Methods: We investigated an eleven-year-old Moroccan consanguineous female patient with suspicion of genetic myopathy. Immunohistochemistry on muscle biopsy was not available. Informed consent was obtained from the patient's guardian before blood sampling and molecular analysis were performed. We firstly screened for the recurrent c.525delT mutation on exon 6 of the *SGCG* gene by direct Sanger sequencing. Second, we searched for recurrent mutations in exon 3 of the *SGCA* gene and finally, next-generation sequencing (NGS) analysis was performed for our patient.

Results: Next-Generation Sequencing revealed a novel homozygous in frame mutation in exon 6 of *SGCB* gene NM_000232.5(*SGCB*):c.919_921delGCT (p.Cys307del). This variant has never been reported in public databases. The accession number from Clinvar database is SCV002525924.

Conclusion: The application of next generation sequencing technology, when available in medical practice, is nowadays very useful for the molecular diagnosis of heterogeneous diseases such as muscular dystrophies, to set up a precise diagnosis for patients and to provide appropriate genetic counseling to families.

Conflict of Interest: None declared.

EP11.017 Genetic pattern of SMN1 and NAIP genes in Moldovian SMA patients

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Background: Spinal muscular atrophy (SMA) is a genetic disorder caused by deletions in *SMN1* gene. The *NAIP* gene has been shown that is hemizygously deleted in ~50% of SMA type I cases. The aim of this study was to analyse the status of the NAIP gene, in Moldavian SMA patients.

Methods: A total of 105 Moldavian patients suspected for SMA and 107 unrelated healthy individuals were enrolled in the study. The molecular genetic methods used were PCR-RFLP and MLPA.

Results: From 105 patients, 50 were confirmed with SMA. In this group were identified in 8 patients (16%) with a homozygous deletion of exon 5 of the *NAIP* gene, 4 patients (8%) had a

heterozygous status, and 2 (4%) had duplications. In the rest of the patients (55), the SMA were not confirmed but homozygous deletion of exon 5 of the *NAIP* gene was established in one patient (2%), 3 patients (5%) had duplications and one patient had 5 copies of the *NAIP* gene. In the 107 healthy controls, one patient (1%) was identified with a homozygous deletion of exon 5, *NAIP*.

Conclusion: It has been shown that the frequency of deletions in the *NAIP* gene is higher in the SMA patient group compared to the control grou, with a significant relationship the *p-value* < 0.00001 (significant at p < .05). As follows, the genetic profile of NAIP gene are asociated with SMA and its characteristic for the population of the Republic of Moldova also.

Conflict of Interest: None declared.

EP11.018 A novel cryptic splice donor due to synonymous variant in VPS13A as an underlying cause of a chorea-acanthocytosis in a large family

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Background: Chorea-acanthocytosis (CHAC) is a form of neurological disorder associated with acanthocytosis of the red blood cells. Generalized weakness, involuntary movements, dystonia, and chorea are the characteristic features of the disease. The neurological features of CHAC resemble those of Huntington disease. Mutations in *VPS13A* have been identified as the underlying cause of CHAC.

Materials: In this study, we recruited a large consanguineous family with multiple individuals initially diagnosed as having Huntingtin's disease. Genomic DNA extraction, followed by whole exome sequencing, was performed to identify the genetic defects underlying the disease. Hypothesis-free, unbiased analysis of the sequence data identified a synonymous variant (NM_001018037.2; c.5040C>T) in *VPS13A*. The potential pathogenicity of the identified synonymous variant was predicted using various splice site algorithms.

Results: The synonymous variant was consistently predicted to be a cryptic splice donor site that may lead to aberrant pre-mRNA splicing. Analyses of patient-derived RNA showed activation of a cryptic mid-exon splice donor, leading to frameshift. The variant was confirmed in all other affected and unaffected individuals using Sanger sequencing and qPCR.

Conclusion: Synonymous variants of *VPS13A* as an underlying cause of CHAC have not been previously reported. Our findings provide the first direct evidence of the involvement of a synonymous variant of VPS13A in CHAC. Moreover, this study highlighted the importance of including the VPS13A gene in the screening of individuals presenting with Huntington's phenotype.

Funding: The Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia funded this research work through project number 442/70.

Conflict of Interest: Sulman Basit Taibah University, Principal Investigator of Deanship of scientific research grant, Supplies and equipments, Majed Alluqmani Taibah University, Yasir Naseem Khan: None declared.

EP11.019 A complex case of spinocerebellar ataxia type 7 in Bulgarian patient confirmed by fragment analysis

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Background: Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant neurodegenerative disease. It's clinically characterized by progressive cerebellar ataxia associated with cone and retinal dystrophy. SCA7 is a rare subtype of SCA caused by a cytosine-adenine-guanine (CAG) repeat expansion in the ataxin-7 (*ATXN7*) gene.

Methods: Here we present a case of 23-years-old male with vision and balance problems who was referred for genetic counseling. The ophthalmology and neurology evaluation showed affected visual acuity and color vision, macular retinal degeneration and progressive ataxia syndrome. MRI of the brain revealed cerebellar atrophy. The patient was tested with next generation sequencing (NGS) panels (341 genes related with retinopathies and 427 genes for ataxia), and TruSight One kit including 4813 genes (clinical exome) analyzed with MiSeq/Illumina. Fragment analysis for *ATXN7*-CAG repeats was performed subsequently on ABI 3130 analyzer.

Results: NGS analysis didn't show clinically relevant genetic variants. The clinical exome sequencing revealed one variant of unknown significance with paternal origin. *ATXN7* fragment analysis detected two alleles – one normal (with 12 CAG repeats) and one expanded (with 51 CAG repeats). The obtained result is associated with the clinical manifestation of SCA7.

Conclusion: The identification of a heterozygous abnormal CAG trinucleotide repeat expansion in *ATXN7* by fragment analysis established the diagnosis spinocerebellar ataxia type 7. Therefore, fragment analysis should be used as a first step in patients with such symptoms.

Conflict of Interest: None declared.

EP11.020 DMD and beyond: Molecular modifiers that alter disease progression in patients with Duchenne Muscular Dystrophy

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Background/Objectives: Duchenne muscular dystrophy (DMD) is the most common and severe pediatric muscular dystrophy. There is great variability in phenotype severity and the course of the disease among patients, even when carrying the same molecular alteration. Recent studies point to trans-acting SNPs in *SPP1, LTBP4, CD40* and *ACTN3* as modulators of DMD phenotype. Our aim was to characterize and validate these genetic modifiers in a DMD argentine cohort. Additionally, a systematic review was performed to understand their current validation status.

Methods: 60 DMD patients were classified into two extreme groups: 1. Patients with loss of ambulation <11years (severe phenotype; $n = 30$); 2. Individuals who remained ambulant or lost ambulation at ages ≥15years (mild phenotype; $n = 30$). Reported SNPs in *SPP1, LTBP4, CD40* and *ACTN3* were evaluated by PCR-Sanger

sequencing. Statistical analysis of the differences between groups was performed by Chi-square test.

Results: Only the *ACTN3* SNP showed significant differences between groups. In the systematic review, no SNP was validated in every study nor on every tested cohort.

Conclusion: The obtained results indicate that the *ACTN3* SNP is a modifier of DMD progression, while the other ones couldn't be validated in our population. Similar results were observed in the systematic review, which suggest ethnicity effects or differences in the standard-of-care between cohorts. It is important to understand the effect of these modifiers on the DMD clinical variability, as it could translate into improved disease prognosis and personalized treatments, as well as, a more accurate design and evaluation of clinical trials.

Conflict of Interest: Chiara Mazzanti: None declared, Micaela Carcione: None declared, Leonela Luce: None declared, Macarena Bollana: None declared, Carmen Llames Massini: None declared, Triana Visconti: None declared, Alberto L. Dubrovsky: None declared, Lilia Mesa: None declared, Florencia Giliberto FG has been awarded a research grant from PTC Therapeutics.

EP11.021 Genetic heterogeneity in muscular dystrophies and congenital myopathies: data from multigene WES-based genetic studies

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Hereditary myopathies comprise a group of genetically heterogeneous diseases, characterized by anomalies in muscle structure and/or function, with estimated prevalence of ~4:100,000 for congenital myopathies (CM) and 19.1-25.1:100,000 for muscular dystrophies (MD). The mutational spectrum of genes related with myopathies, derived from diagnostic studies by WES-based multigene panels is presented.

The information from our database from patients (2016-2022) with clinically relevant variants in genes associated with MD (either congenital or "progressive" forms) and CM was reviewed.

A total of 106 patients with at least one variant classified as pathogenic or likely-pathogenic, harboured variants in 28 different genes. Among these patients, 24 were possibly compatible with CM (most frequent genes: *RYR1* and *NEB*) and 13 with congenital MD (most frequent genes: *COL6A2* and *COL6A1*); whereas 69 had a progressive MD (most frequent genes: *SGCA*, *ANO5*, *DYSF*, *LAMA2* and *TCAP*).

Overall, 76 patients had autosomal recessive forms (44 compound heterozygotes and 32 homozygotes), whereas 16 patients showed autosomal dominant inheritance. A total of 8 hemizygotes were identified in our cohort with defects in *DMD*.

Interestingly, the pathogenic variant NM_000426.3(*LAMA2*):-c.2461A>C (p.Thr821Pro), previously associated to "atypical" late-onset *LAMA2*-related MD, was identified in 6 out of 106 patients. The total number of cases with this variant is now 24, so far all with Portuguese ancestry.

These results illustrate the genetic heterogeneity typically found in these diseases and the interplay of genes underlying both muscular dystrophies and myopathies. In such patients, analysis by WES-based multigene panels is recommended to optimize diagnostic yields and time to diagnosis.

Conflict of Interest: None declared.

EP11.022 Sudden respiratory failure requiring long-term assisted ventilation in a child with SEPN1-related myopathy

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Background: Congenital *SEPN1*-related myopathy (OMIM: 606210, ORPHA 97244) is a slowly progressive childhood-onset congenital muscular disease characterized by delayed motor development, short stature, low body weight, long slender neck, flat retracted thorax, spinal rigidity, and axial weakness. Till the puberty, patients develop scoliosis, and respiratory failure requiring long-term assisted ventilation. SEPN1-related myopathy occur in carriers of biallelic mutations in *SELENON* (*SEPN1*) gene, encoding a glycoprotein that is localized in the endoplasmic reticulum.

Methods: The material for the study was DNA isolated from the peripheral blood leukocytes of a symptomatic patient using automated Maxwell system (Promega). The whole exome sequencing was performed using Twist Human Core Exome kit (Twist Bioscience). The results and segregation analysis were confirmed by Sanger sequencing.

Results: Here, we present 5-years-old child who was tested due to short stature, decreased body weight, frontal bossing, lumbar hyperlordosis and progressive amyothophy. At the age of 7 years, when progressive spinal rigidity and scoliosis were observed, he suddenly developed respiratory failure. After the first respiratory decline the patient started to require long-term assisted ventilation. Rapid whole exome sequencing revealed that the child carries two heterozygous variants in *SELENON* gene (NM_020451.3: p.Asp322ArgfTer30, p.Asn238LysfsTer63) which are the genetic cause of *SEPN1*-related myopathy. The bioinformatics analysis identified these variants as pathogenic according to the ACMG recommendation. The family segregation analysis showed that parents are heterozygotes carriers of single variant within the *SELENON* gene.

Conclusions: Rapid whole exome sequencing is an effective diagnostic tool for patients with muscle weakness with concomitant early respiratory insufficiency.

Conflict of Interest: None declared.

EP11.023 Pilot study for the implementation of newborn screening for spinal muscular atrophy in Valencia

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Background/Objectives: Spinal muscular atrophy (SMA) is a disease characterized by biallelic pathogenic variants in the *SMN1*

gene, being the homozygous deletion of exon 7 of *SMN1* responsible for SMA in 95% of cases. *SMN1* encodes the SMN protein whose deficiency causes progressive weakness and proximal muscle paralysis.

Newborn screening (NBS) for SMA would allow the detection of newborn who carry the homozygous *SMN1* deletion, candidates to receive the therapies available in the earliest stage of the disease or presymptomatic, conditions in which treatment is more effective.

Methods: We performed a screening trial at La Fe University and Polytechnic Hospital between October 2021 and February 2023. The screening was conducted using DNA extracted from dried blood spots (DBS) with a multiplex quantitative polymerase chain reaction assay targeting the position c.840C in the exon 7 of *SMN1* to detect homozygous *SMN1* deletion.

Results: A year and a half NBS population-based prospective study is being carried out at La Fe University and Polytechnic Hospital in Valencia. To date, 5540 DBS samples from two hospitals in Valencia have already been analyzed, without any positive result.

Conclusions: The expected results of this project are the determination of the viability of newborn screening for SMA in our region and the estimation of the prevalence of the disease in The Valencian Community with 35,000 births/year. In the coming months, it is expected to extend the study to all the Hospitals in the Valencian Community.

Grant References: Novartis Gene Therapy, PerkinElmer, Mutua Madrileña Fundation, CP22/00028, ACIF/2021/057.

Conflict of Interest: Alba Berzal-Serrano Full, ACIF/2021/057, Elena Aller Full, Teresa Jaijo Full, Lidon Carretero-Vilarroig Full, Belén García-Bohórquez Full, Cinta Navarro-Moreno Full, Inmaculada Pitarch-Castellano Full, Dolores Rausell Full, José Vicente Marcos-Tomás Full, Sandra Ruiz-Aja Full, Gema García-García Full, CP22/00028, José M. Millán Full, IP.

EP11.024 Novel homozygous variant of unknown significance in patient with spinocerebellar ataxia

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Background: Autosomal recessive spinocerebellar ataxia is a heterogeneous group of progressive hereditary disorder characterized by retardation psychomotor development with manifestation in young age principally. We report the case of 12-year-old girl with consanguineous parents. From the first year of life she demonstrates speech and motor development delay. Currently she has ataxia and cognitive impairment, her phenotype includes macrocephaly, hypertelorism, epicanthus, and broad terminal phalanges of the thumbs and toes. MRI revealed degenerative changes in the hemispheres and cerebellar vermis. Many different genetic tests have been carried out, including gene panels and whole mtDNA sequencing. The causative variant was not found and for this reason whole genome sequencing (WGS) was performed for the proband and parents.

Methods: TrioWGS (PE150, enzymatic- and PCR-free protocol, DNBseq-T7 (MGI)) with Sanger sequencing (ABI3500) was performed.

Results: WGS revealed homozygous variant chr6:86275726T > C (hg19/GRCh37) in 5 intron of SNX14 gene. There is no frequency data at gnomAD, dbSNP. A deep learning-based tool SpliceAI predicted a new donor splice site (c.462-589A > G, NM_153816.6, SpliceAI Δ score 0.63). This variant is classified as variant of uncertain significance (ACMG-PM2 criterion). Proband's parents are heterozygous carriers of chr6:86275726T > C. Pathogenic variants in the SNX14 gene have been described in autosomal recessive spinocerebellar ataxia type 20 (OMIM:616354).

Conclusion: Our data suppose this variant of uncertain significance in SNX14 gene is causative variant for analyzed clinical case due to TrioWGS. Functional analysis is in process.

Grant References: The research was supported by non-profit organization Charity Fund for medical and social genetic aid projects «Life Genome».

Conflict of Interest: None declared.

EP11.025 Clinical variability of a homozygous missense COQ4 variant in individuals with spastic paraplegia and initial multiple-sclerosis-like presentation questioning its deleteriousness and/or specificity of the COQ4-related phenotype

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Biallelic pathogenic *COQ4* variants cause primary Coenzyme Q10 Deficiency. Approximately 50 relevant individuals have been reported, demonstrating variable phenotypes, including cognitive and motor delay, episodic regression, seizures, stroke-like episodes, spasticity, tetraparesis, dystonia, ataxia, dysarthria and other manifestations with distinct clinicoradiological subphenotypes proposed. Recently, some individuals with adult onset ataxia-spasticity and milder course were reported.

We describe two individuals, originating from a village in Cyprus, investigated for spastic paraplegia with onset at the end of their second decade. Both presented weakness and fatiguability of lower extremities, somewhat episodic and fluctuating in nature. Additional features included brisk knee/ankle reflexes, absent/difficult to elicit abdominal reflexes and variable sensory abnormalities. Initial presentation prompted consideration of multiple-sclerosis, not further confirmed. Differences were noted in MRI images with occipitoparietal stroke-like lesions in the most severely affected patient, who progressively became wheelchair-dependent from the age of 21 years, presented deterioration of his dysarthria and developed dysphagia. The other remains ambulatory at 63, with slow progression of his spastic paraparesis and subtle increases in FLAIR signal around the occipital horns. Analysis of a spastic paraplegia CES-based panel revealed

homozygosity for a *COQ4* variant in both (NM_016035.5:c.164G>T/p.Gly55Val).

Review of the literature revealed homozygosity for this variant in two sibs from Turkey with significantly more severe phenotype of childhood-onset slowly progressive ataxia and spasticity, impaired cognition and epilepsy, and notable improvement following CoQ10 administration.

Given previous limited lines of evidence for the deleteriousness of this variant, detailed phenotyping, segregation and metabolic studies are scheduled to support or refute its pathogenicity.

Conflict of Interest: None declared.

EP11.026 Novel COL12A1 variant as a cause of familial Bethlem myopathy 2

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Background: Bethlem myopathy 2 (BTHLM2), also known as myopathic type Ehlers-Danlos syndrome, is caused by heterozygous mutation in the collagen XII gene (*COL12A1*). The disease manifests mainly with hypotonia, mild muscular weakness, joint hyperlaxity, proximal joint contractures and finger contractures.

Methods: We describe a family affected by this rare collagen XII-related myopathy with a novel variant in *COL12A1* gene. A male proband aged 25 months presented with hypotonia, stooped posture, kyphosis, joint hyperlaxity, pes planovalgus, failure to thrive and hydronephrosis. His mother had bilateral congenital hip dislocation, hypotonia, failure to thrive in infancy and she has joint hyperlaxity and flexion finger contractures. Clinical exome sequencing was performed in the subjects using Illumina TruSight One Kit.

Results: A novel heterozygous splicing variant, at position c.8578-1G > C of *COL12A1* gene, was identified in our proband. This variant was inherited from his mother. According to ACMG classification, this variant is likely pathogenic. It is absent in gnomAD database. It hasn't been reported in the ClinVar or LOVD database.

Conclusion: To our knowledge, these are the first cases of BTHLM2 caused by c.8578-1G > C variant in *COL12A1* gene. Our findings expand the variant spectrum for *COL12A1* and the phenotypic spectrum for BTHLM2.

Grant References: This study was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project "Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials".

Conflict of Interest: Katarina Vulin Equipment was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project "Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials", but NO real conflict of interest, Ljubica Odak Equipment was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project "Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials", but NO real conflict of interest, Lana Lončar: None declared, Ana Tripalo Batoš: None declared, Ana-Maria Meašić Equipment was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project "Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials", but NO real conflict of interest, Adriana Bobinec Equipment was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project "Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials", but NO real conflict of interest, Ivona Sansović Equipment was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project "Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials", but NO real conflict of interest, Leona Morožin Pohovski Equipment was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project "Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials", but NO real conflict of interest.

EP11.027 The high incidence of SMA detection with initial step of newborn screening in Ukraine

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Background/Objectives: Spinal muscular atrophy – the most common monogenic neuromuscular pathology with an autosomal recessive type of inheritance, the frequency of heterozygous carriers for which is 1 in 40-60 people, was included in the list of pathologies of mass neonatal screening in Ukraine.

Methods: The analysis of the *SMN1* gene was performed using the "Biocore® SMA/TKID plus" diagnostic kit. The biological material for DNA extraction was DBS (dried blood spot) applied to DBS cards. 16104 newborns were examined.

Results: The neonatal screening for SMA was performed in 7 regions of Western Ukraine since October, 2022. Among 16,104 samples of dried blood spots were 15,999 were normal for SMN amplification in the first RT-PCR, 105 samples were repeated and 5 cases were re-called for second blood spot. 4 cases of SMA were confirmed after additional tests. Diagnosis was confirmed by amplification of the 7th and 8th exons of the *SMN1* and 2 genes followed by cleavage of the PCR products with restriction endonucleases and MLPA analysis for *SMN1* and 2 copy number. Deletions of the 7th and 8th exons of the *SMN1* gene were found in the homozygous state in four cases.

Conclusions: The method of newborn screening of spinal muscular atrophy using RT-PCR was initiated into practice. The high initial frequency of SMA is 1 to 4026 newborn in neonatal screening is established.

Conflict of Interest: None declared.

EP12 Multiple Malformation/Anomalies Syndromes

EP12.001 Mandibulofacial dysostosis with intellectual disability associated with terminal 21q22.3 deletion and 8q24.3 duplication: delineation of phenotype and review of literature

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Background: We report a 31-year-old female patient born from consanguineous, healthy, parents, referred for mandibulofacial dysostosis with intellectual disability (ID) and prior normal lymphocyte karyotyping. Genetic counselling was indicated as the proband's brother was expecting a child.

Methods: The clinical file of the proband and family history were reviewed. Molecular karyotyping using a Cytoscan750k SNP-array (Affymetrix) was applied. FISH analysis was performed in relatives. Literature review was performed based on the identified chromosomal anomalies.

Results and Discussion: The proband's phenotype included: mild-moderate ID, seizures, bilateral cleft lip-palate, scoliosis, cyphosis, sloping shoulders and dysmorphic features (down-slanting palpebral fissures, broad nasal bridge, malar hypoplasia, external ear anomalies, microretrognathia). Family history included anorectal malformation and postnatal demise in a maternal uncle, and a paternal cousin with ID, cleft lip-palate and language delay. The paternal grandmother had 13 pregnancies: 5 babies died in infancy. Molecular karyotyping in the proband identified a 5.7Mb terminal heterozygous duplication at 8q24.3 and a terminal 4.2 Mb heterozygous deletion at 21q22.3, a cryptic unbalanced chromosomal rearrangement, inherited from her balanced carrier father, as shown by FISH. The balanced chromosomal rearrangement was also present in one of her brothers, enabling genetic counselling and prenatal testing for the ongoing pregnancy. Only one case, with an imbalance involving the same OMIM-Morbid genes, also inherited from a balanced carrier father, has been reported. The phenotypes of the 2 patients will be compared. Future cases will help to delineate the gene dosage/phenotype correlation of this rare chromosomal disorder.

Conflict of Interest: Leila Zahed Responsable scientifique, Cliniques Universitaires Saint Luc, Armelle Duquenne Cliniques Universitaires Saint Luc, Anne De Leener Cliniques Universitaires Saint Luc, Nicole Revencu Cliniques Universitaires Saint Luc, Eric Olinger Cliniques Universitaires Saint Luc.

EP12.002 Expanding MNS1 heterotaxy phenotype

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Background: First described in 1994 as a spermatogenesis gene, *MNS1* (meiosis-specific nuclear structural protein-1 gene) encodes a structural protein implicated in motile ciliary function and sperm flagella assembly. To date, two different homozygous *MNS1* variants were recently associated to autosomal recessive visceral heterotaxy with male infertility (OMIM# 618948) in seven individuals from four consanguineous family. Known phenotype includes heterotaxy from laterality defect of visceral organs to situs inversus totalis, congenital heart and spleen defects, mild to severe primary ciliary dyskinesia symptoms (bronchiectasis, recurrent infection of upper airways and chronic cough) and male infertility.

Methods: A French individual was a female born at term with normal prenatal ultrasounds and birth parameters. A complete situs inversus was diagnosed at birth without neonatal complications or organ defect. Genome sequencing identified two compound heterozygous variant (p.(Gln203AlafsTer5) maternally inherited and p.(Cys23*)) paternally inherited. Now aged 17, her clinical evolution included asthma and recurrent upper airway infection, dental agenesis of seven permanent teeth with preserved deciduous dentition, severe bilateral myopia, leg asymmetry, bilateral foot clinodactyly and thin fingers. She has normal intellectual development with no facial features. A collaborative call was proposed via GeneMatcher.

Results: We obtained one positive match for two-sibling foetus with *MNS1* homozygous variant p.(Ala475Pro). Autopsy revealed normal growth (2/2), facial features (2/2), genital anomalies (1/2), complex cardiac defect (2/2) and situs inversus with abdominal (2/2) and pulmonary (1/2) heterotaxy.

Conclusion: These news cases further delineate the clinical description of *MNS1* patients. We hope to build a larger cohort.

Conflict of Interest: None declared.

EP12.003 Clinical and diagnostic characterization of craniosynostosis: A study of 20 cases

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Background: Craniosynostosis is a condition characterized by the premature fusion of one or more cranial sutures, occuring in approximately 1 in 2,000 to 2,500 live births (1).This diverse and complex malformation can be syndromic or non-syndromic (2). Research has identified several genes, including *FGFR2, FGFR3, TWIST1*, and *EFNB1*, that are commonly mutated in individuals with craniosynostosis (2).

Methods: In this study, we report a serie of 20 Moroccan patients referred to the medical genetics consultation for craniosynostosis. The combination of clinical and radiological signs allowed us to identify various diagnoses of syndromic craniosynostoses.

Results: Our cohort comprised of 20 individuals, with a male majority. The average age of the patients at the time of consultation was 2 years, ranging from 4 days to 14 years old.

The most frequently diagnosed syndromes were Apert and Crouzon Syndrome, followed by Antley Bixler, Saethre-Chotzen, Fronto-nasal dysplasia, and finally Carpenter and Pfeiffer syndrome.

Conclusion: This research highlights the importance of a comprehensive clinical evaluation and the use of genetic testing in the diagnosis of syndromic craniosynostosis. These findings contribute to a better understanding of this condition and may inform future research.

Conflict of Interest: None declared.

EP12.004 CDKN1C gene in Beckwith-Wiedemann syndrome: variant databases and literature review

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Background: Beckwith-Wiedemann Syndrome (BWS; OMIM #130650) is an autosomal dominant and rare human genomic disorder. The main causes are alterations in methylation pattern in the 11p15 *locus*, presence of pathogenic monoallelic variants in the maternal allele of the *CDKN1C* gene, paternal uniparental disomy and cytogenic alterations. The sequencing of the *CDKN1C* gene is a second-tier investigation that not infrequently is neglected within the diagnostic process. Consequently, it is relevant to concentrate on BWS caused by *CDKN1C* variants in order to improve the diagnostic accuracy of this disorder.

Objectives: Descriptive compilation of the *CDKN1C* gene's pathogenic and likely pathogenic variants linked to BWS using bioinformatic resources and revising literature. Attempt to enhance the genotype-phenotype correlations already in place by connecting the existence of pathogenic variants in the *CDKN1C* gene to particular clinical manifestations of BWS.

Results and Conclusion: Currently, more than 100 variants reported in *CDKN1C* gene meet the selected criteria of association with the syndrome and clinical significance according to the ACMG classification. Several discrepancies exist within information published in the databases and the pathogenicity criteria assigned by ACMG, emphasizing the need for constant updating of the databases as well as implementation of segregation and functional studies to eliminate ambiguities regarding variants of uncertain significance. It is challenging to make inferences about associations between variants and clinical features since there are few phenotypes available associated with the reported variants.

Grant References: UIDB/00215/2020; UIDP/00215/2020; LA/P/0064/2020

Conflict of Interest: None declared.

EP12.005 Craniosynostosis in individuals with Kabuki syndrome

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Introduction: Kabuki syndrome (KS), first described in 1981, is a clinically recognizable syndrome characterized by growth failure, psychomotor delay, congenital heart disease, and other complications, as well as the facial features that give it its name. *KMT2D* and *KDM6A* were identified as causative genes in 2010 and 2012, respectively, allowing molecular genetic diagnosis.

The phenotype of KS is very diverse, and clinical management based on molecular diagnosis is important. Craniosynostosis (CS) in genetically diagnosed KS have been sporadically reported, its frequency is unknown, and it has not been considered as a major manifestation of KS. We studied the occurrence of CS in individuals with molecularly confirmed KS from medical records.

Methods: Of 50 individuals with KS (including only clinical diagnosis) who visited our department from 2017 to 2022, genetically diagnosed individuals were included in this study.

Details of genetic testing, three-dimensional computed tomography (3D-CT) evaluation, diagnosis of CS, and surgery for CS were reviewed from medical records.

Results: We confirmed the molecular diagnosis for 43 individuals (41 with *KMT2D* and 2 with *KDM6A*). Twenty-seven of them underwent 3D-CT for evaluation of CS, and 20 (46.5%) were diagnosed with CS, 9 of whom required cranioplasty.

Conclusion: In this study, 46.5% of patients with molecularly confirmed KS had CS (the rate was even higher when limited to those who underwent 3D-CT, with 74.1% having CS).

The results of this study suggest that CS is one of the major complications of KS and that proper evaluation of CS is important in their management.

Conflict of Interest: None declared.

EP12.006 MED12-related Hardikar syndrome: case report

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Introduction: Hardikar syndrome (HS) is an X-linked dominant rare multiple congenital malformation syndrome. Cardinal features of HS include cleft lip/cleft palate, biliary anomalies, liver disease, intestinal malrotation, pigmentary retinopathy, and a congenital heart defect. Neurodevelopment is normal. The heterozygous mutations in the MED12 gene were identified in female patients with HS. However, only 12 patients have been described in the literature to date.

Materials and methods: We present a case of a two-month-old girl. She was referred to the Specialized Center of Medical Genetics at the National Children's Specialized Hospital "Okhmatdyt" of the Ministry of Health of Ukraine in Kyiv because of biliary atresia, cleft lip, and congenital heart defect (aortic coarctation, muscular ventricular septal defect, left ventricular non-compaction, right coronary artery-to-right ventricle fistula). A physical exam revealed mild non-specific dysmorphic facial features. We have assumed multiple diagnoses, such as: Alagille syndrome. The patient had a normal female karyotype 46, XX. Next Generation Sequencing (NGS) analysis was done, focusing on 1292 genes associated with clinical features.

Results: NGS revealed a heterozygous variant (c.3488dup, p.Asp1164Glyfs*3) in exon 25 of the MED12 gene (OMIM #300188), categorized as Pathogenic. This variant is absent from the population database GnomAD.

Conclusion: This study is proof of molecular genetic testing in patients with multiple congenital malformations. Early diagnoses led to personalized care for patients and accurate genetic counseling for their families. Our study further expanded the genotypic spectrum and genotype-phenotype correlations of Hardikar syndrome.

Conflict of Interest: Viktoriia Cherevashko full-time, Vira Galagan full-time, Olha Zhurakhovska full-time, Maryna Tsyhankova full-time.

EP12.007 Somatic mosaic variants of PIK3CA-related overgrowth syndrome

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Background/Objectives: PIK3CA-related disorders (PRDs) are a group of genetic diseases caused by somatic mosaic variants in the *PIK3CA* gene. The protein produced by *PIK3CA* is known to regulate the growth and division of cells. Changes in PRDs generally occur in the post-zygotic phase and can therefore lead to severe overgrowth spectrum and vascular malformations in various parts of the body.

Methods: Since 2015, two patients with PRD have been detected in Estonia. Individuals were analyzed with Sanger Sequencing and Next Generation Sequencing (NGS) using Illumina TruSight One/One Expanded gene panel and Agilent SureSelect XT V5 Human exome sequencing kits.

Results: A 6-year-old boy presented with macrocephaly (+5.5SD), facial and legs hemangiomas, low set ears, high palate, large hands and feet and mild to moderate intellectual disability. DNA from cultured fibroblasts from the patient's skin was sequenced by NGS gene panel, and a heterozygous somatic de novo *PIK3CA* pathogenic gene variant p.Gly914Arg was found (23%).

A 7-year-old girl was born with right macrodactyly of T2-3. The patient's tissue of affected toe was initially analyzed for a pathogenic *AKT1* gene variant p.Glu17Lys due to the clinical suspicion of Proteus syndrome. Instead, a heterozygous mosaic *PIK3CA* pathogenic gene variant p.Gln546Lys was detected by the NGS gene panel (10%).

Conclusion: Due to varying levels of somatic mosaicism, it is difficult to detect the presence of PRD-related pathogenic variants in biopsied tissues and cells. These two cases highlight the importance of testing affected tissue(s).

Funding: Estonian Research Council grant PRG471.

Conflict of Interest: None declared.

EP12.008 An extremely rare case of unilateral focal dermal hypoplasia associated with ventricular septal defect in male

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Background: Focal dermal hypoplasia (FDH) is a rare mesoectodermal dysplasia caused by mutations in PORCN gene (Xp11.23). As an X-linked dominant disorder, it is rarely found in males. It mainly affects the skin, eyes, skeleton and teeth, while abnormalities of internal organs are uncommon. Ventricular septal defect has not been previously described. Among 250 reported cases, only ten had a unilateral presentation, of which just two were males. We present a rare case of FDH due to its rarity and novel feature.

Case report: Male newborn was presented to our clinic with multiple anomalies. He showed left facial hypoplasia, left eye microphthalmia and iris and chorioretinal colobomas, ventricular septal defect, hydronephrosis of the left kidney, syndactyly of the left hand and focal dermal hypoplasia on the left leg. There was no similar case in his family. Clinical suspicion of mosaic FDH was made. Exome sequencing, performed in Qgenomics using DNA from peripheral blood, revealed a novel nonsense pathogenic variant c.90G>A in PORCN gene in mosaic form, which was verified by Sanger sequencing and found to be de novo. Now, at the age of four years, this boy has normal development, the heart defect is spontaneously closed and, in addition to previous features, he shows onychodystrophy and irregular dentition.

Conclusion: Our case is an extremely rare unilateral presentation of FDH in male, associated with ventricular septal defect that hasn't been described in previous reports, due to a novel variant in PORCN gene that expands the knowledge of phenotype-genotype correlations of FDH.

Conflict of Interest: None declared.

EP12.009 Children mortality caused by congenital anomalies in Lithuania: 2010-2021

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Background: Congenital anomalies cause a large portion of infant and child deaths worldwide. According to recent studies, they account for about 20% of neonatal and infant deaths in more developed countries. Mortality rates during childhood are considerably lower than in infancy and are less investigated.

Methods: We studied the mortality of children aged 0-19 caused by congenital malformations, deformations and chromosomal abnormalities (ICD-10-AM codes Q00-Q99) in Lithuania during 2010-2021. Data was aquired from the State Registry of Death Cases and Their Causes. Mortality was analyzed separately for infants, children aged 1–4, and children aged 5–19.

Results: All-cause mortality decreased by an average of 54% in all age groups. Congenital anomalies caused 34% of deaths under 1-year-old in 2010. This number remains similar over time, with 27.4% of deaths in 2021. The proportion remains relatively constant because a decrease in deaths from congenital anomalies corresponds to a lower total mortality rate. Congenital anomalies remained Lithuania's second most common cause of infant deaths. In 1-4 and 5-19 year-old groups, an increase from 11.1% to 29.4% and from 5% to 10.5%, respectively, was noticed over the same period. The number of deaths caused by congenital anomalies remained similar; therefore, their relative contribution to total mortality rates has increased.

Conclusion: The proportion of deaths due to congenital anomalies is increasing in older children in Lithuania. That could be explained by lower mortality rates in other disease groups. However, further and more detailed studies are needed to monitor these changes.

Grant References: none.

Conflict of Interest: None declared.

EP12.010 Identification of two new splice site mutations in the PIGN gene contributing to Fryns syndrome

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Background: The Fryns syndrome (FS) is a multiple congenital anomaly syndrome with different multisystemic malformations such as congenital diaphragmatic hernia, pulmonary hypoplasia,

craniofacial dysmorphic features in combination with malformations of the central nervous system such as agenesis of the corpus callosum, cerebellar hypoplasia and enlarged ventricles.

Case: We present a non-consanguineous Northern European family with two recurrent cases of Fryns syndrome: a 2.5-month-old boy with cleft palate, left renal aplasia, right pelvic kidney with megaureter, duodenal stenosis, micropenis, facial dysmorphic signs, muscular hypotonia, failure to thrive and cerebral seizures. The fetus of a former pregnancy showed a similar complex developmental disorder with singular umbilical artery, hydrops fetalis, cardiac defect (truncus arteriosus communis, ventricular septal defect, aortic isthmus stenosis), right diaphragmatic hernia, enlarged ventricles and agenesis of the corpus callosum.

Methods: Morphological examination, Whole Exome Trio analysis and alternative splicing analysis were performed.

Results: A Whole Exome Trio analysis revealed two likely splicing-affecting disease-causing mutations in the *PIGN* gene: a synonymous mutation c.2619G>A, p.(Leu873 =) affecting the last nucleotide of exon 29 and a 30bp-deletion c.996_1023+2del (NM_176787.5) protruding the intron of exon 12, both lying in trans in the affected patients. The truncating effect was confirmed via visualization of alternative splicing on an agarose gel.

Conclusion: Given the role of *PIGN* gene in a multiple congenital anomaly syndrome in combination with the molecular and clinical findings of both patients, we assume that both biallelic splice-affecting variants result in a Fryns syndrome phenotype.

Conflict of Interest: None declared.

EP12.011 Advancing towards a human brain organoid model of Schaaf-Yang syndrome

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Background/Objectives: Schaaf-Yang syndrome (SYS) is an ultra-rare neurodevelopmental disorder caused by truncating mutations in *MAGEL2*. The most common features of SYS include neurodevelopmental delay, sleep disorders, joint contractures and facial dysmorphism. An important setback when studying neurodevelopmental disorders is the difficulty in obtaining relevant human cell models. Our aim is to model SYS and elucidate the cellular bases of the pathology through the generation of iPSCs from patients for their subsequent derivation into brain organoids.

Methods: We have reprogramed iPSCs from skin fibroblasts from 4 lines of SYS patients and 2 controls through an mRNA-based non-integrative approach. Using a characterized patient iPSC line we have generated cortical and ventral brain organoids following an adaptation of previously described protocols.

Results: We have generated 6 lines of iPSCs derived from patient and control fibroblasts. To characterize the iPSCs, each line's identity, genomic stability and pluripotency have been verified. With a patient iPSC line, we have successfully generated cortical and ventral brain organoids. Additionally, at day 60 of differentiation we established forebrain assembloids in order to study neuronal migration. Moreover, we created an ImageJ macro that allowed us to perform an automatic morphometric characterization of the organoids. To further study both organoids and assembloids, immunohistochemistry experiments are being performed at different time points.

Conclusion: The generation of this novel relevant model of Schaaf-Yang syndrome will be useful in future studies to unravel the cellular bases of the pathology and as a platform to test treatments.

Grant references: CIBERER-M.BRAIN; PID2019-107188RB-C21.

Conflict of Interest: None declared.

EP12.012 Alteration of RAS pathway phosphorylation in Noonan syndrome patients carrying hypomorphic variants in two NS genes

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Background/Objectives: Noonan syndrome (NS) is a multi-systemic disorder, showing an autosomal dominant inheritance and characterized by variable expressivity. Generally, the genetic mutation affects one of a subset of RAS pathway genes, even if it is not possible to provide molecular diagnosis for 20-30% of patients. This finding suggests that additional genes or further genetic/epigenetic mechanisms are involved in NS pathogenesis. Recently, we proposed a digenic inheritance of subclinical variants in two NS patients, negative after mutation analysis, as an alternative NS pathogenic model. A CGH array study also excluded the 6p interstitial deletion reported as associated to NS spectrum. Each NS patient showed two hypomorphic variants of RAS pathway genes co-inherited from both their healthy parents, that we hypothesized to generate an additive effect.

Methods: Here we report the phosphoproteome and proteome analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS) performed on the immortalized peripheral blood mononuclear cells (PBMCs) from the two trios.

Results: Our results indicate that the two unrelated patients show overlapped profiles in both protein abundances and their phosphorylation levels, not reached by their parents. IPA software predicted RAS-related pathways as significantly activated in the two patients. Interestingly, they remained unchanged or only slightly activated in both patients' parents.

Conclusion: These findings suggest that the presence of one subclinical variant in the healthy parents can activate RAS pathway below the pathological threshold, which instead can be exceeded by the additive effect for the co-presence of two sub-clinical variants causing NS, according to our hypothesis.

Grants: Academic fund.

Conflict of Interest: None declared.

EP12.013 Severe familial Opitz G/BBB syndrome caused by MID1 gene duplication

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Background/Objectives: X-linked Opitz G/BBB syndrome (XLOS) is a genetic disorder characterized by a congenital midline malformation syndrome associating hypertelorism, genitourinary abnormalities, laryngo-tracheal abnormalities, imperforate anus,

cleft lip and palate and congenital heart defects. Variable *MID1* gene mutations were reported as causative of XLOS with missense, nonsense, splice site variants or small intragenic deletions/insertions. However, large or whole-gene deletions/duplications are unusual. Here, we report a new case of familial XLOS caused by a large intragenic duplication detected using array CGH analysis.

Patient & Methods: The proband is a son of non-consanguineous Saudi parents, born preterm at 34 weeks of gestation and presenting hypertelorism, Patent Foramen ovale (PFO), glandular hypospadias, anorectal malformation and developmental delay. The mother has a familial history of three males presenting similar clinical findings with a pedigree compatible with an X-linked inheritance.

Genetic investigation was performed using 180K Agilent oligonucleotides array CGH analysis according to the manufacturer's instructions.

Results: Array CGH revealed the presence of an hemizygous Xp22.2 duplication of 224 Kb into *MID1* gene involving the exons 2 to 7. arr[GRCh37] Xp22.2(10470307_10694513)x2.

Conclusion: Mutations in *MID1* gene are responsible for defects in the development of embryonic midline structures causing major and minor findings of X-linked Opitz G/BBB syndrome. However, there is no clear genotype-phenotype correlation between the type of mutation and the severity of clinical findings. To elucidate this correlation we report here a new familial case of a large duplication into *MID1* gene associated in affected males with the severe form of the syndrome.

Conflict of Interest: None declared.

EP12.014 A first case of rare MORFAN syndrome confirmed after almost 30 years

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Background/Objectives: We present a case of patient that first visited our department at the age of 18 months for facial dysmorphism, mild intellectual deficiency, overgrowth and hypoglycemic seizures. Eva Seemanová described this patient in 1993 [1] as a new syndrome called MORFAN. It's an acronym for **M**ental retardation, pre- and post-natal **O**vergrowth, **R**emarkable **F**ace, and **A**canthosis **N**igricans. At that time, molecular base was not known. This patient returned to our department again in his 33 years, this time also with several meningiomas.

Methods: Patient phenotype was reevaluated with anthropometric examination and 3D facial scan. Sequencing of genes related to Stickler syndrome, NF2 and AKT2 was performed.

Results: Variant of unknown significance in COL9A1 was found, but it is inherited form the mother. De novo variant c.49G>A (p.Glu17Lys) in AKT2 gene was found.

Conclusion: This gain of function variant found in AKT2 gene was already described in patients with severe fasting hypoglycemia [2] and one patient with similar phenotype [3]. We can now assume that MORFAN syndrome is confirmed in this first described patient after 30 years.

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Grants: Supported by MH CZ DRO (FNM 64203, project 6003).

Conflict of Interest: None declared.

EP12.015 Exploring the role of the SHH gene in development: A case report of two children with Variant of Uncertain Significance

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Background: The *SHH* gene (MIM *600725) encodes a protein that is involved in establishing cell fates at several points during embryogenesis.

In this case report, we describe two children with a variant of uncertain significance in the *SHH* gene.

Methods: Individuals were examined at Medical Genetics and Prenatal diagnostics department of Latvian Children's Clinical University Hospital. Written consent was obtained from all participants.

Results: The clinical geneticist consulted the first child due to holoprosencephaly. DNA testing revealed variant of unknown significance (VUS) c.427G>C,p.(Gly143Arg) in the *SHH* gene. Child died before the age of one year. Variant segregation revealed that the probands' father is the carrier of the variant. Phenotyping of both parents was made – father had hypotelorism, epicanthal folds and cleft palate. Prenatal consult was done for family, but further testing was not offered. The second child was born with unilateral choanal atresia, currently progressive microcephaly, and developmental delay. Second child underwent clinical-exome sequencing, which revealed the same variant in the *SHH* gene.

Conclusion: The variant was not previously described and was absent from gnomAD. It is localized in the hotspot of missense variants, and pathogenic variant is described in the same codon. From clinical point of view, variant should be classified as likely pathogenic. Pathogenic variants in *SHH* gene have variable expressivity. This poses a challenge in prenatal diagnosis, even more when the variant classification is not straightforward.

Findings of this case report highlight the importance of *SHH* variants evaluation and developing a better understanding of the genotype-phenotype correlation.

Conflict of Interest: None declared.

EP12.016 Zhu-Tokita-Takenouchi-Kim (ZTTK) syndrome: the first prenatal diagnosis

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Background/Objectives: Zhu-Tokita-Takenouchi-Kim syndrome (ZTTK) is an autosomal dominant condition associated with pathogenic variants in the SON gene. It causes intellectual disability and various malformations: thinned corpus callosum, hypotonia, facial dysmorphia, musculoskeletal and genitourinary alterations. We report the first case with prenatal diagnosis.

Methods: Case report of a male patient, 2 years and 6 months old, first child of non-consanguineous parents. It was a planned pregnancy, which evolved with placental abruption in the first trimester and ultrasound findings of mild ventriculomegaly, thinning of the corpus callosum, unilateral hydronephrosis on the right. Amniocentesis was performed for karyotype and exome. He was born by cesarean section measuring 45 cm and weighing 2530 g, with mild respiratory distress. Ventricular communication,

laryngomalacia and dysmorphisms were detected: bilateral epicanthus, elongated palpebral fissure, low set ears and hypoplastic columella. Today, he has hypotonia and global developmental delay, contractures in his knees and wrists, and epilepsy.

Results: Karyotype is 46,XY [20]. Exoma showed pathogenic variant in the SON gene c.2847_2854del p.(tyr949*) in heterozygosis, not previously described. The family received genetic counseling during prenatal care and the child has clinical support and global stimulation since birth.

Conclusion: The investigation of prenatal morphological findings is essential for better management of congenital conditions. Early diagnosis allows adequate guidance to families and, in this case, greater knowledge of the natural history of a rare condition. This report presents a variant not previously described in the literature.

Conflict of Interest: None declared.

EP12.017 Inherited novel CDK8 variant associated with syndromic intellectual developmental disorder

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Background: Cyclin-dependent kinase 8 (CDK8) is part of a regulatory kinase module that regulates the activity of the Mediator complex. The Mediator complex goes on to regulate RNA polymerase II activity consequently affecting transcriptional regulation. Thus, inactivating mutations of the gene within the kinase module cause aberrant transcriptional regulation and a condition, named as CDK8-related intellectual developmental disorder with hypotonia and behavioral abnormalities.

Objective: Identification of the genetic underpinning responsible for the clinical presentation as well as parental inheritance.

Methods: Blood samples were collected from the family with written informed consent. DNA extraction, next generation sequencing, and data analysis were performed in a targeted exome to identify the causal gene and the inheritance.

Results: Herein, we describe a novel likely pathogenic heterozygous *CDK8* variant C.599G > A, p.(Arg200Gln) in a patient transmitted from the mother with a first time reported parental inheritance. Clinical presentation of the child is within the described clinical spectrum for CDK8-related syndromes, but also includes undocumented progressive contractures of the hips and knees as well as scoliosis. This phenotype was different in the mother, highlighting the heterogenous presentation for the same variant, even within the same family.

Conclusion: The described clinical presentation coincides with the notion of a Mediator complex pathology or MED12/MED13L/CDK8 related syndrome with new phenotypic features, variable expression and documents for the first time a parental inheritance.

Grant References: New Brunswick Health Research Intuition, Centre de Formation Medicale du Nouveau Brunswick.

Conflict of Interest: BEN AMOR MOUNA new Brunswick health research foundation, Centre de formation medicale du nouveau Brunswick, dominique comeau: None declared, jenna beliiveau: None declared.

EP12.018 Quadruple mosaicism associated with neurodevelopmental delay and short stature: a new case reporting a complex mosaicism

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Background: Chromosomal structural variants described for chromosome 18 are diverse with several cases of deletion, duplication, isochromosomes, dicentric or ring configurations having been documented. The presence of these anomalies together as a complex mosaicism is rare not only because of the molecular findings but also because of the variable phenotypic presentation.

Methods: We describe an 8-year-old patient with neurodevelopmental delay, language impairment and short stature with mild dysmorphism not configurating a syndromic gestalt. Routine copy number variation study by aCGH, and a subsequent conventional karyotyping were performed. Informed consent was obtained.

Results: Microarray analysis demonstrated a deletion of both ends of chromosome 18, suggesting a ring configuration that was confirmed by karyotyping, observed in 48% of the metaphases. Another three cell lines were detected, with normal masculine chromosomal complement in 33% of the metaphases; a chr18 monosomy in 12%; and in the 7% remaining a dicentric 18 trisomy.

Conclusion: A quadruple mosaicism was found in a patient, probably as a multiple-step event during development, with neurodevelopmental delay, language impairment and pathological short stature as the main clinical features. Karyotyping together with array analysis resolved the diagnosis and mechanism in this case, both congruent with the phenotype. This case presents a novel report of co-ocurrence of three peculiar chromosomal abnormalities pertaining to chromosome 18.

Grant references: Cero grants.

Conflict of Interest: None declared.

EP12.019 Genetic diagnosis of dup15q syndrome in a group of patients from Serbia

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Dup15q syndrome is caused by chromosome abnormalities that result in at least one extra copy of a 15q11.2-q13.1 region. Its prevalence can be 1 in 5,000 individuals in the general population, while it is 10 times more common in those affected with developmental delay, ASD, hypotonia, epilepsy, and others.

In most cases, the diagnosis of this syndrome is established by the detection of the extra copies arising by an isodicentric 15q11.2-q13.1 supernumerary chromosome – idic(15) resulting in tetrasomy. Otherwise, the cause can be an interstitial duplication that typically includes one extra copy of 15q11.2-q13.1 within chromosome 15, resulting in trisomy of this region.

The aim of this study was to find genetic cause underlying dup15q syndrome. In the Laboratory of Medical Genetics (Mother and Child Health Care Institute `Dr. Vukan Cupic`) 555 children were tested for microdeletion syndromes (February 2015 -February 2023) using MLPA. Dup15q was found in eight patients – four (4/8) have three copies while the other four (4/8) have four copies of this region. Additional karyotype, FISH and MS MLPA analyses in patients with four copies showed supernumerary marker chromosome 15 which confirmed tetrasomy of the 15q11.2-q13.1 region in these cases (maternal origin).

In this paper, the authors will discuss the importance of multimethod approach in the genetic diagnosis of the dup15q syndrome along with its significance for patient management and genetic counseling of families at risk.

Conflict of Interest: Maja Cupurdija full time, Marijana Miskovic full time, Sanja Cirkovic full time, Danijela Radivojevic full time, Nina Ilic full time, Tanja Lalic full time, Bojana Dobric full time, Marina Djurisic full time.

EP12.020 A novel heterozygous nonsense variant in the FOXG1 gene causing FOXG1-related encephalopathy

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Background: FOXG1-related encephalopathy (FE) is a severe neurodevelopmental disorder with microcephaly and brain malformations. It is caused by heterozygous variants in a single exon gene, *FOXG1*, which encodes a forkhead transcription repressor. A recent study suggests that truncating *FOXG1* variants in the N-terminus and the DNA-binding forkhead domain (FHD) present more severe phenotypes.

Subject and methods: We report a 14-year-old boy born to healthy parents with severe global developmental delay, absent speech, poor eye contact, generalized epilepsy, microcephaly, pachygyria, sleep disorders, spasticity, scoliosis, and strabismus. He did not achieve developmental milestones. Clinical exome sequencing was performed in the subject using Illumina TruSight One Kit.

Results: A novel heterozygous nonsense variant, c.625G>T (p.Glu209Ter) in FHD of *FOXG1* gene was identified. According to ACMG classification, the variant is pathogenic. Six in silico prediction scores are in favor of the damaging effect of the variant.

Conclusion: To our knowledge, this is the first case of FE due to c.625G>T variant in *FOXG1*. The phenotype of the patient is in accordance with phenotypes of other reported subjects carrying truncating mutations in FHD of *FOXG1*. Based on in silico prediction scores, highly conserved amino acid sequence of FHD, and specific phenotype in subject, we consider c.625G>T as a likely pathogenic cause of FE. This variant will contribute to the mutational spectrum of FE.

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EP12.021 Genetic heterogeneity and somatic mosaicism in Cornelia de Lange Syndrome

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Introduction: Cornelia de Lange syndrome (CdLS) is a developmental disorder characterized by distinctive facial appearance, growth retardation, intellectual disability and hirsutism. De novo mutations in *NIPBL* gene are found in the majority of patients with somatic mosaicism detected in a significant proportion. Other genes belonging to the cohesin complex (*SMC1A*, *SMC3, HDAC8, RAD21*) are also mutated in moderate forms of CdLS. Somatic mosaicism has been described for all of these genes, in blood, buccal cells and fibroblasts.

Methods: We tested *NIPBL* gene by sanger sequencing in 111 patients and *NIPBL, SMC1A, SMC3, HDAC8, RAD21, ANKRD11* and *KMT2A* genes by deep next-generation sequencing in 78 patients.

Results: Mutations were identified in *NIPBL* (89 patients, 91%), *SMC1A* (3, 3%), *SMC3* (2, 2%). In 13 patients, *NIPBL* mutations (15%) were absent from leukocytes and only found in buccal cells or fibroblasts, 7/13 patients carried a mutant load below 25% in the mutant tissue. We also identified mutations in *ANKRD11* (2 patients) and *KMT2A* (2 patients) in patients with typical CdLS.

Conclusion: Our results highlight the need to study multiple tissues and to use high sensitive technologies in order to improve the diagnosis of CdLS.

Conflict of Interest: None declared.

EP12.022 Genitourinary malformations as possible features of SETD5-related syndrome

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Introduction: Mutations in genes encoding components of the epigenetic machinery cause a group of chromatin modifying disorders that share core phenotypic features including growth retardation, intellectual disability and craniofacial dysmorphism. These include SETD5-related neurodevelopmental syndrome caused by heterozygous loss-of-function variants in SETD5, encoding a histone-lysine N-methyltransferase, and showing clinical overlap with other chromatinopathies such as Cornelia de Lange or KBG syndrome.

Case Report: A 43-year-old man was referred for an undiagnosed condition of mild intellectual disability and genitourinary malformations. Unilateral cryptorchidism was recorded at birth and surgically repaired. He underwent speech and infant psychiatry therapy for language and school difficulties. Craniofacial dysmorphism included bitemporal narrowing, short, webbed neck, low hairline, synophrys, deep-set eyes, dysmorphic ears, tubular nose, long philtrum, and narrow palate. Teletelia, cubitus valgus and multiple vertebral blocks were recorded. At adult age, congenital genitourinary anomalies were diagnosed including right-side renal agenesis with a remaining ureteral lump, vesicoureteral reflux and thickening of the prostatic urethra.

Results: Exome sequencing revealed a de novo heterozygous variant c.1197del, p.(Val400Trpfs*50) in the SETD5 gene, not reported in GnomAD and scored as pathogenic by dedicated tools.

Conclusion: We describe genitourinary defects in a patient with SETD5-related neurodevelopmental syndrome possibly expanding the phenotypic spectrum of this rare chromatinopathy. Although more data are needed to confirm such association, clinical and instrumental assessment of the kidneys and urinary tract may be relevant in SETD5-mutated individuals for appropriate management and follow-up.

Grant References:
Ricerca corrente 2022 to FB

Conflict of Interest: None declared.

EP12.023 Variable clinical findings related with the deletion size in 22q11 microdeletion syndrome

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Background/Objectives: 22q11.21 microdeletion is a well-known contiguous-gene-deletion-syndrome that occurs through the rearrangement of low-copy repeat regions(LCR). The aim of this study is to investigate the correlation between different LCR in the patients diagnosed with 22q11.21 microdeletion and their clinical presentation, and to emphasize the rare clinical findings of the syndrome.

Methods: The findings identified in 34 patients with 22q11.21 microdeletion-syndrome confirmed by microarray(Infinium-CytoSNP-850K BeadChip,Illumina) were divided into five-main-groups as cardiologic, endocrinologic, immunological, dysmorphic and neurodevelopmental. Deletions were also grouped as proximal, central,and distal according to the deletion lengths*.

Results: Most of the deletions(29 out of 34) were localized within the proximal-region. One patient had deletion within the central-region, and 4 patients within the distal-region, respectively. While there were findings related to at least 2 main-systems of the syndrome in patients with proximal-region, only 7 patients had a conotruncal-heart-defect. A patient with central-deletion had only neurodevelopmental-findings. All 4 patients with distal-deletion were tested for dysmorphic-findings, and only 2 had cardiac-valvuler-anomalies. Only 11 out of 34 patients were prediagnosed with 22q11.21 microdeletion-syndrome before testing, and all of them had deletions in the proximal-region. Furthermore, two different patients in the proximal-LCR group had unusual findings like isolated-bilateral-sensorineural-deafness and intentional-tremor, respectively.

Conclusion: 22q11.21 microdeletion-syndrome's clinical-presentation isn't uniform even in the same genetic imbalances. Although it is known that the patients in the proximal-LCR-group showed a more apparent-phenotype, only 38% of these patients had a clinical-prediagnosis. Therefore, more studies are needed to find factors other than deletion-size in the variety of clinical-findings.

Count: 247 words

Conflict of Interest: None declared.

EP12.024 Novel variant p.(Pro429Ser) in the DHCR7 gene in combination with pathogenic variant p.(Trp151*) causing Smith-Lemli-Opitz syndrome

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Smith-Lemli-Opitz syndrome (SLOS) is a well-known autosomal recessive multiple congenital malformation and mental retardation syndrome caused by homozygous or compound heterozygous pathogenic variants in the *DHCR7* gene. A number of pathogenic variants have been described in SLOS patients, but the p.(Pro429Ser) variant has not yet been reported.

Our patient underwent an amniocentesis due to the positivity of the integrated screening with a high risk of M.Edwards, M.Patau and SLOS and a previous ultrasound finding of a fetal hygroma colli in the 13th week of gestation. The aCGH profile of the foetus was normal - arr(X,Y)x1,(1-22)x2. The spectrophotometric determination of 7-dehydrocholesterol in the amniotic fluid was negative. A DNA analysis revealed pathogenic variant c.452G>A p.(Trp151*) and variant of unknown pathogenicity c.1285C>T p.(Pro429Ser) in the *DHCR7* gene. Each variant was present in one of the parents. The p.(Pro429Ser) variant had not previously been described in the variation databases and was rated inconsistently by the prediction programs – SIFT (v6. 2. 0): Tolerated, MutationTaster (v2013): disease causing, PolyPhen2: probably damaging. The fetal ultrasound findings in the 20th week of pregnancy were normal. The parents decided to continue the pregnancy.

After birth, the child had a typical facial dysmorphia, camptodactyly, syndactyly of the toes, a wide neck and hypotonia. The spectrophotometric determination of 7-dehydrocholesterol in a blood sample was positive. Psychomotor development of the child is delayed.

This case demonstrates the pathogenicity of the p.(Pro429Ser) variant of the *DHCR7* gene.

Conflict of Interest: None declared.

EP12.025 A new case of Rasmussen syndrome: bilateral external auditory canal atresia and congenital vertical talus caused by a small 18q22.3-q23 microdeletion involving TSHZ1 and SMIM21

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Background: In 1979, Rasmussen et al., described a family with autosomal dominant congenital external auditory canal atresia (EACA) and variably expressed bilateral foot anomalies, including congenital vertical talus (CVT). Both EACA and CVT occur in isolated and syndromic forms and can co-occur in 18q deletion syndrome. Feenstra et al., (2011) demonstrated that 18q22.3-q23 microdeletions in one family resulted in EACA and CVT and that *TSHZ1* haploinsufficiency in this region causes EACA with incomplete penetrance. Further, Mark et al., (2013) identified 18q22.3-q23, specifically *TSHZ1* and *SMIM21*, as the critical region for CVT via haploinsufficiency on review of overlapping microdeletions.

Methods: Chromosomal microarray analysis (CMA) was performed on proband peripheral blood DNA.

Results: We report a 5-year-old girl with congenital bilateral EACA and CVT. She also presents with dysmorphic facial features, abnormal dentition, mild myopia, mild global developmental delay, and possible premature adrenarche. CMA revealed arr[GRCh37] 18q22.3q23(72997677_73851810)x1, an 854 kb deletion that involves the majority of the *TSHZ1* coding sequence and completely overlaps *SMIM21*, a gene of unknown function.

Conclusion: The simultaneous finding of EACA and CVT in our patient and 4 previously reported individuals with 18q22.3-q23 microdeletions from 2016-2022 confirms that deletions in this

region cause Rasmussen syndrome. To our knowledge, this deletion is the smallest reported in this region and confirms that the critical region for these phenotypes in 18q deletion syndrome comprises *TSHZ1* and *SMIM21*. While *TSHZ1* underlies EACA, it remains unclear if loss of one or both genes causes CVT or other foot anomalies.

Conflict of Interest: None declared.

EP12.026 Application of Bead-Chip technology and exome sequencing in the genetic analysis of pediatric patients with congenital anomalies of the kidney and urinary tract (CAKUT)

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Background/Objectives: Congenital anomalies of the kidney and urinary tract (CAKUT) are the most common cause of chronic kidney disease among children and adults younger than 30 yr and occur in 3-6 of 1000 live birth (1).

Pathogenic variants in more than 50 genes have been reported with an autosomal dominant or, more rarely, autosomal recessive inheritance (2).

Furthermore, a significant number of CAKUT patients carry copy number variants (CNVs) overlapped with known syndromes. Using SNP-array technology is particularly promising in pediatric patients exhibiting syndromal phenotypes, in whom microdeletions or microduplications affecting more than a single gene are a likely cause.

Methods: We performed CNVs analysis on 86 probands with CAKUT using the Infinium BeadChip (*Illumina*). The SNP array platform includes 850000 selected single nucleotide polymorphisms (SNPs) spanning the entire genome with enriched coverage for 3,262 genes of known cytogenetics relevance. Furthemore, trio WES analysis on the same probands and their parents is in progress to identify single nucleotide mutations.

Results: Based on the preliminary analysis, SNP-array platform disclosed a 17q11.2 pathogenetic deletion of 5 Mb, including the *HNF1B* gene, associated with renal cysts and diabetes syndrome (OMIM #137920), 8 CNVs which may be pathogenetic and 4 with uncertain significance (CNV-VUS).

Conclusion: Often CAKUT is the first sign of a complex systemic disease, so an early molecular diagnosis can help the physician to identify other subtle clinical manifestations, affecting the management and prognosis of patients.

References:

1)Vivante A et al. 2014.
2)Hwang D-Y et al. 2014.

Grants: Italian Ministry of Health.

Conflict of Interest: None declared.

EP12.027 Chromosome 14q32 abnormalities in patients with clinical Silver-Russell suspicion

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Background: Silver-Russell (SRS) and Temple syndromes (TS14) are imprinting disorders characterized by fetal and postnatal growth retardation and feeding problems. Clinical overlap is observed especially in infancy to early childhood. Typical for TS14 weight gain and premature puberty are observed later in a patient's life. SRS is mainly caused by hypomethylation of *H19/IGF2*:IG-DMR at the 11p15 or upd(7)mat, in a small number of patients other abnormalities, e.g. in chromosome 14q32 or upd(20)mat, have been found. TS14 is associated with defects in the 14q32 region.

Patients and Methods: A group of 25 patients with SRS suspicion and with excluded 11p15 defects and upd(7)mat underwent further molecular investigations. Analyses by methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) including imprinted loci on chromosomes: 6q24, 7p12, 7q32, 11p15, 14q32, 15q11, 20q13 were applied.

Results: Analysis of several imprinted loci revealed molecular alterations affecting *MEG3*:TSS-DMR in the 14q32 corresponding to TS14 in two patients. *MEG3*:TSS-DMR loss of methylation and maternal UPD of chromosome 14 [upd(14)mat], confirmed by microsatellite analyses and CGH + SNP microarray, were identified.

Conclusion: Clinical overlap between SRS and TS14 sometimes makes difficult the correct clinical diagnosis, especially in young children. Methylation/copy number analyses for the 14q32 region should be considered in children suspected of SRS but with no defects at the 11p15 or upd(7)mat. MS-MLPA is a valuable tool allowing simultaneous analyses of imprinted loci involved in genetic etiology of SRS and TS14.

Grant References: The study was partly supported by CMHI project S180/2019.

Conflict of Interest: None declared.

EP12.028 CHARGE syndrome: Phenotypic and molecular spectrum in Malaysian patients

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Background/Objectives: CHARGE syndrome is an autosomal dominant multisystem disorder due to pathogenic variants in the *CHD7* gene. Clinical presentation is highly variable, and includes typical, partial and atypical forms.

Methods: In this retrospective cohort study, we identified 16 individuals with *CHD7* pathogenic or likely pathogenic variants confirmed by whole exome sequencing or gene panel testing and analyzed their clinical characteristics in relation to the current clinical diagnostic criteria.

Results: Molecular analysis of the *CHD7* gene identified 11 nonsense, 3 frameshift and 2 splice site variants, out of which 7 were novel mutations (c.5973_5975delATTinsG, c.4995G>A, c.2723G>A, c.5782C>T, c.5095_5096insG, c.1932_1935del, c.5210+5G > C). Clinical features noted in our cohort include coloboma (50%), choanal atresia or cleft palate (38%), external, middle or inner ear anomalies (100%), hearing impairment including cranial nerve dysfunction (75%), cardiovascular or tracheoesophageal malformations (88%), hypothalamo-hypophyseal dysfunction and genital anomalies (56%), developmental delay (100%), feeding difficulties (94%) and renal/skeletal/limb anomalies (69%). Only 5/16 (31%) fulfilled the diagnostic criteria proposed by Blake and Prasad. According the Verloes criteria, 2/16 (12%) were typical CHARGE, 1/16 (6%) partial CHARGE and 9/16 (56%) atypical CHARGE while the other 4

(25%) did not fulfil the diagnostic criteria. All 16 individuals fulfilled the clinical criteria as proposed by Hale et al.,

Conclusion: Individuals with *CHD7* pathogenic mutations have a wide clinical spectrum with most of our cohort having the atypical CHARGE presentation. Our study supports the importance of the including the *CHD7* pathogenic variant in the clinical diagnostic algorithm.

Grant references: None.

Conflict of Interest: None declared.

EP12.029 Variant first, phenotype second: Committing Clinical Genetic sacrilege

Caoimhe McKenna[1], *Katie Kerr*[2], *Diya Thomas*[2], *shirley heggarty*[3], *william wright*[3], *padraig hart*[3], *Helen Savage*[4], *beth green*[4], *Natalie Trump*[4], *lisa soeters*[3], *Cheryl Flanagan*[3], *gillian rea*[1], *Shane McKee*[1], *Amy Jayne McKnight*[2]

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The Northern Ireland Regional Genetics Service recruited 442 probands to the 100,000 Genomes Project. A Genomics England algorithm filtered variants based on HPO terms, PanelApp and segregation. Locally, clinicians reviewed these filtered variants, and submitted any for classification where they felt the gene-disease association was a plausible fit, regardless of molecular characteristics. Following this "first pass", 82 patients received a diagnosis (18.5%), 360 remained undiagnosed.

We utilised a variant prioritisation tool (Exomiser) to review the top five 'exomiser variants' in the undiagnosed patients. A clinician reviewed basic meta-data for each of the top five variants, identifying those which appeared "molecularly hot", and only then making a comparison with the patient's phenotype. Plausible variants were submitted for formal classification.

We identified variants of interest in 64 patients (18%). To date, formal classification has been undertaken for 41 patients, of which 25 received a molecular diagnosis (61%). Reasons for "missed" diagnoses on first pass included: incomplete HPO terms; variants in genes which were not "green" at the time of panel application; erroneous segregation information; and gene-disease associations which were incorrectly discounted by clinicians.

We propose that traditional filtering of variants led by panels and purported inheritance contributed to a significant number of missed diagnoses in our cohort. Our "variant first, phenotype second" approach increased diagnostic yield. This approach may also improve efficiency, focusing interpretative efforts on variants with the potential to reach a pathogenic classification. We propose that prioritising variants, using increasingly sophisticated computational tools, will be the future of diagnostic discovery.

Conflict of Interest: None declared.

EP12.030 A 43 years-old patient with Cornelia de Lange Syndrome with NIPBL gene mutation and a mild phenotype

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Background: Cornelia de Lange Syndrome (CdLS) is a rare genetic and multisystem disease with a prevalence of 1:30 000 live births. The patients present several physical, cognitive, and behavioral phenotypes. The life expectancy of patients with CdLS varies between childhood and puberty, but recently, with the advancement of technology, patients in adulthood are being reported.

Methods: Here we report a female patient at 43 years old. Exome sequencing was performed with paired-end libraries on Illumina HiSeq 2500 platform. Reads were aligned using NOVOALIGN, variants were called using GATK annotated using ANNOVAR. Exonic and intronic variants within 30 bp from exon–intron boundaries were examined.

Results: A pathogenic de novo mutation was detected in a *NIPBL* gene splice site (NM_133433.3:c.7410+4A > G:p.Lys2422_Glu2470del), thus confirming the CdLS diagnosis. In addition to her advanced age, the patient has a higher-than-expected cognitive development compared to most patients with CdLS. She developed synophrys and dry skin in childhood, but these features disappeared with age. The patient presents arched eyebrows, thin lips, long philtrum, and minor limb malformations (syndactyly and finger bends). Her phenotypic characteristics do not interfere severely with her lifestyle.

Conclusion: Few cases of CdLS patients over 40 years old have been described. Thus, the reported patient is an atypical case of CdLS due to her advanced age and non-severe characteristics. The present work contributes to helping understand the variability of CdLS since a patient with NIPBL mutation can present only mild phenotype and a long life expectancy.

Grants References: #2019/21644-0 and #2022/09582-1, FAPESP.

Conflict of Interest: None declared.

EP12.031 A case 5p- syndrome with atypical findings due to paternal t(5;20) rearrangement

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Introduction: 5p- syndrome (Cri-du-Chat syndrome- CdCs) is a contiguous gene syndrome resulting from deletions with different breakpoints, which encompasses a critical region located between p15.2 and p15.3. of chromosome 5p.

Herein, we report a case of 5p deletion in a newborn girl with with unusual clinical findings whose father is a balanced t(5:20)(p15;p13) carrier. The case has 5p15.33-pter deletion and is trisomic 20p13-pter. In addition to the classic CdCs findings, the patient has thymic aplasia and lymphopenia.

Materials and Methods: G-banding was performed in patient and her parents. FISH was performed to define the chromosomal regions involved and microarray was carried out in order to define deletion size in our patient.

Results: The karyotype and FISH analyses of the proband revealed 5p deletion. Paternal chromosome analysis was 46,XY, t(5:20)(p15;p13). Microarray confirmed a 23 Mb deletion on chromosome 5p15.33p14.3, and 13 Mb gain on chromosome 20p13p12.1.

Conclusions: CdCs is a contiguous gene syndrome, phenotypic findings vary according to the gene content of the deleted region and context of genomic imbalances. To the best of our knowledge, thymic aplasia and lymphopenia were not previously reported in CdCs. Deleted region included 56 genes which some of them are known to be related to classic 5p- findings but functions of other genes and interactions between them and

related pathways remain to be elucidated. Furthermore partial trisomy 20p may contribute clinical findings in this case. With better understanding of gene dosage effect of unbalanced chromosomal segments will help to clarify genotype-phenotype correlation in these patients.

Conflict of Interest: None declared.

EP12.032 Upgrading an intronic TMEM67 variant of unknown significance to likely pathogenic through RNA studies and community data sharing

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Background: An Ashkenazi Jewish couple was referred for genetic counselling following a previous pregnancy termination due to posterior encephalocele and enlarged kidneys. The index pregnancy also exhibited several fetal abnormalities, including enlarged kidneys with cystic dysplasia and abnormal cerebellar morphology, suggestive of Joubert syndrome.

Methods: Trio exome sequencing (ES) was performed and analyzed in-house. The potential effect of the paternal intronic variant on mRNA splicing was studied using RNA extracted from parental peripheral blood mononuclear cells (a fetal sample was unavailable); *TMEM67* exons 11-14 were amplified for subsequent analyses.

Results: Trio exome sequencing revealed compound heterozygosity for variants in *TMEM67*: a known pathogenic (P) maternally-inherited variant, in trans with a paternal intronic variant of unknown significance (VUS). RNA analysis revealed that the intronic variant creates a cryptic acceptor splice site in intron 12, leading to insertion of 22 bp and causing a frameshift with a premature stop codon. This analysis enabled the reclassification of the intronic variant to likely pathogenic (LP).

Conclusions: Our study highlights the importance of pursuing further testing using RNA analysis when a VUS is observed in trans with a P/LP variant in genes compatible with the fetal phenotype, especially in recurrences. In our case, this resulted in a clear resolution allowing precise genetic counseling regarding recurrence risk and reproductive options for future pregnancies. Since the submission of this variant classification to the Franklin Community, this intronic variant has been detected in trans with other pathogenic *TMEM67* variants in two additional fetuses manifesting with a ciliopathy phenotype.

Conflict of Interest: None declared.

EP12.033 Study of the spectrum of rare mutations leading to Noonan syndrome and other RASopathies in the Russian Federation

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Background: Noonan Syndrome is a group of genetically heterogeneous diseases caused by mutations in genes encoding proteins with roles in the RAS-MAPK signaling pathway. It is known as an important cascade of signal transmission from the cell membrane to the nucleus. This cascade is involved in cycles of cell differentiation, growth, ogranogenesis, synaptic plasticity, etc. Previously it was thought that about 50% of hereditary forms of NS are caused by mutations of the *PTPN11* gene.

Materials and Methods: 463 samples of unrelated probands were investigated. In our laboratory. It was probands with incoming diagnoses like Noonan Syndrome, LEOPARD Syndrome, Costello Syndrome, etc. The search for mutations was carried out using the mass parallel sequencing (MPS) method using a custom panel including the *NRAS, RIT1, SHOC2, CBL, PTPN11, HRAS, KRAS, A2ML1, SOS2, SPRED1, MAP2K1, NF1, MAP2K2, PPP1R13L, SOS1* genes.

Results: Results are obtained for 459 samples. 188 pathogenic or probably pathogenic variants have been found. This includes 80 pathogenic variants in the non-*PTPN11* genes, which is 43% among all the identified variants. The most common genes are: *SOS1* and *BRAF* (7.4% each), *SHOC2* (6.9%), *NF1* (6.4%). Other genes take 14,4% it is *RIT1*, *KRAS*, *MAP2K1*, *RAF1*, *SPREAD1*, *HRAS*, *LZTR1*, *CBL* and *NRAS*.

Conclusions: the world statistics: in 50% of cases, the diagnosis belonging to the class of Rasopathies is due to a mutation in the *PTPN11* gene. We have other data. For Russian population there is 57.4% for *PTPN11* and 43% for other genes.

Conflict of Interest: None declared.

EP12.034 Gene panel testing in RASopathies: is it sufficient?

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Background/Objectives: The RAS/mitogen-activated protein kinase (MAPK) pathway plays a crucial role in cell differentiation, growth, and senescence. Heterozygous pathogenic variants in genes regulating this pathway are known to cause a group of rare developmental syndromes called RASopathies. These disorders display numerous overlapping phenotypic characteristics, making clinical diagnosis quite challenging. This study aims to assess the diagnostic value of NGS gene panel testing for RASopathies.

Methods: Fifteen pediatric patients suspected of RASopathy were referred for genetic testing. DNA sequencing was carried out on an Ion Torrent S5 NGS platform using an Ampliseq RASopathy gene panel covering 14 genes: *A2ML1, SPRED1, RAF1, RIT1, SHOC2, SOS1, PTPN11, CBL, NRAS, MAP2K2, KRAS, HRAS, BRAF* and *MAP2K1*. Variant calling and interpretation of pathogenicity were performed according to the ACMG criteria using Ion Reporter v.5.18 and Qiagen Clinical Insight Interpret 9.0 software. Additionally, trio whole-exome sequencing using an Illumina NGS platform was done in one patient who tested negative on the RASopathy gene panel.

Results: Sequencing data identified clinically significant pathogenic variants in **33% (5/15)** of the analyzed patients: two variants in the ***BRAF*** gene **(c.1455G>C and c.1460T>G)**, and another three variants in the genes ***RAF1*** **(c.483T>G),** ***MAP2K1*** **(c.389A>G)** and ***PTPN11*** **(c.922A>G)**. Trio whole-exome sequencing revealed an ultra-rare de novo pathogenic variant **c.2935dupG** in the ***BICRA*** gene associated with Coffin-Siris syndrome (OMIM #619325).

Conclusion: Gene panel testing is a useful and cost-efficient approach in the evaluation of common RASopathy-associated genes. However, in some cases, broader supplementary analyses are needed for further clarification and confirmation of diagnosis.

Conflict of Interest: None declared.

EP12.035 Coexisting conditions modifying phenotypes of patients with 22q11.2 Deletion Syndrome

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Background: 22q11.2DS is the most common chromosomal microdeletion syndrome with a prevalence of 1:2148 live births. 22q11 deletion is a result of non-allelic homologous recombination between high identity low copy repeats (LCRs). That leads to a diminished dosage of nearly 60 genes. The characteristics of this syndrome is highly variable phenotypic severity with many common and rare features, but none appear to be fully penetrant and each exhibits variable expressivity. It remains unknown why individuals with deletion of the same size, present such a wide range of phenotypes. The aim of our study was to investigate how often the additional variants in the genome, can affect clinical variation, among patients with the recurrent deletion.

Methods: To examine the presence of additional variants, affecting the phenotype we performed microarray in 82 prenatal and 77 postnatal cases and exome sequencing in 86 postnatal patients with 22q11.2DS

Results: Within those 159 patients where array was performed, 5 pathogenic and 5 likely pathogenic CNVs have been identified, outside of the 22q11.2 region. This indicates that in 6.3 % cases, additional CNVs most likely contribute to the clinical presentation. Additionally, exome sequencing in 86 patients revealed 3 pathogenic (3.49%) and 5 likely pathogenic (5.81%) SNVs and small CNV.

Conclusion: Our study demonstrates that secondary diagnoses should be taken into account in all patients with 22q11.2DS, and the extension of diagnostics with genome-wide methods can reveal other clinically relevant changes in patients with 22q11 deletion syndrome.

Grant References: OPUS NCN 2020/37/B/NZ5/03337 to BN.

Conflict of Interest: None declared.

EP12.036 Clinical and molecular findings in three Albanian families with KBG syndrome caused by mutation of ANKRD11 gene

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KBG syndrome, due to ANKRD11 alteration is characterized by dysmorphic facial features, macrodontia, skeletal anomalies, short stature and neurologic involvement that may include seizures and intellectual disability.

The variable expressivity of KBG syndrome makes it challenging to establish genotype phenotype correlations.

We aim to report three unrelated families with KBG syndrome caused by ANKRD11 gene pathological variants and to evaluate the clinical spectrum of KBG syndrome.

Whole exome sequencing performed on the DNA of the patients identified pathogenic heterozygous variants in ANKRD11 gene and the diagnosis of KBG syndrome was made in three Albanian families.

The first family, a 12-year-old boy and his father presented intellectual disability. Exome sequencing revealed a heterozygous pathogenic variant c. 2273_2274del p. (Leu758Glnfs*23) in the ANKRD11 gene in both of them.

The second family, a 10 months old boy, presented bulbous nose, hypertelorism, prominent ears, narrow palate, hypotonia and seizures. His mother was clinically unaffected but exome sequencing revealed an ANKRD11 heterozygous pathogenic variant c.6792dup p. (Ala2265Argfs*8) in both of them.

The third family, a 1-year-old girl, presented optic atrophy, seizures, hypotonia. Exome sequencing revealed a likely pathogenic heterozygous variant c.4895_4907dup, p. (Asp1636Glufs*9). Her father presented macrodontia and her first degree relative, 23 years old, intellectual disability, seizures, and macrodontia.

To the best of our knowledge, this is the first report of KBG syndrome in Albania caused by a mutation in ANKRD11 gene. Our findings provide evidence on inter and intra familial variability and NGS-based approaches for sequencing will improve the detection of the syndrome.

Conflict of Interest: None declared.

EP12.037 A milder phenotype in a patient with a homozygous variant in the ZNF699 gene: a case report

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Background/Objectives: DEGCAGS syndrome (Developmental Delay, Gastrointestinal, Cardiovascular, Genitourinary, Skeletal) is a severe autosomal recessive neurodevelopmental disorder due to mutations in the *ZNF699* gene that encodes a large nuclear zinc-finger protein. Variants in this gene were found in 14 patients, all presenting a severe phenotype. Childhood death may occur.

We report the clinical observation of a patient harboring a novel frameshift variant of the *ZNF699* gene and presenting with a mild phenotype.

Methods: We performed a whole-exome sequencing (WES) on the proband's lymphocyte DNA.

Results: The proband is a three-year-old girl born to consanguineous parents, who was followed up for developmental delay, growth retardation and facial dysmorphism. Family history revealed early deaths from encephalopathy of unknown etiology in two cousins. Pregnancy was complicated by oligohydramnios and intrauterine growth retardation. The patient had anemia and leucopenia. Currently, she presented microcephaly, epicanthal folds, wide mouth, retrognathia, syndactyly and hypotonia. Brain MRI revealed a corpus callosum hypoplasia and global hypomyelination. Cardiac and abdominal ultrasounds were unremarkable.

WES showed a homozygous variant on *ZNF699* gene: NM_198535.3:c.1272_1273del(p.Cys424TrpfsTer11). This variation was never described in GnomAD, ClinVar, or literature, and was

considered as probably pathogenic (PVS1, PM2) according to the American College of Medical Genetics and Genomics criteria.

Conclusion: This case emphasizes the role of pangenomic methods such as WES in the genetic investigation of rare syndromes and is an example of how variants in the same gene can be associated with different phenotypes. More cases need to be studied to establish a possible genotype-phenotype correlation.

Grant References:

Conflict of Interest: None declared.

EP12.038 The novel variants detected in TSEN2 gene as a possible molecular background of ultra-rare pontocerebellar hypoplasia type 2B

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Background: Pontocerebellar hypoplasia type 2B (PCH2B, OMIM# 612389) is an ultra-rare autosomal recessive disorder characterized by progressive microcephaly from birth combined with severe neurologic manifestations. Manifestation includes hypotonia or spasticity, delayed psychomotor development, seizures and various brain defects. The disorder is caused by biallelic disruption of *TSEN2* gene.

Material and methods: In this report we present a female patient of Polish origin, child of the non-consanguineous parents. The patient presented microcephaly, axial hypotonia, peripheral spasticity, seizures and early respiratory insufficiency. Neuroimaging showed: simplified gyral pattern, cerebellar hypoplasia and enlarged cisterna magna.

Results: Whole exome sequencing detected compound heterozygosity for in trans *TSEN2* variants: c.1268T>C (p.Leu423Ser) and c.141_143del (p.Asn48del). Both variants were never described in association with PCH2B. Leu423Ser was classified as variant of uncertain significance and Asn48del as likely pathogenic according to ACMG guidelines.

Conclusions: The phenotype of the patients is consistent with the clinical presentation of PCH2B, therefore we suggest that detected variants in *TSEN2* gene are disease-causing. Given the shortage of data regarding this condition, clinical and molecular findings described here expand the molecular and clinical spectrum of PCH2B.

Conflict of Interest: None declared.

EP12.039 A rare mosaic form of ring chromosome 22 syndrome with a non-specific phenotype: a case report from paediatric practice

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Background: Constitutional ring chromosomes are rare structural abnormalities that result from breakpoints in both arms, followed by fusion of the proximal ends into a ring with loss of distal material. Ring chromosome 22(r(22)) is associated with a variable clinical phenotype, including developmental delay, severe speech disability, growth retardation, microcephaly, hypotonia, and dysmorphic traits. In addition to congenital anomalies, risks for specific cancers have been reported. The incidence of the r(22) is estimated to be<1:1000000 live births.

Methods: We present the case of a 3 years old boy with facial dysmorphism, cerebral development anomalies and global developmental delay. Combined analyses of chromosomal morphology by karyotype and FISH investigations were used for testing of our index patient and his parents. To characterize the identified ring chromosome in the proband, we performed FISH experiments using probes particular for chromosome 22, specifically wcp22, DiGeorge *TBX1* region and 22q13.3 region.

Results: Metaphase analysis of the G-banded chromosomes showed a mos 46,XY, r(22)(p11.2q13)[27]/46,XY[3]. Subsequent targeted FISH analysis for chromosome 22 confirmed the origin of the ring chromosome and indicated the deletion of the 22q13.3 region, with the loss of at least the telomeric end of *SHANK3* gene.

Conclusions: Ring chromosomes are usually identified using G-banding, which is still the basic step in multiple abnormalities syndromes diagnosis. Karyotyping also reveals the mosaic status, and molecular cytogenetic techniques should be applied to confirm the origin of the ring chromosome. Early diagnosis should be pursued in order to provide medical and social assistance by a multidisciplinary team.

Conflict of Interest: None declared.

EP12.040 The phenotypic spectrum of Lithuanian and Polish cohorts with overgrowth

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Introduction: Overgrowth syndromes (OGs) are a heterogeneous group with a diverse phenotype. The most common features of OGs include the increased height and occipitofrontal circumference (OFC) (>2 SD), dysmorphic features, and intellectual disability.

Results: Patients with OGs (N = 108) from Lithuanian and Polish cohorts were recruited for this study. The patients were assigned into 3 groups: isolated (4%), syndromic (67%), and asymmetric overgrowth (26%), respectively. They all had similar phenotypic features. More than 64% of them had psychomotor development delay or intellectual disability, 56% - had prominent dysmorphic features and 45% - had congenital defects of different organ systems. Asymmetry of lower extremities growth was most prominent (80%). Other clinical features in the groups included tumours, pigmentary anomalies, and joint laxity, while skeletal anomalies, and neurological symptoms were the most common. Genealogy analysis indicated that more than half (58%) of patients had a positive family history of cancer.

Conclusions: Based on the phenotypic analysis, the majority of the OGs cohort had the same "classic" phenotypic features as described in the literature. More detailed family history analysis revealed a potential risk of various types of neoplasms in this

group. However, further work is needed to confirm this hypothesis.

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Conflict of Interest: None declared.

EP12.041 Natural history of spinal involvement in Neurofibromatosis type 1: clues from "reverse follow-up"

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Background/Objectives: Spinal involvement, which is the presence of one or more spinal neurofibromas, is a possible manifestation in Neurofibromatosis type 1 (NF1), affecting up to 40% patients. Among these patients, we identify subjects with bilateral neurofibromas involving all spinal roots (SNF) and subjects showing multiple neurofibromas involving only some roots (MNFSR). Even if several studies have recently focused on this aspect, longitudinal data on the spinal phenotype are still scarce. Our aim is to shed light on natural history of spinal NF1.

Methods: We conducted a monocentric, observational, retrospective study on a wide cohort of adult NF1 patients. We selected the patients with spinal involvement, both SNF and MNFSR, and identified those with at least two follow-up spinal MRIs.

Results: 553 patients underwent spinal MRI (326 females, 227 males, age 18-69). A spinal involvement has been detected in 179 patients (85 females (26%), 93 males (41%)). 126/179 patients had at least two MRIs (follow-up range 1-31 years), 43 with SNF and 83 with MNFSR. 49/126 (39%) patients showed disease progression during follow-up (mode 7 years), with increased number of spinal neurofibromas, particularly 15 moved to SFN group from MNFSR or negative MRI.

Conclusion: Spinal involvement is a relevant complication in NF1. In our cohort it has been found to be a frequent manifestation and males are the most affected. However more studies are needed to deepen and characterize the clinical evolution of spinal NF1.

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Conflict of Interest: Claudia Cesaretti Full-time, Collaborator; Grant References: RF-2016-02361293 supported by Italian Ministry of Health, Beatrice Conti Full-time, Federico Grilli Full-time, Silvia Casale Full-time, Paola Riva Full-time, Collaborator; Grant References: RF-2016-02361293 supported by Italian Ministry of Health, Viviana Tritto Full-time, Collaborator; Grant References: RF-2016-02361293 supported by Italian Ministry of Health, Maria Cristina Ibba Full-time, Collaborator; Grant References: RF-2016-02361293 supported by Italian Ministry of Health, Antonia Valentina Genovese Full-time, Monica Sciacco Full-time, Manuela Caroli Full-time, Marica Eoli Full-time, Principal investigator; Grant References: RF-2016-02361293 supported by Italian Ministry of Health, Sabrina Avignone Full-time, Federica Natacci Full-time, Collaborator; Grant References: RF-2016-02361293 supported by Italian Ministry of Health.

EP12.042 Clinical heterogeneity of Charge syndrome from extremely severe expression in critically affected newborns to mild phenotype – clinical and molecular analysis of 20 cases from one genetic center

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Introduction: CHARGE syndrome is a neurodevelopmental disorder characterized by colobomas, heart defects, choanal atresia, retarded growth/development, genital hypoplasia and ear defects/deafness. Patients with CHARGE syndrome usually carry a pathogenic variant in the CHD7 gene.

Patients and methods: We present clinical evaluation of 20 patients (including 2 familial cases) aged from 3 weeks to 31 years with CHARGE syndrome. The molecular analysis has been performed with exome or targeted NGS (2 and 13 cases) or by Sanger method (4 cases; familial ones).

Results: All patients had dysmorphic features specific for CHARGE syndrome. In all but one, congenital defects were present: 76% patients had heart defect (ASD, VSD, ARSA, Fallot's tetralogy), 59% - cranial nerve paralysis, 47% - deafness, 40% - eye colobomas, hypogonadism or speech delay, 30% - intellectual disability, semicircular canals' agenesia/hypoplasia, choanal atresia or cleft lip/palate. Two newborns died with severe swallowing and breathing difficulties. Mothers of 2 patients had mild dysmorphic features and squint (1pt). Molecular analysis revealed the presence of pathogenic, loss of function variants in the CHD7 gene.

Conclusions: Our analysis demonstrate clinical heterogeneity of CHARGE syndrome. Severe clinical expression is due to heart or cranial nerves defects. The last were present in more than half of patients suggesting the role of CHD7 protein in cranial nerve development or function. Targeted sequencing is effective in the diagnosis of CHARGE syndrome, especially when the clinical presentation is unusually severe or mild.

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Conflict of Interest: None declared.

EP12.043 Evaluation of clinical and laboratory findings of individuals with Down syndrome: 11 years of experience of a single tertiary centre

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Background: Down syndrome occurs in 1 in 800 live births and is the most common genetic cause of intellectual disability. Of all Down syndrome cases, 96% of are regular type, 3-4% are

translocation type and 1-2% are mosaic type, meanwhile <1% of cases develop due to partial trisomy. Individuals with Down syndrome are at increased risk for congenital heart diseases, thyroid disorders, celiac disease, atlantoaxial instability, hearing loss, otitis media, sleep apnea, transient abnormal myelopoiesis, and acute leukemia.

Materials and Method: In this study, we analyzed 264 individuals with Down syndrome whose first admission to our clinic were between the dates 01.01.2010-31.12.2020. Prenatal, natal, and postnatal histories, and demographic characteristics along with clinical, laboratory and radiological findings were evaluated retrospectively.

Results: We found that 97% of individuals with Down syndrome had regular type, 2.3% had translocation type and 0.7% had mosaic type Down syndrome. Congenital heart disease was detected in 71.8% of them with secundum atrial septal defect being the most common. Sleep apnea was found in 95.6% of individuals who underwent polysomnography in correlation with the history of otitis media. History of recurrent pneumonia was found in 12.5% of individuals with Down syndrome which correlates with the findings of congenital heart disease, high congenital heart disease class and immunologic parameter impairment. Various central nervous system anomalies, including cerebral atrophy and ventriculomegaly, were found in 33% of individuals with Down syndrome.

Discussion: Down syndrome is a multisystemic disorder which requires multi-disciplinary follow up with regular intervals.

Conflict of Interest: Dorukcan Alkan Full, Hacettepe University (until 01.03.2023), Pelin Simsek-Kiper Full, Hacettepe University, Gulen Eda Utine Full, Hacettepe University, koray boduroglu Full, Hacettepe University.

EP12.044 Clinical spectrum in patients with PIK3CA-related overgrowth syndrome (PROS)

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Introduction: PIK3CA-related overgrowth spectrum (PROS) is a group of rare disorders characterised by segmental/focal overgrowth which is usually caused by somatic mutations in the PIK3CA gene.PIK3CA gene encodes p110 alpha protein, which is a subunit of an enzyme called phosphatidylinositol 3-kinase (PI3K). PI3K/AKT signalling pathway is critical for cellular growth and metabolism. Somatic activating mutations in the PI3K/AKT/mTOR pathway underlie heterogeneous segmental overgrowth phenotypes.

Cases: We present three patients with PROS. First patient showed after birth with large head circumference, postaxial hexadactyly, syndactyly andnaevusflammeus on face. Because of hydrocephalus, ventriculoperitoneal shunt was performed. Psychomotor development has been delayed. Heterozygous pathogenic variant (mosaicism) in the PIK3CAc.2176G > A, p.(Glu726Lys) was detected. Second patient was born by c-section because of prenatal diagnosed enlarged right cerebral hemisphere. Physical examination showed right cheek and foot enlargement. Brain MRI showed right hemimegalencephaly. Seizures began in infancy despite the antiepileptic therapy and severe developmental delay was noticed. Surgical hemispherectomy was done because of drug-resistant seizures.Genetic analysis is in progress. Third patient was born after a normal pregnancy. After birth, left-sided hemihypertrophy, skin capillary malformations and syndactyly were noticed. Brain MRI showed enlarged left side with tertiary gyration. Psychomotor development has been delayed.

Conclusion: It is important to consider mutations in the PIK3CA gene during the evaluation of the patients with a combination of clinical features suspected to PROS. The mosaicism of a pathogenic variant may lead to a spectrum of clinical phenotypes ranging from mild to severe, depending on the tissues affected.

Conflict of Interest: Marija Vidakovic full-time, Ivan Lehman full-time, Sanda Huljev Frkovic full-time.

EP12.045 Novel genotypic and phenotypic spectrum of PIGP deficiency associated to multiple congenital anomalies

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Background/Objectives. Glycosylphosphatidylinositol-anchored proteins (GPI-APs) are involved in multiple physiological process including embryogenesis and neurogenesis. The initial stage of GPI-APs biosynthesis is mediated by *PIGA, PIGC, PIGH, PIGP, PIGQ, PIGY*, and *DMP2* genes, which have been linked to a wide spectrum of phenotypes depending on the gene damaged. To date, the *PIGP* gene has only been related to Developmental and Epileptic Encephalopathy 55 (OMIM #617599) in just seven patients.

Methods. A detailed medical history was performed in two affected siblings with a polymalformative syndrome. Genomic DNA was performed using whole-exome sequencing. Sanger sequencing and CytoScan 750K SNP array were performed in all participants to confirm detected variants.

Results. One patient presented dysmorphic features, congenital anomalies, hypotonia and epileptic encephalopathy as described in *PIGA*, *PIGQ* and *PIGY* deficiencies. The other one was a fetus with a severe malformation disorder at 17 weeks of gestation whose pregnancy was interrupted. Both were compound heterozygous carriers of pathogenic variants in *PIGP* gene: NM_153682.3:c.2T>C (p.Met1?) and a 136 Kb deletion in 21q22.13 affecting the entire *PIGP* gene.

Conclusion. Further characterization of physiologic role and functional consequences of the type of variant in the *PIGP* gene are needed. Our results expand the clinical phenotype associated to *PIGP* gene and propose a novel Multiple Congenital Anomalies-Hypotonia-Seizures syndrome associated to GPI biosynthesis defect.

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Membrado Full, Alfonso Caro Llopis Full, Marta Domínguez Martínez Full, Francisco Martinez Full.

EP12.046 Oculo-auricular syndrome in a new multiplex family does not link to HMX1 or its downstream enhancer

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Background: Oculo-auricular syndrome (OCACS, #612109) is an autosomal recessively inherited disease, featuring congenital ocular (microphthalmia, microcornea, cataracts, coloboma, nystagmus, retinal dystrophy) and ear anomalies (abnormal external ear cartilage, underdeveloped ear lobes). Biallelic loss-of-function of H6-family homeobox 1 (HMX1) is the only known mechanism associated with this rare malformation syndrome. Copy number variations in the conserved downstream enhancer region (ECR) of *HMX1* causes isolated microtia in humans, mice, and sheep. Here, we describe a new multiplex family with an OCACS phenotype, without pathogenic variations in *HMX1* or its downstream ECR.

Methods: All coding and noncoding regions of *HMX1* were Sanger sequenced. WGS analysis was performed in two affected and one healthy siblings. Identity-by-descent (IBD) analysis was conducted within all family members (three affected and two unaffected siblings, and first-cousin parents) by SNP-array.

Results: All three affecteds presented with congenital eye anomalies comprising microphthalmia, microcornea, and cataracts; and dysplastic low-set ears with underdeveloped lobules. Less common findings were iris coloboma, chorioretinal defects, nystagmus, and facial hyperhydrosis. The latter is a previously undescribed finding in OCACS. Sanger sequencing showed no pathogenic variants in *HMX1*. We failed to identify a candidate gene through segregation of the variants within the homozygosity regions, detected by trio-based WGS analysis.

Discussion: We expand the limited literature on the ultra-rare OCACS phenotype. Since causative variants in *HMX1* and its ECR have been ruled out, our report paves the way for further investigations on a possibly novel gene contributing to the genetic etiopathogenesis of congenital ocular and auricular malformations.

Conflict of Interest: Umut Altunoglu Koç University (Faculty member), Koç University Hospital (physician), Mert Kaya PhD student, n. bilge satkın Koç Unviersity Hospital Genetic Diseases Evaluation Center, Aida Bertoli-Avella CENTOGENE GmbH, Esra Börklü Koç Unviersity Hospital Genetic Diseases Evaluation Center.

EP12.047 DNA methylation suggesting the extension of the clinical spectrum of the SETD2-related disorders to a syndromic multiple tumor phenotype

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Introduction: *SETD2* is a gene playing an essential role in epigenetic regulation. Pathogenic *SETD2* variants are responsible of neurodevelopmental disorders (SETD2-NDDs), that include various degrees of intellectual disability (ID), macrocephaly, brain malformations, and generalized overgrowth. A specific phenotype has been reported in association with a single pathogenic variant, p.Arg1740Trp, which leads to microcephaly, congenital malformations, eye findings, severe failure to thrive, epilepsy and profound ID. To date, about thirty patients have been described in the literature. A distinctive epigenetic signature in peripheral blood leukocytes has been identified. We report here a patient with a phenotype distinct from SETD2-NDDs.

Methods: The patient's phenotype distinctive facial features, and multiple tumor histories including a sacral osteoblastoma at age 7, a benign femoral bone tumor at age 17, a peritoneal pseudomyxoma at age 27, a hypophyseal macroadenoma and a low-grade optochiasmatic glioma at age 37 years. WAIS IV revealed a heterogeneous profile but could exclude ID. We performed a trio exome sequencing analysis and a DNA methylation study by EpiSign™ assay.

Results: Exome sequencing revealed a de novo *SETD2* heterozygous Variant of Unknown Significance p.Ser1658Leu. The episignature was compatible with the SETD2 reported episignature.

Conclusion: These results show the interest of DNA methylation studies in the discussion of pathogenicity of variants, and thus possibly expanding the clinical spectrum of disease genes. Given the implication of somatic SETD2 variants in benign and malignant tumors, the implication of this SETD2 constitutional variant in the tumor history of the patient is questioned.

Grant References: Extrican project.

Conflict of Interest: None declared.

EP12.048 Expansion of the symptoms associated with bi-allelic variants in TRAPPC12

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Background: Bi-allelic variants in *TRAPPC12* were first described in children with progressive encephalopathy and brain atrophy (1). The associated syndrome is named early-onset progressive encephalopathy with brain atrophy and spasticity (PEBAS, OMIM 617669). To the best of our knowledge five families with eight children have been described. Herein, we report on a further family and expand the clinical spectrum possibly associated with this syndrome.

Probands: The family presented in the 12th week of the first pregnancy with increased nuchal translucency. Subsequently a diaphragmatic hernia, partial agenesis of the corpus callosum (ACC), retrognathia and shortened long bones were noted. The pregnancy was terminated in the 22nd week.

In the second pregnancy early hygroma colli, diaphragmatic hernia, microcephaly, ventriculomegaly, complete ACC, vermis hypoplasia, short long bones, retrognathia and congenital heart defect (truncus arteriosus communis) were diagnosed. The pregnancy ended by spontaneous fetal demise in the 33rd week. The parents are first degree cousins.

Methods: Exome based sequencing of both fetuses and the mother was performed. Findings were verified by Sanger sequencing in both parents and fetuses.

Results: In both fetuses homozygosity for the variant NM_016030.6:c.1677+5G > A in *TRAPPC12* was diagnosed. Both parents are heterozygous.

Conclusion: The pathogenic variant reported herein has already been diagnosed together with a different variant in fetuses affected by hydrocephaly (2). We propose that the phenotype associated with pathogenic variants in *TRAPPC12* also comprises major malformations like diaphragmatic hernia and congenital heart defect.

(1) Milev et al., 2017. PMID: 28777934.
(2) Gass et al., 2020 PMID: 32347653.

Conflict of Interest: None declared.

EP12.049 First European case of Noonan syndrome-like disorder with loose anagen hair-2 caused by the recurrent c.146C>G missense variant in PPP1CB: broadening the clinical spectrum

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Noonan syndrome-like disorder with loose anagen hair (NSLH) is a rare disease part of the RASopathies. It is clinically close to NS, but it is characterized by additional distinctive findings, such as ectodermal abnormalities, among which loose anagen hair is most notably, a higher incidence of intellectual disability and hyperactive behaviour. Heterozygous pathogenetic variants in the *SHOC2* gene on the chromosome 10q25 are responsible for the Noonan syndrome-like disorder with loose anagen hair-1 (NSLH1), which includes the majority of the cases of NSLH, while heterozygous pathogenetic variants in the *PPP1CB* gene on the chromosome 2p23 are responsible for the Noonan syndrome-like disorder with loose anagen hair-2 (NSLH2), which is arisen in the last few years. They both result in an increasing activation of the Ras/mitogen-activated protein kinase (MAPK) pathway.

Herein, we describe a 7.4 year-old girl with height and weight below the 3rd centile, absolute macrocephaly, Noonan typical facial features and slow-growing hair. No other ectodermal abnormalities were observed. Her motor development is referred delayed. She presents learning disabilities and hyperactivity. Her echocardiogram was normal. A multigene NGS panel for RASopathies identified a de novo heterozygous missense pathogenetic variant in *PPP1CB* (NM_206876.1), c.146C>G; p.Pro49Arg.

To the best of our knowledge, this is the first reported case of NSLH2 in Europe and the fourteenth reported case of NSLH2 with the same recurrent variant. Because of the smallness of the number of patients with a *PPP1CB*-related disorder, delineate a genotype-phenotype correlation is difficult. Our findings expand the present data about it.

Conflict of Interest: Antonella Pizza: None declared, Elia Marco Paolo Minale: None declared, Danilo Venturino: None declared, Luigia De Falco Full-time, eloisa evangelista Full-time, Paola D'Ambrosio Full-time, Vincenzo Nigro Full-time, antonio fico Full-time, Carmelo Piscopo Full-time.

EP12.050 Chromosome 22q11.2 inherited microduplication associated with a severe phenotype with limb defects

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Background/Objectives: The 22q11.2 microduplication syndrome (OMIM: 608363) results in a highly variable phenotype, ranging from normal or milder phenotype to multiple phenotypic alterations, including mental retardation/learning difficulties, delayed psychomotor development, facial dysmorphisms, microcephaly, and heart and urogenital abnormalities. Few patients may show minor hand and foot anomalies, such as clinodactyly of the fifth fingers and hypoplastic nails. Rhizomelic and mesomelic shortening of the limbs have already been described.

Case studied: A 6-month-old male patient with facial and corporal asymmetry, absence of the right radius, subluxation of the right elbow, shortening and bowing of the ulna at right, butterfly vertebrae, kyphosis, lumbar thoracic scoliosis, ribs fusion, heart defect, pulmonary hypertension, ocular hypertelorism, ear anomaly, and macrostomia.

Results: Karyotype and cytogenomic analyses revealed a 22q11.2 2.8 Mb duplication, inherited from the father as follows: 46,XY.rsa 22q11.2(P250)×3.arr[GRCh37] 22q11.21(18,640,729_21,465,659)×3 pat. Apart from the duplication, whole exome sequencing revealed two variants in the *FREM2* gene, each one inherited from a parent (c.1837C>T:p.R613C, and c.2770_2772del:p.924_924del (GRCh37)). Whole genome sequencing confirms the 22q11.21 duplication with a minimum size of 2.2Mb: NC_000022.11:g.(?_18906320)_(21064168_?)[DUP] (GRCh38), also present in the father. The missense and deletion variants in *FREM2* were classified as VUS, and the phenotype of the patient could not be associated with *FREM2*-related disorders.

Conclusions: The severe clinical features of the patient may be attributed to the clinical variability of the 22q112 microduplication syndrome, less probably to the *FREM2* variants, but also to the simultaneous presence of both genomic variations.

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Conflict of Interest: Beatriz Carvalho Nunes: None declared, Henrique Garcia Silveira: None declared, Giovana Manilli Toccoli: None declared, Natalia Nunes: None declared, Vera Ayres Meloni: None declared, Jihye Kim: None declared, Maria Isabel Melaragno FAPESP 2019/21644-0.

EP12.051 A rare case of 17q23.1q24.2 duplication with bladder-exstrophy-epispadias complex

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Introduction: High gene density, the presence of dosage-sensitive genes, excess of segmental duplications (SD), and relative abundance of short interspersed nucleotide elements (SINE) are considered the cause of a relatively high proportion of disease-associated copy number variants mapping to chromosome 17.

Methods: We report a boy born at term with IUGR, BW 2150 g, BL 48 cm, Apgar score 9,9. Multiple congenital anomalies, including bladder exstrophy, epispadias, microcephaly, and ventricular septal defects, were observed at birth. He had a severe developmental delay. Molecular karyotyping was performed on the SurePrint G3 Human CGH 8 × 60k microarray platform with an average probe spacing of 41 kb (Agilent Technologies, Santa Clara, CA, USA).

Results: Microarray analysis revealed a gain in DNA copy number involving a 9 Mb segment (chr17: 57913469-66960450; hg19) in the 17q23.1q24.2 region. According to the literature, the patients with this duplication, in addition to foot deformities, have developmental and speech delay, behavioural problems, and possible epilepsy. Rare reports of these patients suggest that duplication of dosage sensitive genes TBX2, TBX4 and TANC2 are most likely responsible for the observed phenotype.

Conclusion: The main clinical features of the patients with duplications in the 17q23.1q24.2 region are developmental and speech delay, anomalies of the corpus callosum, and foot anomalies. To the best of our knowledge, Bladder-Exstrophy-Epispadias Complex (BEEC) as the severe end of the uro-rectal malformation spectrum has not been previously described in the patients with duplication 17q23.1q24.2.

Conflict of Interest: None declared.

EP12.052 A de novo missense variant in the MAF gene causes a milder Aymé-Gripp phenotype

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Aymé-Gripp Syndrome (AGS) is an ultra-rare disease caused by a pathogenic variant in the MAF gene that affects multiple systems of the body. It is characterized by peculiar facial traits often defined as Down-like, combined with bilateral early cataracts, sensorineural hearing loss, and variable neurodevelopmental abnormalities.

The majority of the patients described so far are carrying a de novo missense variant and all of them show a complex phenotype that requires multiple clinical management especially for hearing loss and cataracts that are recurrent features of this condition.

Since AGS has been recently discovered and since still few cases have been reported, the phenotypic spectrum is limited to the severe form of the disease and we are still making a big effort to expand the genotypic and phenotypic spectrum.

Here we report the peculiar case of a 6-year-old girl carrying a de novo missense pathogenic variant in the MAF gene (NM_005360: c.161C>G p.Ser54Trp) identified through clinical exome. She presented short stature, suggestive facial features, intellectual disability, and fine skeletal and ectodermal abnormalities. The clinical report recorded a pericardial effusion at birth.

Since then, we reviewed the literature and we realized that while hearing loss is regularly represented since birth, cataracts are often present in childhood but may also occur later.

To our knowledge, she is the first patient to show a milder phenotype of this condition without cataracts or deafness displayed.

Through our experience, we encourage educated clinical guesses in suspecting rare diseases even if crucial features are missing.

Conflict of Interest: None declared.

EP12.053 Coffin-Siris syndrome – case report

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Background: Coffin-Siris syndrome is a rare genetically heterogeneous disorder that is characterized by distinctive craniofacial, skeletal abnormalities and intellectual impairment.

Methods: 6 y.o. girl had been admitted for evaluation of coarse facial features, mild intellectual impairment and delay in growth development. She presented herself with antimongoloid palpebral fissure, hypertelorism, low ear set and pinna abnormalities, thick lips, microretrognathia and gothic palate. On examination of the skeletal features - short stature (under the third percentile), cubitus valgus, boad chest with widely spaced nipples. Delay in bone maturity was assessed. Negative family history for hereditary abnormalities. Chromosomal analysis, MLPA and panel sequencing of 249 genes had been performed.

Results: Chromosomal analysis showed cultural mosaicism with partial deletion of the ninth chromosome. Negative result from microdeletion/microduplication analysis was received. A panel sequencing was then performed and a pathogenic variant, c.1096C>T (p.Arg366Cys) was identified in SMARCB1.

Conclusion: This particular mutation is associated with Coffin-Siris syndrome, autosomal dominant rhabdoid tumor predisposition syndrome 1 (RTPS1), and schwannomatosis. The currently available evidence indicates that the variant is likely pathogenic and most likely to be inherited de novo. Although the mutation leads to loss-of-function of a tumor-supressor gene and it is assumed that it can also follow autosomal recessive pattern of inheritance. If the CSS-causing pathogenic variant should been identified in an affected family member, prenatal testing of a pregnancy at increased risk and preimplantation genetic testing are possible. SMARCB1- related conditions such as autosomal dominant RTPS1 and schwannomatosis should be taken into consideration for future follow-up.

Conflict of Interest: None declared.

EP12.055 A first report of three siblings with severe morbidity due to a FOXA2 mutation inherited from a healthy mother

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Introduction: Developmental disorders of the pituitary gland leading to congenital multiple pituitary hormone deficiencies (MPHD) may present in isolation or as part of a multisystemic disorder. Forkhead box A2 (FOXA2) is a transcription factor that has a crucial role in embryonic organogenesis including the pituitary gland. Six patients with de novo heterozygous pathogenic variants in the *FOXA2* gene were previously described. All the patients were diagnosed with multiple pituitary hormone

deficiencies and in addition with variable and diverse extra-pituitary manifestations.

Case Presentation: The proband presented with congenital MPHD, absent anterior pituitary gland on MRI and in addition, atrial septum defect with pulmonary valve stenosis and patent ductus arteriosus, a midgut malrotation and gallbladder septation; A male sibling was diagnosed with congenital MPHD and anal stenosis. A third pregnancy of the parents ended with an induced abortion of a male fetus due to sonographic findings of situs inversus and an atrioventricular septal defect.

This is the first familial report of three siblings with severe morbidity due to likely pathogenic *FOXA2* variant. The variant NM_021784.5: c.769C>A; p.Arg257Ser was inherited from a healthy mother.

Conclusion: our report demonstrates the pleiotropic effects of the *FOXA2* gene and the variability of *FOXA2*-related morbidity, even among siblings. The autosomal dominant mode of inheritance may mimic a number of inheritance modes, in variants of incomplete penetrance or significant clinical variability, or in rare cases of parental mosaicism.

Conflict of Interest: None declared.

EP12.056 Efficiency of in-house next generation sequencing panel in the diagnosis of RASopathies and other dysmorphic syndromes with short stature

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Introduction: RASopathies are well known group of congenital disorders characterized by the presence of dysmorphic features, short stature, congenital heart defects, delayed psychomotor development, skeletal defects and cryptorchidism. However, these findings are not specific for this group of disorders only and may overlap with other dysmorphic syndromes with growth deficiency.

The aim of the study was to assess the efficiency of in-house designed gene panel in the diagnosis of RASopathies and other dysmorphic syndromes with overlapping features.

Patients and methods: The study included 386 patients with the initial diagnosis of RASopathy as well as patients with Coffin-Siris syndrome, Cornelia de Lange syndrome, Kabuki syndrome, KBG syndrome, Rubinstein-Taybi syndrome, Wiedemann-Steiner syndrome and other clinical diagnosis (17, 21, 36, 14, 18, 5 and 23 patients, respectively). The analysis was performed using KAPA HyperCap Workflow and NextSeq550 instrument for library sequencing.

Results: Together 520 patients were analyzed using targeted sequencing. In 177 (34.0%) individuals, the initial diagnosis was confirmed including 130 patients with RASopathy diagnosis and mutation present in the RAS/MAPK signaling pathway genes (efficiency 33.7%). In 24 patients, pathogenic variant in the gene related to other dysmorphic syndrome was found (4.62%). In additional 103 individuals (19.8%) variants of unknown significance were found and required clinical assessment as well as molecular analysis of other family members.

Conclusion: The targeted next generation sequencing seems to be a useful tool as a first tier test for dysmorphic syndromes with short stature and can be performed before complex analyses.

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Conflict of Interest: Monika Gos PI (NCBR), Biogen, Novartis - lectures, Olga Malinowska: None declared, Anna Abramowicz: None declared, Aleksandra Landowska: None declared, Mateusz Dawidziuk: None declared, Sylwia Rzonca: None declared, Tomasz Gambin: None declared, Jerzy Bal: None declared.

EP12.057 A mild female case of ZC4H2-associated rare disorder

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Introduction: Heterozygous/hemizygous loss-of-function variants in *ZC4H2* gene were initially associated with Wieacker-Wolff Syndrome (WWS; MIM#314580), a neurodevelopmental disorder affecting the central and peripheral nervous systems. Cardinal to this pathology are the congenital contractures of variable severity from arthrogryposis multiplex congenita to mild camptodactyly. Additional features include global developmental delay/ intellectual disability (GDD/ID), facial and bulbar weakness, and skeletal abnormalities. About 80 patients with variants in *ZC4H2* have been reported, including male and female patients, consistent with X-linked dominant inheritance.

Case report: 16-year-old female, no relevant family history. Presented with masticatory difficulties, mild motor and language delay, and autism spectrum disorder. Evolved with motor discoordination and muscular weakness, without ID. At observation, she had mild short stature, central obesity, distal muscular hypoplasia, hypomimic face, strabismus, ptosis of the left eye, bilateral hand camptodactyly, limited knee extension, short feet with wide spaced toes and small halluces. Whole exome sequencing uncovered a heterozygous variant in *ZC4H2* gene: c.601C>G, p.(Pro201Ala). Parental segregation showed the variant was de novo.

Discussion and conclusion: A pathogenic variant was described in the same codon in a family with 5 severely affected boys; one female with severe ID, short stature and multiple contractures; and 6 females with a milder phenotype. Variability might be explained by the pattern of X-inactivation in heterozygous females, and led to the renaming of WWS as *ZC4H2*-associated rare disorders (ZARD), to include milder phenotypes such as our patient. ZARD should be suspected in GDD/ID with contractures.

Conflict of Interest: None declared.

EP12.058 The DNA methylation landscape of Coffin-Siris syndrome type 2

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Background: Coffin-Siris syndrome type 2 belongs to chromatinopathies, i.e., disorders resulting from disease-causing variants of chromatin structure and function components.

Materials and methods: One male patient affected with Coffin-Siris syndrome type 2, and one male control were recruited in this study. The patient harbored a pathogenic variant in the *ARID1A* gene, which was identified using whole exome sequencing. To reveal changes in the methylation profile, we have performed whole genome methylation sequencing using NEBNext Enzymatic Methyl-seq kit on genomic DNA isolated from both individuals. The analysis was performed in duplication.

Results: Bioinformatic analysis allowed us to visualize differences in methylation profile in the patient affected with Coffin-siris syndrome type 2 and age-, sex-matched healthy control.

Conclusion: We have unrevealed the episiganture of Coffin-Siris syndrome type 2 using whole genome methylation sequencing. This finding broadens the current knowledge regarding the syndrome and sheds light on its potential treatment perspectives.

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Conflict of Interest: None declared.

EP12.059 The clinical and molecular analysis in a group of 172 probands with clinical diagnosis of a RASopathy: genotype - phenotype correlation

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Background/Objectives: The RASopathies represent one of the most prevalent groups of dysmorphic syndromes. Each RASopathy exhibits a wide phenotypic variability but as a group share many overlapping features. This is due to the fact that their etiopathogenesis involves mutations in many of the RAS/MAPK signaling pathway genes. The aim of the research was to characterize the clinical picture of patients in order to attempt to establish a correlation between phenotypic expression and the nature of the molecular defect.

Methods: A group of 172 probands with clinical diagnosis of a RASopathy group disease was enrolled into the study, including 155 probands with Noonan syndrome. Comprehensive clinical and molecular analysis were performed as well as statistical investigation.

Results: Variants in genes related to the pathogenesis of RASopathy were found in 93.6% of patients. It was shown that the presence of *RAF1* gene mutations in patients with Noonan syndrome correlates with a significantly higher incidence of hypertrophic cardiomyopathy. The p.Ser257Leu variant appears to be most significantly associated with this cardiac defect. Familial forms of RASopathy were identified in 30 (17.4%).

Conclusion: Results of the study allowed for the elaboration of a diagnostic algorithm. The use of such an appropriate algorithm would contribute to the simplification of the diagnostic procedure, reducing its costs and conducting it in accordance with current medical knowledge. The variable expression of clinical features may suggest the existence of other, previously unknown molecular mechanisms or other regulatory mechanisms playing a role in the pathogenesis of the RASopathies.

Conflict of Interest: None declared.

EP12.060 Variable phenotype in KAT6B related disorders

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Background/Objectives: Heterozygous pathogenic variants in the *KAT6B* gene result in Say-Barber-Biesecker-Young-Simpson variant of Ohdo Syndrome (SBBYSS), Genitopatellar Syndrome (GPS) and Intermediate type, characterized by phenotypic variability. Although the phenotypic spectrum of KAT6B disorders is broad including hypotonia, developmental delay/intellectual disability, feeding difficulties, congenital heart defects, hearing loss and dental anomalies, both SBBYSS and GPS have their own distinct characteristic features. SBBYS syndrome has typical morphological features such as blepharophimosis, ptosis, epicanthus and telecanthus while GPS presents with hoarse facial features, skeletal anomalies, corpus callosum agenesis and genital abnormalities.

Methods: We report two new cases of SBBYSS. Both presented with blepharophimosis ptosis and epicanthus. Neither patient 1 (Pt1) nor patient 2 (Pt2) faced other medical problems. Pt1 presented with mild developmental delay whilst Pt2 had severe mental retardation. Whole exome sequencing (WES) revealed two de novo pathogenic variants in exon 3 and in exon 18 of *KAT6B* gene in Pt1 and in Pt2 respectively.

Results: Although pathogenic variants in exon 3 of KAT6B gene have been related with mild phenotype of SBBYSS, pathogenic variants in exon 18 have been related with severe form of SBBYSS. The pathogenic variant in exon 3 (Pt1) is a novel one. However, it is correlated with milder phenotype.

Conclusion: The use of WES has been proven powerful in expanding the genotype- phenotype correlation of rare congenital diseases such as KAT6B related disorders. It is cost and time effective by avoiding additional testing and it allows informed reproductive choices.

Grant references: None.

Conflict of Interest: None declared.

EP12.061 Long overdue diagnosis for Turner syndrome: evaluation of two cases

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Introduction: Turner syndrome (TS) is a phenotypically heterogeneous chromosomal disorder characterized by complete or partial loss of the second sex chromosome. Despite the presence of various phenotypic findings, the age at diagnosis in TS delay in many cases. In this presentation, two cases diagnosed after the age of 40 were evaluated in terms of their clinical aspects and the depth of the problems that occur when left untreated.

Material and Methods: G banding and FISH analysis were performed from peripheral blood.

Results: The karyotype of case 1 was determined as mos 46,X,del(X)(p11.2)[28]/45,X[12] and FISH analysis showed 55% single X. In the G band/ FISH analysis of case 2, 45,X[72].nuc ish(DXZ1x1,D18Z1x2)[188].nuc ish(DXZ1,D18Z1)x2[12] chromosome structure was detected.

Conclusions: Although both cases had typical TS findings, they were diagnosed at age 49 and 41, respectively. Hypothyroidism, osteoporosis, fatty liver, hypergonadotropic hypogonadism, and bilateral hearing loss were common health problems of our cases. In addition, case 1 had hypertension, prediabetes, and case 2 had type 1 diabetes. The presence of untreated hearing loss in both cases had a negative impact on the follow-up and treatment of endocrine diseases. Cases with TS have a much more severe potential to be affected if they are not followed up/treated in terms of diseases

involving many systems that may occur. Because of this risk, the diagnosis of TS should not be delayed. In fact, there are many phenotypic findings that can be clues for early diagnosis in TS.

Conflict of Interest: None declared.

EP12.062 An intermediate SMARCA2-related phenotype with non-compaction hypertrophic cardiomyopathy

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Monoallelic pathogenic variants in SMARCA2 (SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 2, MIM#60014) cause two overlapping disorders, Nicolaides-Baraitser Syndrome (NBS, OMIM # 601358) and Blepharophimosis-Impaired Intellectual Development Syndrome (BIS, OMIM # 619293). Although two phenotypes share findings such as hypertelorism, growth retardation, short stature and intellectual disability, they are distinguished from each other by features such as sparse hair, propitotic down-slanting eyes, broad nasal tip and prominent interphalangeal joints in NBS, and blepharophimosis, up-slanting eyes, pinched nose and milder neurologic involvement in BIS

Our patient, a 13.5 years old boy, was referred to us because of short stature, left ventricular non-compaction hypertrophic cardiomyopathy and attention deficit-hyperactivity disorder. He had growth retardation, triangular face, hypertelorism, short up-slanting palpebral fissures, broad flat nasal bridge and tip, short philtrum, thick lips, prominent ears, chin crease, and prominent proximal interphalangeal joints. He had received growth hormone therapy from 5 to 10 years of age and was discontinued after the diagnosis of non-compacting hypertrophic cardiomyopathy. Clinical exome sequencing revealed a mono-allelic truncating variant in SMARCA2: c.680_681del, p.Gln227ProfsTer31 (rs1491177433), which was not reported in a similar phenotype till now.

Although SMARCA2 causes two different recognizable phenotypes. It can cause intermediate phenotypes like our case. To date, hypertrophic cardiomyopathy has been reported in only one patient with partial SMARCA2 gene deletion and prominent NBS phenotype. Our case is unique with his intermediate phenotype and accompanying non-compaction cardiomyopathy caused by a truncating frameshift variant.

Conflict of Interest: None declared.

EP12.063 Velocardiofacial syndrome (VCFS) - determining the frequency of positive diagnosis using existing guidelines for genetic testing

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Background: Microdeletions of chromosome 22q11.2 causes VCFS, a syndrome associated with more than 180 clinical features. Accurate targeting of molecular diagnosis to those at high risk in middle income countries is essential. Monteiro et al criteria for justification of 22q11.2 deletion testing include cardiac, palatal, neurocognitive and dysmorphic features graded according to frequencies observed in affected cases and organized as three columns. Individuals scoring one column 1, two column 2 OR one column 2 and two column 3 features or four column 3 features are recommended testing.

Objective: Determine frequency of 22q11.2 deletion among Sri Lankan children fulfilling criteria for diagnostic testing.

Methods and materials: Thirty-seven children fulfilling the criteria recruited for a study to develop cost effective genetic testing for 22q11 deletion with informed consent from parents (Ethical approval from LRH, Colombo). Genomic DNA extracted from blood was used for developing semiquantitative and quantitative PCR assays and validated using FISH and whole exome sequencing.

Results: 22/37 were males. Mean age 155.7 months (range 72-504). Nine children (24%) identified with 22q11 deletions. Among 8 with a column 1 feature, 25% had deletion. Among the 27 with column 2 features, 22% had a deletion. There were 2 cases with column 3 features, of whom 1 had a deletion. Among deleted cases, 7/8 had both palatal and cardiac anomalies. Six had dysmorphic features.

Conclusion: Although the guideline is useful, ethnic and age specific refinement may improve its predictive value.

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Reference: Eur J Pediatr (2013) 172:927–945.

Conflict of Interest: Deepthi De Silva University of Kelaniya, Collaborator for research grant, Dinali Ranaweera Research student, Romesh Gunasekera Consultant surgeon, Duminda Samarasinghe Consultant cardiologist, Shehan Perera Consulatant cardiologist, Nirosha Panchananthan research student, Sumudu Rangika Samarasinghe Resesarch scientist, kajan muneeswaran Research student, Siyath Gunewardene University of Colombo, Vaz Gnanam Credence genomics, Vishvanath Chandrasekharan Sri Lanka Biotechnology institute.

EP12.064 Phenotype-based approach can solve cold cases: the paradigm of a mosaic form of Rubinstein Taybi Syndrome

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Background: Rubinstein–Taybi syndrome 1 (RSTS1, #180849) is a rare chromatinopathy caused by mutations in *CREBBP* gene. It's a highly heterogeneous condition characterized by intellectual disability, typical facial features and enlarged thumbs and halluces. A phenotype-based approach to disorders of the epigenetic machinery is nevertheless challenging, due to a broad phenotypical overlap.

Case report: The patient is a 5-year-old female with sacral dimple, growth in the lower limits for age, OFC on 3rd centile and mild developmental delay. She also presented Duane syndrome and recurrent urinary tract infections, due to a dilation of pyelocaliceal cavities. No other abnormalities were reported. Typical RSTS facial features, including medially sparse and arched eyebrows, broad nasal root with tubular nose, prominent columella, high arched palate and retrognathia were noticed together with mildly broad thumbs and halluces.

Genetic analysis: a first Trio Whole Exome Sequencing (WES) resulted negative for pathogenic variants. Under a strong clinical

suspect of RSTS, a targeted re-analysis revealed a de novo truncating variant in *CREBBP* gene (NM_004380.3: c.2012C>A, p.(Ser671Ter)) in 11/114 reads, with an estimated mosaicism of 10%. The variant was absent in international databases and classified as probably damaging according to ACMG guidelines. A mosaicism rate of almost 20% was confirmed on a second DNA sample extracted from patient's buccal mucosa.

Conclusion: targeted phenotype-based approach applied to high-depth NGS may improve WES' diagnostic yield in mild and mosaic conditions.

Grant References: Supported not financially by ERN ITHACA.

Conflict of Interest: None declared.

EP12.065 CRISPR/Cas9 mediated disruption of an evolutionary conserved putative enhancer in the mab21l2 locus induces developmental eye anomalies in Xenopus tropicalis

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Background&Objectives: Genetic variants that affect *cis*-regulatory elements (CREs) have been shown to lead to a variety of human diseases. Here, we aimed to characterize a putative enhancer of *MAB21L2*, localized in a 113.5kb non-coding homozygous deletion identified in a proband with anophthalmia, micrognathia and microcephaly.

Methods&Results: One putative enhancer (CRE14) within the corresponding 39kb region in *Xenopus (X.) tropicalis* containing a conserved binding site for OTX2, a transcription factor (TF) involved in eye development, was identified using genome-wide multi-omics data. Furthermore, binding of Otx2 to CRE14 was confirmed by ChIP-seq in mouse embryonic stem cells. During early *Xenopus* development CRE14 showed the epigenetic marks of a poised enhancer at mid-gastrula stage (NF-St.10.5), and of an active enhancer in early-neurula (NF-St12.5), the stage where expression of *mab21l2* is initiated and the eye is specified. CRISPR-mediated disruption of the Otx2 binding site (CRE14-crispants), as well as modeling of the proband deletion (del-crispants) resulted in diminished levels of *mab21l2* transcripts and in structural eye defects, primarily ocular coloboma. Moreover, 2D and 3D phenotyping of affected eyes using in toto light-sheet microscopy and image reconstruction using a deep neural network application indicated a significant decrease in both volume and sphericity of retina in CRE14-crispants compared to the control group.

Conclusion: We showed for the first time a CRISPR/Cas9-mediated *X. tropicalis* disease model targeting a specific TF binding site in a non-coding CRE and applied sensitive and accurate phenotyping of the eye defects via light-sheet imaging and deep neural network-mediated anatomical reconstruction.

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Conflict of Interest: None declared.

EP13 Cancer Genetics

EP13.001 AS-CMC: a pan-cancer database of alternative splicing for molecular classification of cancer

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Alternative splicing (AS) is a post-transcriptional regulation that leads to the complexity of transcriptome. Despite the growing importance of AS in cancer research, the role of AS has not been systematically studied, especially in understanding cancer molecular classification. Herein, we analyzed the molecular subtype-specific regulation of AS using The Cancer Genome Atlas (TCGA) data and constructed a web-based database, named AS-CMC (Alternative Splicing for Cancer Molecular Classification). Our system harbors three analysis modules for exploring subtype-specific AS events, evaluating their phenotype association, and pan-cancer comparison. The number of subtype-specific AS events were found to be diverse across cancer types and some differentially regulated AS events were recurrently found in multiple cancer types. We analyzed a subtype-specific AS in exon 11 of *MAP3K7* (mitogen-activated protein kinase kinase 7) as an example of pan-cancer AS biomarker. This AS marker showed significant association with the survival of patients with stomach adenocarcinoma. Our analysis revealed AS as an important determinant for cancer molecular classification. AS-CMC is the first web-based resource that provides comprehensive tool to explore biological implications of AS events, facilitating the discovery of novel AS biomarkers.

Conflict of Interest: None declared.

EP13.004 Establishing a Northern Ireland retinoblastoma database to aid familial screening

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Background/Objectives: Northern Ireland is a small geographic location but with large, extended families. Subsequently familial screening for conditions such as retinoblastoma is often considerably more sizeable than for centres on the UK mainland. All Northern Ireland retinoblastoma patients were previously seen in Dublin, with test requests sent out to various centres including Toronto, and as such bypassing our local genetics laboratory. A retinoblastoma service has now been set up in Belfast, with referrals in from relatives, often one or two generations removed from the initial proband. Our aim was to link together families, to source historic genetic results, to guide screening going forward and to identify individuals who could benefit from up-to-date genetic testing and counselling.

Methods: Probands were identified and links made to relatives who have requested screening. Genetic reports were sourced and

appropriately linked. Screening guidelines from other UK centres were consulted to guide our local implementation of similar.

Results: A database was established of all probands and relatives, easily linked to the appropriate genetic reports. This resource provides both the Genetics and Ophthalmology teams with the essential information, easily accessible in the clinic setting.

Conclusion: The creation of this database enables both teams to have a live, up-to-date resource of all retinoblastoma families in Northern Ireland. This will impact on development of local screening guidelines and reduce unnecessary, intensive screening in relatives. It will also aid genetic counselling, including reproductive options and risk of secondary cancers and help identify patients who could benefit from newer genetic testing.

Conflict of Interest: None declared.

EP13.005 Identification of candidate variants for renal and ovarian cancers in association with adenomyosis utilizing whole exome sequencing in a large family

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Background/Objectives: Adenomyosis is defined by the presence of endometrial-like tissue inside the myometrium. Overlapping molecular mechanisms have been identified among various cancers and adenomyosis, however these remain far from complete. We aimed to identify novel candidate genes involved in adenomyosis-related cancer pathogenesis utilizing a large family that contains both conditions.

Methods: 19 women from a three-generation family were recruited. Ultrasonographic visualization was utilized in adenomyosis diagnosis. Two members received renal and ovarian cancer diagnoses through pathological confirmations and were subjected to whole exome sequencing(WES) on Illumina NextSeq550 system. Bioinformatic analyses and variant prioritization at MAF < 1% were performed on Genomize SEQ and Pairend platforms utilizing their proprietary algorithms. Cancer-related variants were selected in accordance with their known/predicted functions in the literature.

Results: The patient with renal cancer was diagnosed with adenomyosis, while ovarian cancer patient described adenomyosis-related symptoms prior to her cancer treatment. WES analysis revealed novel heterozygous deleterious variants in four genes *DNAH5*, *TTLL4*, *MTMR8*, and *MUTYH*. *DNAH5* and *TTLL4* are associated with microtubule organization, which are crucial in cancer progression. The *MUTYH* encodes a DNA repair enzyme. Autophagy associated with *MTMR8* gene ensures sustainable energy source to maintain homeostasis under stressful conditions like tumor microenvironment.

Conclusions: Four candidate genes identified might be involved in adenomyosis-related renal and ovarian cancers. Segregation analysis of the variants will guide patients in the long-term follow-up. Replication of our results in independent cohorts or by functional studies, will contribute to new biomarkers for the conditions.

Grant References: Istanbul University Scientific Research Projects Coordination Unit(38579)

Conflict of Interest: Sevcan Aydın Istanbul University Scientific Research Projects Coordination Unit(38579), nura fitnat topbas selcuki University of Health Sciences Turkey, Istanbul Sisli Hamidiye Etfal Training and Research Hospital, Obstetrics and Gynecology, istanbul, Turkey, engin oral Bezmialem Vakif University, Obstetrics and Gynecology, istanbul, Turkey, pinar yalcin bahat University of Health Sciences Turkey, Istanbul Kanuni Sultan Suleyman Training and Research Hospital, Department, Obstetrics and Gynecology, istanbul, Turkey, Feyza Tuncer Istanbul University, Aziz Sancar Institute of Experimental Medicine, Genetics, istanbul, Turkey, Istanbul University Scientific Research Projects Coordination Unit(38579).

EP13.006 Germline mutation in the CDC73 gene in a woman with osteitis fibrosa cystica ("brown" tumor) and hyperparathyroidism

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Background: We present the case of a 46-year-old woman with complaints of pelvic pain, muscle weakness and excessive diuresis; initial diagnosis was fracture of the pubic bone.

Methods: Histopathological and laboratory examinations, as well as computed tomography, were performed.

Results: Serum calcium levels were 4.02mmol/l, parathormone - 1433.2pg/ml. Computed tomography showed a well-demarcated mass on the right lobe of the thyroid gland with a size of $2.5 \times 1.5 \times 1.5$ cm. Bone biopsy showed osteitis fibrosa cystica. Histopathology of thyroid mass showed parathyroid cancer with invasion of the parathyroid capsule, thyroid gland, and microvascular invasion (T2N0M0). After next generation sequencing using cancer gene panel, we detected a germline mutation in the *CDC73* gene, generating a premature translational stop signal - c.376C>T (p.Arg126*). The gene is associated with autosomal dominant hyperparathyroidism-jaw tumor syndrome (HPT-JT), parathyroid carcinoma (PC), and familial isolated hyperparathyroidism, collectively referred to as *CDC73*-related conditions.

Discussion: *CDC73*-related conditions are characterized by primary hyperparathyroidism, which occurs in over 70% of cases. It is typically caused by benign parathyroid adenomas, though it can also be due to an underlying PC. Additional features include ossifying jaw fibromas, uterine fibroids, renal cysts, hamartomas and Wilms tumor. Over 75% of sporadic PC have biallelic somatic inactivation/loss of *CDC73* as well.

Conclusion: Parathyroid carcinoma is a rare malignancy accounting for less than 1% of all parathyroid tumors. The detection of a germline mutation in the *CDC73* gene is of paramount importance because this mutation can be transmitted by 50% chance to the offspring of the proband.

Conflict of Interest: None declared.

EP13.007 SMAD4 mosaicism with juvenile polyposis and Rendu-Osler phenotype : a case report

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Juvenile polyposis is a rare disease characterized by multiples

hamartomatous polyps in the gastrointestinal tract, associated with pathogenic variants of BMPR1A and SMAD4. A combined syndrome of juvenile polyposis and hereditary hemorrhagic telangiectasia (HHT) might be present in individuals with heterozygous SMAD4 pathogenic variant. Mosaic variant in colorectal cancer (CRC) and polyposis susceptibility genes has already been described, such as APC, PTEN and STK11. However, mosaic variant of SMAD4 associated with juvenile polyposis has never been described before.

We report a sporadic case of a 30-year-old man with caecum adenocarcinoma, peritoneal carcinosis and 10 juvenile colonic polyps. He presents epistaxis since childhood.

NGS analysis of genes involved in inherited CRC and polyposis, including SMAD4 and BMPR1A, was performed on DNA extracted from the patient's blood and revealed no pathogenic variant.

Somatic NGS analysis identified the SMAD4 variant: NM_005359.5:c. 1600C > T, p.(Gln534*) in two different polyps in 28% and 7% of NGS reads respectively, with 15-25% of tumoral cells. This variant is located in the last exon and entail a truncated protein, removing C-terminal part with two functional domains.

NGS data for the blood sample and healthy colic tissue has been reviewed and revealed the variant at a very low frequency (1% of reads), explaining why it was not found before.

These results are consistent with a likely pathogenic mosaic variant of SMAD4, concurring with the phenotype.

This case highlights the diagnosis challenge to detect mosaic variant, in order to offer the appropriate clinical management of the patient and familial counselling.

Conflict of Interest: None declared.

EP13.008 A population-based analysis of RET gene pathogenic variants in medullary thyroid cancer patients in the Republic of Belarus

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Background: Germline *RET* pathogenic variants (PVs) are involved in the development of hereditary form of medullary thyroid cancer (MTC). The disease phenotype is associated with localization of PVs and their transforming potential. The aims of this study were to evaluate the prevalence and spectrum of gene *RET* PVs in a large series of MTC in the Republic of Belarus.

Methods: Genomic DNA was isolated from 361 blood samples obtained from patients with MTC treated at the Republican Center for Thyroid Tumors, Belarus. Sanger sequencing of *RET* gene 5, 8, 10, 11, 13-16 exons was carried out in the genetic department of the Republican Molecular-Genetic Laboratory of Carcinogenesis.

Results: The frequency of gene *RET* PVs in the studied cohort was 14,9% (54/361). More often PVs located in 11 (29,6%), 13 (27,8%) and 10 (14,8%) exons. However, the most studied PV C634R accounted for only 50% of all PV in the 11 exon. In the 13 and 14 exons PVs were presented mainly by Y791F (11/15; 73,3%) and V804M (5/6; 83,3%) respectively. Mutations in *RET* exon 16 represented 7,4% of all detected PVs (3 cases M918T and 1 case R912P).

Conclusion: The spectrum of the mutated *RET* exons in the Republic of Belarus is similar to that in the West Slavic group of countries (Czech Republic, Slovenia, Poland). Another population feature in Belarus is the low incidence of MEN2B syndrome. Lower frequency of hereditary MTC in our cohort indicates the probability of PV localization in non-studied exons.

Grant References: R&D Grant №20192717.

Conflict of Interest: None declared.

EP13.009 Experiences and needs for those with hereditary cancer syndromes carriers: "What they say on internet or in hereditary supportive groups"

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Background/ Objectives: Previous research have looked at lifestyles of people affected by hereditary cancer syndromes but there is limited evidence of interventions focused on health promotion and on the main sources of support and information for these groups. This study aims to identify carriers' experiences and needs.

Methods: Hereditary breast & ovarian alterations or Lynch syndrome carriers were invited to participate in individual semi-structured qualitative interviews, based on Hermeneutic paradigme. Interviews explored questions about their lifestyle, information and support sources and their experiences with healthcare providers. Interviews were recorded and transcribed for reflexive thematic analysis. Patient involvement panel provided input on study design and theme analysis.

Results: Twenty-two people (eight previvors and fourteen survivors) with genetic alterations from ten European countries participated. Common experiences were similar regardless of the country and accessibility to testing and screening. Specific themes identified included: "perception of lack of understanding from healthcare professionals", "Lack of specific information on individual actions" "Patient groups as main emotional support". Internet and patient groups were considered as important support and information.

Conclusion: Participants described that their diagnosis pathway and genetic counsellor information was good. They feel that although having same risk as one oncology patient they are not treated as them and they do not have the same support, unless diagnosed. This result suggest that healthcare professionals such oncology professionals and primary care professionals need better training & education about genetics and cancer and more abilities to manage these persons.

Grants: ISONG.

Conflict of Interest: Celia Díez de los Ríos de la Serna ISONG grant for PhD programme, EONS Education Working Group member, Paz Fernandez-Ortega: None declared, Teresa Lluch-Canut: None declared.

EP13.010 Polygenic scores for cancer

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Background: Polygenic scores (PGS) could contribute significantly to the prevention, identification and management of cancer. There is substantial PGS research and development underway in a variety of cancers.

Methods: We undertook a review of the literature including grey literature, and identification of ongoing clinical trials. This was followed by an appraisal of the research and development of PGS based applications for cancer, their potential use in healthcare, as well as policy and implementation considerations.

Results: We identified that there is considerable research ongoing to determine if PGS can contribute to stratified screening strategies in cancer screening programmes, by improving their clinical effectiveness and efficiency. Trials are underway to determine the role that stratified screening using comprehensive risk prediction models (that include PGS) could have in such programmes. PGS for rarer cancers are also being developed, including those with a limited number of known risk factors and biomarkers. Whilst there are significant issues still to be addressed, such as generalisability, there are indications that PGS can improve risk prediction in some cancers and in some clinical contexts.

Conclusions: The development, validation and regulation of new tests, as well as infrastructure needs and evidence of clinical utility are required before implementation becomes possible. PGS could have potential in specific contexts and for specific purposes but further sustained research and translation efforts are required to adequately assess their potential role in improving cancer prediction, prevention and management.

References: Polygenic scores for Cancer. PHGF 2022.

Conflict of Interest: None declared.

EP13.011 Incidence of the ASXL1 gene in myeloid panel testing of AML and MDS patients

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Background/Objectives: Additional sex combs-like 1 (*ASXL1)* gene is one of the most frequently mutated genes in a number of myeloid neoplasms, including myelodysplastic syndromes (MDS, 14–23%), acute myeloid leukemia (AML, 5–17%), myeloproliferative neoplasms (MPN, 5–11%), and chronic myelomonogenous leukemia (CMML, 40–49%). Mutations in this gene are always associated with poor prognosis. Additionally, *ASXL1* mutations are frequently detected in clonal hematopoiesis (CH), also known as clonal hematopoiesis of indeterminate potential (CHIP), a precursor state for hematologic neoplasms with somatic mutations when there are no diagnostic criteria for hematologic malignancies. In this study, we retrospectively analyzed myeloid panel test results between 2020 and 2022 to find out the incidence of *ASXL1* mutations in AML and MDS patients.

Methods: We performed next-generation sequencing on 84 genes that are related to myeloid syndromes using the Invitae-Archer FusionPlex® Myeloid Kit.

Results: In total, 69 (84%) of 82 patients had somatic pathogenic variants that could be associated with diagnosis, treatment, and prognosis, while 13 (16%) did not. A pathogenic variant was detected in the ASXL1 gene in 15 patients out of 69 positive patients. The c.1934dup (p.Gly646TrpfsTer12) variant was found to be the most common among *ASXL1* mutated patients.

Conclusion: The presence of the c.1934dup (p.Gly646TrpfsTer12) variant in 47% of our study was significant, as it was the most frequently observed variant in the *ASXL1* gene in the literature.

Grant References: Asada, Shuhei, et al., "The role of *ASXL1* in hematopoiesis and myeloid malignancies." Cellular and Molecular Life Sciences 76 (2019): 2511-2523.

Conflict of Interest: Hilal Karakoyun Sapiens Genetics Diagnostic Center, Seren Yirmibes Group Florence Nightingale Healthcare, Ceyda Milas: None declared, Kanay Yararbas Florence Nightingale Hospital and TC Demiroğlu Bilim University, Deniz Gören Sahin Group Florence Nightingale Healthcare and TC Demiroğlu Bilim University, Ceyhan Sayar Sapiens Genetics Diagnostic Center, Yazgi Yucel Sapiens Genetics Diagnostic Center, Mutlu Arat Group Florence Nightingale Healthcare.

EP13.012 Analysis of Plasma Metabolites in SDHx Deficient Cancer Syndromes

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Phaeochromocytomas and paragangliomas (PPGL) are associated with a germline pathogenic variant (GPV) in ~40% of cases, most commonly in the succinate dehydrogenase subunit genes (*SDHx*). Individuals with a GPV are offered lifetime tumour surveillance but penetrance is incomplete (e.g., ~20% for SDHB GPVs). Individuals identified to have a PPGL and a GPV in *SDHx* often have an absence of family history. Serum biomarkers might enable risk stratification and targeted screening in those with GPVs. We sought to identify plasma metabolites for potential use as predictive and/or prognostic biomarkers.

Plasma samples were collected prospectively from a clinically well-phenotyped patient cohort (Cambridge, UK) with *SDHx* and *non-SDHx* variants. We performed liquid chromatography/mass spectroscopy analysis of polar compounds profiling 124 enrolled patients (age 17 – 81, mean 45). 49.6% individuals had a GPV, of which 47.5% had a current benign/metastatic tumour and 27.9% had a previous tumour. Succinate levels were significantly raised in *SDHx* carriers compared to *non-SDHx* carriers ($P < 0.0001$) (Sen 71.4%, Spe 81.4%). Furthermore, succinate levels correlated with tumour size for SDH deficient (dSDH) benign tumours ($r = 0.6239$, $P = 0.0726$) and metastatic dSDH disease burden ($P < 0.0001$). Untargeted metabolites (2300 detected features) were ranked based on discriminatory ability (e.g, differentiating dSDH from proficient SDH tumours and patients with tumours from controls). Attempts to identify metabolites significantly different between dSDHx tumours (metastatic/benign) are in progress. Findings will be confirmed with additional patient samples and comparison to a *SDHB* knockout mouse model (Zhuang Lab, NIH) to validate candidate biomarkers for genetic predisposition and tumour development, progression and recurrence.

Conflict of Interest: None declared.

EP13.013 The landscape of variants in the RB1 gene underlying heritable retinoblastoma

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Purpose: Retinoblastoma (Rb) is the most prevalent childhood ocular cancer, with an incidence of one per 15,000 - 20,000 live births, and is generally caused by genetic alternations in the *RB1* gene. Since data regarding African Rb studies is scarce this study set out to determine the germline variant spectrum of the *RB1* gene in South African patients with heritable Rb.

Methods: *RB1* gene sequence analysis was performed using a next-generation sequencing (NGS) gene panel to catalogue all variants observed in the *RB1* gene. The NGS data was additionally analyzed for copy number variants to detect potential deletions/duplications which were then validated by multiplex ligation-dependent probe amplification.

Results: The likely causal pathogenic variant was detected in 88% of the cases screened thus far. Frameshift variants (35.7%) were the most frequently detected variants, followed by nonsense (32.2%), splice-site (21.4%), and copy number variants (10.7%). In total, 32 unique, benign variants have been identified in 80 controls and 27 unique, causal variants have been identified in 32 cases. The causal variants identified include nine novel single nucleotide variants and three novel multi-exonic deletions.

Conclusions: This is an optimistic first step towards implementation of a genetic diagnostic service for Rb in South Africa. Rb genetic testing will allow accurate risk prediction for siblings and children of affected individuals; and will alleviate the trauma, risk, and burden of copious retinal examinations performed on mutation-negative minors.

Grant References: National Health Laboratory Service Research Trust (Grant004_94861); Faculty of Health Science Start-up Emerging Researcher Award (SERA).

Conflict of Interest: None declared.

EP13.014 Germline DNA repair gene mutations in a cohort of high-risk prostate cancer patients

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Background/Objectives: DNA repair genes play a decisive role in maintaining genomic stability. Pathogenic variants in these genes have been associated with an increased risk of developing prostate cancer as well as with the response to certain treatments. The objective of this study is to determine the frequency of these variants in a cohort of 677 high-risk prostate cancer patients from our population.

Methods: 677 high-risk prostate cancer patients were included. Genetic analysis was performed from peripheral blood DNA using next-generation sequencing technologies. DNA repair genes included were: *ATM*,*BRCA1*,*BRCA2*,*BRIP1*,*CHEK2*,*FANCA*,*MLH1*, *MSH2*,*MSH6*,*NBN*,*PALB2*, *PMS2*,*PTEN*,*RAD51C*,*RAD51D*,*STK11* and *TP53*. Other analyzed genes were *CDH1*, *EPCAM* and *HOXB13*. The identified variants were classified according to American College of Medical Genetics guidelines and specific gene classification guidelines as well.

Results: The overall rate of pathogenic variants in the cohort was 8,71% (59 of 677). These variants were mainly identified in *ATM* (13 of 59; 22,03%), *BRCA2* (10 of 59; 16,95%) and *CHEK2* (9 of 59; 15,25%). The prostate cancer variant *HOXB13* (NM_006361.5): c.251G>A; p.(Gly84Glu) was identified in three patients.

Conclusion: Our results suggest that DNA repair genes are useful prostate cancer predisposition biomarkers. The clinical utility of these findings, including targeted therapies, will become increasingly important as genetic testing becomes more widespread.

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Conflict of Interest: None declared.

EP13.015 Genetic determinants of response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer identified by whole exome sequencing

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Background/Objectives: The cornerstone in the treatment of locally advanced rectal cancer (LARC) is neoadjuvant chemoradiotherapy (nCRT) followed by total mesorectal excision. Reliable predictors of response to nCRT in LARC remain an unmet need in colorectal cancer research. This study used high throughput DNA analysis to investigate genetic differences between highly responsive tumors and tumors resistant to nCRT.

Methods: Whole-exome sequencing of samples from five patients with good response and two patients with resistance to nCRT was performed. An in-house developed algorithm using SQLite Database created in SQLite Expert Professional software was used to identify genetic differences between good and poor response. The interactions between the involved genes were visualised using the String tool. The consequences of mutations were analysed using the Variation Effect Prediction tool. Functional predictive scores were determined using the ProtVar.

Results: The analysis discovered 15 InDels and 202 SNVs exclusively present in tumors with resistance to therapy, mainly in genes involved in cell cycle regulation, adhesion, and migration. On the other hand, 9 InDels and 122 SNVs were exclusively present in good response, in genes involved in extracellular matrix remodelling and immunity. Six discovered variants, mainly frameshift and nonsense mutations, had high deleterious prediction scores: CAPN2 rs17599, COL5A2 rs145404046, ROS1 rs529156, SYNM rs5030699, MAVS rs7262903 and CLIC6 rs13049028.

Conclusion: The study has identified six variants that could be used to predict poor response to nCRT. They can be further tested in the clinical practice with the aim of identifying patients that should avoid this type of treatment.

Conflict of Interest: None declared.

EP13.016 AIP mutations in Portuguese patients with young-onset isolated pituitary adenomas

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Background/Objectives: Mutations in the aryl hydrocarbon receptor interacting protein (*AIP*) gene cause Familial Isolated Pituitary Adenomas (FIPA). *AIP* mutations have also been found in patients with apparently sporadic pituitary adenomas, particularly in young patients with large adenomas. The aim of this study was to investigate the prevalence of these mutations in sporadic pituitary macroadenomas before the age of 40 years, in paediatric patients with micro or macroadenomas, and in familial cases of pituitary adenomas.

Methods: The *AIP* gene was sequenced in 199 patients with sporadic pituitary macroadenomas diagnosed between the ages of 18 and 40 years, 24 paediatric patients and 11 familial pituitary adenomas.

Results: Heterozygous pathogenic variants in *AIP* were identified in 3 (1.5%) patients with sporadic pituitary macroadenomas diagnosed between 18 and 40 years and in 1 (4.2%) paediatric patient. No mutations were found in familial cases. These consisted of two already known mutations (p.Arg81* and p.Leu115Trpfs*41) and two novel mutations (p.Glu246* and p.Ser53Thrfs*36). All four patients had growth hormone-secreting adenomas diagnosed between the ages of 14 and 25 years.

Conclusion: The frequency of *AIP* mutations in this cohort was lower than in other studies. Previous reports may have overestimated the contribution of *AIP* mutations due to the inclusion of variants of uncertain significance. The identification of novel *AIP* mutations expands the known spectrum of genetic causes of pituitary adenomas and may help understand the role of *AIP* mutations in the molecular mechanisms underlying pituitary tumorigenesis.

Grant References: Portuguese Foundation for Science and Technology (PTDC/MEC-MET/29489/2017, UIDB/00709/2020 and SFRH/BD/147160/2019).

Conflict of Interest: None declared.

EP13.017 The expression profile of ABCB11 gene in hepatocellular carcinoma and its association with clinical outcomes

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Background/Objectives: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy that occurs predominantly in patients with underlying chronic liver disease and cirrhosis. ABCB11 encodes Bile Salt Export Pump (BSEP), a member of the ABC transporter family, which is the main hepatocellular transporter responsible for secretion of bile acids into the bile canaliculus. Emerging data have implicated that the expression and function of BSEP are altered in hepatocellular carcinoma. We aimed to systematically analyze ABCB11 expression and its prognostic role in HCC using various open databases.

Methods: Comparison of HCC and matched normal tissue gene expression data form ABCB11gene was performed with the UALCAN and GEPIA. The promoter methylation levels were examined by UALCAN. Correlation between ABCB11 expression and patient survival was evaluated with OncoLnc. ABCB11 genetic alterations in HCC were also explored using cBioPortal.

Results: ABCB11 expression is significantly down-regulated in the clinic-pathological characteristics (cancer stages, tumor grade, nodal metastasis status, and histological subtypes) examined in HCC patients compared to normal counterparts. ABCB11 promoter methylation level in HCC was lower than that in normal liver tissue. Clinically, low expression of ABCB11 was correlated with shorter overall survival in HCC patients ($P = 0.02$). Analysis of the TCGA Liver Hepatocellular Carcinoma dataset (*TCGA's Pan*-Cancer Atlas) revealed a low mutation (1.16%), amplification (0.58%) and deep deletion (0.58%) frequency in HCC.

Conclusion: These observations indicate that ABCB11 may function as a potential tumor suppressor gene. In HCC patients, ABCB11 expression levels may serve as a prognostic predictive marker.

Grant references: none.

Conflict of Interest: None declared.

EP13.018 Features of immune checkpoint gene expression during metastasis and differentiation of gastric cancer

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Background/Objectives: One of the promising innovations in gastric cancer (GC) therapy is the use PD-1/PD-L1 immune checkpoint (IC) inhibitors. However, the proportion of patients who respond to treatment is not large enough. The investigation of other ICs will contribute to improving the effectiveness of treatment. The aim was to study the expression profiles of the IC genes during the progression of GC.

Methods: 101 pairs of the gastric tumor and adjacent normal tissue were examined: GC without metastases (70 patients) and GC with metastases (31 patients). The average age of patients was 62 years. The gene expression level was measured by RT-PCR.

Results: The expression profiles of the *ADAM17, PVR, CD274, CD276, CEACAM1, IDO1, TDO2, LGALS3, LGALS9, HHLA2* genes were analyzed. ROC analysis revealed the association of *TDO2* and *LGALS3* expression with distant metastasis ($p = 0{,}024$; $p = 0{,}031$, respectively). The association was verified using Fisher's exact test. In metastatic GC, *TDO2* expression increases, while *LGALS3* expression decreases. According to the ROC analysis, *IDO1* and *LGALS9* expression is associated with the degree of tumor differentiation. At a low degree of tumor differentiation, the expression level increases.

Conclusion: A new association was found between IC gene expression, clinical manifestation and pathological features of GC including distant metastasis and degree of tumor differentiation. In GC with distant metastases, *TDO2* gene expression increases, which may indicate a potentially increased effectiveness of TDO2 inhibition in metastatic GC. The data obtained are important for the development of prognostic markers and new drugs.

Conflict of Interest: None declared.

EP13.019 Evaluation of anticancer effects of MAPK6 siRNA loaded PLGA nanoparticles in breast cancer

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Background/Objectives: Breast cancer is the most commonly diagnosed cancer in women and causes many deaths. Current treatment methods are far from the desired level in providing effective treatment with minimum side effects. It is known that the *MAPK6* gene is overexpressed in breast cancer cells. Therefore,the possible anticancer effects of suppression of *MAPK6* expression via MAPK6 siRNA-loaded PLGA nanoparticles on the MCF-7 breast cancer cell line were investigated as a treatment option.

Methods: MAPK6 siRNA loaded,scrambled siRNA loaded and blank PLGA nanoparticles were synthesized. Afterward,characterization of nanoparticles was performed. After applying different concentrations of nanoparticles to MCF-7 cells in cell culture,IC_{40} values were determined by MTT assay. *MAPK6* and reference gene *GAPDH* expression levels were analyzed by qRT-PCR. MAPK6 protein level was determined by ELISA. After 24,48 and 72 hours of treatment,cell migration was evaluated by wound healing assay,cell proliferation was evaluated by immunohistochemical PCNA antibody staining,colony formation ability was evaluated by clonogenic assay,and apoptosis was evaluated by flow cytometry.

Results: It was determined that MAPK6 siRNA-loaded nanoparticles significantly decreased the migration,proliferation and colony formation ability of MCF-7 cells and significantly increased apoptosis of MCF-7 cells compared to the control groups.

Conclusion: In conclusion,it was determined that post-transcriptional suppression of *MAPK6* gene with MAPK6 siRNA-loaded nanoparticles in MCF-7 cells produced an anticancer effect. The results show that MAPK6 may be a candidate therapeutic target in breast cancer. Our findings should be verified by further in vivo analysis.

Grant References: The study was supported by Yıldız Technical University,SRP Unit (Project:FYL-2022-4887).

Conflict of Interest: None declared.

EP13.020 Complex diagnostic path of a patient with basal cell nevus syndrome

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Background/Objectives: The basal cell nevus syndrome (BCNS) is characterized by multiple basal cell nevi, jaw keratocysts, lymphomesenteric cysts, ovarian fibromas, and various developmental anomalies. Its prevalence ranges from 1:19,000 to 1:164,000. The disease is caused by mutations in the *PTCH1, PTCH2, a*nd *SUFU* genes. Patients with BCNS require specialized monitoring and treatment.

Methods: We describe a clinical case of BCNS in a 29-year-old patient with multiple basal cell nevi, voluminous irregularly colored formations with uneven contours ranging in diameter from 1-8 cm on the head, neck, and lumbar region.

Results: Computed tomography revealed multiple odontogenic keratocysts. Surgical excision of basal cell nevi was performed between the ages of 16 and 28. At the age of 28, due to voluminous formations, an atypical resection of the stomach with lymphadenectomy and bilateral adnexectomy was carried out. After histological examination, tumor growth was not detected.

Phenotype of the patient differed from her family members and resembled BCNS.The geneticist referred the patient to molecular genetic testing.

Results of NGS analysis revealed a c.1208_1209del nucleotide variant, in the *PTCH1* gene (NM_000264.5, p.Tyr403CysfsTer33). This variant has not been previously reported in population databases, induces a shift in the reading frame, and is classified as pathogenic according to ACMG criteria. Thus, the c.1208_1209del mutation in the *PTCH1* was referred to as causative, and the diagnosis of BCNS was confirmed.

Conclusion: Molecular genetic analysis enables an accurate and timely diagnosis, the development of a personalized monitoring strategy, as well as avoidance of arduous and intricate procedures.

Grant References:

Conflict of Interest: None declared.

EP13.021 The influence of the number of microsatellite markers and sequencing depth on the ability to identify mismatch repair deficient tumours

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Background/Objectives: Analysis of somatic mutation patterns is widely used to infer exposure to exogenous and endogenous mutagenic influences. This raises the question of the amount of sequence data required to detect factors of interest. A common use of such analyses is the identification of increased microsatellite instability to uncover mismatch repair (MMR) defects in tumours and normal tissues. Here we explore the impact of varying sequencing depth and the number of loci analysed on the ability to detect MMR deficient colorectal tumours.

Methods: We analysed publicly available amplicon sequencing data on 24 short monomorphic microsatellites (up to 12 bp in length, PMID 31471937) using artificial neural networks. The data were split in a training (99 samples) and a test set (95 samples),

Results: We show that, at a sequencing depth of 200, pairs of mononucleotide repeats can achieve discrimination between MMR proficient and deficient colorectal tumours similar to those obtained with the original panel, with accuracies above 97% and ROC AUCs in excess of 99% in the test set. Sets of 8 microsatellites can achieve similar results at a sequencing depth of 12.

Conclusion: Our results show that the inclusion of convolutional layers in the network can facilitate identification of MMR deficiency in tumour material. They also indicate that sequencing depth can compensate for target number, but they also suggest that, for a fixed total number of reads per sample, increasing

sensitivity by increasing the number of targets is more efficient than by increasing per target sequencing depth.

Conflict of Interest: Mauro Santibanez Koref named as inventor on patents covering the microsatellite instability markers analyzed: WO/2018/037231 (published March 1, 2018), WO/2021/019197 (published February 4, 2021), and GB2114136.1 (filed October 1, 2021)., Joseph Law: None declared, Richard Gallon named as inventor on patents covering the microsatellite instability markers analyzed: WO/2018/037231 (published March 1, 2018), WO/2021/019197 (published February 4, 2021), and GB2114136.1 (filed October 1, 2021)., Ethan Teare: None declared, Ivan Santibanez Koref: None declared, Rachel Phelps: None declared, John Burn named as inventor on patents covering the microsatellite instability markers analyzed: WO/2018/037231 (published March 1, 2018), WO/2021/019197 (published February 4, 2021), and GB2114136.1 (filed October 1, 2021)., Michael S. Jackson named as inventor on patents covering the microsatellite instability markers analyzed: WO/2018/037231 (published March 1, 2018), WO/2021/019197 (published February 4, 2021), and GB2114136.1 (filed October 1, 2021).

EP13.022 NOTCH pathway and ARID1A downregulation in High-grade ovarian cancer tissues

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Background: High-grade ovarian cancer (HGSOC) is the second deadliest gynecologic malignancy in the world. HGSOC has no viable biomarkers intended for diagnosis. NOTCH pathway and chromatin remodeling genes are amongst the most dysregulated in gynecologic cancers. The aim of our study was to investigate *ARID1A* and NOTCH pathway gene expression in gynecologic cancer tissue samples.

Methods: 51 gynecologic tumors (32 HGSOC, 9 other OC cases, 1 endometrial cancer, and 9 benign ovarian tumors) were investigated for *ARID1A*, *NOTCH1-4*, *CTNNB1*, and *FBXW7* mRNA expression analysis using qPCR. *GAPDH* expression was used for normalization.

Results: The expression of all genes analyzed was significantly downregulated in HGSOC samples when compared to benign controls. *CTNNB1* was the best separator of HGSOC cases from controls with AUC = 0.93, also significantly correlating with higher FIGO stages ($p = 0.20$, Mann-Whiney t-test). *ARID1A* downregulation correlated with large operational findings ($p = 0.22$, Mann-Whiney t-test) as well as showing a tendency for shorter PFS (HR:3.4, 95% CI: 1.1-10.2).

Conclusion: *ARID1A* and NOTCH pathway gene downregulation could be potential diagnostic and predictive biomarkers for HGSOC. Further studies are needed to validate the biomarkers in a larger sample cohort.

Conflict of Interest: None declared.

EP13.023 Evaluation of Fn3 as a potential carrier of cytotoxic molecules for the treatment of mesothelin overexpressing cancers

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Background/Objectives: The overexpression of mesothelin (MSLN) in many malignancies makes it an ideal target for personalized therapy. Antibody-drug conjugates targeting MSLN were developed for therapeutic purposes but with reduced efficacy. Here, we generated an MSLN-overexpressing cell line to evaluate the binding between MSLN and a small peptide-based scaffold derived from the tenth domain of the human fibronectin type III (Fn3) to use as a theranostic tool for MSLN-overexpressing cancers.

Methods: MSLN-overexpressing cells were generated from the MSTO-211H cell line using the pcDNA 3.1(+) plasmid. The clonal selection was performed by fluorescence-activated cell sorting (FACS), and MSLN expression was evaluated with flow cytometry. To evaluate its binding with MSLN, Fn3 was incubated with wildtype MSTO and MSLN-overexpressing clones, and the binding was assessed by flow cytometry. Immunofluorescence was carried out to confirm MSLN-Fn3 interaction and to investigate its cellular localization.

Results: Flow cytometry analyses confirmed the MSLN overexpression (10-fold increase) in MSTO clones. The evaluation of the Fn3-MSLN binding resulted in a 35-fold higher signal in the MSLN-overexpressing clones compared with the wildtype MSTO. Immunofluorescence staining showed the Fn3-MSLN binding on the cell surface.

Conclusion: This work revealed that MSLN-overexpressing cells could allow an accurate assessment of Fn3-MSLN binding affinity. Our data support the binding between Fn3 and MSLN, encouraging further experiments to deepen the knowledge about the Fn3-MSLN interaction. Lastly, Fn3 could be evaluated as a carrier of cytotoxic molecules and radionuclides to elucidate its role as a theranostic agent.

Grant References: AIRC (IG-25708).

Conflict of Interest: None declared.

EP13.024 Adaptive Nanopore sequencing to determine pathogenicity of BRCA1 exonic duplication

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Background/ Objectives: *BRCA1* and *BRCA2* are tumor suppressor genes that have been characterized as cancer predisposition genes for the development of hereditary breast and ovarian cancers among

other malignancies. The molecular diagnosis of this predisposition syndrome is based on the detection of inactivating variants of any type in those genes. But in the case of structural variants, functional consequences can be difficult to assess using standard molecular methods, as the precise resolution of their sequence is often impossible with short-read Next Generation Sequencing techniques.

Methods: It has been recently demonstrated that Oxford Nanopore long-read sequencing technology can accurately and rapidly provide genetic diagnoses of Mendelian diseases, including those linked to pathogenic structural variants. Nanopore sequencing technology coupled with adaptive sampling target enrichment allows a fast library preparation, followed by 48 hours of bioinformatically-targeted sequencing. It allows a significant enrichment of genomic regions of interest, without any need for amplification steps. Downstream bioinformatics analysis is performed using NanoCliD, a custom bioinformatics pipeline (https://github.com/InstituteCurieClinicalBioinformatics/NanoCliD) developed in our Institute.

Results: We report the accurate resolution of a germline duplication of exon 18 to 20 of *BRCA1* using Nanopore sequencing with adaptive sampling target enrichment. We observed it was a tandem duplication disrupting *BRCA1* so this variant could be classified as pathogenic within a timeframe of ten days, compatible with any surgical or treatment decision.

Conclusion: This case provides a proof-of-concept that Nanopore adaptive sampling is a highly efficient technique for the investigation of structural variants of tumor suppressor genes in a clinical context.

Conflict of Interest: None declared.

EP13.025 Intratumoral transcriptomic heterogeneity correlates with tumor location in colorectal cancer

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Background/Objectives: Colorectal cancer (CRC) is the most prevalent cancer worldwide. Patients affected by CRC exhibit strong differences in prognosis and response to treatment, with 20% are metastatic at diagnosis and non-treatable. Here, we describe the molecular findings of transcriptome signatures and intratumoral heterogeneity.

Methods: The cohort consisted of total RNA-seq of colorectal tumors and adjacent non-malignant colon tissue from 136 patients. In silico differential analysis and functional enrichment analysis were performed to identify altered pathways between conditions affecting biological functions. We evaluated CRC transcriptome deconvolution exploiting in silico single cell signatures on six major cell types (epithelial cells, fibroblasts/endothelial cells, myeloid cells, T/natural killer (NK)/NK T lymphocytes, B lymphocytes/plasma cells and mast cells) to detect the fractions and distribution of tumour-infiltrating cells in our CRC samples.

Results: Differential analysis revealed 129 over and 281 down expressed genes between tumor compared to normal tissue implicated in 26 up and 17 down regulated pathways (FDR < 0.05). Localization analysis highlighted 11 up-regulated pathways in left-sided versus rectum and 68 down in right-sided versus rectum. Deconvolution permitted the estimation of cells composition, with 10 subpopulations being over-represented and 9 under-represented in tumor compared to normal tissue.

Conclusion: Analysis showed that CRC exhibits heterogeneity of pathways depending on tumour location, such as inflammatory status, metabolism, oxidative stress and treatment response. Our results allowed to identify transcriptional signatures and their regulation, as well as to elucidate oncogenic pathways in the tumour microenvironment and intratumoural cellular heterogeneity involving functional diversification and environmental adaptation.

Grant References: Intesa Sanpaolo B/2020/0094.

Conflict of Interest: None declared.

EP13.026 Detection of constitutional mismatch repair deficiency in two suspected Lynch syndrome cases with first cancer diagnoses in their late 30s and early 40s

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Background/Objectives: Lynch syndrome (LS) and constitutional mismatch repair deficiency (CMMRD) are cancer predisposition syndromes caused, respectively, by mono- or bi-allelic germline pathogenic variants affecting a mismatch repair (MMR) gene. LS is associated with adult gastrointestinal and genitourinary tract cancers, whereas CMMRD is associated with childhood or adolescent high-grade glioma, haematological malignancies, and LS-spectrum cancers. The phenotypic overlap between these syndromes is under-explored.

Methods: Genetic analyses were conducted according to standard procedures. Constitutional microsatellite instability (cMSI), a diagnostic-hallmark of CMMRD, was analysed by the method of Gallon et al., (https://doi.org/10.1053/j.gastro.2022.12.017).

Results: Case 1 was diagnosed with a colorectal cancer aged 42 years, followed by additional colorectal tumours, and an ovarian cancer aged 52 years, and has an LS family history. Case 2 was diagnosed with an endometrial cancer aged 38 years, and has consanguineous parents and three siblings with cancer diagnoses in their 30s. Both were investigated for LS.

A homozygous *MSH6* likely-pathogenic variant, c.3226C>T p.(Arg1076Cys), was found in case 1. Case 2 was found to be homozygous for *MLH1* c.306G>A p.(Glu102 =), a variant of uncertain significance (VUS). Transcript analysis showed *MLH1* c.306G>A causes "leaky" exon 3 skipping. In both cases, cMSI analysis confirmed a CMMRD diagnosis.

Conclusion: Late onset CMMRD is a differential diagnosis to LS. Screening of further suspected LS patients with compound heterozygous or homozygous MMR VUS for cMSI will likely identify additional late-onset CMMRD cases, which are needed to better understand this phenotype with implications for MMR variant classification and patient management.

Grant References: NA.

Conflict of Interest: Richard Gallon Richard Gallon is a co-inventor on a patent covering the microsatellite instability markers analyzed: GB2114136.1 (filed October 1, 2021)., Carlijn Brekelmans: None declared, Marie Martin: None declared, Vincent Bours: None declared, Simon Schnaiter: None declared, Eric Legius: None declared, Hilde Brems: None declared, Katharina Wimmer: None declared.

EP13.027 Genetics Referral of Ovarian Cancer Patients: Referral Rates at a Publicly Funded Hereditary Cancer Program Between 2010-2019

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Background: Ovarian cancer has a poor 5-year-survivability, and up to 23% of cases are due to pathogenic variants across multiple genes. As a result, guidelines have recommended genetics referral for all patients since 2010. However, previous North American reports demonstrate that referral rates are dramatically lower than expected.

Objectives/Methods: BC's Hereditary Cancer Program (HCP) has implemented (1) GENONC, a referral pathway including medical oncologist-led genetic testing, and (2) GENOVA, a physician-targeted education program for gynecologic cancers. To assess the impact of these initiatives, we identified all patients >18-years-old referred to HCP for ovarian cancer between 2010-2019. Using publicly available provincial data, we calculated annual referral rates, average wait times between diagnosis and referral (time-to-referral), and proportions of referral sources.

Results: Referral rates increased between 2010-2019 (68% in 2019, compared with 40% in 2010). Time-to-referral also decreased, from an average of 659 days in 2010, to 89 days in 2019. Most physician-led referrals were initiated by oncologists (81%), but also by family physicians and surgeons (11% and 6% respectively).

Conclusion: This is the first comprehensive review of BC ovarian cancer referral rates, specifically in context of HCP-led initiatives such as GENONC and GENOVA. Although oncologists provided most referrals, family physicians and surgeons were also involved. While referral rates and time-to-referral improved from 2010 to 2019, ovarian cancer is still under-referred. Future campaigns should continue to target the above physician groups.

Grant References: GENOVA received a clinical innovation grant from BC Cancer Foundation. Dr. Roston is supported by the Clinician Investigator Program (University of British Columbia).

Conflict of Interest: None declared.

EP13.028 BRCA1 and BRCA2 genes analysis in male patients, our laboratory experience

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Background/Objectives: Germline pathogenic/likely pathogenic variants (PVs) in *BRCA1* and *BRCA2* (*BRCA*) genes confer an increased risk of breast and ovarian cancer. Male subjects harbouring *BRCA* PVs represent an underestimated and understudied group of patients. The lack of consistent data on cancers arising in these subjects didn't allow the realization up to now of evidence-based clinical guidelines for surveillance and prevention for them. The aim of this study is to describe our cohort of 216 male patients affected by different types of cancer and/or with a family history of breast and ovary tumours (HBOC), investigated for *BRCA* genes, to define new basis for a correct surveillance and, therefore, prevention, specifically dedicate to this group of subjects.

Methods: A cohort of 216 patients with a personal history of cancer and/or family history of HBOC, referred to our laboratory (2017-2022), was analysed for variants in *BRCA* by NGS.

Results: We identified germline *BRCA* PVs in 60 (27.8%) patients, whereas 149 (70%) subjects didn't show variants. In our cohort, 128 (59.3%) patients developed cancer, in particular in breast and prostate. 15 (11.7%) of these patients showed *BRCA* PVs with a prevalence of variants in the *BRCA2* gene (66.7%) compared to the *BRCA1* gene (33.3%). No *BRCA* variants were found in 107 (83.6%) cancer subjects. PVs were identified in 45 (20.8%) healthy subjects with a family history of HBOC.

Conclusion: Our study can improve the knowledge about the management of male patients with *BRCA* PVs and lead to future screening and surveillance recommendations.

Conflict of Interest: None declared.

EP13.029 The spectrum of pathogenic germline nucleotide variants in gastric cancer patients of Kyrgyz origin

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Background: Gastric cancer (GC) is the third most common cause of cancer-associated mortality worldwide. Most GC cases are classified as sporadic, with a limited proportion (up to 10%) demonstrating familial accumulation. Hereditary forms account for 1-3% of GC cases. The identification of GC-associated genetic variants via next-generation sequencing (NGS) provides substantial benefits for both patients and their families. We conducted the first systematic NGS-based analysis in Kyrgyzstan to determine the frequency of hereditary GC in patients with gastric cancer.

Methods: The study cohort included 113 patients with diagnosed GC from Kyrgyzstan. The age of patients was 57.6 ± 8.9. NGS analysis of genomic DNA was performed using custom KAPA HyperChoice MAX enrichment panel (Roche, USA), that included 111 hereditary cancer-associated genes.

Results: Pathogenic/likely pathogenic variants (PV/LPV) were identified in 7 of 113 individuals (6,2%). In 3,5 % (4/112) of patients, heterozygous PV/LPV were identified in high-penetrance genes (TP53, POLD1, RET, BRCA2) and 2,7 % (3/113) of patients carried heterozygous mutations in genes associated with autosomal recessive conditions: PALB2, FANCA, FANCD2. A combination of two pathogenic heterozygous variants in RET and BRCA2 genes was found in a 60-year old patient. We have not identified any genetic variants in hereditary GC-associated genes: CDH1, STK11, SMAD4, BMPRIA, APC, MLH1, and others.

Conclusion: Our study encompassed the general population. The use of specific selection criteria would increase the number of identified genetic variants in hereditary GC-associated genes. Further research is required to determine the clinical relevance of the genetic variants identified in the current study.

Conflict of Interest: None declared.

EP13.030 Osteosarcoma without prior retinal affection in a patient with compound heterozygous RB1 pathogenic germline variants, a case within a family context of hereditary retinoblastoma

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Hereditary retinoblastoma is a hereditary cancer syndrome with autosomal dominant inheritance. This syndrome usually causes retinal tumors in carriers of germline pathogenic variants in RB1, which is a suppressor tumor gene. It is well established that patients with germline pathogenic variants in RB1 have an increased risk to develop second primary malignancies including in decreasing order of frequency osteosarcoma, soft-tissue sarcoma, melanoma and epithelial carcinoma (mostly lung and bladder). Aversely the incidence of osteosarcoma as primary tumor without prior retinal affection has not been delineated. We report the case of a male patient, 18 years old, son of consanguineous parents, who consult to Oncology Department with osteosarcoma diagnosis affecting his right leg who require amputation and chemoteraphy treatment. Pathologic anatomy confirmed high grade femoral osteosarcoma (G3). Patient received oncologic genetic counselling because a familial context of several affected members with retinoblastoma or malignant peripheral nerve sheath tumor. Multipanel germline sequencing study revealed two heterozygous likely pathogenic and pathogenic variants both locating in coding 20 exon: c.1964A>G (p.Tyr655Cys) and c.1961T>A (p.Val654Glu), in the same chromosome, in RB1 gene correspondingly. This report again raises the questions about intrafamilial variable expression, the risk that confer low penetrance variants and parental origen from inherited variant. Regardless of familial context, this finding reaffirm the need to expand germline testing including RB1 gene sequencing in cases with osteosarcoma as primary tumors.

Conflict of Interest: None declared.

EP13.032 Usefulness of a predictive model for Polygenic Hereditary Cancer detection

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Background: A hereditary component of cancer disease is mostly based on the presence of germline pathogenic variants mainly in high penetrance genes which differ according to the diverse syndromes. However, no pathogenic variant is detected in some patients who are strong for hereditary cancer due to the strong family history.

Methods: A total of 101 cancer susceptibility genes was analysed by Next Generation Sequencing in 48 breast cancer and colorectal cancer patients selected by their strong family history. Simultaneously, we developed a predictive model for helping us to integrate the different phenotypic variables, as type of cancer, age of cancer onset and family history, with those variants which were identified.

Results: Twelve of the samples (25%) resulted carriers of two or more simultaneous germline variants in predisposition cancer genes which were not previous described or classified as uncertain as well as conflicted of significance. In combination with their phenotype and family history information, the use of our predictive model allowed us to determine their contribution in disease development of these patients.

Conclusion: Our results support a polygenic model, in which aggregation of multiple low and moderate penetrance gene variants are contributing to some hereditary cancer disease development when no clear deleterious variants are found. The inclusion of this polygenic hereditary cancer cases will improve the clinical management of this type of patients and their families, enabling genetic counselling and their inclusion in prevention cancer programs.

Grants: Predoctoral fellowship co-financed by European Social Fund and Junta de Castilla y León (EDU/842/2022).

Conflict of Interest: None declared.

EP13.035 Experience in multigene panel testing for inherited cancer-predisposition syndromes from a Spanish tertiary referral hospital

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Background/Objectives: Diagnostic yield of inherited cancer-predisposition syndromes has increased due to the discovery of new susceptibility genes and the development of available technology. The aim of this study is to assess the genotypic spectrum of inherited cancer-predisposition syndromes in a cohort of Spanish patients and the utility of reanalysis of unsolved cases in clinical care.

Methods: Retrospective review of 2209 individuals with personal or family history of cancer subject to referral from 2020 to 2022. Targeted genes were captured with a Custom Hereditary Cancer Solution kit (SOPHiA GENETICS) and bioinformatic analysis was performed by SOPHiA-DMM platform.

Results: Germinal pathogenic variants were identified in 10.86% of individuals. Among them, Hereditary Breast and Ovarian Cancer (HBOC) seems to be the most frequent syndrome (70.83%). However, its global diagnostic yield is lower than syndromes with more specific phenotypes such as Li-Fraumeni Syndrome or Lynch Syndrome (diagnostic rate 10% versus 29.41% and 21.38% respectively).

Additionally, 525 of the 2209 cases were actually reanalysis. Among them, deletereous variants were identified in 10.1% of the cases. 93.94% of them were cases of HBOC individuals. This is related with the inclusion of newly associated genes beyond BRCA1 and BRCA2 since the original analysis, being ATM (10.24%), PALB2 (10.24%), CHEK2 (7.23%) and BRIP1 (6.02%) the most prevalent.

Conclusion: Our results are consistent with the diagnostic yield described in literature. This study highlights the value of reanalysis

of genetic test results and the usefulness of a multigene testing strategy for the diagnosis of inherited cancer-predisposition syndromes.

Conflict of Interest: None declared.

EP13.037 It's a MANS world

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MBD4-associated neoplasia syndrome (MANS), first described in 2021, causes an autosomal recessive cancer predisposition disorder, from loss of function variants in the base excision repair protein MBD4. Core elements include colonic polyposis and acute myeloid leukaemia, however tumour spectrum is unclear. We report two families with novel germline pathogenic variants in MBD4, resulting in MANS.

Family 1. Two sisters presented with multiple malignancies. One had uveal melanoma aged 30, dysplastic colonic and duodenal polyps aged 37, and died from acute myeloid leukaemia (AML) aged 42. Her younger sister had a uveal melanoma aged 38, multiple colonic adenomatous polyps aged 35, meningioma aged 41, recurrent ocular melanoma aged 45, breast cancer aged 50 and multiple schwannomas. This family was reported in Palles et al., AJHG 2022.

Family 2. A 40 year old man had multiple colonic polyps from late 30's. He had a bone marrow transplant for AML aged 24. DNA was obtained from cultured fibroblasts.

Germline APC, MUTYH, Lynch genes (F1 + F2); CDKN2A, BAP1, BRCA 1/2 (F1); BMPR1A, NTHL1, POLD1, POLE, PTEN, SMAD4, STK11 (F2) all normal

Affected members of both unrelated families were found to be heterozygous for the same two loss of function MBD4 variants – c.939dup p.(Glu314fs)/c.1688T>A p.(Leu563Ter).

These cases highlight the role of MBD4 in cancer predisposition. Further studies are required to identify the full spectrum of cancer predisposition, and screening guidelines remain empiric. The diagnosis of MANS may indicate the use of checkpoint inhibitors. MBD4 should be included in polyposis and early onset AML gene panels.

Conflict of Interest: None declared.

EP13.038 Hereditary gastric cancer: single gene or multigene panel testing? A mono-institutional experience

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Background: Pathogenic variants (PVs) in specific cancer predisposition genes increase gastric cancer (GC) risk. Identifying high-risk individuals (HRIs) is crucial for targeting preventive strategies. Recent evidence suggests that multigene panel testing (MGPT) improves HRI identification.

Methods: Fifty-four GC patients were consecutively referred for genetic counselling and single-gene testing (SGT) of one or more genes according to their personal (PH) and/or family history (FH). Fifty patients underwent SGT for unknown PVs and four patients for known PVs. Thirty-six with an available blood sample underwent MGPT. We used Fisher's exact test to compare carriers and non-carriers according to MGPT results.

Results: Nine patients (9/54;16.7%) turned out to be PV carriers. SGT for unknown mutations detected seven PVs (7/50;14%): one in *BRCA1* (2%), two in *BRCA2* (4%), three in *CDH1* (6%), and one in *MSH2* (2%), plus one (2%) variant of unknown significance (VUS). MGPT identified two additional PVs in hereditary GC candidate genes (*ATM* and *RAD51D*) and at least one VUS in 15 patients. Comparing PV carriers and non-carriers according to MGPT results, we observed a statistically significant difference in PVs between patients with and without FH for GC (5/17:29.4% *vs*. 0/19:0%; p-value:0.016) or Lynch-related tumours (4/11:36.4% *vs*. 1/25:4%; p-value:0.02). *CDH1* and *MSH2* emerged involved in early-onset diffuse and later-onset intestinal GCs, respectively.

Conclusions: Genetic counselling and SGT remain central for genetic GC risk assessment. MGPT appears a capable tool for HRI identification, especially in patients with FH for GC or Lynch-related cancers, although leading to challenging results in 47.2% (17/36) patients.

Conflict of Interest: None declared.

EP13.039 Searching for germinal variants of BRD4 epigenetic gene in Polish prostate cancer patients - preliminary results

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Introduction: The epigenetic variants are present in all human cancers and associated with genetic alterations to drive a cancer phenotype. Thus, we searched for germinal variants in *BRD4* epigenetic gene in Polish prostate cancer patients.

Material: The material of investigation was DNA from 97 men with prostate cancer (PC) from all over Poland. The median age of patients at PC diagnosis was 60,4 years (45-76).

Methods: NGS and Sanger sequencing.

Results: In 10/97 (10,3%) PC patients 8 variants of analyzed gene were detected. These were 5 missense variants, 2 silent variants and 1 splicing variant. Bioinformatic analysis of all changes was performed using Franklin or VarSome databases. The c.2170A>C (p.Ser724Arg), c.605C>G (p.Ala202Gly), c.3301G>A (p.Val110Met) and c.689C>T (p.Pro230Leu) were predicted as VUS (variants of uncertain significance). The c.3057C>G (p.Pro1019 =) was predicted as likely benign. The c.2300C>G (p.Ser767Cys) and c.3690C>T (p.Ala1230 =) were classified as benign. The c.4020+30G > T was predicted as benign and probably not involved in RNA splicing. Three PC patients were carriers of

c.2300C>G, two were carriers of c.3690C>T and one PC patient was the carrier of both c.4020+30G > T and c.2300C>G variants.

Conclusions: The results of the preliminary investigation point at the need to study germinal variants of *BRD4* epigenetic gene to help fully understand the pathogenesis of prostate cancer, identify men at PC high risk and predict the disease recurrence risk after radical prostatectomy.

This study was supported by the fund of the Collegium Medicum Nicolaus Copernicus University, Bydgoszcz, Poland.

Conflict of Interest: None declared.

EP13.040 Comparative quantification of cell-free DNA in Diffuse Large B-cell Lymphoma patients with fluorimetry (Qubit) and Bioanalyzer for estimation of minimal residual disease in Indian patients

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Introduction: Determination of cfDNA quality & quantity is essential for MRD analysis by NGS for various malignancies. Qubit-fluorometry and Electrophoresis-based instruments(e.g. Bioanalyzer) are two commonly used methods for this purpose. Qubit performs fluorometric quantification of cfDNA, while Bioanalyzer allows to visualize concentration of cfDNA by size. Presently, there is lack of data on comparative merits between these methods. We compared cfDNA estimation using Qubit with Bioanalyzer in lymphoma patients.

Materials and methods: A total of 41 blood-samples from patients of large B-cell lymphoma were collected into BD-Vacutainer K2EDTA tubes. Plasma was separated within 2-hours and stored at –80°C. cfDNA was extracted using a MagMAX™ Cell-Free DNA isolation kit. The extracted cfDNA was quantified using two different methods: Qubit (Qubit 4 Fluorometer - Invitrogen) and automated electrophoresis based method (Bioanalyzer).

Results: The estimates of cfDNA by Qubit (median, 3300pg/µl) were higher than those by bioanalyzer (median, 3246.06 pg/µl). We believe that due to inclusion of higher molecular weight-range, the median value of cfDNA in Qubit analysis was higher. With Spearman correlation coefficient of 0.523, we found a strong positive correlation in cfDNA quantification between Qubit and Bioanalyzer. No statistically significant difference was observed between these two-quantification method with Bland Altman test & Mann Whitney-U test ($p = 0.199$, $p = 0.553$).

Conclusions: In our study, both methods had positive correlation for estimation of cfDNA in paired samples from patients. However, using Bioanalyzer was more expensive and cumbersome. Hence, Qubit may be a better option due to its ease-of-use, shorter processing time, and low cost.

Conflict of Interest: Gaurav Prakash PGIMER, PGIMER, INTAS, Samsung Bioepis, Anu Kumari PGIMER, Anupriya Kaur PGIMER, PGIMER, Priyanka Srivastava PGIMER, PGIMER, Amanjit Bal PGIMER, PGIMER, Pankaj Malhotra PGIMER.

EP13.044 "Music to my ears" or "a slap in the face": patient experiences of tumour genomic profiling and the impact of results

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Background: Tumour genomic profiling (TGP) shows increasing clinical utility in informing personalised cancer management plans. Results may also indicate potential germline cancer-predisposition pathogenic variants. Such variants carry wide-ranging implications, requiring confirmation and consequent supports. Evidence remains limited on the real-world experiences of patients in this setting and their support needs- including informational support to promote patient autonomy and informed consent- within this distinct setting in Australia.

Aim: To explore the experiences of patients who have received TGP results.

Methodology: Semi-structured interviews were recorded with patients who received results from TGP during their clinical care. Interviews explored participants' experiences of TGP, including their expectations and understanding, and their perceptions of results. Transcripts were thematically analysed using a reflexive phenomenological approach. These were coded and key themes identified.

Results: The analysis of nine transcripts found participants perceived consenting to TGP as an insignificant decision, yet attached great significance to their results regardless of result type. The timing and context of TGP impacted participants' decision-making prior to consenting, subsequent reactions to their results, and their ability to recall details of both. Additionally, participants found it difficult to contend with the implications of potential or actual germline pathogenic variants for their family members.

Conclusion: While participants reported feeling satisfied with the informational support received on TGP from their oncologists, TGP occurred during times of increased stress, and this impacted the real-world implementation of patient autonomy and informed consent. The data highlight the need for further support of patients' psychosocial needs in this setting.

Conflict of Interest: Zarae Davis Peter MacCallum Cancer Centre and The Royal Melbourne Hospital (part time), Shelby Taylor Peter MacCallum Cancer Centre and The Royal Melbourne Hospital (full time), Linda Cicciarelli Austin Health (part time), University of Melbourne (part time), Alison Trainer Peter MacCallum Cancer Centre and The Royal Melbourne Hospital (full time).

EP13.045 Transcriptoma analysis of laryngeal papilomas

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Backround: Laryngeal papillomatosis is a benign neoplastic disorder caused by chronic infection with low-risk human papillomavirus (HPV) types 6 or 11. The aim of the study was to define the transcriptional profile of laryngeal papilomas compared to normal non-HPV infected laryngeal mucosa.

Methods: Twenty-four laryngeal papiloma samples were included into the study. The control group included 17 samples of healthy laryngeal mucosa from patients with a vocal cord cyst

served as controls. RNASeq libraries were prepared using either high or low RNA input kit and sequenced. Differential gene expression and HPV gene expression was analyzed using the DeSeq2 tool.

Results: In 70% of samples, HPV6 was detected while HPV11 was found in 22% of them. The HPV type-specific gene expression showed that *E2, E4, E6* and *E7* genes of both HPV6 and HPV11 were most strongly expressed in laryngeal papilomas while expression of *E5b, L1* and *L2* genes of both viruses was low. No HPV gene expression was detected in normal mucosa samples. We did not detect transcription of any other viruses in papiloma samples. Altogether, 1360 differentially expressed genes between the papiloma and control groups were found.

Conclusions: We have characterized the transcriptional profile of laryngeal papilomas compared to normal non-HPV infected laryngeal mucosa. There was a high concordance of HPV6 and HPV11 virus detection between the polymerase chain reaction and RNASeq methods.

Grant References: This work was supported by the Ministry of Health of the Czech Republic IP/RVO-FNOs/2019.

Conflict of Interest: Pavlína Plevová Collabolator, Michal Vasinek Collaborator, Martin Formanek Principal investigator, Karol Zelenik: None declared, Pavel Hurnik Collaborator, Jakub Mrázek: None declared, Marketa Homolova: None declared, Petr Broz: None declared, Iveta Bystronova: None declared, Pavlina Kusnierova: None declared.

EP13.046 Genes associated to PD-L1 network are potential glioblastoma biomarkers

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Background: Glioblastoma is the most common primary brain tumor with exceptionally low survival rate, as most patients die within 2 years of diagnosis. The main factors contributing to treatment failure are lack of reliable biomarkers and efficient therapy. In our research, we analyzed gene expression of the PD-L1 network, one of the most de-regulated immune pathways in cancer, as potential glioblastoma biomarkers.

Methods: Network was constructed using Cytoscape, with STRING PubMed query and STRING protein query set to 0.7 confidence. Gene expression was analyzed using CGGA (mRNA-seq_693 dataset), TCGA (TCGA GBMLGG dataset retrieved from GlioVis), and Rembrandt (retrieved from GlioVis). Survival analysis of GBM patients was determined based on optimal cutoff, calculated by surv_cutpoint function in R (version 4.1.1), using package survminer.

Results: Enrichment data analysis shows that the genes are mainly involved in immune system processes. Next, we selected genes that were 1) overexpressed in glioblastoma compared with tumors of lower grade glioma, 2) overexpressed in glioblastoma compared with normal brain tissue and 3) their higher expression was associated with poorer overall survival. The genes that met all three criteria in all three datasets were *CASP4*, *CD44*, *CD163*, *CD276*, *RAB42*, *STAT3* and *HOXD13*.

Conclusion: Our results suggest that the discovered genes may be novel biomarkers and therapeutic targets for glioblastoma.

Grant references: This work was supported by Slovenian Research Agency (Z3-2649 project grant, Z3-4510 project grant, and P1-0390). This work was also supported by CELSA project Generation of nanobodies against immunomodulating checkpoint receptors in glioblastoma tumor cells.

Conflict of Interest: None declared.

EP13.047 Implementation of an algorithm for the detection and management of low allelic fraction variants detected in germline by NGS in hereditary cancer studies

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Background/Objectives: Hereditary cancer syndromes are a clinical entity characterized by an increased risk of developing cancer due the presence of heterozygous germinal variants in cancer susceptibility genes. Constitutional mosaic variants can affect both somatic and germinal cells, and thus can be passed to the offspring and have important consequences for genetic counselling. Constitutional mosaicism in cancer related genes has been previously described. It is detected as low variant allele frequency (VAF) variants (<30%) that are generally overlooked during the prioritization process. We propose an algorithm to deal with this potential mosaic findings identified in germline by Next Generation Sequencing (NGS).

Methods: First, the algorithm must be able to rule out false positives results arising from sequencing and alignment artefacts. Second, it must be able to discriminate between somatic mosaicism in peripheral blood leukocytes (PBL) and constitutional mosaicism. For it, our proposed protocol may include testing and quantifying several tissues derived from additional germ layers. Low invasive samples such as buccal swab or urine are recommended. And finally, it includes tumor testing for the variant to establish its role in tumor development.

Results: The implementation of this algorithm in 2020 in our laboratory allowed us to detect three cases of mosaic variants in cancer susceptibility genes.

Conclusion: Detection of mosaicism constitutes a diagnostic challenge, laboratories should define a diagnostic algorithm in order to avoid misinterpretations since failure to detect mosaicism may result in inappropriate cancer risk assessment, inappropriate clinical management and a substantial difference in recurrence risk assessment.

Conflict of Interest: None declared.

EP13.048 Real-world evidence data for detection of EGFR mutations in cf-DNA from metastatic NSCLC patients

Maria Michelli[1], *Ioanna Giannopoulou*[1], *Eirini Roupou*[1], *Ilenia Chatziandreou*[1], *Niki Prekete*[1], *Theofilos Kaparis*[1], *NIKOLAOS KAVANTZAS*[1], *Angelica a Saetta*[1]

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Background/Objectives: EGFR mutation analysis in circulating cell-free tumor DNA (cf-DNA) from plasma constitutes a minimally invasive alternative approach for NSCLC patients without adequate tumor tissue for molecular analysis or for monitoring of lung cancer progression.

Methods: We examined 558 blood samples from patients with NSCLC and 133 matched FFPE tumor tissues using Cobas ® EGFR mutation v2 (IVD). 17 blood samples were also re-analyzed using Idylla™ cf-DNA mutation test.

Results: EGFR mutations in cf-DNA were detected in 27% of the samples. The most common mutations were deletions in exon 19

(67%), followed by point mutations in exon 21 (26%). A resistance mutation, p.Thr790Met was found in 22% of the mutant cases. The presence of mutations in primary samples reached 10% whereas for follow up samples the percentage of mutations was 52%. The overall concordance of EGFR mutation status in plasma and tumor was 75% and the sensitivity 55%, specificity 100%, PPV 100%. Using Idylla cf-DNA mutation test, the specificity and PPV was 100% whereas sensitivity reached 50% and the concordance of EGFR mutation status in plasma between the two methods was 64%.

Conclusion: The above analysis adds to current data indicating that EGFR mutation testing in cf-DNA has high specificity and PPV, using either of the two different mutations tests. The PPV indicates that EGFR mutations could be reported with certainty. However, the sensitivity of these tests is not optimal yet, indicating that ideally a biopsy should be obtained for patients with an EGFR mutation-negative cf-DNA test.

Grant References: no.

Conflict of Interest: Maria Michelli full, Ioanna Giannopoulou full, Eirini Roupou full, Ilenia Chatziandreou full, Niki Prekete full, Theofilos Kaparis: None declared, NIKOLAOS KAVANTZAS full, Angelica a Saetta full.

EP13.050 Implementation of PIK3CA biomarker testing for breast cancer patients targeted therapy: evidence for best practice

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Background/Objectives: Breast cancer is the most prevalent cancer worldwide and there is a need for new therapeutics and biomarkers for advanced disease. PIK3CA mutations are common in breast cancer mainly affecting exons 9 and 20 encoding the helical and kinase domain respectively. This study aims to compare Next-generation sequencing (NGS) and Sanger sequencing for PIK3CA mutation detection in exons 9 and 20 as a predictive biomarker in breast cancer patients with HR+ advanced disease.

Methods: 160 samples (145 FFPE tissue and 15 plasma cases) from women previously diagnosed with breast cancer were examined through NGS and Sanger sequencing. The same isolated DNA from the relevant breast cancer tissues was used for both experimental methods to avoid any biased results.

Results: 53 cases were only analysed through Sanger sequencing due to exclusion from NGS analysis according to the pre-analytical criteria or non-informative result. 6% of the mutations were detectable only with NGS. The most frequent mutations found were p.E545K and p.H1047R in exons 9 and 20 respectively.

Conclusion: The two methods showed an overall 94% concordance. Thus we conclude that they are both useful in detecting SNVs such as those in the PIK3CA gene. However, NGS is superior in cases in which several biomarkers are required simultaneously for treatment decisions whereas Sanger sequencing could provide results in cases excluded from NGS due to quality criteria or for single gene analysis. Thus they can be considered complementary in clinical practice depending on specific requirements.

Grant References: No.

Conflict of Interest: None declared.

EP13.051 Correlation between HPV DNA testing and cytological examination of cervical samples

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Background/Objectives: Human papilloma virus (HPV) is a dsDNA virus, with more than 200 haplotypes and is one of the most common sexually transmitted viruses, worldwide [1,2]. Despite the fact that there are many screening programs and public awareness campaigns, as well as many molecular detection methods available, still remains one of the most common causes of cervical cancer [2]. The aim of this study was to explore possible correlations between a positive HPV DNA test with cervical lesions detected in a parallel Pap test performed.

Methods: A total of 2964 thin prep samples were collected from females in Northern Greece in order cytological examination of cervical cells (PAP test) and HPV DNA test to be performed. HPV DNA presence examined by real-time PCR using a commercial kit.

Results: Out of 2964 cases, 544 (18.35%) were found positive for at least one of the HPV high-risk haplotypes tested. Concerning cytological examination of the above samples, only 71 (2.39%) resulted in a Test Pap with pathological findings. Remarkably, out of 544 samples positive for one of the HPV haplotypes examined, only 36 (6.61%) were found with an abnormal Pap test.

Conclusion: These results show that HPV infection occurs despite a normal cytological examination, emphasizing the importance of HPV DNA test as a screening method for prevention of cervical cancer.

Grant References:

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2. Okunade, K.S. (2020) 'Human papillomavirus and cervical cancer', Journal of Obstetrics and Gynaecology.

Conflict of Interest: None declared.

EP13.052 Case report of duration of response in HER2-positive NSCLC patient

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Introduction: Oncogenic driver mutations have become important targets for molecular therapies in many cancers. While HER2 mutations are well studied in breast cancer, their role in non-small cell lung cancer (NSCLC) remains largely unconfirmed, hindering the development of standard of care for such patients. HER2 mutations are found in 1-2% of lung adenocarcinomas, making their potential therapeutic role worth studying in more detail.

Patient and methods: A 59-year-old female patient with a history of breast cancer and a long remission period of more than 20 years is presented. She was diagnosed with NSCLC in early 2020 and treated with Afatinib as a first-line treatment. The patient's tests showed negative results for EGFR and ALK mutations, PD-L1 < 1%, but a positive result for HER2(+).

Results: The patient achieved a duration of response (DoR) period of 21 months, which is much longer than expected with other treatment regimens. Currently, the patient is receiving second-line treatment with immunotherapy (Atezolizumab) due to progression of the disease.

Conclusion: This case report provides anecdotal evidence that HER2 may play a potentially positive role in achieving more effective treatment of NSCLC. However, additional evidence is needed to confirm these findings and develop effective HER2-based therapeutic protocols.

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Conflict of Interest: None declared.

EP13.053 Spectrum of BRCA Mutations and Identification of novel founder mutation in BRCA2

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Objectives: The prevalence and effect of *BRCA1* and *BRCA2* mutations in Middle Eastern population is not fully explored. To study the prevalence of *BRCA1* and *2* mutations, we established a molecular database for all patients with personal history of breast cancer. An extensive review of the spectrum of *BRCA1* and *2* mutations was undertaken to study the following objectives: identification of *BRCA 1* and *2* founder mutation, prevalence of BRCA1 and 2 mutations in patients attending the cancer genetics clinic at Sultan Qaboos Comprehensive Cancer Care and Research Centre.

Methods: Genomic DNA was prepared from the peripheral blood leukocytes of at least one affected woman from each family. Comprehensive cancer panel of 128 genes was used where next generation sequencing and deletion/duplications of the genes was performed using multiplex ligation dependent probe amplification MLPA.

Results: Mutations in *BRCA1* and *BRCA2* were found in 30 (5%) of the 592 families studied. 22 mutations in *BRCA2* and 8 mutations in *BRCA1* were identified. A founder mutation was identified in four un-related families in BRCA2 (c.2588 dup). No phenotype/genotype correlations were noted.

Conclusion: Importance of screening patients with early onset breast cancer for *BRCA1* and *2* mutations to enable personalized screening and therapy for affected and at risk related members of the family. Identification of a founder mutation in *BRCA2* (c.2588dup), knowledge of founder mutations can help to develop cost effective genetic screening strategies for Omani patients which ultimately reduces cost and turn-around time.

Conflict of Interest: None declared.

EP13.054 TRAIL apoptotic pathway is significantly deregulated in breast cancer

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Background/Objectives: There are many anticancer therapeutic approaches seeking to activate TRAIL-mediated apoptotic pathway, due to its antitumor cell selectivity. First and second generation therapies targeting TRAIL pathway in clinical trials failed to meet the expectations of the preclinical data[1]. Our aim was to investigate the relative mRNA expression of key components of the pathway in order to underline its deregulation in breast cancer as a causative parameter for low efficacy/resistance of the abovementioned therapies.

Methods: Relative mRNA levels of TRAIL pathway genes (*TRAIL, DR4, DR5, DcR1, DcR2, FADD, TRADD*) were examined in 94 breast cancer tissues by RT-PCR/ΔΔCt. Activation of caspases 3/8 was evaluated in subset of cases by IHC. Statistical analysis was performed by SPSSv.25 software.

Results: Most cases (31-64%) presented reduced mRNA levels of the examined genes, while elevated levels were observed in 5-16% of the cases. Different co-expression patterns emerged with *DR5/DR4* demonstrating the strongest linear correlation ($R = 0{,}634$, $p < 0{,}001$). Clinicopathological characteristics (age, pPrognostic stage, molecular subtype) displayed statistical correlations with relative mRNA levels of various examined genes. Interestingly, active caspase3 statistically correlated with the HER2-enriched molecular subtype ($p = 0{,}032$).

Conclusion: The mRNA expression of key components of TRAIL pathway is significantly deregulated in breast cancer, indicating complex regulation mechanisms and different mRNA expression patterns. The correlation of relative mRNA levels with clinicopathological parameters supports their importance as potential predictive biomarkers for TRAIL targeting therapies.

References: Di Cristofano et al. (2023) *Biochem Soc Trans* https://doi.org/10.1042/bst20220098.

Grants: No funding.

Conflict of Interest: None declared.

EP13.055 Somatic APC mosaicism as a cause of adenomatous polyposis

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Background/Objectives: Inherited pathogenic/likely pathogenic (P/LP) germline variants in *APC*,*MUTYH*,*POLE*,*POLD1*,*NTHL1*, and *MSH3* genes, as well as somatic mosaicism in *APC* gene, are well-established causes of adenomatous polyposis of colon. Familial adenomatous polyposis (FAP) is an autosomal dominant colorectal cancer (CRC) predisposition syndrome. FAP is cause of less than 1% of all CRC cases and is characterized by the development of multiple (hundreds-to-thousands) adenomatous polyps of colon from an early age. This study aimed to determine the prevalence of somatic mosaicism in the *APC* gene as the cause of FAP in patients without known germline P/LP variants in polyposis-associated genes.

Methods: Between 2019 and 2022, 46 patients were tested for presence of somatic mosaicism in *APC* gene at Institute of Oncology Ljubljana. Our inclusion criteria were ≥10 adenomatous polyps found across multiple colonoscopies in patient with no causative germline variants detected. Testing was performed by selecting two or more polyps from different colon segments. DNA was extracted from FFPE and NGS-sequencing was performed using Illumina_TruSight_Tumor_170_DNA.

Results: Among the 46 tested patients somatic *APC* mosaicism was detected in 4 patients (8.7%). Testing was unsuccessful due to poor tissue sample quality in 3 patients (6.5%).

Conclusions: *APC* mosaicism was detected in 8.7% of patients with unexplained adenomatous polyposis, allowing us to provide conclusive diagnosis. Since the percentage of identified somatic *APC* mosaicism cases depends on the inclusion criteria larger cohorts and more studies are needed to accurately define most optimal inclusion criteria. Further studies are also needed to determine optimal number of tested polyps for reliable results.

Conflict of Interest: None declared.

EP13.056 Identification and characterization of clinically relevant variants in not-routinely screened cancer genes: Is it time to reconsider current molecular oncology investigations?

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Background/Objectives: Several variants in oncogenes are used for cancer prevention and targeted therapies. Unfortunately, for several neoplasms there are not clinically actionable variants (CAV). Furthermore, current genetic tests explain about 5-10% of total neoplasms while a familial association is observed in about 30% of cases. In this study we looked for CAV, in genes not routinely tested (NRTG), to identify new clinically actionable targets.

Methods: We performed Illumina TruSight Oncology 500 (TSO-500) tumor genomic profiling assay, which analyzes 523 cancer genes, on 62 cancers distributed as: 20 thyroid cancers, 13 colorectal cancers, 10 melanomas, 10 breast cancers, 9 prostate cancers. Pathogenic-Likely Pathogenic variants were reported according to ACMG-AMP guidelines (Richards et al., 2015), TierI-TierII variants according to AMP-ASCO guidelines (Marilyn et al., 2017). We compared the percentage of CAV between genes currently recommended for therapy (OncoKB) and prevention (NCCN/NIH /AIOM guidelines), and the NRTGs in the panel.

Results: We identified at least one CAV in 95% (59/62) of cases. Notably, 56% (77/138) of P-LP variants, and 72% (108/150) of TierI-TierII variants were in NRTG. VUS and Tier III in NRTG was respectively 89% (637/712) and 79% (770/975).

Conclusions: NRTG screening increased diagnostic yield in predisposing genes (61 to 138 P/LP variants) and in genes used for therapeutic purposes (42 to 150 TierI-II variants). Our data suggest that the extension to other cancer genes could increase the effectiveness of genetic test in somatic and germline setting. The identification of new CAV would improve the management and clinical follow-up of cancer patients.

Conflict of Interest: None declared.

EP13.057 Pathogenic variants in genes involved in homologous recombination and mismatch repair among prostate cancer patient

Vida Stegel[1], *Petra Škerl*[1], *Vita Setrajcic Dragos*[1], *Gasper Klancar*[1], *Anja Zagožen Klasinc*[1], *Alenka Bombač*[1], *Gregor Hertl*[1], *Vesna Vogric*[1], *Ksenija Strojnik*[2], *Aleksander Novaković*[2], *Ana Blatnik*[2], *Mateja Krajc*[2], *Srdjan Novaković*[1]

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Background/Objectives: Prostate cancer (PC) is the most common cancer in men in the Slovene population (SLORA 2019). In metastatic castration-resistant prostate cancer (mCRPC) tumor genotyping as well as germline genotyping of genes involved in homologous recombination repair (HRR), or mismatch repair (MMR) can be performed to guide treatment decisions related to PARP or immune-checkpoint inhibitors.

Methods: In three years (2020 to 2022) 330 PC patients, regardless of tumor stage, were tested with NGS for germline pathogenic variants (PV) in BRCA1/2, PALB2, CHEK1/2, ATM, RAD51B/C/D, BRIP1, BARD1, FANCL, NBN, CDK12, MLH1, MSH2, MSH6, and PMS2 gene. Additionally, 210 FFPE tumor tissue samples from mCRPC patients were genotyped with NGS.

Results: Among the PC patients who underwent germline genotyping, PV in HRR genes were detected in 9.0% of patients; 2.7% in BRCA2, 1.5% in BRCA1, and 5.3% in other HRR genes. Tumor genotyping was successful in 86.0%. Among genotyped tumors, PV was detected in 27.0% (10.0% in BRCA2, 0.5% in BRCA1, 8.8% in ATM, 4.6 % in CDK12, 2.3% in other HRR genes, and 3.7% in MMR genes). In 25.0% of cases, PV was detected in at least two HRR or MMR genes. The majority of tumors with PV in MMR genes had co-existing PV in BRCA genes.

Conclusions: Tumor genotyping resulted in a higher PV detection rate than germline genotyping, therefore resulting in a higher number of possible targets for targeted therapy.

Conflict of Interest: None declared.

EP13.058 Interplay between epithelial-mesenchymal transition, cancer stem cell markers and extracellular matrix components in colorectal cancerogenesis

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Background: Certain molecular pathways of colorectal cancer (CRC) development are well understood; however, these genetic aberrations do not explain malignant transformation and development of metastases. Another challenging aspect is intra-tumour heterogeneity (ITH). Our bioinformatics analysis of publically available microarrays data suggest involvement of epithelial-mesenchymal transition (EMT), cancer stem cells (CSCs) and extracellular matrix (ECM) components in CRC cancerogenesis and ITH.

Methods: For analysis of CRC development, 47 patients were included (70 samples: normal mucosa, adenoma, early CRC, advanced CRC with and without lymph node metastases); and for ITH analysis 19 patients were included (63 samples: invasive front, centre of primary tumour; lymph node and liver metastases). RNA was isolated from formalin-fixed paraffin-embedded (FFPE) tissue, mRNAs and miRNAs were validated using qPCR, protein using immunohistochemistry on FFPE tissue slides. Meta-analysis of publicly available sequencing data was performed to confirm results.

Results: We observed differential expression of EMT markers (*miR-200* family, *ZEB2*), ECM components (*DCN, FN1, SPARC, SPON2, SPP1*) and CSC markers (*L1TD1, SLITRK6, SOX2, ST6GALNAC1, TCEA3*) in CRC cancerogenesis and ITH. We confirmed interplay between EMT and CSC (*miR-200-SOX2*), and EMT and ECM (*miR-200c-DCN*). Meta-analysis of publicly available sequencing data confirmed involvement of CSC, ECM and EMT in CRC cancerogenesis and ITH, and identify interplay between EMT and CSC (*miR-200b-PDGFD*).

Conclusion: Our results suggest that there is an interplay between EMT, CSC and ECM in CRC development and progression. EMT, CSC markers and ECM components contribute also to ITH.

Grant references: Slovenian Research Agency (ARRS) J3-1754, P3-0054.

Conflict of Interest: None declared.

EP13.059 Evaluation of the proliferation marker Ki-67 and the IDH mutation in glioblastoma

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Background: Glioblastomas are the most commonly malignant central nervous system tumor (14.5% of all primary brain tumors) [1]. Despite therapeutic management, the prognosis still poor with an average survival of 14.7 months [2].

The aim of this study is to evaluate the presence and frequency of the main alterations characterizing Moroccan patients with glioblastoma.

Methods: This is a retrospective study of 126 patients with glioblastoma diagnosed at the University Hospital Ibn Rochd of Casablanca between 2016 and 2019. Immunohistochemistry was conducted with two antibodies: Ki-67 and IDH1R132H. We performed PCR and SANGER to study *IDH 1/2* genes mutations in glioblastomas.

Results: In the studied population, there was a male preponderance with male/female ratio of 1.47 (75 males and 51 females). The mean age of patients with glioblastoma at diagnosis was 49.7 years ranged from 6 to 81 years. Ki-67 immunostaining was found in 71.4% of patients. The use of anti-IDH1 antibody shows cytoplasmic and diffuse positivity in 13.4% of glioblastoma cases. The Arginine-Histidine mutation at the analogous residue 132 of the *IDH 1* gene was detected in all 17 immunohistochemically marked cases. No case presented an *IDH2* gene mutation.

We examined clinicopathologic parameters and we sought to determine whether there was a correlation between these status and overall survival in 126 patients (Still in process).

Conclusion: Glioblastomas are heterogeneous tumors. A systematic search for molecular alterations, in particular those of IDH, may lead to improved treatment and management of patients with glioblastoma.

References:
1. https://doi.org/10.1093/neuonc/noaa200.
2. https://doi.org/10.7150/jca.32909.

Conflict of Interest: None declared.

EP13.060 A case of a familial RCC-associated constitutional chromosomal rearrangement involving chromosome 3

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Background: Familial renal cell carcinomas (RCCs) are genetically heterogeneous malignancies characterized by frequent bilateral occurrence, multi-focality and early onset, as compared to sporadic cases. The most common histopathologic subtype of sporadic and familial RCC is clear cell carcinoma (ccRCC) with the von Hippel-Lindau disease being the most frequent cause. Constitutional translocations, involving chromosome 3, are recognized as a rare cause of inherited predisposition to RCC.

Case Report: A 35-year-old man was referred to urologic department for RCC. CT revealed 22 bilaterally positioned renal multiple tumors. Patient was subjected to a complete nephrectomy. Tumor histology analysis showed a presence of bilateral ccRCC with metastatic spreading to lung and gut. Family history was positive: patient's mother had unilateral RCC, while other affected members had bilateral RCC (mother's sister and brother and brother's daughter). Based on familial history, Multi-Cancer Panel involving 84 genes was performed and no pathogenic variants were found. Also, classical karyotyping analysis was performed. Chromosome G-banding showed a male karyotype with an apparently balanced translocation between long arms of chromosomes 3 and 10 (46,XY,t(3;10)(q21;q11). Array CGH analysis did not find the existence of chromosomal breakpoints.

Conclusion: This case report emphasizes the importance of combining different methods of genomic diagnosis taking into an account advantages and limitations of each technique. Such approach will most certainly reveal the genetic cause of RCCs in some families with a positive disease history.

Grants: EU, ER Operational Programme Competitiveness, and Cohesion, grant agreement No. KK.01.1.1.01.0008, Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials.

Conflict of Interest: None declared.

EP13.061 Translocation t(19;21): a novel translocation linked to acute myeloid leukemia

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Introduction: Pure erythroid leukemia (PEL) is a rare and aggressive subtype of acute myeloid leukemia (AML) characterized by a neoplastic proliferation of immature erythroblasts. It is a heterogeneous entity amongst AML, may occur at any age and accounts for approximately 1% of all AML diagnoses.

Case Report: A 78-year-old man was referred to CHTMAD´s emergency service with sudden onset of pain in the left shoulder and lumbar region, accompanied by asthenia, anorexia, weight loss of 11 kg and nocturnal hypersudorrhea. Complementary tests reveal 7.40 g/Dl haemoglobin, 38,000 platelets, 5.77 leucocytes and a peripheral blood smear showing minor poikilocytosis, some erythroblasts, confirmed thrombocytopenia and rare myelocytes. Myelogram with slight hypercellularity due to erythroid hyperplasia and immunophenotyping showed a normocellular marrow with presence of 68% erythroid line cells with abnormal immunophenotypic features. The study was suggestive of pure erythroleukemia diagnosis. Conventional cytogenetic analysis revealed a translocation involving chromosomes 19 and 21, in 19/21 metaphases examined. The patient started chemotherapy with Azacitidine and support therapy with blood derivatives, but he died one month later.

Discussion and Conclusion: The present case had a rare t(19;21)(q13;q22) detected by conventional cytogenetics. In the literature, there are only two cases described, both with AML diagnosis and fatal outcome.

Although, PEL is a subtype of AML with poor response to typical AML therapy, as well as the need for further cases to fully understand the clinical behaviour and prognosis of t(19;21), we may infer that this translocation is linked to AML and is associated with poor prognosis.

Conflict of Interest: None declared.

EP13.062 Whole exome sequencing in patients with familial polyposis

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Background/Objectives: Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer death. Most CRCs begin as growths on the mucosal surface of the colon or rectum, called polyps, and some types of polyps can evolve into cancer. Most CRCs arise sporadically from acquired somatic genomic alterations, while 5% are associated with hereditary cancer syndromes caused by inherited germline mutations, like *APC*, *MUTYH* or DNA repair genes. This project aims to investigate why some familial polyposis lack germline mutations in high penetrance genes.

Methods: We selected 7 patients with familial polyposis and without germline mutations in high penetrance genes. Genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) polyps and normal adjacent tissues using the QIAamp DNA FFPE tissue kit (Qiagen). Whole exome sequencing (WES) was performed on NovaSeq6000 system (Illumina). Mutations present exclusively in dysplastic tissue were selected.

Results: A total of 290 somatic mutations were identified: 232 single nucleotide variants, 39 deletions and 19 insertions. 60% of polyps have pathogenic somatic mutations in *APC*, while the remaining 40% have neither relevant nor recurrent known pathogenic mutations, suggesting that the cause for these polyps could be in epigenetic pathways or non-coding RNAs.

Conclusions: WES has become an increasingly useful approach for detection of novel cancer related genes. A larger number of polyps should be studied to reach clear conclusions, in addition to exploring epigenetic pathways and non-coding RNAs that could be involved in the evolution of polyposis.

Grants: Study funded by Fundación Mutua Madrileña.

Conflict of Interest: None declared.

EP13.063 A novel pathogenic germline variant c.1596G>A in the CDH1 gene identified in Russian patient

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Background: Gastric cancer (GC) is one of the most common malignancies worldwide. Hereditary forms of GC account for 1-3% cases. Hereditary diffuse GC (HDGC) is an autosomal dominant cancer syndrome caused by germline mutations in the CDH1 gene. HDGC is associated with a high risk of diffuse GC and lobular breast cancer. To date, 321 clinically significant variants have been registered in the CDH1 gene.

Methods: We present a clinical case of a 41-year-old male patient with diffuse GC. A proband's genomic DNA analysis was performed by NGS. The libraries were prepared using the KAPA Hyper Prep Kit (Roche). A custom panel for selective enrichment of 44 genes' coding regions, including CDH1, was used. NGS was performed on the MiSeq platform (Illumina).

Results: A truncating heterozygous variant of CDH1(NM_004360.5) chr16:68819310G > A (c.1596G > A, p.Trp532Ter) in exon 11 was identified. To assess the segregation of this variant in a family, proband's sibs and son were tested by Sanger sequencing; a c.1596G>A variant was not detected in their samples. The proband's parents refused from genetic testing. This variant is not reported in genomic databases. Based on the ACMG criteria, this variant was classified as pathogenic, thus a patient was diagnosed with HDGC.

Conclusions: The present study demonstrates the efficacy of NGS in identifying novel clinically significant genetic variants associated with HDGC. Given the refusal of proband's parents from DNA diagnostics, it is challenging to assess the penetrance of the c.1596G>A variant, since the "de novo" nature of the variant can't be ruled out.

Conflict of Interest: None declared.

EP13.064 Whole-exome sequencing to identify candidate genes associated with rectal bleeding post-radiotherapy in prostate cancer patients

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Background/Objectives: The objective of this study is to identify, by a whole-exome association study (WES), candidate genes associated with rectal bleeding as a side effect in prostate cancer patients (PrCa) treated with radiotherapy (RT).

Methods: We conducted a case-control study using an extreme phenotypes design. From the PrCa RADIOGEN cohort ($n = 1113$, 3DRT) 30 patients with severe rectal bleeding (cases; CTCAE grade ≥3) and 30 matched patients without any toxicity (controls; CTCAE = 0 in all toxicities) with at least 5 years of follow-up were selected. DNA libraries from peripheral blood pre-RT were constructed with SureSelect-All-Exon-V6 + UTR(Agilent) and sequenced on the NovaSeq6000(Illumina). Genomic variants were detected by GATK and annotated with ANNOVARv.2019. Variants

with frequency ≤0.001 in GnomAD_AF_NFE(v2.1.1) were prioritized. For the association analysis, we carried out the optimal unified test (SKAT-O), using SKAT-R-package and including age-at-radiotherapy and rectum-mean-dose as covariates. Multiple test corrections were performed.

Results: Three genes (*MMRN1*, *C8orf82*, and *PKP2)* reached a p-value $\leq 5 \times 10^{-4}$. Interestingly, the protein (multimerin-1) encoded by the *MMRN1* gene is a factor *V*-binding-protein, carrier for platelet factor *V/Va*, improves platelet adhesion and may limit platelet and plasma factor *Va*-dependent thrombin generation. No genes remained significant after multiple test corrections.

Conclusions: We have identified candidate genes that may be associated with severe rectal bleeding in prostate cancer patients treated with RT. Validation of these results will be performed in independent cohorts.

Grant References:
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Conflict of Interest: None declared.

EP13.065 Identification of 29-gene signature as diagnostic blood biomarker for patients with colorectal cancer

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Background/Objectives: Despite the advances in cancer therapies and raising awareness, colorectal cancer (CRC) continues to be among the most malignant tumors. Therefore, it is of utmost importance to detect the disease at its earliest stages reliably in order to reduce the risk of mortality and to have better disease outcome.

Methods: Genome-wide gene expression profiling datasets from tissue and blood samples of CRC patients were used to conduct an integrated omics investigation of CRC in order to identify the potential diagnostic gene signature. Additional transcriptomic datasets from CRC patients and healthy controls are also used to validate the diagnostic potential of the discovered gene signature.

Results: The integrated omics analysis revealed a 29-gene signature that is significantly expressed in the blood and tissue of CRC patients in comparison to healthy controls. The diagnostic capability of the gene signature was validated by using transcriptomic datasets. Unsupervised principal component analysis and hierarchical clustering using the 29 genes clearly distinguished patients from healthy controls. Moreover, the examination of the expression of gene markers in blood was further confirmed and revealed significantly higher/lower levels of expression in the tumor compared to normal (p-value < 0.01).

Conclusion: Our integrated omics analysis may provide a reliable methodology to diagnose patients with CRC non-invasively and lead to improved diagnosis and early detection of patients with CRC.

References: None.

Grants: None.

Conflict of Interest: None declared.

EP13.066 Novel cancer-associated germline variants in patients with breast, ovarian or colorectal cancers and suspected hereditary cancer syndromes

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Background: Whole-genome sequencing (WGS) can detect all types of cancer-associated genetic variants. We have focused on novel (undescribed) cancer-associated genetic variants in Moscow, Russia.

Objective: to identify and characterize undescribed cancer-associated germline variants.

Methods: 1800 WGS (with Sanger sequencing validation for all significant findings) for 1800 patients with suspected hereditary cancer syndromes (HCS) were performed. The variant classification was made according to ACMG criteria.

Results: We have revealed 226/1800 patients with HCS (pathogenic germline variants in the following genes: BRCA1, BRCA2, APC, MLH1, MSH2, MSH6, PMS2, PTEN, TP53). In 9/226 patients, 9 novel variants were identified (table).

Table. Novel causative germline genetic variants in HCS patients

Gene	Variant (WGS, hg38)	Gender	Age	Diagnosis
APC	chr5:g.112839372C > T c.2929C>T (p.Gln977Ter)	Female	37	Colorectal cancer
MLH1	c.293G>C (p.Gly98Ala)	Female	47	Ovarian and endometrial cancer
MLH1	c.1921dup (p.Leu641fs)	Male	37	Colorectal cancer
PMS2	c.327del (p.Ala110LeufsTer2)	Female	39	Colorectal cancer
MSH6	c.1964T>A (p.Leu655Ter)	Female	40	Breast cancer
MSH2	c.1857dup (p.Val620CysfsTer24)	Male	36	Colorectal cancer
BRCA1	c.31_34del (p.Val11LysfsTer11)	Female	46	Ovarian cancer
BRCA2	c.8827C>T (p.Gln2943Ter)	Female	58	Bilateral breast cancer
BRCA2	c.8437G>T (p.Gly2813Ter)	Female	34	Breast cancer

Conclusion: WGS is an important instrument to discover all possible causative genetic variants including unknown. We report information on 9 novel variants in HCS syndromes that will help specialists in the interpretation of genomic data and in the management of oncological patients in the future.

Grants: Moscow City Health Department financial support.

Conflict of Interest: None declared.

EP13.067 Preliminary results for non-invasive DNA Methylation test for colorectal cancer (CRC) screening

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Introduction: High-performance, non-invasive screening is a requirement in CRC as early detection of precancerous lesions might be promising for prevention, better management and treatment. Colonoscopy, the actual gold standard in CRC screening, is invasive and often avoided by patients.

CRC carcinogenesis is attributed to a series of genetic mutations and epigenetic alterations. Promoter DNA methylation is the most prevalent epigenetic alteration that has been linked to all CRC stages.

Purpose: Evaluation of the clinical performance of ADHFE1, SDC2, and PPP2R5C methylated genes in stool DNA as biomarkers for early detection of CRC.

Methods: 20 FFPE samples diagnosed with CRC MSI-H were used for validation. 5 stool samples self-collected at home of healthy people 45-56 years old without a CRC family history were extracted and used for the multiplex methylation specific qPCR, on Light Cycler System, to detect abnormal CRC gene biomarkers. Colour compensation kit was used to correct the spectral overlap for reliable results.

Results: All biomarkers were methylated to all FFPE patients which confirms the relate between the methylated genes and the procedure of CRC carcinogenesis. To one sample from healthy people SDC2 was methylated which agree with his diagnostic colonoscopy where 2 polyps non-cancerous were present and indicates the high sensitivity of this method for precancerous lesions. The other 4 samples had no methylated markers and negative colonoscopy.

Conclusion: This stool-DNA methylation test seems to be an indicated and effective tool for early-stage specific detection of CRC and it should be evaluated to a larger number of samples.

Conflict of Interest: MARIA CHATZIDAKI Full, Georgia Thodi Full, Katherine Anagnostopoulou Full, Eleni Molou Full, Stamatina Poula Full, Stefania Antonopoulou Full, Dimitra Petrakaki Full, Nikolaos Kentepozidis Full, Yannis Dotsikas Full, Yannis Loukas Full.

EP13.068 Implementation of the pediatric cancer predisposition unit (UPGeCI) to improve diagnosis and folow-up of children and families with a genetic predisposition to cancer

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Germline mutations in cancer-predisposing genes are detected in approximately 10-12% of children with cancer. The diagnosis of pediatric cancer predisposition syndromes (pCPS) caused by these genetic mutations is essential for the correct management, follow-up, and genetic counseling of patients and their families. However, the identification of pCPS in daily practice is not routinely undertaken by the lack of experts and specialized units focused on this group of complex and heterogenous pathologies without recognizable clinical phenotypes. In this study, we present the experience of a multidisciplinary cancer predisposition unit (UPGeCI) focused exclusively on pediatric cancer patients. Between 2018 and 2022, 171 patients were screened at the UPGeCI, of whom 166 (97%) patients met criteria to perform a genetic study following the Jongmans and Ripperger recommendations. As a result, 83 patients (48.5%) were diagnosed with CPS and underwent genetic counseling; 54 of them (63%) were retinoblastoma patients carrying a mutation in the RB1 gene. We show that implementing a specialized pCPS unit in the routine care of a public hospital is feasible, leading to an improvement in the diagnosis, management, and outcome of children and families with genetic cancer predisposition.

Conflict of Interest: None declared.

EP13.070 Identification of subnetwork markers with diagnostic and prognostic potential for women with breast cancer

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Introduction: Breast cancer is a major health problem in the world and is the most common cause of cancer death among women. Early diagnosis and treatment are critical to provide a better management of the disease. The integration of gene expression profiles and protein–protein interaction (PPI) network provides a better understanding of the molecular architecture of diseases. A disease subnetwork or module consists of linked genes or proteins that share mutations, biological processes or expression variations which can be associated with the disease. It has been shown that subnetwork biomarkers are more reliable in disease diagnosis than single biomarker genes.

Materials and methods: We clustered gene networks into subnetworks and defined activity scores for each significant subnetwork. We then used these scores as feature values to build a classifier using k-nearest neighbor (KNN) algorithm. We validated diagnostic and prognostic potential of the identified subnetwork markers using independent datasets.

Results: We identified four significant subnetworks. Unsupervised principle component analysis (PCA) clearly distinguished breast cancer patients from normal controls in multiple whole-genome expression datasets. KNN classification model achieved 97% predictive accuracy, 98% sensitivity, 94% specificity, and AUC of 96% in discriminating patients from controls. Finally, we confirmed the prognostic significance for recurrence free survival using on two transcriptomic datasets with over 3,000 patients.

Conclusions: Our results suggest that subnetwork markers derived from integrated analysis of gene network with genomic analysis for breast cancer may lead to improved diagnosis, prognosis and therapeutic options.

Grant: Dr. Colak received seed funds from KFSHRC (RAC#2110006).

Conflict of Interest: Dilek Colak King Faisal Specialist Hospital and Research Center, King Faisal Specialist Hospital and Research Center (RAC#2110006 to DC), Achraf Elallali: None declared, Ibrahim Kaya: None declared, Olfat Alharazi: None declared.

EP13.072 Lack of BCR-ABL T315I Mutation in Tunisian Patients with Imatinib-Resistant Chronic Myeloid Leukemia

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Chronic myeloid leukemia (CML) is a hematopoietic stem cell (SC) neoplasm characterized by an acquired genetic alteration ; the t(9;22)(BCR-ABL1) fusion gene.

Emergence of point mutations within the BCR-ABL gene particularly T315I mutation in the ABL kinase domain mutations have been implicated in the resistance to the BCR-ABL inhibitor

imatinib mesylate, for up to 20% of all clinically observed resistance[1,2].

The purpose of this study is to determine the prevalence of the BCR-ABL T315I mutation in Tunisian CML patients with imatinib resistance.

A retrospective cross-sectional study included 30 patients diagnosed with CML. a Sanger sequencing was performed using a high fidelity DNA polymerase.

None of the CML patients have the T315I mutation.

Our findings suggest that T315I mutation is uncommon in Tunisian CML resistant patients.

A comprehensive mutational analysis of others genes and epigenetic factors are mandatory to elucidate the molecular mechanisms that lead to kinase inhibitor resistance.

1.Elsir Khai, H., Ahmed Moha, B., Yousef Nou, B. & Ali Waggia, H. Prevalence of BCR-ABL T315I Mutation in Different Chronic Myeloid Leukemia patients Categories. *Pak. J. Biol. Sci*. **25**, 175–181 (2022).

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Conflict of Interest: None declared.

EP13.073 Genetic alterations in the BCR-ABL1 fusion gene related to imatinib resistance in chronic myeloid leukemia patients

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Background/objectives: Current first line therapy in CML is based on the use of imatinib, a potent tyrosine kinase inhibitor, which has significantly decreased mortality rate. However, approximately 30% of CML patients showed resistance to imatinib, mainly caused by the presence of point mutations in the *BCR-ABL1* kinase domain. Thus, the aim of this study was to identify genetic alterations related to imatinib resistance through next generation sequencing platforms.

Methods: Study population was composed of 22 patients diagnosed with CML showing no clinical response to imatinib. Total RNA was used for cDNA synthesis, and a fragment encompassing kinase domain of *BCR-ABL1* gene was amplified using a nested-PCR approach and Sanger and next generation sequencing were used to detect genetic alterations. Variant calling was done using haplotype Caller, whereas fusion breakpoint identification was carried out using the STAR-Fusion software.

Results: After sequencing analysis, mutations F311I, F317L, and E450K were detected in three different individuals. Additionally, single nucleotide variants (SNVs) in *BCR* (rs9608100, rs140506 and rs16802) and *ABL1* (rs35011138) gene were detected in two other patients. The e14a2 and e13a2 transcripts were observed in 11, and 9 patients, respectively, whereas the coexistence of both transcripts was identified in only 1 patient. Co-expression of e14a2 and e14a8 transcripts was also observed in one participant.

Conclusions: Our data suggest that SNVs and co-expression of different types of *BCR-ABL1* transcripts could participate in cellular resistance to imatinib.

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Conflict of Interest: None declared.

EP13.074 Evaluation of TNC, ULBP1 and COL18A1 as prospective pancreatic cancer biomarkers in PANC1 cell Line

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One of the leading causes of cancer-related deaths is pancreatic adenocarcinoma and specific prognostic and treatment-predictive biomarkers are urgently needed for decision-making. We determined TNC, COL18A1, and ULBP1 as promising prospective biomarkers. With respect to these targets, PANC1 cells under basal conditions were compared to different gemcitabine incubation-times (48,72,96 hours) and exposure-doses (2.5,5,15 μM).

Target gene expressions were analyzed using qRT-PCR (TNC, ULBP1, COL18A1, ZEB1). Cell lysates and upper medium sections were compared by using LUMINEX (TNC, ULBP1, COL18A1) and ELISA (CA19-9 and ZEB1) platforms. ULBP1 protein was demonstrated confocal microscopy in cultured cells.

The most suitable IC50 value was determined as 5 μM for 72-hour incubation. All of the expression levels of our target genes increased at this condition, where the change in ULBP1 and TNC was more prominent. Comparing cell lysates of basal conditions and 5 μM gemcitabine dose for 72-hour incubation time in LUMINEX platform, COL18A1 and ULBP1 levels were determined to be increased. Similar trends were observed for upper medium section. As control biomarkers, ZEB1 and CA19-9 were shown to be increased in both cell lysates and in upper medium sections by using ELISA. ULBP1 protein was demonstrated in PANC1 cells under these experimental conditions in confocal microscopy. These preliminary results indicate that TNC, ULBP1 and COL18A1 are promising biomarkers for pancreatic cancer research.

Conflict of Interest: None declared.

EP13.076 Molecular and phenotypic evaluation of individuals and families with Multilocus Inherited Neoplasia Alleles Syndrome

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Multilocus Inherited Neoplasia Alleles Syndrome (MINAS) is a rare condition characterized by the presence of two or more germline pathogenic variants (GPV) in cancer predisposing genes. Despite recent advancements in the field, the clinical implications of this condition on patients' phenotypes remain largely unknown. This study aims to provide a clinical characterization of patients and families with MINAS, and to evaluate tumor molecular features to characterize relationships between variants and tumors. MINAS cases were identified retrospectively from approximately 7,500 individuals with genetic testing data from A.C.Camargo Cancer Center (Brazil). Clinical, histopathological, and family history data were collected, and loss of heterozygosity (LOH) analysis was performed on tumor DNA. A total of 34 MINAS patients were identified, with a prevalence of female patients (28/34) with breast cancer (BC) (20/34). The most frequently genes with GPV were: BRCA2 (9), MUTYH (7), CHEK2 (7), BRCA1 (5), TP53 (4) and ATM (4). 59% of patients had variants in two autosomal dominant genes and

18% had GPV in two dominant high penetrance genes, such as BRCA1 and TP53. Interestingly, 5/7 BC cases with one of the GPV occurring in BRCA1 or PALB2 had non-triple negative BC. LOH analysis showed loss of the wild-type allele of at least 1 altered gene in 5/10 tumors, both in expected and unexpected gene/tumor-type combinations. In conclusion, our study provides a comprehensive clinical and molecular characterization of a rare group of patients with MINAS, with the goal of advancing our understanding of the phenotypic consequences of this condition.

Conflict of Interest: None declared.

EP13.077 Analysis of HIF-1α and HIF-2α inhibitory compounds in oral cavity and pharyngeal cancer cell lines

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Background/Objectives: The hypoxic microenvironment in tumors leads to the activation of genes that promote tumor angiogenesis, mesenchymal-epithelial transition, and metastasis. Hypoxia-Inducible Factor (HIF) is the crucial gene activated by hypoxia, and this overexpression is associated with the progression and aggressiveness of cancer. The objective of this study was to analyze the inhibition of HIF through compounds as a therapeutic strategy to prevent tumor development in head and neck cancer.

Methods: Protein data bank and the ZINC15 database were used to obtain the molecular protein structures of HIF-1α and HIF-2α, and compounds molecular structures, respectively, as Topotecan, Acriflavine and Resveratrol. Molecular Docking was performed to evaluate the compound-ligand interaction. The compounds IC50 values were measured using cytotoxicity, and cell viability assays (GraphPad Prism software). qPCR and Western Blotting were used to analyze HIF-1α and HIF-2α gene and protein expression in HN13 and FaDu cell lines treated, respectively.

Results: Topotecan showed better binding energy with the structures of HIF-1α and HIF-2α in Molecular Docking. After cell treatment with this compound were observed significant decreases in HIF-1α and HIF-2α expressions, and Acriflavine caused a decrease in HIF-1α expression, both results in HN13 cell line. Resveratrol caused an increase in the expressions of HIF-1α and HIF-2α in the two evaluated cell lines.

Conclusion: Topotecan and Acriflavine shows effectiveness in inhibiting the expression of HIFs. Thus, they are compounds indicated for use as target therapy aimed at inhibiting HIFs for head and neck cancer.

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Conflict of Interest: Bianca Barbério Bogdan Tedeschi CAPES(Financial code 001), Tiago Henrique: None declared, Marcia Maria Urbanin Castanhole-Nunes: None declared, Rosa Sayoko Kawasaki-Oyama: None declared, Gabriela Helena Rodrigues-Fleming: None declared, Ana Paula Simedan Villa: None declared, Ludimila Leite Marzochi: None declared, Vitoria Scavacini Possebon: None declared, Juliana Garcia Oliveira-Cucolo: None declared, Erika Cristina Pavarino: None declared, Eny Maria Goloni-Bertollo FAPESP #2018/26166-6; #2018/24825-2CNPq #310987/2018-0.

EP14 Genome Variation and Architecture

EP14.001 Unexpected inheritance patterns in a large cohort of patients with a suspected Bardet-Biedl or Alström syndrome

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Background: Ciliopathies are rare genetic diseases caused by a dysfunction of the motile or the primary cilium. Among them, Bardet-Biedl syndrome (BBS) is characterized by retinitis pigmentosa (RP), polydactyly, obesity, renal anomalies and cognitive impairment. Alström syndrome (AS) is characterized by the absence of polydactyly, early RP, diabetes, deafness and heart disease. For 20 years, we have collected clinical and molecular data from more than 800 individuals suspected of having a ciliopathy (BBS = 770 and AS = 76). Their mode of inheritance is mostly autosomal recessive with biallelic pathogenic variants inherited from the parents (>24 BBS genes and one for AS, i.e. ALMS1). However, exceptions exist such as uniparental disomy (UPD) or the appearance of a de novo pathogenic variant in trans of an inherited pathogenic variant. These two genetic mechanisms are supposed to be extremely rare and few data are available in literature regarding ciliopathies.

Methods: We investigated >800 individuals suspected of ciliopathy and performed a literature review of UPD and de novo variants in ciliopathies.

Results: Within 624 individuals with a molecular diagnosis, we identified five UPD uncovering an inherited pathogenic variant and five pathogenic variants of de novo appearance (in trans of another pathogenic variant). Moreover, from these ten cases, we reported on 15 different pathogenic variants of which five are novel.

Conclusion: We demonstrated a relatively high prevalence of UPD and de novo variants in a large cohort of ciliopathies and warn on the importance to identify such rare genetic events especially for genetic counseling.

Grant: PHRC N5514, CREGEMES.

Conflict of Interest: None declared.

EP14.002 Cohort analysis of new SPAST mutations in SPG4 patients and implementation of functional studies toidentify the pathogenic mechanism caused by splicing mutations

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Background: Pure hereditary spastic paraplegia (SPG) type 4 is caused by mutations of SPAST gene.

This study aims to analyze SPAST mutations in SPG4 patients

Methods: We performed NGS panel in 105 patients carrying a clinical phenotype corresponding to upper

motor neuron syndrome, in silico analysis for splicing mutations and in vitro minigene assay.

Results: Pathogenic mutations in SPAST were identified in 12 patients (11,42 %), 5 missense, 3 frameshift,and 5 splicing variants.Then, we focused on the patients carrying splicing mutations by using a combined approach of in silico and in vitro analysis through minigene assay. For two splicing variants, (i.e. c.1245+1G > A and c.1414-2A > T), functional assays confirm the types of molecular alterations suggested by the in silico analysis (loss exon 9 and exon 12). In contrast, the splicing variant c.1005-1delG differed from what was predicted (skipping exon 7), and the functional study indicates the loss of frame and formation of a premature stop codon. The variant c.1537-8T > G identified in two families, represents a "non-canonical splice-site mutation", since it falls at +8 from the exon 14, and it was classified as benign. Minigene assay and RNA analysis highlighted pathogenic intronic retention, thus allowing to reclassify this variant as pathogenic.

Conclusions: The present study evidenced the high frequencies of splice variants in SPG4 patients and indicates the relevance of functional assays added to in-silico analysis for the correct identification of the molecular mechanisms produced by these variants.

Conflict of Interest: Rosangela Ferese full, principal investigator, collaborator and consultant, Simona Scala full, Federica Sammarone part-time, Antonio Suppa part-time, Rosa Campopiano full, Francesco Asci part-time, Alessandro Zampogna part-time, Maria Antonietta Chiaravalloti full, Annamaria Griguoli part-time, Marianna Storto full, Alessia Lombardi part-time, Luana DiPilla part-time, Milena Cannella full, Alba Di Pardo full, Emiliano Giardina full, principal investigator, Stefania Zampatti part-time, Mirco Fanelli Full, Stefano Gambardella Full, principal investigator, collaborator and consultant.

EP14.003 Genome integrity variation effects of ionising radiation in the Lithuanian Chornobyl catastrophe clean-up workers

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Background/Objectives: Ionising radiation (IR) is a well-known factor that predisposes cells to mutagenesis, cancerous processes, inflammation, oxidative stress, apoptosis, or DNA repair. However, the genetic factors to maintain the integrity of the genome after IR are not fully defined. Relatively healthy Lithuanian Chornobyl catastrophe clean-up workers (LCCW) are a unique study group. We hypothesize that this group might have a specific genomic variation which determines their reaction to IR. Thus, we aim to perform the analysis of candidate genes that might be involved in genome integrity maintenance.

Methods: We compiled a list of 26 candidate genes representing different molecular mechanisms or pathways (apoptosis, tumour suppression, inflammation, oxidative stress, DNA homologous recombination repair) potentially related to the genome and cell integrity after exposure to high-dose IR. The study group included 40 male LCCWs. The control group consisted of 27 unrelated, self-reported healthy males of Lithuanian descent. Variants of the genes were retrieved from whole-genome short-read sequencing data. Candidate gene association analysis was performed using PLINK v1.9 software.

Results: The study identified statistically significant ($p \leq 0.05$) associations in genes *IFNGR2, IFNG-AS1, RAD51B, ATMIN*, and *CASP3* in six genomic regions 21q22.11 and 21q22.12, 12q15, 14q24.1, 16q23.2, 4q35.1 respectively.

Conclusion: Analysis showed that variation in the genes responsible for inflammation, DNA homologous recombination repair, and apoptosis might have specific effects on maintaining genome and cell integrity after exposure to high-dose IR.

Grant References: This study is a part of the ADAPT (agreement No. S-MIP-20-35) project, funded by the Research Council of Lithuania (LMTLT).

Conflict of Interest: None declared.

EP14.004 The interplay between genetic and environmental factors in shaping the gut microbiome of Familial Mediterranean Fever (FMF) patients

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Objective: FMF is an autosomal recessive autoinflammatory disease affecting people originating from the Mediterranean Sea. The vast clinical expressivity of the disease is related to the allelic heterogeneity of the *MEFV* gene, and also to the environment. This study aims at investigating the role of host genetics and environmental factors, mainly diet and physical activity, in shaping the composition of gut microbiota of FMF patients, and subsequently in modifying the severity of the disease.

Methods: Thirty genetically diagnosed FMF patients, 30 heterozygous patients and 30 healthy controls will be enrolled in the study. The *MEFV* gene will be genotyped by Sanger sequencing. Polymorphisms in various modifying genes (*IL-17*, *IL-18*, *JAK2* and *STAT3)* will be studied by PCR-RFLP. The analysis of gut microbiota will be done using the 16S ribosomal RNA gene sequencing. The dietary habits and physical activity status will be assessed by the Prime Screen validated questionnaire and the International Physical Activity Questionnaire (IPAQ), respectively.

Results: We expect to have a poorer microbiota in FMF patients, and major shifts in some bacterial populations, mainly those belonging to the order of *Clostridiales*, as compared to controls. Patients carrying the *M694V* mutation, along with variations in susceptibility genes, a low physical activity, and poor dietary habits will have the most significant microbiota alterations. They will also exhibit the most severe phenotype and probably the highest resistance to colchicine.

Conclusion: The manipulation of gut microbiota, through targeting genetic and environmental factors, can help in the treatment and management of FMF.

Conflict of Interest: None declared.

EP14.005 Molecular characterization of a new CYP21A2 allele and classification of its pathogenicity

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Background: The *CYP21A2* gene, coding for 21-Hydroxylase (21-OH), is located on 6p21.3 within the major histocompatibility complex, and integrated in a cluster of genes (*RP1, C4A, C4B, TNXB*) and pseudogenes (*RP2, CYP21A1P, TNXA*). This genomic region is variable in size and gene copy number. Due to the high homology between genes and their pseudogenes, recombination is common, deletions, insertions and duplications are frequent. The great diversity of this cluster and rare alleles contributes to additional difficulties on molecular analysis and pathogenicity classification.

Methods: The *CYP21A* cluster was characterized using genomic DNA obtained from four healthy brothers (parents not available). Two long-PCR products, specific for each *CYP21A2* copy of a trimodular allele (with two *CYP21A2* copies), and for a normal/bimodular allele present in this family, were characterized by Sanger cycling sequencing and MLPA (MRC-Holland, P050-C1 kit).

Results: The molecular studies revealed that one sister, who asked for genetic counselling, has a very rare trimodular allele, with two *CYP21A2* genes. One of these genes has a deletion covering exons 4 to 7 and an insertion of exons 4 to 7 of the pseudogene (*CYP21A1P*) which has the pathogenic variants c.518T>A, c.710T>A, c.713T>A, c.719T>A, c.844G>T and c.923dupT, all in phase. This alteration can be described as: *CYP21A2*ex4_7delins*CYP21A1*Pex4_7.

Conclusion: The developed molecular approach, which was specifically designed for this family and included segregation analysis of all brothers, allowed the characterization of a new *CYP21A2* trimodular allele that, even containing six pathogenic variants, is non-pathogenic as it also has (in phase) a normal *CYP21A2* copy.

Conflict of Interest: Susana Gomes Employment at full-time, JOANA MARGARIDA ROSMANINHO SALGADO Employment at full-time, Jorge M Saraiva Employment at full-time, João Gonçalves Employment at full-time.

EP14.006 novel homozygous SCP2 mutation is found during the whole genome sequencing of non-obstructive azoospermia Saudi infertility patient

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Non-obstructive azoospermia (NOA) is the most common cause of having no sperm during ejaculation due to inadequate gonadotropin production or intrinsic testicular impairment. If the testes fail to produce sperm it is called hypogonadism or primary testicular failure. In this case, the patient who has a high level of FSH has been severed from primary testicular failure for 15 years. He has no dysmorphology nor congenital malformations. Usually, physicians require genetic testes to rule out the cause of Non-obstructive azoospermia (NOA) is genetic. Both patients' Karyotype and Y-chromosome microdeletions (YCM) are normal. However, he has a family history of male infertility. Therefore, this leads to performing whole-genome sequencing (WGS) since it identifies any change in the patient's DNA by sequencing coding and non-coding regions. The results show variants of certain and uncertain significance, which the latter is discussed in this article. Therefore, the aim of this study is to mention the possibility of a novel genetic variant which is a homozygous SCP2 mutation causing Non-obstructive azoospermia in a Saudi infertility patient.

Conflict of Interest: None declared.

EP14.007 Cell cycle dynamics and chromosomal aberration hotspots in induced pluripotent stem cells under replication stress

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Background: Elevated basal level of replication stress is a feature of pluripotent stem cells (PSCs), that can contribute to their numerical and structural chromosomal instability. Defining the repertoire of replication stress-sensitive genomic regions, or common fragile sites, is essential for clinical and fundamental applications of iPSCs. Published estimations of genotoxic effects of exogenous replication stress in PSC are contradictory due to variability of experimental conditions, with no clarity in the duration of the cell cycle under replication inhibitors.

Methods: EdU was introduced to iPSCs for 21-48 hours simultaneously with Aphidicolin and Caffeine, visualized on cytogenetic metaphase slides using click-reaction. Custom FISH probes were made using nick-translation labeling of long-range PCR amplicons.

Results: Using EdU labeling of replication, we characterized the dynamics of mitotic entry by asynchronous cells exposed to replication inhibitors. IPSCs manifested higher chromosome breakage rate under replication stress compared to lymphocytes under optimized timing of experiments. Mapping of induced breaks on G-banded chromosomes revealed three iPSC-specific cFS, *FRA3A*, *FRA20E* and *FRAXD*, that have not been reported in other tissues and exhibited <1% threshold frequency in lymphocytes. Using locus-specific FISH probes targeted to the borders of candidate extremely large genes, we performed fine mapping of these cFSs.

Conclusion: Uncovering the dynamics of the cell cycle in iPSCs under exogenous replication stress will facilitate further research of genotoxicity in PSCs. We enlarged the list of molecularly mapped cFSs in this cell type and revealed potential somatic instability of clinically relevant genes during early embryogenesis and in vitro cell expansion.

Conflict of Interest: None declared.

EP14.008 Cytogenetic mapping aphidicolin-sensitive fragile sites and mitotic DNA synthesis regions in human induced pluripotent stem cells

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Background/Objectives: Replication stress predisposes to numerical and structural chromosomal aberrations in induced pluripotent

stem cells (iPSCs), which can confer altered functionality and tumorigenic potential. Common fragile sites (cFS) are tissue-specific genomic hotspots of replication stress-induced chromosomal aberrations, which have not been mapped in iPSCs. cFS can be detected as recurrent breaks or gaps on metaphase chromosomes. To prevent mitotic errors, under-replicated DNA undergoes mitotic DNA synthesis (MiDAS) which is an alternative molecular marker of cFS.

Methods: Replication stress was induced by treatment with Aphidicolin and Caffeine for 24h. For MiDAS region detection, cells were synchronized with RO-3306, treated with EdU and colcemid, followed by standard cytogenetic slide preparation. MiDAS regions were detected using the Click-iTPlus EdU Cell Proliferation Kit. G-banding was done with Actinomycin D and DAPI.

Results: Previously we characterized the repertoire of cFS in iPSC line derived from a cystic fibrosis patient. To exclude potential patient-specific chromosomal breaks, we refined cytogenetic cFS mapping using the cell line from a healthy unrelated donor. Most active and tissue-specific cFSs were defined showing a unique pattern of replication stress markers. Co-localization of cFS with previously reported recurrent chromosomal aberrations in iPSCs was observed indicating the role of replication stress in their formation. We characterized aphidicolin-inducible MiDAS regions in both cell lines and compared their locations with chromosomal breaks at cFSs. Most MiDAS regions coincided with cFSs even without breakage further supporting mapping results.

Conclusion: Our study provides the first whole-genome analysis of common fragile sites and MiDAS regions in iPSCs.

Conflict of Interest: None declared.

EP14.009 Pre-mRNA splicing defects caused by CLCN5 and OCRL mutations associated with Dent disease

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Dent disease is a rare X-linked tubulopathy that affects almost exclusively males. Approximately 60% of cases are caused by mutations in the *CLCN5* gene (Dent disease type 1) while 15% of cases are caused by *OCRL* mutations (Dent disease type 2). Patients show low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis and nephrolithiasis, and eventually develop end-stage renal disease. In this study, we present clinical and genetic data of twelve new cases. Also, using a minigene system and RT-PCR we analyzed the effect of exonic and intronic mutations on pre-mRNA splicing. These mutations were selected using several prediction tools. Affected subjects presented the typical phenotype of Dent disease. Eight patients showed *CLCN5* mutations while the rest revealed mutations in *OCRL*; nine of these mutations were novel. Results of the minigene assay showed that three *CLCN5* exonic mutations and two *OCRL* intronic mutations affecting canonical splice sites altered the respective mRNAs. In conclusion, our results provide new data on the mutation spectrum of Dent disease and emphasize the importance of assessing at the mRNA level the effects of *CLCN5* and *OCRL* mutations.

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Conflict of Interest: None declared.

EP15 Cytogenetics

EP15.001 Mosaic derivative chromosomes at chorionic villi sampling are markers of cryptic disease-causing rearrangements in fetal tissues: report of further three cases

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Background: We have previously shown that mosaic derivative chromosomes detected at chorionic villi sampling (CVS) are rather difficult to interpret due to their instability and different distribution along the trophoblast-mesenchyme-fetal axis (1)

Methods: Three prenatal diagnosis with karyotype, array-CGH and FISH analysis

Results: Three cases with cytogenetic discrepancies in the different feto-placental tissues, as follows: 1) presence of add(6)(p25) in cytotrophoblast, with normal chorionic villi (CV) mesenchymal cells. Array-CGH and FISH analysis on amniocytes showed a translocation derivative chromosome 6 with a microdeletion at 6p25.1 (4.2 Mb) and a microduplication at 19q13.43 (300 kb); 2) presence of an homogeneous add(14)(p11.1) in the CV mesenchyme with two different translocation derivatives 14 in amniocytes, resulting in partial trisomy 8p23.3p23.1 (about 7 Mb) and partial trisomy 8p23.1 (2 Mb), respectively; 3) presence of mosaic der(15)t(2;15) in CV mesenchymal cells with normal karyotype in cytotrophoblast. Array-CGH and FISH analysis confirmed mosaic partial trisomy 2q14.1q37.3 (129 Mb), disclosing a cryptic adjacent terminal deletion in 15q26.2q26.3 (about 7 Mb). The same deletion was revealed in the seemingly normal karyotype both in cytotrophoblast and in the second mesenchymal cell line.

Conclusion: Derivative chromosomes detected as mosaics at CVS indicate instability of the chromosomal rearrangement, which will be further modified in the fetal tissues by positively selecting the most viable cell lines. Those lines, however, still carry disease-causing unbalances and should be carefully searched and evaluated during prenatal genetic counselling.

Reference:

1. Pittalis et al. Am J Med Genet A (2013)

Conflict of Interest: None declared.

EP15.002 Copy number variations inherited from a complex rearrangement uncovered by cytogenetic techniques

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Background/Objectives: A newborn male presented with several dysmorphic features, hypotonia, feeding difficulties and respiratory distress. He has two healthy sisters and his mother had one previous termination of pregnancy because of brain anomalies in the fetus. Chromosomal SNPs microarray (CMA) was requested.

Methods: CMA was performed following standard procedure and copy number variations (CNVs) were identified. G-banded karyotype and metaphase fluorescence in situ hybridization (FISH)

studies were then perform on the proband and parental samples to determine the structure and inheritance of these CNVs.

Results: A 30.2 Mb terminal duplication of the short arm of chromosome 9 and a 2.5 Mb terminal deletion of the long arm of chromosome 13 were identified. Moreover, a 4.6 Mb interstitial deletion of uncertain significance was also detected on the long arm of chromosome 13, just proximal to the terminal deletion breakpoint. Genotype and phenotype correlation suggest that the terminal duplication and deletion are causative of the observed clinical findings. Karyotype and metaphase FISH studies using probes specific to sequences on chromosomes 9 and 13 revealed that all CNVs were inherited from an apparently balanced but complex rearrangement in the father.

Conclusion: This case study extends the clinical characterization of this chromosomal rearrangement. It also highlights the contribution of the current microarray and conventional methods as well as the potential that Optical Genome Mapping would offer in elucidating this complex rearrangement.

Conflict of Interest: None declared.

EP15.003 Detection of chromosome 12 polysomy in soft tissue neoplasms using MDM2 and CEP12 Fluorescence In-Situ Hybridization (FISH) probes

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Background: MDM2 amplification is a hallmark of sarcomas, including atypical lipomatous tumor (ALT)/well-differentiated liposarcoma (WDL), dedifferentiated liposarcoma (DDL), intimal sarcoma and low grade osteosarcoma. Histologic overlap between different subtypes requires MDM2 FISH for differential diagnosis. Infrequently, chromosome 12 polysomy, defined by increased MDM2 and CEP12 copies (ratio <2.0), is observed. This study sought to elucidate the histological features of soft tissue neoplasms showing chromosome 12 polysomy.

Methods: 784 consecutive soft tissue neoplasms were retrospectively studied for MDM2 and CEP 12 copy numbers using FISH assays from Jan 2020-Nov 2022. Results were classified as non-amplified (ratio <2.0; MDM2 and CEP12 < 4.0), amplified (ratio ≥2.0), low polysomy (ratio <2.0; MDM2 ≥4.0- < 6.0) and high polysomy (ratio <2.0; MDM2 ≥6.0).

Results: MDM2 amplification occurred in 18.9% ($n = 148$), low polysomy in 2.2% ($n = 17$) and high polysomy in 1.4% ($n = 11$). In low polysomy group, myxofibrosarcoma ($n = 5$) and undifferentiated pleomorphic sarcoma ($n = 4$) comprised 56.3%. Rhabdomysarcoma, malignant peripheral nerve sheath and undifferentiated spindle sarcoma were found exclusively in this group. In contrast, leiomyosarcoma ($n = 3$) and pleomorphic spindle sarcoma ($n = 3$) were seen in 54.5% of the high polysomy group. Other subtypes detected include pleomorphic myxoid sarcoma, pleomorphic liposarcoma, spindle cell neoplasm. Polysomy was not observed in ALT/WDL, DDL, osteosarcoma or lipoma.

Conclusion: Most common histological subtypes showing polysomy include myxofibrosarcoma, undifferentiated pleomorphic sarcoma, leiomyosarcoma and pleomorphic spindle sarcoma. As polysomy may be a distinct finding in soft tissue neoplasms, its presence warrants highlighting in clinical reports, lest the MDM2 non-amplification result is construed as false-negative.

Conflict of Interest: Tse Hui Lim Full time, Alvin Soon Tiong Lim Full time, Sim Leng Tien Full time, Yvette Yee Shan Yeap Full time, Yit Jun Ng Full time, Josephine Kah Kee Ho Full time, Cheng Har What Full time, Rebekah Xin Ying Loo Full time, Sathiyamoorthy Selvarajan Full time.

EP15.004 Are QF-PCR and CMA adequate to address high risk NIPT result for Sex Chromosome Aneuploidies (SCA)?

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Background: A case of female fetus showed high risk for Turner syndrome (TS) by non-invasive prenatal test (NIPT). Subsequent chromosomal analysis found mosaic 45,X/47,XXX. Quantitative Fluorescence Polymerase chain reaction (QF-PCR) and chromosomal microarray analysis (CMA) showed no abnormality. This report highlights the importance of performing chromosome analysis or fluorescence in-situ hybridization (FISH) following findings of high risk for sex chromosome aneuploidies (SCA) via NIPT.

Methods: QF-PCR and CMA were performed from DNA extracted from uncultured amniotic fluid. Chromosome analysis was performed on 55 G-banded metaphases derived from three independent cultures. FISH was performed on uncultured amniotic fluid using centromeric DNA probes for chromosome X (DXZ1).

Results: Chromosome analysis performed revealed a mosaic karyotype with 44(80%) metaphases showing 45,X and 11(20%) metaphases showing 47,XXX. FISH showed 43% showing monosomy X and 54% showing trisomy X signals. QF-PCR and CMA did not detect the aneuploidy due to the overall X chromosome normal dosage.

Conclusion: X0 cell line was found to have a growth advantage over XXX cell line in vitro from the observed results derived from chromosome analysis and FISH. The X/XXX ratio of 43%:54% resulted in <10% mosaicism, which is beyond the detection limit for QF-PCR and CMA. Despite the growing use of CMA over conventional karyotyping, diagnosis of mosaic TS, which may present with mild clinical features, will be missed if chromosome analysis or FISH were not considered for such cases.

Conflict of Interest: Min Hwee YONG Full Time, Alexis Sihui Wang Full Time, Teck Wah Ting Full Time, George S. H. Yeo Full Time.

EP15.005 Obesity in turner syndrome: comparison between pure and mosaic 45,X monosomy

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Background/Objectives: Turner syndrome (TS) is a multisystem syndrome characterized by a complete/partial and pure/mosaic monosomy X. The aim of our study was to study the impact of chromosomal formula in the obesity associated with TS.

Methods: In a retrospective study, a cohort of 26 youth and adults harboring a complete/partial and pure/mosaic monosomy X was collected. Clinical, hormonal and cytogenetic data were analyzed. The BMI, the waist circumference and the degree of truncal adiposity were compared according to the cytogenetic formula using logistic regression analysis.

Results: Monosomy X was complete and pure in three cases, complete with an intermediate mosaic (6 to 15/50 ; 45,X cells /analyzed cells) in two cases, complete with a very low mosaic (3 to 5/50 ; 45,X cells /analyzed cells) in 12 cases, partial with an X or Y structural abnormality and with or without a 45,X cell line in 9 cases (delXp, delXq, dupXq, isoXq, isoYq, and rX). BMI, waist circumference and visceral adiposity were significantly more prevalent in pure and complete monosomy X ($p < 0.001$) compared with the other chromosomal formulas of TS. The body shape and the truncal fat distribution were specific and pathognomonic of the pure 45,X TS.

Conclusion: Women with pure monosomy X were significantly more obese compared to women with mosaic monosomy X. Our results underline the pathognomonic syndromic obesity of the complete and pure TS (with a higher BMI, a higher waist circumference and a particular truncal fat distribution).

Grant References : None.

Conflict of Interest: Nouha ABDELMOULA full-time, Balkiss Abdelmoula part-time.

EP15.006 A rare congenital chromosomal aberration t(3;5)(q21;q34) associated with mosaic Turner syndrome – case report

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Background: Turner syndrome (TS) is a genetic disorder that affects 1/2500 lives-born females and is related to haploinsufficiency of genes resulting from complete or partial loss of the second sex chromosome. Mosaic TS makes up about 30% of cases. TS clinical features are heterogeneous, most common symptoms are short stature and ovarian failure. Reciprocal translocations are the most common structural rearrangements (1/500–625 live-births). The coexistence of autosomal translocations with abnormalities of the X chromosome, are rarely reported.

Case presentation: We present a patient 14y4m old with short stature, mild intellectual disability and seizures until 7 years of age. Cytogenetic analysis made for proband revealed mosaic TS with isochromosome Xq and reciprocal translocation between chromosome 3 and 5: mos 45,X,t(3;5)(q21;q34)[38]/46,X,i(X)(q10),t(3;5)(q21;q34)[2]. MLPA subtelomeric MRC Holland P036 - Mix 1 analysis shows deletion of SHOX region and VAMP7 (in PAR2). To determine if the translocation was inherited or de novo, her parents are karyotyped (in progress).

Conclusion: To the best of our knowledge there are only 12 cases reported of TS patients with structural alterations in autosomal chromosomes. This is the first case reported with translocations (3;5) and mosaic TS with isochromosome Xq, confirmed by conventional cytogenetics. Clinical features of our patient are closely related with the loss of one X chromosome, however mild intellectual disability can be likely explained by autosomal genes. The presence of a familial translocations generally is a common finding, thus indicating the need for familiar testing for further genetic counselling.

Conflict of Interest: Oana Popa "C.I.Parhon" National Institute of Endocrinology, Sorina Violeta Schipor "C.I.Parhon" National Institute of Endocrinology, Aura Madalina Boboc "Carol Davila" University of Medicine and Pharmacy.

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EP15.007 Karyotype never dies

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Background: Optical genome mapping (OGM) can unify three conventional cytogenetic techniques: karyotyping, FISH and CGH-arrays. OGM can solve limitations of each of them and detect alterations with greater sensibility in less time.

Methods: It was performed an OGM to two patient with endocrine pathology (delayed puberty with uterine hypoplasia and short stature). Rare Variant Analysis pipeline were used with GRCh38 as the reference genome.

Results: We had detected two deletions in heterozygosis, one in Xq21.1q28 of 72.6Mb and the other in Xp22.33p11.21 of 55.1Mb. In both cases, it was classified as pathogenic because it is compatible with a variant of Turner syndrome.

This type of deletions are usually observed in complex chromosomal rearrangements, so karyotype and FISH were performed. Both patients had a mosaicism of two cell lines in a proportion of approximately 50%: one of them was 45,X0; and the other line had: in first patient a isodicentric X chromosome of the short arm, and in second patient an isochromosome of the long arm.

Conclusion: Because of the level of mosaicism and the gaps in the centromeric region of the reference genome, the data shown in OGM could be mistakenly interpreted as a heterozygous deletion of one arm of the X chromosome instead of the real chromosomal rearrangements.

OGM provides in a very short time a genetic diagnosis that is useful for clinical decisions. Nevertheless, a conventional karyotype is necessary in some cases to determine exactly how the chromosomal rearrangement is to give a full reproductive diagnostic.

Conflict of Interest: Carmen María Amor Llamas: None declared, Ana María Gómez García: None declared, Ana Martín Martín: None declared, Laura Rodero Jurado: None declared, Beatriz Ruiz Gil: None declared, Jesús Pozo Román: None declared, Maria Güemes Hidalgo: None declared, Gabriel Angel Martos Moreno: None declared, Manuel Ramirez Orellana: None declared, Ana Isabel Quinteiro García: None declared, Nelmar Valentina Ortiz Cabrera part-time: NIMGenetics, Barbara Fernández Garoz: None declared.

EP15.008 Optical Genome Mapping. Contribution to the Etiological Diagnosis of Developmental disorders : experience of the Nantes Genetic Department on over 60 individuals

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The Optical Genome Mapping (OGM) technique is available for a few years with Bionano protocol. It allows the identification of SVs and CNVs in different applications, such as patients in diagnostic odyssey, presenting malformative syndromes with or without intellectual disability.

The Nantes University Hospital started using the Bionano OGM in hematological and constitutional diseases a few months ago. We report our experience in the genetics department.

OGM technique was settled with 10 known chromosomal abnormalities and we included 60 patients for a solo genome optical mapping. Most patients had a phenotype characterized by developmental disorders.

We used fresh, or frozen blood, sometimes even blood that had undergone a thaw/refreeze cycle at -80°C.

We identified significant chromosomal rearrangements in all known control samples, and in over 50% of the 60 samples tested: Inversion, Insertion, Deletion, Duplication/Triplication, Translocation, mosaic Ring Chromosomes. We identified 4 rearrangements retrospectively « visible » in Karyotype, CGHa or by the genome study but not previously detected by the classic algorithms. We detected interesting CNVs for at least 6 other patients: deletions and insertions linked to candidate genes or at distance in regulatory regions (ex. inversion and *FOXG1*).

This experience has generated practical questions of technical or medical nature: running time on Saphyr scanner, delay in reporting results, reconstruction of complex rearrangements, ISCN used, polymorphisms database, control techniques to be used, limits and guidelines.

Our experience is positive for this complementary approach in MSDI etiological diagnosis, very useful for genetic counseling.

Grant from hematological Nantes service

Conflict of Interest: Martine Doco-Fenzy ACLF and FFGH board in France, Olivier Pichon: None declared, Mathilde Nizon: None declared, Emilie Landais: None declared, Poirsier Céline: None declared, Sandra Mercier: None declared, Bertrand Isidor: None declared, Benjamin Cogne: None declared, Tony Yammine: None declared, Kamran Moradkhani: None declared, Faezeh Vasheghani Farahani: None declared, Sylvie Odent: None declared, Sylvie Jaillard: None declared, Mylène Beri: None declared, Nathalie Bednarek: None declared, Laetitia Lambert: None declared, Agnes Guichet: None declared, celine bonnet: None declared, Florence Demurger: None declared, Clemence Jacquin: None declared, Aziza Belkayat: None declared, Myriam Essid: None declared, Molka SEBAI: None declared, Marc-Antoine Belaud-Rotureau: None declared, Yannick Le Bris hematology, stéphane bezieau inserm.

EP15.009 A gonadal dysgenesis syndrome with dysgerminoma showing lymphocyte culture 46 XY and a mosaic 46 XY/46 XX/47 XXY at tumor tissue level

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Introduction: Gonadal dysgenesis syndrome is a rare sex development disorder characterized by presence of abnormal sex chromosomes, gonadal or anatomic sex development abnormality.

Methods: A 17-year-old phenotypically female patient presented with a history of primary amenorrhea and delayed puberty with abdominal distention diagnosed with standard karyotyping, Fluorescence In Situ Hybridization (FISH) analysis and Formalin fixation and paraffin embedding (FFPE) histological tissues. Both Cytogenetics and histology laboratories analyses were performed at the Department of Pathology and Laboratory medicine, King Abdulaziz Medical City, Ministry of National Guard, Riyadh, Saudi Arabia.

Results: On clinical examination, patient has breast development, pubic and axillary hair, and normal external genitalia. A pelvic MRI revealed presence of large solid pelvic mass. Patient underwent right salpingo-oophorectomy with pathological features of low stage dysgerminoma without other germ cell components. Subsequent total hysterectomy and left salpingo-oophorectomy showed hypoplastic left ovary lacking primordial follicles and oocytes. Furthermore, lymphocyte culture chromosome analysis revealed 46, XY karyotype with presence of SRY gene on FISH analysis. Subsequent, FFPE FISH analysis revealed a mosaic 46 XY in 58%, 46 XX in 22% and 47 XXY in 22% respectively.

Conclusions: Adolescent primary amenorrhea patients may be at risk of having abnormal genetic karyotype and developing ovarian malignancies. Early genetically and pathological investigation is highly recommended that might help in better counseling and better patient care management.

Conflict of Interest: None declared.

EP15.010 A complex 9p24 cryptic rearrangement segregating with heart defect in five generations unraveled using a combined genomics approach of chromosomal microarray, FISH, whole-genome sequencing and optical genome mapping

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Background: Structural variants (SVs) pose a challenge to detect and interpret, but their study provide novel biological insights, besides molecular diagnosis underlying rare diseases. As new technologies are developed, SVs can be detailed, leading to further advancements.

Objectives: The aim was to resolve a 9p rearrangement segregating with a congenital heart defect in a dominant pattern

in a family through five generations, by applying a combined genomic analysis.

Methods: Samples from affected individuals with congenital pulmonary artery and aortic stenosis were evaluated. The analysis involved multiple techniques to investigate genomic variants, including karyotype, chromosomal microarray analysis (CMA), FISH, whole-genome sequencing (WGS), and optical genome mapping (OGM).

Results: A complex 9p duplication were disclosed by CMA, composed of three segments, two at 9p24.3 (partial sequences of *DOCK8* and *KANK1*), and one at 9p24.2. FISH analysis showed a duplicated segment mapped inverted on chromosome 9 itself. OGM detected a deletion between the two distal 9p24.3 duplications, in addition to an inversion disrupting *SMARCA2*, adjacent to the 9p24.2 duplication, and inserted in 9p24.3. WGS confirmed duplications and revealed that two of them were juxtaposed to each side of the distal 9p24.3 duplication, in an inverted position.

Conclusion: The combined approach allowed a deep characterization of this complex rearrangement. OGM data did not called 9p duplications, evidencing a limitation of this technique. Disrupted genes (*DOCK8*, *KANK1* and *SMARCA2*) did not explain the phenotype, and further analysis is required to link the rearrangement with the heart defect.

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Conflict of Interest: None declared.

EP15.011 Partial trisomy 19p: Case report and a literature review

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Partial trisomy of the short arm of chromosome 19 (19p) is a rare chromosomal abnormality with significant clinical implications. We report a case study of a five-year-old boy with multiple anomalies including microcephaly, bilateral hydronephrosis, dysmorphic features, mild bilateral hearing loss, failure to thrive and global developmental delay. His mother was first referred due to abnormal antenatal findings such as oligohydramnios and severe intrauterine growth retardation. Amniocentesis along with chromosomal microarray analysis (CMA) were performed. Genetic analysis showed approximately 6.8 kb copy number gain of the short arm of chromosome 19 at bands 19p13.3p13.2. Cytogenetic analysis of the mother showed an apparently balanced translocation t(13;19) which resulted in abnormal chromosomal segregation during meiosis I and producing gametes with unbalanced chromosomes. To our knowledge, this is among the first reports of partial trisomy 19p as a result of maternal reciprocal translocation. The cytogenetic and clinical findings of our patient is discussed in this report in relation to previously reported cases of patients carrying this mutation.

Conflict of Interest: Reema Abualnaja: None declared, Zohor Azher Medical genetics department, Umm Al-Qura universiry

EP15.012 Cryptic rearrangement in an apparently balanced translocation: the role of molecular cytogenetic techniques

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Background: De novo chromosomal translocations are occasionally associated with chromosomal breakage-and-fusion processes. These rearrangements may be cryptic and more complex than initially suspected and may involve several chromosomal breaks that can lead to DNA loss or gain.

Methods: Classical and molecular cytogenetics (G band, subtelomeric FISH, chromosome painting, CGH-microarray, OMG).

Results: We present a 6-year-old girl with ASD and developmental delay. The CGH-array described a deletion at 12q21.33 and another at 13q33.3 that included the *ATP2B1* gene. This deletion may explain the neurodevelopmental disorder. Patient's karyotype showed a translocation between chromosomes 12 and 13 with breakpoints difficult to determine by conventional cytogenetics, and did not match the CNV regions described in the array study. FISH analysis with chromosomal painting for chromosomes 12 and 13 confirmed that only these two chromosomes were involved in the rearrangement, but the sizes of the translocated fragments indicated different breakpoints than those shown by the deletions. Subtelomeric FISH study confirmed the presence of the telomere from chromosome 13 translocated to chromosome 12.

Conclusions: The presence of several CNVs in the same patient may be due to complex processes of chromosomal breaks and reunions that may lead to duplications and deletions, as well as disruption of genes or regulatory regions. This case demonstrates the need to combine different cytogenomic techniques for the correct characterization of highly complex anomalies. The emerging technique of optical genome mapping can be useful in these highly complex rearrangements.

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Conflict of Interest: Mar Xunclà Full time Hospital Universitari Vall Hebron, Natàlia Rey Full time Hospital Universitari Vall Hebron, Neus Castells Full time Hospital Universitari Vall Hebron, Collaborator PI20/01767, Alberto Plaja Full time Hospital Universitari Vall Hebron, Principal investigator PI20/01767, Irene Valenzuela Palafoll Full time Hospital Universitari Vall Hebron, Anna Maria Cueto-González Full time Hospital Universitari Vall Hebron, Principal investigator PI20/01767, María Serrano Full time Hospital Universitari Vall Hebron, Pedro Antonio Martínez Full time Hospital Universitari Vall Hebron, Lourdes Trobo Full time Hospital Universitari Vall Hebron, Maria Àngels Rigola Part-time Universitat Autònoma de Barcelona (UAB), Elena García-Arumí Full time Hospital Universitari Vall Hebron, Eduardo Tizzano Full time Hospital Universitari Vall Hebron.

EP15.013 Characterisation of a complex rearrangement of a chromosome 20 by array CGH

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Introduction: We report a patient with a complex partial deletion and duplication involving 20p and partial duplication of 20q. The common phenotype of affected patients includes mild facial dysmorphia, delayed development, and speech difficulties.

Materials and methods: The patient is a boy with bilateral cleft lip and palate, and dysmorphia. Molecular karyotyping on

proband was performed on the SurePrint G3 Human CGH 8 × 60k microarray platform with an average probe spacing of 41 kb (Agilent Technologies, Santa Clara, CA, USA). To confirm deletion of 20p13 and the duplication of 20q12q13.33, MLPA analysis was performed, using subtelomeric sets (P036 and P070).

Results: Microarray analysis revealed a complex 1.3 Mb deletion of 20p13(212390_1532079), an interstitial 3 Mb duplication of 20p12.2p12.1(9967816_13029401), and a 25 Mb duplication of 20q12q13.33(39122831_64258081). 20p13 microdeletion syndrome has been described in the literature, and is associated with delayed development, mild-to-moderate intellectual disability, epilepsy, and dysmorphia. Deletions of SOX12 and NRSN2 genes are associated with developmental and speech delay. The duplicated region 20p12.2p12 includes the following morbid genes: JAG1, MKKS and SNAP25. The correlation between the deletion of the 20p13 region, the duplication of the 20p12.2p12.1 region, and the phenotype in our patient is complicated by the presence of the 20q12q13.33 duplication.

Conclusions: To clarify the chromosome architecture, it is necessary to include karyotype and FISH analysis of the patient and his parents. The identified chromosomal changes deepen our understanding of this genomic region and suggest that a long-term follow-up of the patient cognitive ability is needed.

Conflict of Interest: None declared.

EP16 New Technologies and Approaches

EP16.001 Exploring the future prospects of a Dutch NGS-based newborn screening by investigating technical possibilities of targeted NGS, WES and WGS

Gea Kiewiet[1], *Eddy de Boer*[1], *Dineke Westra*[2], *Els Voorhoeve*[3], *Rebecca Heiner-Fokkema*[4], *Francjan van Spronsen*[5], *B. Sikkema-Raddatz*[1], *Marcel Nelen*[6]

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There is an ongoing demand for expansion of the Dutch newborn screening (NBS). The use of NGS-based genetic testing has been recognized as promising. We aimed to compare different NGS approaches (targeted panel (tNGS), WES and WGS) for application in NBS.

DNA was extracted from dried blood spots (DBS) from 50 patients with genetic variants associated with an inherited metabolic disorder (IMD) and 50 control samples. Sequencing was performed following standard diagnostic procedures. Hundred IMD-related genes were analyzed. Different data-filtering strategies were applied: 1) to detect only (likely) pathogenic variants ((L)P) and 2) to detect both (L)P variants and VUS in an autosomal recessive gene with one (L)P variant. Variants left after filtering were compared with known variants in the samples to define true/false positives (TP/FP) and true/false negatives (TN/FN). Coverage and turn-around-time of the different approaches were compared.

The coverage of the NGS approaches was similar and of diagnostic quality. Results were obtained within 5 days.Three to four samples failed for sequencing (tNGS/WGS:3, WES:4). For tNGS, WES and WGS the number of TP out of 50 patient samples were 33, 31, 31 respectively, and 40, 40, 39 including VUS. FN were 14, 15, 16 respectively, and 7, 6, 8 including VUS. Remaining FN were mainly samples with a homozygous VUS. All control samples were TN.

All three NGS approaches are suitable for reliable variant detection in DBS. However, data analysis strategies need to be further optimized to reduce FN results. Follow-up studies are needed before implementation.

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Conflict of Interest: Gea Kiewiet: None declared, Eddy de Boer: None declared, Dineke Westra: None declared, Els Voorhoeve: None declared, Rebecca Heiner-Fokkema Advisory committee Newborn Screening for Metabolic Diseases of the Dutch Society for Pediatrics, Francjan van Spronsen Advisory committee Newborn Screening for Metabolic Diseases of the Dutch Society for Pediatrics, Program committee for Newborn Blood Screening of the National Institute for Public Health and the Environment, B. Sikkema-Raddatz: None declared, Marcel Nelen: None declared.

EP16.002 Technical assessment of different extraction methods and Transcriptome profiling of RNA isolated from small volumes of blood

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The analysis of the human transcriptome provides a window to understand gene regulation and genome plasticity. However, gene expression profiling can only be of value when the RNA under study is representative of the starting material. Recently, finger-stick blood collection systems have allowed a less invasive and quicker collection of peripheral blood for the analysis of the human transcriptome, offering practical advantages.

Automated workflows offer several advantages for large-scale projects, as they increase sample throughput and reduce cost and manual errors.

Here, we have compared the manual RNA isolation of small volumes of blood (Tempus Blood RNA kit) and an automated workflow implemented in-house by using the MagMAX™ for Stabilized Blood RNA Isolation kit on the Hamilton NGS Star platform (Hamilton Robotics). Transcriptome sequencing was performed by using the Lexogen QuantSeq 3′ mRNA-Seq Library Prep FWD kit with unique molecular identifiers.

Samples processed manually had a slightly higher percentage of reads mapping to exonic region, however the samples showed overall consistent body coverage, alignment scores and gene type assignment profiles.

The transcriptomic profiles were driven mainly by the subject assignment rather than by analytical variables, suggesting that the Lexogen QuantSeq 3′ FWD mRNA-Seq is a robust method for gene expression profiling even when adopted for RNA extracted from small volumes of blood (as low as 16 μl).

We expect this study to increase the adoption of automation systems for RNA isolation from small volumes of blood especially in core facility settings where sample throughput and turn-around-time are of critical importance.

Conflict of Interest: None declared.

EP16.003 Long-read sequencing and optical genome mapping enable full characterization of previously unresolved structural variation

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Background: Conventional diagnostic methods often fail in resolving exact breakpoints of structural variants (SVs). We investigated if long-read sequencing (LRS) and optical genome mapping (OGM) can detect and fully characterize previously unresolved SVs.

Methods: Nanopore LRS was performed on six individuals (S1-6) with a de novo SV. Breakpoints could not be determined through conventional methods, resulting in an unexplained phenotype in S1 and S2. SVs include a translocation (S1-2), deletion (S3), triplication (S4) and complex rearrangement (S5-6). Bionano OGM was additionally applied to S1, S2, and S6.

Results: LRS and OGM resolve the interrogated SV and allow for a genetic diagnosis in all cases. A single gene disruption is identified as causal in S1 (*CELF2* - intellectual disability (ID)) and S3 (*MYT1L* – ID). Remarkably, in the remaining four individuals the rearrangement is more complex than anticipated, revealing additional breakpoints and disrupting previously seemingly unaffected chromosomes. Of particular interest is the characterization of the translocation in S2 where a complex event is found to affect the *ZEB2* region, resulting in the diagnosis of Mowat-Wilson syndrome. Furthermore, S6 shows a local chromothripsis explaining infertility and the complex rearrangements in individuals S4 + S5 manifesting with ID are now delineated correctly.

Conclusion: This study highlights the potential of LRS and OGM as a first-tier test for SV identification. We reveal that complex rearrangements are often inaccurately characterized by conventional methods and are more common than expected. LRS further offers single basepair breakpoint resolution allowing precise SV delineation. These benefits ultimately result in increased diagnostic yield.

Conflict of Interest: Griet De Clercq received free supplies from Oxford Nanopore Technologies, received sponsored travel and accommodation from Oxford Nanopore Technologies, Lies Vantomme: None declared, Bert Callewaert: None declared, Sarah Vergult: None declared, Barbara Dewaele: None declared, Joris Vermeesch: None declared, Wouter De Coster received sponsored travel and accommodation from Oxford Nanopore Technologies, Mojca Strazisar: None declared, Tim De Pooter: None declared, Annelies Dheedene: None declared, Björn Menten: None declared.

EP16.004 Use of 3D Facial Analysis for Diagnosis and Treatment Monitoring in Hereditary Angioedema

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Rare diseases pose a diagnostic conundrum to even the most experienced clinicians around the world. Technology could play an assistive role in hastening the diagnosis process. Data-driven methodologies can identify distinctive disease features and create a definitive diagnostic spectrum. The healthcare professionals in developed and developing nations would benefit immensely from these approaches resulting in quicker diagnosis and enabling early care for the patients. Hereditary Angioedema is one such rare disease that requires a lengthy diagnostic cascade ensuing massive patient inconvenience and cost burden on the healthcare system. It is hypothesized that facial analysis with advanced imaging and algorithmic association can create an ideal diagnostic peer to the clinician while assimilating signs and symptoms in the hospital. 3D photogrammetry has been applied to diagnose rare diseases in various cohorts. The facial features are captured at a granular level in utmost finer detail. A validated and proven algorithm-powered software provides recommendations in real-time. Thus, paving the way for quick and early diagnosis to well-trained or less trained clinicians in different settings around the globe. The generated evidence indicates the strong applicability of 3 D photogrammetry in association with proprietary Cliniface software to Hereditary Angioedema for aiding in the diagnostic process. The approach, mechanism, and beneficial impact have been sketched out appropriately herein. This proof of concept blueprint for hereditary angioedema may have far-reaching consequences beyond disease diagnosis to benefit all the stakeholders in the healthcare arena including research and new drug development.

Conflict of Interest: Saumya Jamuar: None declared, Richard Palmer: None declared, Petra Helmholz: None declared, Stuart Lee Takeda Pharmaceuticals, Choo Beng Goh Takeda Pharmaceuticals, Gareth Baynam Grant from Takeda Pharmaceuticals.

EP16.005 A computational approach to design a COVID-19 vaccine against a predicted SARS-CoV-2 variant: high immunogenicity, efficacy and safety of DELLERA vaccine

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Background: Severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2) caused 6,817,478 deaths worldwide since its first identification in 2019. Constant virus evolution and selection of vaccine escape variants still poses a major global health threat and represents the rationale for the tireless design of new candidate vaccines. In this study, we use a computational approach for the design of a novel candidate vaccine for a predicted SARS-CoV-2 variant.

Methods: A total of 393,594 Spike (S) protein genomes of SARS-CoV-2 were analyzed with the aim to find a reference sequence of the virus most widely spread in Europe at the time and predicted (MaxEnt niche-based model) to remain as the dominant clade for the next COVID-19 wave. Therefore, the viral S protein of an UK-Alpha variant of SARS-CoV-2 from a real sample (hCoV-19/England/MILK-B94A53/2020) was selected as the basis for our vaccine design.

Toxicity, immunogenicity and efficacy were evaluated after vaccination (D0, D14, D28) with 1µg and 10µg of Dellera.

Results: Toxicology studies conducted on Sprague Dawley rats showed lack of adverse or toxic effects andtreatment-related deaths. Immunization of BALB/c mice resulted in high titers of functional (neutralizing) anti-RBD antibodies for ancestral and Omicron variants and anti-S1/S2 antibodies for UK-Alpha variant. Moreover, decreased lung pathology in Syrian hamsters challenged with an high intranasal Sars-CoV-2 inoculum suggests robust protective efficacy of Dellera.

Conclusion: Successful preclinical data support Dellera further development as clinical candidate and suggest that our approach represents a promising choice to design vaccines against new predicted variants of SARS-CoV-2.

Conflict of Interest: None declared.

EP16.006 Optical Genome Mapping for the Molecular Diagnosis of Facioscapulohumeral Muscular Dystrophy: advancement and challenges

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is the second most common muscular dystrophy in adults, associated with a D4Z4 microsatellite repeat contraction on chromosome 4. FSHD1 diagnosis is Southern blot (SB)-based, however the protocol is technically challenging, time consuming and requires training in results interpretation. Currently only a few laboratories worldwide offer a full FSHD genotyping work-up and access to FSHD genetic diagnosis in low and middle-income countries (LMIC) is limited. Optical genome mapping (OGM) is a new promising technology to assess genomic structural variants. We aimed to investigate the use of OGM as diagnostic tool in testing FSHD cases from UK and India.

Methods: We aimed to compare traditional techniques such as linear gel (LGE) and Pulsed-field gel electrophoresis (PFGE) Southern blotting. Samples were processed with the Saphyr Genome Imaging Instrument. Data was analysed using the custom EnFocus FSHD analysis. 31 probands with suspected/confirmed FSHD diagnosis were analysed.

Results: OGM was able to confirm the diagnosis of FSHD1 in 22 cases and D4Z4 sizing highly correlates with SB ($p < 0.001$). Two cases were identified as mosaic on the permissive 4qA chromosome by OGM and one case was found with an homozygous 10U D4Z4 contraction. Eight cases were found to be negative with both OGM and SB.

Conclusions: OGM is a promising new technology able to unravel structural variants in the genome and seems a valid tool to diagnose FSHD1. Further data are ongoing to assess OGM efficacy in assessing complex rearrangements.

Grant References: National Brain Appeal, Rosetrees, EJP RD Mobility

Conflict of Interest: Stephanie Efthymiou full time, Venugopalan Y Vishnu full time, collaborator, richard lemmers full time, collaborator, lindsay wilson full time, patrick j van der vliet full time, natalia dominik full time, benedetta perone full time, Rinkle Mishra full time, Alisha Reyaz full time, Tanveer Ahmad full time, Rohit Bhatia full time, Mv Padma Srivastava full time, PI, Stefano Facchini full time, Henry Houlden full time, PI, Andrea Cortese full time, PI, Silvère M van der Maarel full time, PI, Michael Hanna full time, PI, Enrico Bugiardini full time, PI.

EP16.007 PhotoMeDAS: something better than a tape measure for a head with atypical shape

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Background: The analysis of the development of the shape of the skull and face is fundamental for dysmorphological assessment. The interaction of genetic and environmental factors is of enormous importance in cranial development, both prenatally and in the first year of life. Many times the positional cranial deformation can be confused with craniosynostosis. Nowadays, the use of 3D models to evaluate cranial deformation is becoming more common. However, due to high cost, they are not widely implemented in clinical practice.

Materials and Method: One patient is measured over time three times with the fully automatic PhotoMeDAS solution to determine any cranial deformation. The tool is composed of a coded cap, three coded stickers, a mobile app and server-based computation. The data acquisition is carried out during consultation with a mobile application in less than five minutes. After processing the data, a link allows the specialists to download a full report and the 3D model. The report displays the head's shape and automatically determines 21 anthropometric variables and indexes such as the cephalic index, oblique cranial length ratio, towering index and metopic index.

Results: The anthropometric indexes are monitored over time. The report also displays both the actual and the ideal head's 3D models.

Conclusions: The anthropometric variables and indexes help specialists to diagnose better cranial deformations, requiring just a smartphone and a coded cap. In addition, visualising the 3D head's shape and their spatial deformations with an ideal comparable head's shape add value to set eventual diagnosis.

Conflict of Interest: None declared.

EP16.008 OGM reveals the complexity of two constitutional structural chromosomal events

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We applied Bionano OGM (Optical Genome Mapping) to elucidate the structure of two different chromosomal events.

Patient 1: a 2-months-old girl referred for infantile spasms and cerebral anomalies. The arrayCGH detected a gain in the *Dystrophin (DMD)* gene, inherited from her mother, as an incidental finding. A follow-up was done for the mother to evaluate the rearrangement structure with the aim of predicting the type of dystrophinopathy concerning eventual future pregnancies.

OGM allowed to demonstrate how two apparently independent gains on chromosome X, were part of a more complex structural variant leading to the insertion of three different duplicated

fragments within the *DMD* gene. This result allowed to upgrade the prediction in a male carrier of this *DMD* gain from a Becker phenotype to a possible severe Duchenne phenotype.

Patient 2: a 21-years-old male patient presenting with developmental delay, short stature and facial dysmorphisms. The arrayCGH identified four deletions affecting chromosomes 9 and 13. Karyotype and FISH analyses revealed a complex chromosomal event involving these two chromosomes. OGM showed a highly complex event with a total of 21 breakpoints on both chromosomes. We speculate that firstly chromosome 13 underwent chromothripsis followed by the insertion of two rearranged fragments on chromosome 9. Investigations of the 21 breakpoints exclude a breakpoint pathology. Further evaluations are ongoing to identify eventual regulatory anomalies, possibly linked to alteration of TAD structures, on candidate genes.

OGM technology allowed the characterization of complex chromosomal events thereby allowing to provide essential knowledge about their pathogenicity.

Conflict of Interest: None declared.

EP16.009 Models of Lysosomal Storage Diseases

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Background: Lysosomal Storage Disorders (LSDs) are degenerative disorders in which the lysosome plays a pivotal role. Usually of autosomal recessive nature, these multisystemic diseases present great phenotypic and genetic variability. Models of disease have been used in research to help understand the biological significance of new variants, even within sibs. Laboratory animals, yeast and insect models are often used to carry out functional tests and shed light on pathogenic mechanisms. However, they lack the specific genetic background of the patients. Disease models based on human induced Pluripotent Stem Cells (hiPSCs) are derived from patient somatic cells and share the patient's own genetic background providing a great advantage over other types of models.

Methods: We have induced and generated hiPSCs from Gaucher and Fabry patient fibroblasts, two LSDs, methods and results were described in https://doi.org/10.1016/j.scr.2019.101595 and https://doi.org/10.1016/j.scr.2020.101794. Presently, human iPSCs are being used as models for gene editing, relying on prime editing, to correct p.W287X mutation in cell line INSAi002-A.

Results: In the past the above mentioned cell lines replicated different patient cell types. Now we are manipulating these cells by "search-and-replace"prime-editing in order to obtain corrected cells with precise and efficient editing. Gene and protein analysis results will be presented.

Conclusion: Potentially, hiPSCs are a valuable research tool to study disease and to accelerate therapeutic methodologies.

Our aim is to create reliable cell models, clarify mechanisms and attempt therapeutic approaches.

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Conflict of Interest: olga amaral National institute of health Ricardo Jorge, This work received finantial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/00211/2020; and also from FCT PTDC/BIMMEC/4762/2014 and SFRH/BD/118009/2016 and INSA 2017DGH1439, Ana Joana Duarte INSA, This work received finantial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/00211/2020; and also from FCT PTDC/BIMMEC/4762/2014 and SFRH/BD/118009/2016 and INSA 2017DGH1439, ICBAS (University of Porto), PhD student fees used for supplies through the kind support of Professor Rosario Almeida.

EP16.010 The International Consortium on Newborn Sequencing (ICoNS)

Nicolas Encina[1;2], *Amy Brower*[3], *Alessandra Ferlini*[4;5;6], *Nicolas Garnier*[6;7], *Holly Peay*[8;9], *Lilian Downie*[10;11], *Don Bailey*[8;9], *Melissa Wasserstein*[12;13], *Stephen Kingsmore*[14;15], *Wendy Chung*[16;17], *Richard Scott*[18], *David Bick*[18], *Robert Green*[19;20;21]

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Background: Newborn sequencing (NBSeq) has the potential to offer a lifetime of personalized health care and disease prevention that is specific to each individual genome. When fully realized, NBSeq will mark a disruptive transition into personalized medicine and public health. A number of research projects and commercial offerings are already underway to explore the implementation of newborn sequencing in clinical settings.

Objectives: Given the proliferation of clinical implementation efforts, we sought to create the first organization specifically dedicated to communicating and sharing progress and best practices in the implementation of NBSeq. Initial topics for consideration included gene selection for initial panels, variant interpretation, patient communication and planning for clinical follow up.

Methods: Principal investigators from 8 separate groups that were already conducting or imminently planning to conduct NBSeq research began regular meetings and agreed to establish the International Consortium on Newborn Sequencing (ICoNS). ICoNS was created to harmonize activities leading to evidence-based best practices for implementing newborn sequencing.

Results: We have established ICoNS as an alliance of active investigators in NBSeq research with an annual interdisciplinary conference. An initial conference featuring 19 presentations was held in October 2022 in Boston, MA with over 300 attendees from 35 countries, and the next conference is planned for October 5-6, 2023 in London. Discussions of organization and governance are underway.

Conclusion: ICoNS presents an opportunity to gather multi-disciplinary governmental, academic and industry stakeholders and experts from around the world in order to accelerate and harmonize research progress and real-world implementation in NBSeq.

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HHSN275201800005C, Alessandra Ferlini Project Grant Number(s): Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 101034427, Nicolas Garnier Pfizer, Holly Peay HLP has received research funding from the Helmsley Charitable Trust, Juvenile Diabetes Research Foundation, Travere Pharmaceuticals, Sarepta Therapeutics, Muscular Dystrophy Association, The John Merck Fund, Lilian Downie: None declared, Don Bailey Don Bailey has received research funding from the Helmsley Charitable Trust, Juvenile Diabetes Research Foundation, Travere Pharmaceuticals, The John Merck Fund, Melissa Wasserstein MW has received research funding from Abeona Therapeutics, Alexion Pharmaceuticals, the Ara Parseghian Medical Research Foundation, BioMarin Pharmaceutical, Cure Sanfilippo Foundation, Dana's Angels Research Trust, Firefly Fund, Noah's Hope, Orchard Therapeutic, Passage Bio, Sio Gene Therapies, Takeda Pharmaceutical, Travere Therapeutics, and Ultragenyx Pharmaceutical; and consulting fees, speaker fees, research support, and travel reimbursement from Sanofi Genzyme.

Project Grant Number(s): R01HD073292, Stephen Kingsmore Project Grant Number(s): UL1TR002550 R01HD101540, SFK has filed patents related to newborn screening via genome sequencing. Rady Children's Institute has received research funding from the Marriott Foundation, the Rady family, and several pharmaceutical companies., Wendy Chung W.K.C. has received funding from Illumina, GeneDx, and Sanofi., W.K.C. is on the scientific advisory board of the Regeneron Genetics Center and the Board of Directors of Prime Medicine., Richard Scott: None declared, David Bick David Bick has Tier 2 COI for consultancy work with HudsonAlpha Clinical Services Lab LLC, iRepertoire Molecular Lab, and Northwestern Mutual Life Insurance Company and was employed by Smith Family Clinic for Genomic Medicine until October 2021., Robert Green Project Grant Number(s): U19HD077671 U01TR003201, Robert Green is co-founder of Genome Medical and Nurture Genomics., Robert Green has received compensation for advising the following companies: AIA, Allelica, Atria, Fabric, Genome Web, Genomic Life, Grail, Verily, VinBigData.

EP16.011 Ring chromosome detected using T2T reference in Optical Genome Mapping. One technique to rule them all

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Background: Ring chromosome Y (rY) (ORPHA:261529) is a chromosomal aberration, characterized by short stature, partial to total gonadal failure and genital malformations.

Ring chromosome 20 (r20) (ORPHA:1444) is a rare cause of refractory epilepsy, behavioral problems and cognitive impairment in children.

These chromosomal aberration are often in mosaic state. Karyotype is the gold standard for detection of ring chromosomes. Copy number variation (CNV) detection techniques are incapable of ring chromosome detection when there is no CNV at the breakpoints.

Methodology: We tested 3 peripheral blood samples known to have ring chromosomes, 2 with r20 mosaicism of 21 and 35% and one with rY mosaicism of 86%.

Optical genome mapping (OGM) was performed using the Rare Variant Analysis pipeline using the GRCh38 as the reference genome. We re-analyzed the data gathered using the Rare Variant Analysis pipeline with Bionano Solve software with T2T as reference genome.

Results: Using Rare variant analysis with GRCh38 as reference the chromosomal aberrations were not detected. When we ran the Rare Variant analysis using T2T we were able to detect r20 and rY as intrachromosomal translocation in all 3 samples, mosaic % was concordant with karyotype.

Conclusion: OGM has proved to be a reliable technology for the detection of ring chromosomes with associated CNVs (data not shown) and allows the reliable detection of mosaic levels in concordance with standard of care. Improvements in gap filling of the reference genome are necessary to optimize ring chromosome detection using OGM.

Conflict of Interest: None declared.

EP16.012 Optical genome mapping in genetic cold cases with a neurodevelopmental disorder

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Optical genome mapping (OGM) is a new method aiming to fill in the gap between structural variants (SVs) and short-read sequencing techniques. It is based on fluorescently labeled tags marking long linearized DNA molecules to detect genetic variants. The study material was selected based on clinical phenotype that had been remained unsolved in previous studies (ES, WGS, molecular karyotyping). So far, we have completed OGM in 42 families with a genetically unsolved neurodevelopmental disorder (NDD). Ultra-high molecular weight DNA was extracted from frozen blood, fluorescently labeled with DLE-1 and OGM was performed on Bionano's Saphyr instrument. Single-molecule maps were assembled de novo into consensus maps using the Bionano Solve™ data analysis software, followed by SV calling against the hg38/GRCh38 reference. The average filtered molecule N50 was 283kbp and the average effective coverage of the reference was 188x. In 6 of 42 families (14%), we found possible pathogenic SVs in known neurodevelopmental disorder (NDD) genes, including *OPHN1*, *NUP133*, *H3F3A*, *SON*, *PHF8* and *BCL11A*. These variants have been/are currently being validated and reconstructed further with techniques such as long-read sequencing or Sanger sequencing. In several families, we found rare or unique SVs in candidate genes. Variants in known NDD genes or candidate variants of interest missed by exome sequencing mainly consisted of larger insertions (>1kbp), inversions, and deletions/duplications of a low number of exons (1-4 exons). Analysis of additional families is ongoing and results of a larger cohort will be presented at the meeting. Funding: Gertrude H. Sergievsky Center Award, R21 NS123325.

Conflict of Interest: None declared.

EP16.013 Detection of SMA by discrimination of SMN1 and SMN2 using long-read sequencing (ONT) and artificial intelligence (AI)

Chris Kyriakidis[1], *David Galevski*[2], *Gjorgji Madjarov*[1;2;3], *Aleksandar Nikov*[2], *Grzegorz Nowicki*[4], *Rafal Kalka*[1], *Anne Kristine Schack*[1;5],

Karmele Alapont[1;5], Lukasz Krych[5], Marija Chaushevska[1;2;3], Dimitrios Kyriakidis[1], Zoran Velkoski[1], Carmen Garrido Navas[1;6;7]

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Background: Bi-allelic mutations in the *SMN1* gene result in motor neuron degeneration and spinal muscular atrophy (SMA), a progressive, recessive neuromuscular disease with varying presentation in terms of onset and severity. The high clinical and genetic heterogeneity of SMA together with its low prevalence (1 in 10,000 births), makes diagnosis and treatment very challenging. The severity of SMA is associated with the copy number of the survival motor neuron 2 (*SMN2*) gene that is almost identical, in genomic sequence, with *SMN1*. The frequency of unaffected, heterozygous carriers of the *SMN1* mutations is high among Caucasian and Asian populations. Diagnosis of SMA is usually approached by RT-PCR for the *SMN1* gene (sometimes including *SMN2*) after clinical symptoms suggest the condition; however, genetic testing is needed to confirm a positive result (3). This procedure is not only slow and costly, but also inefficient because the clinical symptoms overlap with other neuromuscular diseases, increasing misdiagnosis rates.

Methods: Combination of targeted long-read sequencing (ONT) and statistical analysis using our Phivea® platform allows to discriminate between *SMN1* and *SMN2* as well as to count the number of copies for each gene.

Results: We analysed samples from SMA patients using our Phivea® platform and we were able to identify a point mutation (C > T) that usually occurs in the telomeric region, as well as to count the number of copies for *SMN1* and *SMN2*.

Conclusion: The Phivea® platform can be used as a novel approach to study *SMN1* and *SMN2* mutations to improve diagnostic yield of SMA.

Conflict of Interest: Chris Kyriakidis Employed by gMendel, Owner of gMendel and Phivea platform, David Galevski: None declared, Gjorgji Madjarov Consultant at gMendel, Aleksandar Nikov: None declared, Grzegorz Nowicki Employed by Genxone, Rafal Kalka: None declared, Anne Kristine Schack Employed by gMendel as industrial PhD student, Karmele Alapont Employed by gMendel as industrial PhD student, Lukasz Krych: None declared, Marija Chaushevska: None declared, Dimitrios Kyriakidis: None declared, Zoran Velkoski Employed by gMendel, Owner of gMendel and Phivea platform, Carmen Garrido Navas Consultant at gMendel.

EP16.014 Multiplexing approach for high-throughput cystic fibrosis screening using ONT and artificial intelligence

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Background: Cystic fibrosis (CF), the most common genetic disorder in Caucasians, is caused by mutations in *CFTR*, a gene producing a conductance-regulating transmembrane protein. Diagnosis of CF is usually done by the sweat chloride test (SCT), detecting elevated sweat chloride concentrations. After this, molecular testing confirms the presence of two *CFTR* variants. However, abnormal SCT were found in non-CF individuals due to methodological errors, metabolic issues or skin and sweat gland diseases. Furthermore, sometimes only one *CFTR* mutation is identified, due to the rarity of the second, or to its complexity. Only five CF-causing mutations make up almost two-thirds of all CF alleles (P508del, G542X, G551D, N1303K, and W1282X), but one-third of variants very low in frequency still delay diagnosis.

Methods: Long-read sequencing allows studying *CFTR* variants from a different perspective, improving diagnostic yield. The multilevel multiplexing approach (Torchlex) developed by gMendel® for its Phivea® platform allows reducing price and improving outcomes. We analyzed some *CFTR* variants following the European guidelines for CF testing using the ONT technology and the Phivea® platform.

Results: We detected 8 common *CFTR* mutations (F508del, Y122X, S549R, S549N, G542X, V520F, W1282X and R1162X) and 5T/7T/9T alleles. We are expanding the number of samples to train our Phivea® platform to detect a more comprehensive panel of *CFTR* mutations and we will present its performance for at least the 25 variants recommended by the ACMG.

Conclusion: The Phivea® platform can be used to screen for any *CFTR* variant increasing the diagnostic yield of CF and reducing price.

Conflict of Interest: Gjorgji Madjarov Consultant at gMendel, David Galevski: None declared, Aleksandar Nikov: None declared, Grzegorz Nowicki Employed at Genxone, Rafal Kalka: None declared, Anne Kristine Schack Employed by gMendel as industrial PhD, Karmele Alapont Employed by gMendel as industrial PhD, Lukasz Krych: None declared, Marija Chaushevska: None declared, Dimitrios Kyriakidis: None declared, Chris Kyriakidis Employed by gMendel, Owner of gMendel and Phivea platform, Zoran Velkoski Employed by gMendel, Owner of gMendel and Phivea platform, Carmen Garrido Navas Consultant at gMendel.

EP16.015 Investigation of Optical Genome Mapping Diagnostic Capabilities as a Potential Routine Clinical Test

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Genomic technologies helped to identify genetic diagnoses in many cases. However, despite this success, many still remain undiagnosed. Each of the current cytogenetic methods: karyotyping, microarrays and FISH, have limitations such as resolution, inability to identify balanced events, or being targeted, respectively. Conversely, short-read genome sequencing, which reliably identifies SNVs and INDELs, cannot sensitively identify many of the different types of structural variants (SVs) due to its methodological limitations. *Optical Genome Mapping* (OGM), addresses these limitations by capturing patterns of fluorescent labels within DNA molecules >150 kbp in nanochannel arrays for SV calling.

We have performed validation of OGM using 72 samples for which diagnoses had been achieved with orthogonal methods. In total, OGM was 97% concordant with previous diagnoses only missing one translocation case due to breakpoints being located in centromere and one mosaic variant in an FSHD case. We also tested 20 undiagnosed cases for which microarray and exome sequencing were uninformative.

To select for potential clinically significant variants, we filtered SVs based on quality, size, frequency and gene overlap. We

identified two likely pathogenic deletions involving two clinical genes, *CLTC* and *DHX30*. We also identified several potentially interesting insertions in disease causing genes needing further investigation. Lastly, we identified a translocation involving *SON* gene and mosaic deletion involving *TSC2* gene. Both SVs were diagnostic and were validated via targeted long-read sequencing. In summary, we show that OGM detects large SVs, has high concordance with other cytogenetic methods and can provide diagnosis in negative cases.

Conflict of Interest: Doris Eisenreich Medizinisch Genetisches Zentrum, Hayk Barseghyan Bionano, Illumina, Pacific Biosciences, Hannes Erdmann Medizinisch Genetisches Zentrum, Ariane Hallermayr Medizinisch Genetisches Zentrum, Evgenia Lindt Medizinisch Genetisches Zentrum, Kai Sendelbach Medizinisch Genetisches Zentrum, Udo Koehler Medizinisch Genetisches Zentrum, Teresa Neuhann Medizinisch Genetisches Zentrum, Angela Abicht Medizinisch Genetisches Zentrum, Elke Holinski-Feder Medizinisch Genetisches Zentrum.

EP16.016 Effect of the variance in local allelic spectrum on gene expression regulation

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Background/Objectives: The presence of distal regulatory elements such as super enhancers suggests that stretches of genome, either close or far apart from genic regions, have impact on the regulation of gene expression (GE). Therefore, the genetic variance over an interval compared to a single genetic variant could provide a comprehensive overview regarding the genetic variance on GE.

Methods: We divided a reference human genome into non-overlapping 5kbps intervals and calculated local principal components (LPCs) using common variants (minor allele frequency > 1%) in each interval. A generalized linear model was used to calculate the contribution of LPCs on GE.

Results: With matched genotype and GE data for 48 tissues from Genotype-Tissue Expression project (GTEx V8 release), we found 16,633 genes associated with LPCs within 1.5Mbps of genes. The significant intervals were enriched with regulatory element candidates from ENCODE project (especially with promotor- or enhancer-like element candidates). Overall, the intervals closer to genes had larger effects. On average, the model with LPCs explained larger proportion of GE variance than those with single eQTL. Some genes with significant eQTLs were not associated with LPCs, suggesting differential contribution of SNVs and local allelic spectrum for GE.

Conclusion: We demonstrated an intuitive way to analyze the impact of local allelic spectrum on GE. Although the explained proportion of variance is relatively small, it shows that multiple genetic variants collectively contribute to GE variance across individuals.

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Conflict of Interest: None declared.

EP16.017 Heritability, pQTLs, and environmental influence on proteins involved in age and cardiovascular risk using SomaScan™

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Background/Objectives: Highly multiplex proteomic assays, such as SomaScan™, have enabled a growth in protein quantitative trait locus ("pQTL") studies, which can reveal the underlying mechanisms of genetic disease risks and suggest potential drug targets. Because the proteome is also sensitive to environmental factors, proteomics captures effects that genomics does not.

Methods: We compiled a list of pQTLs from two recent studies measuring the level of > 5,000 proteins—Ferkingstad et al., (2021) identified pQTLs in the Icelandic deCODE study, and Zhang et al., (2022) identified pQTLs and estimated heritability in European and African Americans from the ARIC study. For protein analytes associated and not associated with aging and cardiovascular disease (CVD) endpoints, we compiled heritability and calculated the proportion with cis-pQTLs.

Results: Across the SomaScan v4 assay analytes, about half (48.7%) were cis-pQTL-associated, while 30% had significant heritability in the ARIC study. By contrast, 70% of analytes strongly associated with age or CVD were also pQTL-associated, and 48-50% (depending on ancestry) had significant heritability. Overall, mean heritability across analytes was 13-14% regardless of endpoint association status.

Conclusions: Proteins associated with aging or CVD are more likely to have evidence of genetic influence than proteins not associated with aging or CVD, supporting the use of proteomic data in Mendelian randomization and proteome-wide association studies. However, there are still many proteins that have a strong association with an endpoint, but no cis-pQTL association or low heritability. This suggests that the SomaScan platform captures additional effects of environmental variation on the proteome.

Conflict of Interest: Brendan Epstein SomaLogic employee, Ted Johnson SomaLogic employee, Tina Lai SomaLogic employee, David Astling SomaLogic employee, Michael Hinterberg SomaLogic employee.

EP16.019 Lung organoids as a model for gene therapy with recombinant adeno-associated viral and adenoviral delivery

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Background/Objectives: Organoids are a unique cellular model for studying and developing treatments for various hereditary diseases, such as cystic fibrosis (CF). One of the most promising ways to deliver therapeutic transgenes are recombinant viral vectors. Three adeno-associated viral (AAV) serotypes (AAV5, 6, and 9), as well as adenoviral vector (AdV) serotype 5 were selected, based on their tropism to lung cells. The aim of the work was to evaluate the effectiveness of delivery of the *EGFP* and *mCherry* transgenes to lung organoids (LOs) using selected viral vectors.

Methods: The work was carried out on LOs derived from induced pluripotent stem cells (iPSCs) of a healthy donor and from a CF patient with homozygous F508del mutation in *CFTR* gene. LOs were transduced by three rAAV serotypes with *EGFP* transgene at different MOI, as well as AdV5 with *mCherry* transgene (courtesy of Prof. D. Logunov from Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russia) in various PFU.

Results: rAAV6 and rAAV9 was the most effective for LOs transduction using protocol with the extraction of LOs from the matrix (80% and 35% with MOI 1E10, respectively) as well as, AdV5 (37% with PFU 8E06).

Conclusion: Optimal vectors in optimal doses for efficient transduction of LOs were identified in this work. They can be used in further studies for the delivery of various transgenes, including CRISPR-Cas9 for genome editing in *CFTR* gene for cystic fibrosis treatment.

Conflict of Interest: None declared.

EP16.020 A Customized Array-Cgh for solving unsolvable genetic dIseAses (ACACIA): where are we now?

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Background: The role of copy number variants (CNVs) in pathomechanisms of rare diseases is increasingly recognized, although their detection is challenging. Despite their limitations, commercial array-CGH designs and bioinformatic tools for calling CNVs from NGS data are widely used. We recently developed a high-resolution customized exome-based array-CGH test (ACACIA) to explore CNVs as causative of rare genetic diseases.

Methods: We used ACACIA to test 49 samples from unsolved pediatric patients exhibiting syndromic phenotypes with incomplete or inconclusive molecular diagnosis recruited from the Telethon Undiagnosed Diseases Program and the University of Campania Medical Genetics Unit and Rare Ocular Diseases Center. ACACIA (first release) covered 8,228 known or candidate (pLI>0.5) disease-causing genes with ~1M probes.

Results: WES previously identified heterozygous variants in recessive genes ($n = 18$) and resulted negative in the remaining samples ($n = 31$). In 7/18 patients with a potential recessive disorder, ACACIA identified a heterozygous deletion/duplication in five genes, unmasking the second allelic variant undetected by WES. ACACIA also identified pathogenic CNVs in TBX5 and AUTS2 in two patients with inconclusive WES, allowing molecular diagnosis of Holt-Oram syndrome and intellectual development disorder, respectively. These promising findings prompted us to update AACIA, which now includes exon-based coverage of 9,085 genes.

Conclusions: Our results suggest that, unlike commercial array-CGH designs, ACACIA is able to identify elusive CNVs involving even a single exon. ACACIA could also usefully assist in validating not always reliable findings from bioinformatic tools calling NGS-predicted CNVs. By combining advanced technologies, diagnostic detection rates for unsolved patients can be significantly increased.

Conflict of Interest: None declared.

EP16.022 Characterization of a translocation that disrupts the COL4A5 gene in a girl with Alport syndrome by optical genome mapping

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Background: Two-year-old girl with recurrent hematuria and short stature with a clinical diagnosis of Alport syndrome was referred for genetic studies. The karyotype study detected an apparently balanced reciprocal translocation between chromosomes X and 1. The breakpoint of the X chromosome is located in the q22 band, which is where the COL4A5 gene is located, which causes X- linked Alport syndrome. An optical genome mapping (OGM) study was carried out, which allowed us to map the breakpoints.

Methods: Ultra high molecular weight (UHMW) DNA was extracted from peripheal blood samples and labelling following the manufacturer's protocols. Labeled DNA was loaded on a Saphyr chip and run on a Saphyr instrument. The de novo genome map assembly was performed using BionanoSolve™ and structural variants were called against the human reference GRCh38 assembly. Data were analyzed with Bionano Access™ and Bionano Tools™ on Saphyr Compute Servers (Bionano Genomics).

Results: Analysis by means of OGM allowed us to identify the (X;1) translocation and map the breakpoint of the X chromosome in the COL4A5 gene (chrX:8,454,516; GRCh38) and the breakpoint on chromosome 1 in the RERE gene (chr1:108,574,884; GRCh38). No other structural variants that could have clinical relevance were observed.

Conclusion: In a single trial, the study using optical genome mapping has made it possible to characterize the cut-off point of the translocation with considerable precision and confirm the breakage of the COL4A5 gene, which is the cause of the clinical manifestations in this patient.

Conflict of Interest: None declared.

EP16.024 Long repeat sequence synthesis by single-stranded circular DNA

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Background: The discovery that simple tandem repeats or microsatellites can cause neurological diseases was revolutionary in the field of neurodegenerative disorder studies. Nearly 50 neurological diseases have been identified so far, of which 26 diseases are related to the repeat expansion in coding, non-coding, and 5′ and 3′ UTR regions. Among the repeat expansion-related diseases, spinocerebellar ataxia type 31 (SCA31) is caused by the repeat expansion of pentanucleotide TGGAA in the intron region of the BEAN gene. To study these diseases, transgenic models have been developed using interrupted repeats. However, synthesizing repeat sequences from synthetic oligonucleotides is challenging as they are unstable, lack unique sequences, and exhibit propensity to make secondary structures. Synthesizing long repeat sequence using polymerase chain reaction is also often difficult because of lack of unique sequences.

Methods: Here, we employed a rolling circle amplification technique to obtain seamless long repeat sequences using tiny synthetic single-stranded circular DNA as template.

Results: We obtained 2.5–3 kbp uninterrupted TGGAA repeats, which is observed in SCA31, and confirmed it using restriction digestion, Sanger and Nanopore sequencing.

Conclusion: This cell-free, in vitro cloning method may be applicable for other repeat expansion diseases and be used to produce animal and cell culture models to study repeat expansion diseases in vivo and in vitro.

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EP17 Diagnostic Improvements and Quality Control

EP17.001 Meta-analysis of the diagnostic and clinical utility of exome and genome sequencing in paediatric and adult patients with rare diseases across different ancestries

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Background/Objectives: To conduct a meta-analysis to evaluate the diagnostic and clinical utility of whole-exome sequencing (WES) versus whole-genomes sequencing (WGS) in paediatric and adult patients with rare diseases across different ancestries.

Methods: A meta-analysis was conducted on the diagnostic and clinical utility of WES and WGS, with relevant studies identified from 2011–2021.

Results: One-hundred-and-sixty-one studies across 31 jurisdictions were eligible, featuring 50,417 probands of East/South Asian, Caucasian, Middle Eastern, and African American/Hispanic ancestries. Diagnostic yields of WES (0.38, 95% CI 0.36–0.40) and WGS (0.34, 95% CI 0.30–0.38) were similar ($p = 0.1$). Within-cohort comparison illustrated a 1.2 times odds of diagnosis by WGS over WES (95% CI 0.79–1.83, $p = 0.38$). Diagnostic yield among cohorts of non-Caucasians (0.42, 95% CI 0.39–0.45) was non-inferior to that of Caucasians (0.42, 95% 0.29–0.55) ($p = 0.98$). WGS studies discovered a higher range of novel genes than WES studies, yet the rate of variant of unknown significance between the two did not differ significantly ($p = 0.78$). Among high-quality studies, clinical utility of WGS (0.77, 95% CI 0.64–0.90) was significantly higher than WES (0.44, 95% CI 0.30–0.58) ($p < 0.01$). Only seven WES studies performed cost-effectiveness analysis, with all concluding WES being cost-saving.

Conclusion: This meta-analysis provides an important update to demonstrate similar diagnostic yield between WES and WGS and higher clinical utility of WGS over WES. It highlights the increased awareness of genomic diversity as illustrated by the rise in publications targeting patients of non-Caucasians in 2019–2021, showing non-inferior diagnostic yields.

Grant References: N/A.

Conflict of Interest: None declared.

EP17.002 Application of droplet digital PCR technology for prenatal carrier screening in spinal muscular atrophy

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Background: Spinal muscular atrophy (SMA) is caused by the absence of exon 7 on the SMN1 gene, resulting in muscle weakness, atrophy, and premature death. The severity of the SMA phenotype is influenced by copies of SMN2, a highly homologous gene with SMN1.

Methods: To compare the effectiveness of carrier screening for SMA between ddPCR and standard MLPA technique and assess the cost-effectiveness of ddPCR as a carrier screening test for pregnant women.

Results: The ddPCR results concordant with MLPA for determination of the copy number of SMN1 and SMN2 exon 7. SMN1 was 100% (51/51), and SMN2 exon 7 was 96% (49/51). For SMN2 exon 7, one sample found inconsistent results between MLPA and ddPCR. MLPA found two copies, while ddPCR found one copy of SMN2 exon 7.

Conclusion: The ddPCR indicates an acceptable level of inaccuracy, showing excellent results compared to the MLPA gold standard. Therefore, ddPCR is expected to be useful for carrier screening and prenatal diagnosis of SMA in pregnant women.

Conflict of Interest: None declared.

EP17.003 Data (gold) mining in genomic databases subsequent to intensive prospective bibliographic monitoring: a substantial diagnostic rate

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Purpose: exome sequencing harbors a diagnostic yield ranging from 25% to 60% in rare diseases and regularly identifies novel causal genes. Data reanalysis has demonstrated a strong efficacy to improve diagnosis (additional diagnostic yield ranging from 10.5% to 32%). We applied a reanalysis strategy based on intensive prospective bibliographic monitoring and direct fast request to large genomic databases of unsolved individuals.

Methods: Since January 2019 we regularly submit five keywords of interest (*intellectual disability (neuro)developmental delay/disorder*)) to PubMed for collecting genes newly implicated in (neuro)developmental disorders and/or novel phenotypes. These genes were searched for, in the Solve-RD 23,000 genome and in-house 5,500 exome databases, to identify causal/candidate variants in unsolved individuals.

Preliminary results: during the first twelve months, by grepping 136 genes, mostly (51 %) published in 5 journals (*Am J Hum Genet, J Med Genet, Brain, Clin Genet* and *Genet Med*), we collected 64 candidate variants in 61 unrelated individuals. After biological analysis, Sanger validation and family segregation, we identified causal diagnosis for 21/136 GREPs (15%) in 24/61 unrelated individuals, and variants of unknown significance for 24/136 GREPs (17%) in 28/61 unrelated individuals. The delay between the publication and the novel diagnostic report was on average of 2.1 months (1m-6m).

Conclusion: intensive prospective medical bibliographic monitoring applied to massive genomic databases of unsolved individuals appears highly efficient and less tedious than complete periodical data reanalysis. We will present the expansion of this strategy in 2019-2023 with ever-growing genetic knowledge and discuss its interest to shorten diagnostic odyssey.

Conflict of Interest: None declared.

EP17.004 Why quality control matters - the impact of quality metrics on sequencing results

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Quality control (QC) steps in any workflow help distinguish between high- and low-quality samples before sequencing. The Agilent TapeStation is an automated electrophoresis instrument that can provide objective analysis of DNA samples and generates a DNA quality metric called the DNA Integrity Number (DIN) which is based on the sample size and concentration. The DIN provides a score of 1 to 10 to each sample analyzed and laboratories can establish their own sample quality criteria to employ in their workflows. The German Cancer Research Center (DKFZ) used the TapeStation to analyze thousands of samples over the past years which can be used to determine an optimal DIN threshold for their sequencing applications. The sequencing data QC metrics for each sample was compared with its DIN value to find what an optimal DIN may be. When a DIN of seven was used as the threshold for determining good sample quality, 16% of samples scored below the DIN threshold but still passed data QC metrics after sequencing, indicating the threshold was set too high for their workflow. When the DIN threshold was set to five, only 5% of samples passing data QC metric scored below the DIN threshold. By lowering the DIN threshold from seven to five, 11% of the samples that would have normally been excluded were successfully sequenced. This study showed that by optimizing the DIN threshold, more samples were determined to be of sufficient quality, saving time and resources by allowing for more samples to be sequenced.

Conflict of Interest: Bettina Strauch: None declared, Carsten Maus: None declared, Tim Butler Agilent employee, Isabell Pechtl Agilent employee.

EP17.005 The clustering analysis of 3D facial models in autism spectrum disorder children is strongly influenced by BMI

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Background/Objectives: Autism spectrum disorders (ASD) is one of the most common neurodevelopmental disorders with an estimated incidence of 1/54. Endophenotyping in these patients is very complicated. Hence 3D analysis („gestalt" analysis) of facial traits is a novel promising method.

Methods: In the presented work we performed a cluster analysis of sets of 3D scans of ASD patients (116) and controls (157) using euclidean and geodesic distances.

Results: In a clustering scenario, where all possible distances between selected landmarks were used, the control group is significantly, but not completely separated from ASD cases. Additionally, in the same scenario, the ASD group was partitioned into several sub-groups and open-mouth subjects tend to cluster together. However, the resulting clusters exhibit significant differences in BMI implying that BMI is the main factor determining clustering structure. When using data cleaned from outliers with only a subset of all distances not correlating with BMI, the cluster structure observed above completely disappeared and the controls were not separated from the ASD cases. This conclusion applies to both euclidean and geodesic distances.

Conclusion: In this presentation, we want to emphasize the confounding effect of BMI on the results of cluster analysis and the necessity to carefully control this factor in similar studies.

Grant references: This work was supported by Charles University Grant (GAUK) number 134121.

Conflict of Interest: None declared.

EP17.006 Development of a cell-line-derived cfDNA Prostate Cancer NGS reference material for liquid biopsy and MRD

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Background As prostate cancer is one of the most prevalent cancers in males globally, it is a prime target in oncology diagnostics for improving accuracy of prostate cancer testing assays. Recent advances in the liquid biopsy field enable the detection of variants from small amounts of fragmented DNA, at low allelic frequencies using highly quantitative methods, such as Droplet Digital PCR (ddPCR) or Next Generation Sequencing (NGS) assays.

To support the development of these assays, Horizon has developed a cfDNA Prostate Cancer Panel. The panel is highly

characterized, cell-line-derived and reproducible, designed to control variabilities in each step of the workflow. The product contains 15 clinically relevant mutations involved in the prostate cancer disease progression in cfDNA format.

Methods: Cell-lines that were engineered, using Horizon CRISPR technology to express the variants of interest were used to produce clonal cell population for each mutation.

The genomic DNA was extracted from the cell lines, fragmented to mimic patient sample DNA and blended in defined ratios to yield a multiplex blend containing a range of mutations at varied allele frequencies. All 15 variants were confirmed by Sanger Sequencing and ddPCR.

Results: Respective allelic frequencies were assessed by quadruplicate ddPCR analysis for each variant on the Bio-Rad QX200 ddPCR platform. The results confirmed all the claimed variants at expected allelic frequencies with high reproducibility.

Conclusion: This study confirmed the use of Horizon's Prostate Panel cfDNA as a valuable control for achieving confidence in all the stages of development and validation of liquid biopsy assays.

Conflict of Interest: Mila Pavlova Horizon Discovery, bernice freeman Horizon Discovery, ilaria farace Horizon Discovery.

EP17.007 A comparison of two approaches for autosomal recessive diseases carrier screening

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Background/Objectives: Carrier screening for four autosomal recessive diseases (cystic fibrosis, phenylketonuria, sensorineural hearing loss, alpha-1-antitrypsin deficiency) was performed using real-time PCR (RT-PCR) and next generation sequencing (NGS). RT-PCR screening was performed using a panel consisting of the 115 most frequent pathogenic variants of the *CFTR*, *PAH*, *SERPINA1*, *GJB2* genes, according to literature data based on affected patients. During NGS screening, the sequencing of all exons of four genes was performed. Variants pathogenic or likely pathogenic according to ClinVar, CFTR2, or BIOPKU databases, Russian cystic fibrosis register were analyzed. The aim was to compare these two approaches to carrier screening.

Methods: RT-PCR was performed with QuantStudio 12 K Flex ($n = 1221$); NGS with NextSeq 550 ($n = 1289$). The study was performed on the ESSE-RF population sample and on a cohort of patients with different diagnoses, excluding the studied disorders. Related participants were excluded. Statistical analysis was performed using R 4.1. The two-sided Fisher's exact test was used. A p-value < 0.05 was considered significant.

Results: The carriers' fraction detected in different samples is presented in Table 1. A statistically significant difference was found in the percentage of detected carriers only in *PAH* ($p = 0.009$).

Table 1. Carriers' fraction detected in different samples

Approach	*PAH*	*GJB2*	*SERPINA1*	*CFTR*
RT-PCR	2.05%	6.55%	4.18%	2.05%
NGS	3.80%	8.38%	4.50%	3.18%

Conclusion: Thus, RT-PCR showed comparable performance to that of NGS, and, combined with a lower cost, it can be a competitive tool for carrier screening when an extended variant list is used.

Conflict of Interest: None declared.

EP17.009 Auditing genetic testing for aortopathy at the genomics laboratory in Royal Brompton & Harefield Hospital

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Introduction: Familial aortopathies can be syndromic or non-syndromic. With factors e.g. family history or early onset, best practice is to refer for genetic testing, usually via a multigene next-generation sequencing panel.

Patients carrying deleterious variants in known aortopathy-associated genes may be more prone to aortic dissections. Timely genetic testing guides management to prevent further complications and enables some risk prediction in family members. However, identifying variants of unknown significance (VUS) cannot meaningfully guide management and causes uncertainty to patients, family, and healthcare professionals.

Objectives: To examine the number and nature of aortopathy testing referrals in the UK, and compare identification rates of pathogenic variants, likely pathogenic variants, and VUS between the 63-gene Vasculopathy & Aortopathy (V&A) and 34-gene R125 panels in a laboratory dedicated to aortopathy gene testing (Royal Brompton & Harefield Hospitals, UK).

Materials and Methods: Data from July to December 2019 ($n = 82$) when the V&A panel was used was compared to that from February to July 2021 ($n = 110$) after switching to R125. A chi-squared comparison of proportions test was conducted on the percentages of likely pathogenic/pathogenic and VUS.

Results: The R125 panel detected fewer VUS (9.1%) compared to the V&A panel (20.7%). The difference between pick-up rates of likely pathogenic/pathogenic variants between the 2 panels was not statistically significant.

Conclusion: The R125 panel identified fewer VUS than the V&A panel, while preserving identification rates of (likely) pathogenic variants. This audit highlights the importance of curating diagnostic gene panels to only include genes with strong evidence of association.

Conflict of Interest: None declared.

EP17.010 Comprehensive detection of imbalances in a mosaic state with Array-CGH

Michael Willam[1], *Anna Bertoni*[1], *Christian Schwarm*[1], *Gesine Kitschun*[1], *Gisela Lorenz*[1], *Ira Schwaab*[1], *Stephanie Schwarz*[2], *Berit Kerner*[1]

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Germline deletions of the *CHEK2* gene on chromosome 22q12.1 are associated with a moderately increased risk of carcinoma, particularly breast, prostate, and colon carcinoma. In addition, major somatic deletions of the *CHEK2* gene and other genes on the long arm of chromosome 22 have been described in tumor tissue of patients with breast and colon carcinoma. The detection of those imblances, however, depends on the amount of affected cells, the used method and its sensitivity.

To detect variants in a mosaic state, we performed Array-CGH on artificially created mosaics and we were able to securely detect a 25% mosaic.

Here, we investigated a 46-year old woman affected with triple negative breast cancer. NGS analysis for breast cancer associated genes was performed on genomic DNA derived from EDTA-blood. Our CNV pipeline analysis from NGS data showed a reduced coverage within the *CHEK2* gene. In addition, MLPA analysis detected an approximately 25% reduced signal intensity and therefore confirmed the suspected deletion. To further determine the precise breakpoints of this deletion, we performed Array-CGH and found 2 adjacent deletions on chromosome 22 (one affecting the *CHEK2* gene) with a $\log_2$ ratio of approximately -0.3, which confirms a deletion in a mosaic state.

In summary, we are able to comprehensively detect imbalances in mosaic state by Array-CGH.

Conflict of Interest: None declared.

EP17.011 determining the diagnostic yield of genomic testing in interstitial lung disease in Ireland

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Idiopathic pulmonary fibrosis (IPF) is a fatal, scarring, progressive and irreversible interstitial lung disease. When IPF occurs in more than one first-degree relative it is termed familial pulmonary fibrosis (FPF). Patients with connective tissue disease (CTD) can develop inflammation and scarring in their alveolar cells, which may progress to interstitial lung disease or pulmonary fibrosis. We compared the diagnostic yield of genomic testing when applied to IPF, FPF and CTD and catalogued the genetic landscape of pulmonary fibrosis mutations in Ireland. We recruited and consented approximately 115 patients to the study via the Respiratory and Rheumatology clinics at Beaumont Hospital, Dublin. To date, we have analysed sequence data from 61 patients; 13 with IPF, 12 with FPF and 36 with CTD-ILD. Whole-exome data was obtained from blood-derived DNA and processed using a GATK-V4.2 bioinformatics pipeline. A diagnostic assessment of each variant was conducted according to the criteria of the American College of Medical Genetics and Genomics. We identified a pathogenic *RTEL1* variant [NM_001283009:exon30:c.C2920T] in a family with FPF. We identified a variant of unknown significance in *RTEL1*[RTEL1:NM_001283009:exon14:c.C1189G:p.Q397E] in another family with FPF. No pathogenic/likely-pathogenic variants were identified in the IPF and CTD datasets, although we did identify variants of unknown significance in *RTEL1*, *SFTPA1*, *NAF1* and *ZCCHC8*. These results indicate a diagnostic yield for FPF of 12.5% in the Irish population, although the sample size analysed to date is small. A lack of pathogenic variants in the IPF or CTD groups is consistent with the literature.

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Conflict of Interest: None declared.

EP17.012 Procedure used for flexible scope accreditation under ISO15189 guidelines of a panel of hereditary cancer genes by massive next-generation sequencing (NGS)

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Background: Due to the constant evolution of clinical laboratories and the availability of large panel of NGS genes, it is necessary to describe a methodology in order to accredit these studies for flexible scope according to ISO15189 guidelines, allowing to include in the accreditation the study of new genes without prior approval by Spanish National Accreditation Body (ENAC).

Aim: Verification of a 70-hereditary cancer genes NGS panel.

Methods: Verification study of the Hereditary Cancer Solution panel (Sophia Genetics). For this study, 24 samples (clinical samples containing genetic alterations previously characterized by Sanger sequencing and MLPA and reference samples) were sequenced in two different MiSeq runs. The variants obtained were analyzed using Sophia DDM analysis software. Read quality, variant detection capacity and reproducibility/repeatability were verified.

Results: Verification of reading quality:

	% OBTAINED	% DESIRED
%Mapped readings	>99%	≥95%
Coverage(200X)	>99%	≥90%
coverage uniformity	0%-0,11%	0-1%
%On-target	>80%	≥70%

Evaluation of the detection capability of SNVs and INDELs:

100% of SNVs and 96% of INDELs were detected (a threshold of >95% has been established)

Evaluation of reproducibility/repeatability:

High reproducibility/repeatability of the results.

Conclusions: The verification of the panel, it has been carried out satisfactorily.

These results corresponding to the analytical part, together with the completion of a check list where changes in the pre-analytical, post-analytical and Laboratory Information System (LIS) are revised, allowed us to define a systematic for a flexible scope accreditation following ISO15189 guidelines.

Conflict of Interest: None declared.

EP17.013 Clinical diagnostic yield of trio-WES of congenital disorders in population enriched by consanguineous marriages

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Exome sequencing has accelerated diagnosis of patients with rare heterogeneous Mendelian diseases, in particular neurodevelopmental disorders with added value of analyzing proband and his/her parents (trio-WES analysis). We investigated 97 individuals meeting the following criteria: 1. Developmental delay DQ/IQ < 54 2. Global developmental delay DQ/IQ/ < 70 with epilepsy or a major congenital anomaly. Trio-WES was performed in 95 families and additional two families were analyzed with one parent only. Bioinformatics analysis was aided by Genoox platform (version 2022.4).

Diagnostic yield reached 49.5% (47/97 unrelated families), two families had suspicious variants in two novel candidate genes, and functional analysis is pending. Consanguinity was reported in 43 families. Diagnosis of autosomal recessive conditions was found in 22 families similar to dominant de novo pathogenic alleles in 21 families, in addition, two families had inherited dominant condition, 1 X-linked and 1 mitochondrial inheritance.

Surprisingly, there were no significant differences in the diagnostic yield between consanguineous versus non-consanguineous families, 46% and 50% respectively. Notably, 59.5% of the detected pathogenic variants were extremely rare and absent from databases including ClinVar, GnomAD or relevant literature. Two additional likely pathogenic variants were located in two novel genes which were confirmed as causative by on-going functional studies.

The disparity between the actual versus the expected diagnostic yield rate in consanguineous populations is puzzling. The unmet enrichment of AR genes involved in the genetic background of affected individuals in this population might be explained by cohort bias, mutations located in non-coding sequences and other rare genetic mechanisms.

Conflict of Interest: None declared.

EP17.014 A general framework for validation of variant detection approaches in clinical whole genome sequencing

Jamie Ellingford[1], *Susan Walker*[1], *Eva Serra*[1], *Gabriel Aldam*[1], *Mark Doherty*[1], *Liam Abrahams*[1], *Kevin Savage*[1], *Conor Proud*[1], *Javier Lopez*[1], *Genomics England Research Consortium*[1], *Matt Brown*[1], *Rachael Mein*[2], *Zandra Deans*[3], *Joo Wook Ahn*[4], *Sue Hill*[2], *Richard Scott*[1], *Augusto Rendon*[1], *Dalia Kasperaviciute*[1]

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Background/Objectives: Whole genome sequencing (WGS) has become established as a frontline diagnostic test for individuals with specific rare disease in England. The utility of WGS is dependent on the performance of variant detection methods and the types of variants that can be detected, which are continuously evolving. Updating clinical bioinformatics pipelines to include new techniques and utilities, whilst maintaining standard-of-care, is challenging as it requires extensive testing across diverse variant types.

Methods: We define and quantify essential assessments to inform upgrades to clinical germline WGS bioinformatics pipelines. These approaches are applied across a wide range of areas and variant types to investigate an exemplar upgrade, including: sensitivity/concordance against standard-of-care diagnostic tests; variant precision and reproducibility; external quality assessment; and defining appropriate test sets to assess new utilities.

Results: WGS datasets from >1500 families were included in an exemplar validation (from DRAGENv3.2 to DRAGENv4.0 software) using our general framework. We noted improved: alignments in clinically relevant genes (new complete coding-region coverage ≥20x, $n = 18$); copy number variant (CNV) inheritance status (previously unavailable); *de-novo* indel classification (70% improvement for problematic cases, $n = 10$); large mosaic CNV detection (100% concordant, $n = 3$); and targeted variant calling (e.g. *SMN1/2*).

Conclusions: We have developed a comprehensive and transparent framework for the assessment of alignment and variant calling tools for germline WGS, which informs clinical utility and application for multiple variant types. This general framework can be utilised to benchmark software upgrades, and ensure clarity and standardisation of the evidence-base to inform diagnostic germline WGS approaches.

Grant References: None.

Conflict of Interest: None declared.

EP17.016 Genetic diagnostic approach in nephrotic syndrome to decide on treatment strategy

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Background/Objectives: Nephrotic syndrome (NS) is divided in two categories, steroid-sensitive (SSNS) and steroid-resistant (SRNS), according to response in treatment. Extended WES analysis is necessary for appropriate clinical management.

Methods: In the case presented, the individual tested developed nephrotic syndrome at the age of 19. He took treatment with cortisone, but subsequently was not responding to therapy. At the age of 39 he was tested by Whole Exome Sequencing (WES). WES was performed using the Ion Proton System (Thermo Fisher Scientific). Raw data alignment and variant calling was performed by the manufacturer software and the generated bam file was analyzed by the Ion Reporter software for variant annotation, while in-house analysis and filtering of the annotated vcf file was applied.

Results: The genomic analysis in the filtered variants list showed the heterozygous mutation c.1013_1014ins (p.Val339-Glyfs*) in gene *NPHS2*, which is Likely Pathogenic according to ACMG. *NPHS2* is associated with SRNS type 2. Variant analysis was extended in the non-filtered vcf file, where the heterozygous polymorphism c.686G>A (p.Arg229Gln, p.R229Q) in *NPHS2* was also detected.

Conclusion: The copresence of the mutation c.1013_1014ins with polymorphism p.R229Q in *NPHS2*, in compound heterozygosity, confirms the clinical diagnosis of steroid-resistant NS, in the present case. Coexistence of p.R229Q with a pathogenic mutation in *NPHS2* has been already described in patients with adult-onset SRNS. Therefore, patients with clinical diagnosis of NS should be tested by WES prior treatment and bioinformatics analysis should be performed without filtering, in order to define NS type and avoid unnecessary administration of cortisone.

Conflict of Interest: Vasiliki Chini Head of Clinical Services in InterGenetics - Medicover company, Sofia Sevastidou Full-time Scientific staff in InterGenetics - Medicover, Nikoleta Dounavi Full-time Scientific staff in InterGenetics - Medicover, Sofia Karapanou Full-time Scientific staff in InterGenetics - Medicover, Constantinos Pangalos Clinical Geneticist in InterGenetics - Medicover, Voula Velissariou Scientific & Managing Director in InterGenetics - Medicover.

EP17.017 Mosaicism for a variant in PTCH1 causing Nevoid Basal Cell Carcinoma Syndrome (Gorlin Syndrome)

Inger Norlyk Sheyanth[1], *Mette Sommerlund*[2], *Hanne Vinter*[3], *Jannie Assenholt*[4], *Hans Gjørup*[5], *Lone Sunde*[1], *Malene Lundsgaard*[1]

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Introduction: Nevoid Basal Cell Carcinoma Syndrome (NBCCS) is an autosomal dominant tumor pre-disposing disorder associated with basal cell carcinoma (BCC), odontogenic keratocysts and childhood medulloblastoma. Pathogenic variants in *PTCH1*, *SUFU* or *PTCH2* are found to be the cause in the majority of patients meeting the diagnostic clinical criteria.

Case: A 21-year-old man was referred to the Department of Dermatology because of occupational hand eczema. The patient had previously been operated for an infundibulocystic BCC on the back at the age of fifteen and presented with mild facial NBCCS-like features. Imaging suggested three mandibular cysts and one cyst in the maxilla. Histopathology showed an inflamed odontogenic keratocyst.

None of the patient's relatives had similar findings.

Methods: Massive parallel sequencing of the exons of *PTCH1, SUFU, PTCH2*.

Results: No pathogenic variants were detected in DNA from blood. In DNA from the BCC and from an odontogenic keratocyst, heterozygosity for the pathogenic variant in *PTCH1*: c.2917C>T p.(Gln973*) (NM_000264.5) was identified, along with heterozygosity for one other *PTCH1* variant in the BCC and another in the cyst. The variant *PTCH1*:c.2917C>T p.(Gln973*) was detected in 0 out of 412 reads in DNA from blood.

Conclusion: In patients suspected of hereditary predisposition to disorders where the two-hit mechanism is involved, and with no pathogenic variant detected in blood, sequencing should be repeated in two or more samples from affected tissue.

Conflict of Interest: Inger Norlyk Sheyanth MD, Department of Clinical Genetics, Aalborg University Hospital, Denmark, Mette Sommerlund MD, PhD, Department of Dermatology, Aarhus University Hospital, Aarhus, Denmark, Hanne Vinter Department of Pathology, Aarhus University Hospital, Aarhus, Denmark, Jannie Assenholt MOMA, Aarhus University Hospital, Aarhus, Denmark, Hans Gjørup Center for Oral HEALTH in Rare Diseases, Dept of Dental and Craniofacial Surgery, Aarhus University Hospital, Lone Sunde Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark, Malene Lundsgaard Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark.

EP17.018 Diagnostic yield of karyotyping and aCGH in the genetic diagnostics in patients with developmental disorders

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In Germany patients with developmental disorders (DD) are usually analysed by karyotyping and comparative genomic hybridisation array (aCGH). In the literature, the diagnostic yields of these methods are 3% and 10%, respectively. In this study, we investigated the diagnostic yields of both methods in our lab for 2021 and compared them with the literature. This data is a part of a bigger study which aims to compare the diagnostic yield of standard diagnostics with genome analysis.

All reports of patients with DD, who were tested at our institute in 2021, were sighted and categorised by phenotype. Patients were divided in a group with isolated DD and one with additional features like dysmorphism or malformations. The diagnostic yield has been calculated as the number of likely pathogenic and pathogenic variants divided by the total number of cases.

There were 10 (likely) pathogenic findings in 263 patients, tested by karyotyping, which results in a diagnostic yield of 3,8%. 425 patients were analysed by aCGH, of which 43 had a VUS and 56 a (likely) pathogenic copy number variation. Thus, the diagnostic yield was determined to 13,17%.

The diagnostic yields of karyotyping and aCGH are comparable to the literature data. In the outlook we are planning to compare the summed up diagnostic yields of karyotyping, aCGH and exome sequencing compared to genome analysis.

Conflict of Interest: None declared.

EP17.020 Difficult genetic diagnosis of Diamond-Blackfan Anemia after bone marrow transplantation: DNA from buccal swabs can be almost entirely of donor origin

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Background: A woman consulted at 8 weeks of her second pregnancy requesting prenatal diagnosis. Her 11-year-old son had severe corticosteroid-responsive anemia and bilateral flat thenar eminence and had been diagnosed with Diamond-Blackfan anemia (DBA) at age 4. The mother had iron supplementation since youth. In 2015 mother and son were reported as heterozygous for the pathogenic variant *RPL11*:c.443_444dup, confirming diagnosis of DBA7.

Methods and results: The variant was retested by PCR and Sanger sequencing. The mother was confirmed as heterozygous but the variant was not detected in her son. QC revealed that the DNA sample was from a female, so a new sample was requested. At resampling, the mother reported that the boy had a bone marrow transplantation in 2019 from a female donor.

The variant was consequently retested from a buccal swab but again the sequence was normal. Testing of hair follicle DNA finally confirmed that the boy was a heterozygous carrier of the described *RPL11* variant.

Conclusions: Cheek swabs are rich in epithelial cells and are a common material for genetic testing when blood is not considered appropriate. In this patient after bone-marrow transplantation, the DNA from the cheek swab was almost entirely of donor origin (estimated ≥90%) and the pathogenic variant was not detected.

Consequently, hair follicle DNA should be considered as the preferred "constitutional" sample after bone-marrow transplantation to avoid potential false-negative results.

Finally this family emphasizes the considerable variability of expression of DBA which can complicate diagnosis and genetic counselling related to reproductive options.

Conflict of Interest: None declared.

EP17.021 Implementation of a DNA-methylation based method for pediatric medulloblastoma subgroup classification and clinical practice

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Piedad Alba-Pavón[1], Olatz Villate[1], Itziar Astigarraga[1;5;6], Nerea Bilbao Aldaiturriaga[1;7], Idoia Martin-Guerrero[1;8]

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Background/objectives: Pediatric medulloblastoma (MB) is a very heterogeneous tumor at clinical and molecular level. The development of molecular techniques has enabled to classify MB into four molecular subgroups: WNT-activated, SHH-activated, Group 3 and Group 4, all of them with different mutational and methylation profiles. As each subgroup is associated with different prognosis, the stratification of each patient by risk is crucial to personalize the treatment. The objective was to select a method for the subclassification of MB patients to be applied in clinics based on DNA methylation.

Methods: A systematic search of literature was carried out to choose a suitable methodology using (Pediatric medulloblastoma) and (methylation). The selected methodology was tested in 62 patients. The mutational status of MB related genes (*CTNNB1, APC, TP53, PTCH1, SUFU, SMO, PALB2 and BRCA2*) was performed.

Results: Methylation analysis was able to classify 90% of the samples, of which 5% belonged to WNT-MB, 19% to SHH-MB, 11% to Group 3 and 55% to Group 4. Mutations in *CTNNB1* were found in all WNT-MB samples, whereas in SHH-MBs *TP53* mutations were found in one third of the cases. Centralized molecular review added relevant information in the 83,9% of the cases having clinical impact in terms of diagnosis, prognosis, therapeutic changes and in detection of cancer predisposition syndromes.

Conclusion: Methylation analysis is useful for subclassifing MB by subgroup and adding clinical information, allowing for better risk stratification and improve clinical management of MB patients.

Grant references: Asociación Pablo Ugarte APU (BC/A/14/015), and EiTB Media project (BIO20/CI/013BCB).

Conflict of Interest: None declared.

EP17.022 The utility of clinical exome sequencing in a South African infant cohort

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Introduction: Mendelian disorders, caused by genetic changes in a single gene, significantly affect infant mortality and hospitalisation. Their diagnosis remains a considerable challenge in infant medical management. Clinical exome sequencing (CES) has proven successful as a first- and second-tier diagnostic tool, however, limited investigations into its efficacy in African populations, which hold higher background genetic variation and in which the burden of genetic diseases is largely unknown, have been performed. Our study aimed to develop a clinical WES workflow for the diagnosis of Mendelian disorders in ill infants in the South African state healthcare system and assessed its utility as a diagnostic tool.

Methods: We recruited a cohort of 35 ill infants suspected of having a genetic condition from two state hospitals in Johannesburg, South Africa, with most participants referred for genetic testing by a neonatologist or paediatrician. We performed CES and analysed data using 3 filtering approaches, including a curated gene panel implicated in neonatal- and early childhood-onset conditions, and genes identified to have a higher variant frequency in African populations.

Results: We reported a diagnostic yield of approximately 12% in this cohort; including a case of *NPHS1* nephrotic syndrome, a *COL2A* collagen disorder and 2 cases of severe congenital myopathy - one of these being *STAC3*, an African-specific condition.

Conclusion: Our study demonstrates the clinical utility of CES in the South African healthcare context to reduce the diagnostic odyssey in difficult-to-diagnose patient groups, allowing for improved clinical management of patients and families.

Grant: National Research Foundation Thuthuka 1903204240019

Conflict of Interest: None declared.

EP17.023 Examination of difference in fetal fraction for male and female fetuses

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Background: The fetal fraction (FF) is the key parameter in non-invasive prenatal testing (NIPT). As combination of biological factors and bioinformatics algorithms it provides test sensitivity for fetal aneuploidy and serves for quality control. Different NIPT assays employ different approaches for FF estimation and different genomic regions are utilized. It is a vital issue whether it is possible to draw out more information from FF or increase test efficiency.

Methods: At previous step we studied difference associated with fetal sex and it helped to increase test efficiency by means of sample management. We have doubled our set of samples to verify observed patterns and analyzed 40 000 selected clinical NIPT results. For female fetuses an advanced method for FF measurement based on differentially methylated genomic regions (FF-QuantSC) was employed.

Results: Previously we have revealed FF of female fetuses being lower compared to male and that rate of inconclusive results was much higher (3.63% vs. 0.39% of cases this time). Furthermore, for female fetuses there was a density spike just above the threshold (3.5%-3.6% FF). We are searching for the cause and surmise that it is an intra-sample polymorphism. Chances of gaining a successful test result from the same plasma aliquot and FF variability within the routine protocol were also estimated.

Conclusion: Confirmed findings suggest lowered expediency of repetitive testing in routine NIPT for female fetuses. Test efficiency can be increased taking into account our data by adjustments either in bioinformatics algorithm or in sample management.

Grant references:

Conflict of Interest: None declared.

EP17.024 An advisory on skin xenografts in single cell RNA sequencing

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Background/Objectives: Single-cell RNA sequencing (scRNA-seq) is a relatively novel technique that has established itself as a vital method to acquire detailed information on RNA expression profiles in individual cells or tissues of interest to better understand their biology and function. Our goal was to explore the applicability of scRNA-seq in investigating effects of antisense oligonucleotide-mediated exon skipping in human skin xenografts.

Methods: Xenografts were generated from patient keratinocytes and fibroblasts harboring COL7A1 null variants on immunocompromised nude mice. After harvesting, xenografts were dissociated for use in scRNA-seq. Tissue dissociation is the process of obtaining live single cells in suspension from whole tissue. A dissociation protocol should be optimized for each type of tissue, and yield and viability of the resulting cells verified.

Results: Our optimized dissociation protocol resulted in acceptable cell counts and viability in control skin biopsies and xenografts. As expected, xenograft sequencing data showed a mix of murine and human transcriptome profiles. However, after optimization, and unlike the mouse cells, all human cells presented as low-quality reads. We attributed this to preferential dissociation of mouse tissue and dead cells in the grafts.

Conclusion: We present an advisory on tissue dissociation of xenografts for single cell applications. Dissociating both murine and human cells from xenografts is required for successful processing in the scRNA-seq pipeline. However, we found that both tissues require different dissociation protocols. scRNA-seq from human xenografts may only be possible after careful selection of xenograft tissue only.

Grant References: Dutch Butterfly Child Foundation, Zeldzame Ziektenfonds, ZonMW (90715614).

Conflict of Interest: None declared.

EP17.026 Single center experience with diagnostic whole genome sequencing in 70 patients with rare diseases

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Background: Whole genome sequencing (WGS) is about to become routine in rare disease diagnostics. In addition to a streamlined lab workflow, WGS is free of enrichment biases, lowering the risk of missing variants at difficult to enrich loci and allows extensive variant detection, including structural variants.

Methods: Seventy patients, who presented with neurodevelopmental disorders ($n = 49$), tumor risk syndromes ($n = 3$) or other syndromic disorders ($n = 18$) received WGS. Half of them had previous genetic work up. WGS was done with a 40x median coverage and 150nt paired-end mode. Data was processed with the Illumina Trusight Software Suite.

Results: We identified pathogenic variants in 16 patients, 10 of which previously received WES, increasing the diagnostic yield by 28%. Ten pathogenic variants were either intronic SNVs, CNVs or structural variants that would not have been discovered by WES. WGS analysis was especially expedient in patients with a focused clinical question, e.g. bilateral retinoblastoma or CdL syndrome. Furthermore, WGS enabled the diagnosis of cartilage-hair-hypoplasia with biallelic variants in the RNA-coding RMRP gene that is not enriched in commonly used exome kits. Importantly, WGS enabled exclusion of a recessive disorder in patients with one heterozygous pathogenic variant, in which we could demonstrate the absence of additional rare variants, including intronic regions and UTRs.

Conclusion: Our results demonstrate the diagnostic advantage of WGS by increasing the diagnostic yield by 28%. We also highlight the importance of the thorough clinical evaluation prior to genetic testing. Future characterization of non-coding variants will further improve the diagnostic yield of WGS.

Conflict of Interest: None declared.

EP17.027 Long read sequencing for clinical routine diagnosis of a recessive disease – Proof of concept

Xavier Vanhoye[1], *Pascal Mouty*[1], *Nicolas Bargues*[1], *Nicole Couprie*[1], *Evelyne Fayolle*[1], *Vanna Geromel*[1], *Laure Raymond*[1], *Mohamed Taoudi*[1], *Jean-Francois Taly*[1]

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Long-read sequencing technology is widely used in many research areas and start to be a useful tool in clinical diagnosis mainly regarding infectious disease. Despite the recent improvements of the both wet lab and dry lab aspect, there is nowadays no application in clinical diagnosis for constitutional human disease. However, long read sequencing could be a valuable tool for molecular geneticist, mainly for intronic variant detection and for variant phasing (indispensable information for recessive diseases). As a proof-of-concept, we choose to implement in clinical diagnosis routine a long-read sequencing workflow for MEFV gene. Variants in this gene are responsible of familial Mediterranean fever, a recessive disease with a high prevalence.

We propose a Nanopore-based workflow: amplification of the whole MEFV gene with a long-range PCR, library preparation with the Native Barcoding kit, sequencing on a GridION and in-house pipeline for bioinformatics. In order to reach the ISO15189 certification, we validate extensively this new workflow versus our previous short-reads workflow on 39 samples from patients and 3 samples from external quality assessment scheme.

Among those 42 samples, reproducibility, repeatability, sensibility, and specificity were measured and satisfied the ISO15189 requirements, allowing this workflow to be used in clinical diagnosis routine. To date, this analysis was performed on 69 patients since October 2022. No failure was reported. 4 patients were compound heterozygous for likely pathogenic or pathogenic variant. With the previous method, segregation analyses in parents would have been required for these 4 patients, prolonging time and increasing costs for definitive results.

Conflict of Interest: Xavier Vanhoye Eurofins Biomnis, Pascal Mouty Eurofins Biomnis, Nicolas Bargues Eurofins Biomnis, Nicole Couprie Eurofins Biomnis, Evelyne Fayolle Eurofins Biomnis, Vanna Geromel Eurofins Biomnis, Laure Raymond Eurofins Biomnis, Mohamed Taoudi Eurofins Biomnis, Jean-Francois Taly Eurofins Biomnis.

EP18 Bioinformatics, Machine Learning and Statistical Methods

EP18.004 Regulation of tumor microenvironment (TME) of pancreatic cancer: a mechanistic insight for potential tumor immunotherapeutics

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Background: Pancreatic cancer(PC) is one of the most lethal cancer types worldwide. Although several chemotherapy combinations are applied, T-cell dysfunction in immunosuppressive tumor microenvironment(TME) limits the success of therapeutics. Tumor-derived exosomes(TEXs) are nanovesicles involved in cancer progression and immune-tolerance. The role of exosomes in TME regulation remains to be elucidated. To obtain molecular insight of TEX in TME regulation in PC, comprehensive in silico analyses performed.

Methods: GSE115831 dataset (RNA-Seq data from donor T-lymphocytes and BxPC3(PC cell-line) derived exosomes) analyzed using GREIN platform. 122 differentially expressed genes were uploaded to iLINCS for pathway analyses. By using GEPIA2, gene expression profiles examined in PC and control samples from TCGA and GTEx databanks. Survival graphs depicted by Kaplan-Meier Plotter.

Results: By analyzing RNA-Seq data, we found 122 differentially expressed genes between PC-derived exosome stimulated and control groups. Reactome and KEGG pathway analyses revealed HSF1 dependent heat shock response which contains *HSPA1A* and *HSPA1B* genes that upregulated in PC-derived exosome treated group. These genes were also upregulated in Pancreatic Adenocarcinoma patients from TCGA and GTEx datasets. Importantly, worse survival was observed in PC patients expressing high levels of *HSPA1A* and *HSPA1B*. Overall, PC's immunosuppressive TME could be regulated through exosome mediated HSF1 pathway.

Conclusion: To our knowledge, this is the first study to reveal HSF1-dependent regulation of TME through TEX signaling in PC.Given the crucial role of HSF1 as potential cancer biomarker and therapeutic target in PC, it is noteworthy to further examine the role of exosomal *HSPA1A* and *HSPA1B* in experimental setup.

Conflict of Interest: None declared.

EP18.005 TUBA1C: a potential biomarker for Type II Diabetes Mellitus (T2D) associated cancer

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Background: Cancer is a leading cause of death worldwide. There is an urgent need for the development of biomarkers that could specifically differentiate malignancies from other complex diseases including diabetes. Accumulating evidence suggests that diabetes is a decisive risk factor for the development of pancreatic and liver cancer, although this relationship is complicated and requires more research for underlying molecular mechanisms. This work aims to investigate potential biomarkers for T2D-associated liver/pancreas cancers.

Methods: Differentially expressed genes (DEG) between T2D patient pancreas beta cells and healthy cells were analyzed using single-cell RNA-sequencing data from the GSE81608 dataset. Matched differentially expressed target gene profiles were also evaluated in liver cancer(hepatocellular carcinoma-HCC) and pancreas cancer(PC) patients using TCGA and GTEx datasets. Target gene's prognostic profile was evaluated using the GEPIA2 platform. The effects of DEG on Survival assessed by Kaplan Meier plotter. Related pathway analyses and possible targets were studied by Reactome and KEGG databases.

Results: 48 genes found to be DEG in T2D patients compared to controls. These genes were analyzed in HCC and PC samples(among the top 500 genes) and only 1 gene match was found (*TUBA1C*) (FDR < 0.05). The survival data also showed that *TUBA1C* upregulation in tumor samples was associated with poor overall survival(HCC, PC) and with increased expression from T2D to the malignant stage.

Conclusion: Tubulin Alpha 1c-*TUBA1C* encodes for microtubules and elevated levels of this gene could be an early sign of cancer progression in T2D patients. Overall, it is noteworthy to further examine this gene in detail.

Conflict of Interest: None declared.

EP18.006 A multi-caller pipeline to maximize the output of somatic exome sequencing analysis

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Variations at the nucleotide level in the DNA are usually studied through exome regions, since most variants with known effect are found there. Whole-Exome Sequencing (WES) and bioinformatic programs are used to obtain useful information from it.

The continue increasing of bioinformatic programs available to analyze data obtained from WES and the need of some basic informatic knowledge to use them is a problem researchers are facing, making this type of analyses complex and very time consuming.

As a result from that, we designed a pipeline containing some of these programs in order to help researchers obtain useful data from the exomes in an easier and faster way, while at the same time, increase the confidence and the information obtained with this method by using more than one variant caller. To achieve this objective, we've reviewed the literature regarding software's for analysis of somatic exomes. We found the programs that were the best for each of the different tasks of these pipeline and we also confirmed that by using multiple callers we obtained more variants than using only one, given that different callers use different algorithms which results in different variants called. Furthermore, we prioritized the variants obtained according to the number of callers that found the variant and its quality, what allowed us to better select only the variants of interest. Using benchmarked data, available in the Genome in a Bootle project, and real world data we verified the improvements our pipeline has when compared with other approachs.

Conflict of Interest: None declared.

EP18.007 Machine learning and system biology application to scRNA-seq data analysis

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Background/Objectives: Commonly used methods for scRNA-seq

data analysis allows for precise clustering and cell typing of blood and nerve tissue cells. However, for connective tissues standard data processing appears insufficient. Machine learning and neural networks ensures comprehensive data analysis for cells from subcutaneous adipose tissue.

Methods: In addition to canonical Cell Ranger and the R-package Seurat pipelines we applied machine learning and neural networks. For clustering, we used R-package based on scDeepCluster neural networks, which allows the iterative feedback without adaptive adjustments to feature space; for cell typing - ScCapsNet (CapsNet) neural network. The scCapsNet model extracts specific features (gene expression levels) providing new cell type identification. For precise cell typing, the scCapsNet algorithm mixes the RNA expression levels of two different cell types and uses the scCapsNet model trained with non-mixed data to predict specific cell types in the mixed data.

Results: scDeepCluster application for analysis of MSCs allowed us to identify 8 clusters instead of 6 clusters detected with graph-based clustering algorithm followed by Louvain Modularity Optimization in Cell Ranger count. Using scCapsNet, we revealed additional small clusters with distinctive gene expression profiles and related biochemical processes.

Conclusion: Application of machine learning and neural networks ensures complete disclosure of biological insights in contrast to limited application of standard scRNA-seq data analysis algorithms.

Grant Reference: Russian Foundation for Basic Research (RFBR) 20-015-00402.

Conflict of Interest: None declared.

EP18.008 BinDel: software for detecting clinically significant microdeletions from low-coverage WGS-based NIPT

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Background/Objectives: Clinically pathogenic chromosomal microdeletions cause fetal developmental deficiencies and severe genetic disorders such as DiGeorge or Angelman syndrome. For pregnant women younger than 35 years, the overall prevalence rate of fetal microdeletions is estimated to be approximately one in 2,500 pregnancies, which is more frequent than Down syndrome. Since there are no reliable biomarker or ultrasound screening smethods for microdeletion risk detection for first-trimester screening, a reliable Non-Invasive Prenatal genetic Testing (NIPT) based fetal microdeletion risk screening is crucial.

Methods: We present BinDel, a novel region-aware microdeletion detection R software package developed to infer clinically relevant microdeletions risk from low-coverage whole-genome sequencing NIPT data. We estimated BinDel's accuracy on low-coverage WGS NIPT samples with in-silico-induced fetal microdeletions, known fetal microdeletion samples, and clinically validated aneuploidy samples.

Results: Based on our analysis, we observed that higher fetal DNA fraction (FF) considerably increased the microdeletion detection accuracy, while sequencing coverage became increasingly more relevant as FF decreased. Lower sequencing coverage significantly impacted microdeletion detection accuracy for some of the studied clinically relevant microdeletion regions. Considering microdeletion region-specific characteristics such as region length, region-specific bin size, and regional sequencing data variability can improve the accuracy of detecting corresponding potential microdeletions. Moreover, we observed that full-chromosome fetal aneuploidies could potentially alter the overall expected read count distribution and regional variability, possibly leading to false-positive high-risk microdeletion calls.

Conclusion: BinDel identified each positive control sample as high risk.

Grant References: Enterprise Estonia (EU48695, EU53935); Estonian Research Council (PRG1076), Horizon 2020 innovation grant (ERIN, EU952516).

Conflict of Interest: Priit Paluoja Competence Centre on Health Technologies; University of Tartu, Hindrek Teder Competence Centre on Health Technologies, Andres Salumets Competence Centre on Health Technologies; University of Tartu; Karolinska Institutet and Karolinska University Hospital, Kaarel Krjutškov Competence Centre on Health Technologies; University of Tartu, Priit Palta University of Tartu; Institute for Molecular Medicine Finland (FIMM).

EP18.009 Machine-learning approach for disease prediction improves Genome wide association studies

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Background/Objectives: Genome wide association studies (GWAS) assume there is a correct classification into cases and controls. Nonetheless, misclassification is expected, especially in biobanks that rely on administrative health data. Accounting for misclassification can improve GWAS discovery and development of more predictive polygenic scores.

Methods: To address these limitations, we implemented a gradient boosted classifier (XGBoost) that we used to predict 5 diseases (type 2 diabetes, ischemic stroke, coronary heart disease (CHD), Dementia and Breast cancer) by integrating information on 3828 diagnoses, 499 medications, 25 socio-economic variables. Instead of binary disease labels, the model outputs a continuous probability measure which was used to perform GWAS. The analyses were conducted on N = 317,687 individuals from the FinnGen study.

Results: After predicting disease probability, we perform a GWAS of the predicted values. Results recapitulate a case/control GWAS and in some instances identify new loci (e.g. 3 loci in stroke). Then we performed a GWAS of disease probability only in sub groups of the population and to see, if it represent the original cases and controls we checked the genetic correlation (e.g. rg=0.88, P-value = 3.7e-7,6.3e-14 for stroke and CHD). We also identify established loci, for example APOE in GWAS only among Dementia controls (rs429358, P-value = 1.97e-29). In a GWAS in only cases of CHD we were able to recover 3 loci (p-values = 1.46 e-11,1.47e-11,6e-13).

Conclusion: The possibility to go beyond a case-control model for GWAS analyses opens new venues to the development of more predictive polygenic scores. However, interpretation of new biological results from this approach remains challenging.

Conflict of Interest: None declared.

EP18.010 Team-based approach to using the Face2Gene platform – a Spanish experience

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Background: Advances in genetics have revolutionized all medicine, making personalized medicine possible. In Spain, the medical specialty of Genetics has not yet been approved, which - on many occasions - creates difficulties when it comes to providing adequate assistance in this area. And what we energetically claim.

Objective: Our clinical care, our teaching and research activity increasingly requires networking. In the case of genetics, it is the rule with national and international initiatives. For approximately 10 years we have had an application for health professionals, universal and free, called Face2Gene that uses for facial recognition, machine learning technologies. Since last year, this includes the Gestaltmatcher technology that provides syndrome and patient matches, not just for rare diseases, but for ultra-rare and even undiagnosed diseases. In addition, the patient analysis is facilitated by including clinical findings based on the H.P.O. classification. This has been established as the "lingua franca" for the clinical description of all Human Pathology linked to genetic findings.

Methods: We have been using this platform for the analysis of 1149 patients under the diagnostic facet of Clinical Genetics from different Services and Hospitals, facilitating the submission of clinical data - classified through H.P.O. guiding us to the most probable genes. Therefore facilitating panel selection and the interpretation of genetic results post molecular genetics and advanced cytogenetics testing.

Conclusions: We cannot work outside of new technologies and possibilities that we have within our reach. Network initiatives at different levels are of paramount importance.

Conflict of Interest: None declared.

EP18.011 Building a Human Infertility Genome Database

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Introduction: Infertility is a multifactorial clinical phenotype that approximately affects 10%-15% of the global population and still poses as a common problem for many couples. The work into genomics and infertility is still ongoing to date showing how complexed infertility is in both idiopathic and genomic related diseases. We created and currently analysing a novel SQL database consisting of 50 patients with fertility complications. Preliminary data we provide are cumulative genome annotation data presented as four major datatypes: CNVs, SVs, InDels, and SNPs.

Aim: To identify the chromosome that occurs frequently amongst our datasets within the database for future analysis.

Methods: We categorised the patients into categories: recurrent miscarriage, recurrent implantation failure, advanced maternal age, azoospermia, oligoasthenoteratozoospermia, asthenospermia, poor sperm parameters, and normal sperm parameters that required ICSI. We then identified the most common chromosomal locations with flagged variants amongst the groups. We queried the data to gain an accumulated chromosome count per chromosome for each of the categories and normalised the data.

Results: From our data queries and data normalisation, various chromosomal regions have been flagged up, with chromosome 19 regions most frequently. A review of the literature revealed the need for more research into these chromosomal areas and their relationship to infertility.

Conclusion: Amongst the data within our database, we identified various variants frequently flagged on specific chromosomal regions. Currently, we are continuing working in our approach to uncover genomic locations that relate to human infertility and link those to other genomic studies and databases.

Conflict of Interest: Jack Wieland: None declared, Sarah Buchan Full time - Principle Academic in Immunology, Sioban Sen Gupta Full Time - Associate Professor in Human Genetics, Anna Mantzouratou Full time - Principal Academic In Human Genetics.

EP18.012 Associations between polygenic risk score and COVID-19 susceptibility and severity across ethnic groups: a UK Biobank analysis

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Background/Objectives: COVID-19 manifests with heterogeneity in susceptibility and severity outcomes, with UK Black, Asian and Minority Ethnic (BAME) groups suffering disproportionate burdens. Some variability remains unexplained. Polygenic risk scores (PRS) determine genetic predisposition to disease based on single nucleotide polymorphisms (SNPs). COVID-19 PRS analyses within non-European cohorts are limited. We developed and applied multi-ethnic PRS to understand genetic contribution to COVID-19 variability.

Methods: Two PRS for susceptibility and severity were constructed utilising leading SNPs from the COVID-19 Host Genetics Initiative GWAS meta-analysis, then applied to 447,382 UK Biobank participants. Associations with COVID-19 outcomes were tested using binary logistic regression. Incremental area under receiver operating curve (ΔAUC) validated discriminative power, and incremental pseudo-R^2 (ΔR^2) assessed variance explained.

Results: Compared to low genetic risk, high genetic risk individuals had a greater risk of severe COVID-19 for White (odds ratio [OR] 1.57 95% confidence intervals [CI] 1.42-1.74), Asian (OR 2.88, CI 1.63-5.09) and Black (OR 1.98, CI 1.11-3.53) ethnic groups. Severity PRS performed best within Asian (ΔAUC 0.90%, ΔR^2 0.98%) and Black (ΔAUC 0.60%, ΔR^2 0.61%) cohorts. For susceptibility, high genetic risk was significantly associated with infection risk for the White cohort (OR 1.31, CI 1.26-1.36), but not for Black or Asian groups.

Conclusion: A genetic basis for variability in COVID-19 outcomes was established. PRS showed utility in identifying high-risk individuals for severe COVID-19 across all groups. The multi-ethnic approach allowed applicability of PRS to diverse populations. Further studies using larger non-European sample sizes are required to increase statistical power within BAME populations.

Conflict of Interest: None declared.

EP18.015 Bioinformatics Data Analysis Using Docker in HPC Environment

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Background: Since bioinformatics area emerged, various tools have been developed and applied for supporting bioinformatics analysis. These various tools are developed in different operating systems, kernels or developmental environments. These features caused complex problems in installation or configuration of individual tools and further library dependencies or module compatibility among programs. Because of these problems, it is quite impossible to install and operate various bioinformatic tools in single HPC.

Methods: To support operation of various tools in single HPC by solving these problems, we built a new type of HPC by emerging docker local repository and rootless docker. Almost schedulers did not support docker, we solved this problem using docker repository, SGE environmental variables, and in house scripts. Administrator created and uploaded images containing bioinformatics tools to repository and users performed bioinformatics analysis by using Docker images in repository through scheduler.

Results: We built improved HPC by linking rootless docker and HPC to support analysis using various bioinformatics tools in single environment. Container showed similar or faster performance compared to native environment by the estimation of operation time for individual tools. This result indicated that users can perform bioinformatics analysis using minimal overhead.

Conclusions: The improved HPC suggested that various bioinformatics tools over hundred numbers can be performed in single environment by linking docker and HPC. This new approach provides high portability, resource efficiency, and flexibility to administrator and convenience to users.

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Conflict of Interest: IKSU BYEON Korea Research Institute of Bioscience and Biotechnology (full time), National Research Foundation of Korea(Collaborator), JONG CHEOL YOON Korea Research Institute of Bioscience and Biotechnology(full time), National Research Foundation of Korea(Collaborator).

EP18.016 Analysis of human phenotype ontology terms using ClinPhen, a natural language processing extraction tool, in the Program in prenatal and pediatric genomic sequencing (P3EGS) pediatric cohort

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Background/Objectives: Human phenotype ontology (HPO) terms standardize clinical features and predict causative genes to aid in variant interpretation following exome sequencing (ES). However, the best practices for generating HPO terms are undetermined.

Methods: We performed ES and used Natural Language Processing (NLP) software (ClinPhen) to generate HPO terms for 421 predominantly underserved patients in the P[3]EGS study. 27.8% patients had received 'definitive positive' or 'probable positive' ES results.

Results: The average number of terms extracted/patient was 20.5 ± 12.1 with HPO 'depth' of 10.1 ± 1.8 terms. We did not find a significant association between the number of HPO terms or term depth with a positive diagnosis. Many HPO terms generated by ClinPhen were considered unlikely to be related to the phenotype (e.g. allergies). For patients who had HPO terms extracted manually and with ClinPhen, there was an approximately linear relationship between the log ranking for manual vs. ClinPhen HPO term lists. Manual HPO term extraction outperformed NLP-generated HPO terms for predicting genes with a high probability of a causative association.

Conclusion: We conclude that ClinPhen and other NLP software programs are valuable for generating HPO terms. However, manual extraction of HPO terms with clinician weighting and selection of HPO terms with clinical relevance can improve the ability of the terms to predict underlying causative genes.

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EP18.017 Enhancing applicability of PEDIA by integration of CADA and GestaltMatcher into the variant analysis framework VarFish

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Background: Prioritization algorithms for variants are crucial for the efficient analysis of NGS data and assist in the classification of variants according to the ACMG guidelines. Evidence for pathogenicity can also be inferred from a matching phenotype (PP4 criteria). CADA and GestaltMatcher are algorithms that quantify the phenotypic similarity of a patient to a known disorder by analyzing the clinical features and medical imaging data. PEDIA is an algorithm that combines these feature-based and gestalt-based scores with molecular information into a variant score. We integrated PEDIA into VarFish which is an open source framework for variant analysis and measured the performance in a prospective multi-center study.

Methods: 358 patients from centers of rare diseases with suspected genetic disorders were subjected to exome sequencing, variants were imported into VarFish and filtered for frequency, and quality. The pathogenicity based on molecular information was assessed by CADD scoring. Phenotypic scoring was performed with CADA and GestaltMatcher. Prioritizations of all combinations of scores were evaluated.

Results: In total, 64 different monogenic disorders could be established. The top-10 accuracies were 45% for CADD alone, 61% for CADD + CADA, and 82% for PEDIA (CADD + CADA+GestaltMatcher).

Conclusion: The inclusion of phenotypic scores into variant prioritization can increase performance significantly. All tested components were open source and can be integrated into existing diagnostic frameworks.

Conflict of Interest: Meghna Ahuja Bhasin IGSB, Bonn, Tzung-Chien Hsieh IGSB, Bonn, Alexej Knaus IGSB, Bonn, Peter Krawitz IGSB, Bonn, Pietro Incardona IGSB, Bonn, Alex Schmid IGSB, Bonn.

EP18.018 Use of Elasticsearch-based Business Intelligence tools for manipulation, integration and visualization of biological data

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The emergence of massive datasets exploring the multiple levels of molecular biology has made their analysis and knowledge transfer more complex. Efficient and powerful exploratory tools are needed to fully extract the insights hidden in such massive and heterogenous data so they can then be used to help better understand biological phenomenons. Business Intelligence (BI) tools have been used in many fields has exploratory tools that have numerous connectors to link numerous data repositories with a unified graphic interface. Their graphic interface uses dashboards that give an overview of data and facilitate interpretation for decision makers.

BI tools could be a flexible and user-friendly way of handling molecular biological data with interactive visualizations. However, it is rather uncommon to see such tools use for the exploration of datasets in the biological fields. We believe two main obstacles could be the reason. Firstly, we posit that the way to import data into BI tools are not compatible with biological databases. Secondly, BI tools may not be adapted to certain particularities of complex biological data, namely the size and variability of datasets.

Thus, we have evaluated the dashboards, connectors and visualizations of various BI tools: Elastic Kibana, Siren investigate, Microsoft Power BI, Salesforce Tableau and Apache Superset. This work highlights a comparison of these BI tools onto which massive data management repository engine called Elasticsearch are compatible. Finally, three case studies will be discussed in which these BI tools were applied on biological datasets with different characteristics and their performances measured

Conflict of Interest: None declared.

EP18.019 Cross-species identification of core genes and gene networks associated with animal domestication and human sociability to predict novel biomarkers of autism

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Background/Objectives: Autism is a complex neurodevelopmental condition, characterized by deficit in social interaction and communication, whose aetiology is attributed to gene-environmental interaction. Sociability is essential for the biological competences and health of many animal species including human. Genomic studies identified genes and pathways involved in sociability and communication evolution of domesticated animals showing that some of them are recurrent. Network analysis is crucial to study gene-gene interactions and explain how genetic variation perturbs genetic systems to produce phenotypes. We speculated that the cross-species integration and gene network analysis of these genes could identify the core networks of sociability that could be useful for better understanding of human social behaviour and predicting possible novel biomarkers of autism.

Methods: Genes linked with animal and human social behaviour and communication were extracted from the literature. Genes associated with autism were downloaded from SFARIdb. Molecular interactions were collected from STRING, RNAinter, mirTarBase, and HumanBase for tissue specific analysis (i.e. brain). Gene network analysis was performed by means of dmfind.

Results: We analysed the molecular interactions of sociability genes and identified cross-species evolutionarily maintained genes and networks linked with social behaviour and communication, and predicted novel biomarkers of autism.

Conclusion: Genomics cross-species analysis, performed by bioinformatics tools, is a powerful approach that allows to identify the genes involved in the core traits of a specific function, i.e. sociability. Subsequent gene network analysis allowed us to predict human genes that could represent novel biomarkers and possible therapeutic targets of autism.

Grant references: EU-H2020 GEMMA (ID:825033).

Conflict of Interest: None declared.

EP18.020 Missense3D suite: harnessing the power of 3D protein structures to predict the effect of missense variants

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In 2019, we released the algorithm Missense3D, which predicts the pathogenicity of a missense variant by determining its effect on three-dimensional (3D) structures. Missense3D, which has had >250 citations and >8000 users in the last 12 months only, uses the structures of single proteins. We have now developed Missense3D-PPI and Missense3-TM for the prediction of missense variants located in protein-protein interfaces and transmembrane regions, respectively.

The variants for training and testing were selected from 4 million human missense variants. Experimental structures were obtained from the Protein Data Bank and protein models were generated in-house with our Phyre2 algorithm. The final datasets for Missense3D-PPI included 1,301 interface variants in 441 proteins and 553 experimental 3D complexes, whereas the final datasets for Missense3D-TM included 3,346 transmembrane variants in 746 proteins and 772 protein structures (103 experimental and 669 models). The structural features used to assess the impact of variants were selected based on the biology of protein interfaces and transmembrane regions.

Missense3D-PPI achieved an accuracy of 56% (sensitivity 42% and specificity 78%) and outperformed our Missense3D algorithm for single protein chain ($p = 1 \times 10^{-9}$) but also currently available algorithms for interface variant prediction, such as BeatMusic ($p < 0.003$) and mCSM-PPI ($p = 1.87 \times 10^{-6}$). Missense3D-TM achieved an accuracy of 65% (sensitivity 57% and specificity 82%) and outperformed our Missense3D ($p < 1 \times 10^{-6}$). Missense3D-TM performed similar to the mCSM ($p = 0.05$), which predicts changes in protein folding energetics.

Missense3D-PPI and Missense3D-TM are new valuable tools for variant prediction that will be available from http://missense3d.bc.ic.ac.uk/.

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Conflict of Interest: Alessia David full, Gordon Hanna Full, Cecilia Pennica: None declared, Suhail Islam Full, Michael J E Sternberg Full, Principal investigator on Wellcome Trust and BBSRC grants.

P18.021 GAN-based data augmentation to improve 3D chromatin features identification in Hi-C data

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High-throughput chromosome conformation capture sequencing (Hi-C) has been widely used to understand the three-dimensional (3D) structure of genomes and unravel the principles of chromatin folding. The conformational structures of chromatin, such as topologically associating domains (TADs), provide insight into the connections between regulatory elements and genes linked to human diseases. However, accurately detecting these regions particularly depends on a high resolution of Hi-C data, which is hard to ascertain and thus is less abundant compared with other types of sequencing data. Here, we develop a conditional Generative Adversarial Networks (GANs) model that leverages recent advances in data augmentation using GANs to augment the quality and quantity of Hi-C data to better characterize 3D chromatin structures. Our cGAN-HiC model consists of two competing neural networks (a generator and a discriminator) and uses an adversarial training approach in which the generator and discriminator contest with and learn from each other. We incorporate a combination of convolutional layers in the generator and discriminator of the model. We first pre-train our model on the identified TAD boundaries pooled from publicly available Hi-C data in a majority population in lymphoblastoid cell lines and then fine-tune this model using the remained minority populations. We applied our model to the published Hi-C data and showed its power to significantly improve the quality and diversity of Hi-C data and boost the accuracy of identification for TADs similar to those detected in deeply sequenced Hi-C data.

Grant references: the National Science Foundation of the United States (Award Number: 1750632).

Conflict of Interest: None declared.

EP18.022 The intronic position of a variant influences splice prediction in two machine learning models

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Background/Objectives: Whole-intron sequencing identifies an increasing number of rare intronic variants. This study aimed to evaluate the performance of a neural-network-based (SpliceAI) and a random-forest-based (SPiP) machine learning algorithm on 762 intronic single-nucleotide variants collected from a literature review and supported by functional evidence.

Methods: A dataset of 487 pathogenic and 275 non-pathogenic variants classified into intronic variants located less than 100 nucleotides, excluding positions of natural splice sites (Group1), and variants found more than 100 nucleotides away from the natural splice sites (deep intronic variants, Group2). SpliceAI and SPiP were used for in silico prediction, and McNemar's test was used for comparison.

Results: SpliceAI showed better performance than SPiP for the whole dataset. This difference was caused especially by variants in Group 2 (deep-intronic), while SPiP outperformed SpliceAI in Group 1 variants (see table).

Conclusion: Our results suggest that prediction accuracy could be optimized by choosing the employed machine learning algorithm based on the position of the intronic variant.

Conflict of Interest: Emilia Maneiro Full time, Laura Cazón Full time, Luis De la Higuera Romero Full time, Marlene Perez Barbeito Full time, Rosalía Peteiro Full time, Iria Gómez Díaz Full time, Paula Velez Full time, Maria Sanchez Full time, Anahi Sanluis Verdes Full time, Guillermo Smith Ramos Full time, Xusto Fernandez Full time, Diego Cabrera Argaña Full time, Almudena Amor Full time, María Valverde Full time, Soledad García Hernández Full time, Ivonne Cárdenas Reyes Full time, Martin Ortiz Genga Part-time, Juan Pablo Ochoa Full time.

EP18.023 Finding the right match for your variant – Incompatibility of peptide based databases with standard transcripts in annotation programs

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Clinical variant database annotation comes in two flavors. It is based on a specific nucleotide change at a defined position of the human genome or refers to a transcript/peptide position. Genome based annotation is generally easier to manage. If any processing of variant data is required at all, it can be done by a liftover from one genome to the other. However, this will not consider

Performance measures of splice prediction algorithms

	Dataset (N = 762)		Group1 (N = 419)		Group2 (N = 343)	
	SPiP	SpliceAI	SPiP	SpliceAI	SPiP	SpliceAI
TP	384	420	273	256	111	164
FP	30	23	21	18	9	5
TN	245	252	98	101	147	151
FN	103	67	27	44	76	23
Sensitivity	0.79	0.86	0.91	0.85	0.59	0.88
Specificity	0.89	0.92	0.82	0.85	0.94	0.97
PPV	0.93	0.95	0.93	0.93	0.93	0.97
NPV	0.7	0.79	0.78	0.7	0.66	0.87
Accuracy	0.83	0.88	0.89	0.85	0.75	0.92
AUC ROC	0.83 ± 0.02	0.92 ± 0.01	0.90 ± 0.02	0.83 ± 0.02	0.73 ± 0.03	0.94 ± 0.02

unknown variants that might lead to the same peptide change and therefore should be part of a clinical report. For that reason, peptide based annotation can be considered more comprehensive. Some databases for somatic variants (e.g. jaxCKB, oncokb) only show the variants on peptide level.

Annotation programs like ANNOVAR and VEP usually are run with a predefined database, which in general covers the most recent version of the transcripts available for a specific gene. This version in some cases does not match the coding sequence of variant databases and literature, the latter referring to older versions of the transcript, making it difficult to identify the variants. We will explain this challenge for the gene MYD88 based on NCBI RefSeq annotation. We provide software tools and a workflow to identify and overcome situations of missing transcripts, using NCBI E-utilities and ClustalX. The workflow quickly identifies needed adaptations of ANNOVAR and VEP gene tables for predefined and custom gene panels.

Conflict of Interest: None declared.

EP18.024 Data visualisation of phenotypic similarity networks approach for variant prioritization of unsolved rare disease cases: a preliminary methodological report and proposal of an analysis pipeline

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Background/Objectives Methods: Rare diseases (RD) have a prevalence of not more than 1/2000 in the European population, and are characterised by the difficulty of obtaining a correct and timely diagnosis. A significant proportion of patients suspected to have a genetic RD receive an inconclusive exome/genome sequencing and according to Orphanet, 72,5% of RD have a genetic origin although 35% of them do not yet have an identified causative gene. The Orphanet RD Knowledge Database stores on Orphadata.com, data of known RD annotated with (i) phenotypic features using the Human Phenotype Ontology (HPO) and (ii) genetical information. In the frame of the Solve-RD project, which aims to identify the molecular causes underlying undiagnosed RD, we developed a phenotypic similarity-based variant prioritization methodology by comparing submitted patients' cases (phenotypically annotated with HPO) amongst them and with known RD in Orphanet (ORPHAcodes).

Results: The results could be visualized as similarity networks by using the Cytoscape software platform. Also, based on this methodology, we devised an automated standardized phenotypic-based re-analysis pipeline, which results can be analysed in a web-based portal using Cytoscape JS. End-users can interactively navigate into undirected large condensed networks built on similarity scores, discovering associations-based neighborhoods, apply specific filters, construct clusters/subgraphs (including cases, ORPHAcodes, genes or gene variants) and/or export results.

Conclusion: Beyond this case study, the Cytoscape workflow offers various approaches to facilitate data interpretation in Solve-RD and can be scaled-up to projects dealing with representation of a huge amount of connected data including ontologies (i.e. WikiPathways).

Grant References: Solve-RD.

Conflict of Interest: Maroua Chahdil INSERM-US14-Orphanet: Full time, collaborator Solve-RD, David Lagorce INSERM-US14-Orphanet: Full time, collaborator OD4RD2; EJP RD, ELIXIR-FR; Solve-RD; TEHDaS JA;, Caterina Lucano INSERM-US14-Orphanet: Full time, collaborator OD4RD; OD4RD2; Solve-RD, Emeline Lebreton INSERM-US14-Orphanet: Full time, collaborator Solve-RD, Carolina Fabrizzi INSERM-US14-Orphanet: Full time, collaborator Solve-RD, Mutaz Amin INSERM-US14-Orphanet: Full time, Charlotte Rodwell Part time (90%): INSERM-US14-Orphanet, Collaborator EJP RD; Solve-RD, advisory board: Maladies Rares Info Services, Valerie Serriere-Lanneau INSERM-US14-Orphanet: Full time, collaborator: EJP-RD, OD4RD2, Marc Hanauer INSERM-US14-Orphanet: Full time, collaborator OD4RD2; EJP RD, ELIXIR-FR; Solve-RD; EHDS Pilot 2; TEHDaS JA;, advisory board: Scientific Advisory board national support group "Aide aux Jeunes Diabétiques"., Support group Chairman (Association ENT'RED-paris, réseau enfance diabète), Ana Rath INSERM-US14-Orphanet: Full time, PI OD4RD2; collaborator: EJP RD; Solve-RD; EHDS Pilot 2; TEHDaS JA;, advisory board: ERN-LUNG; ERN-BOND; Share4Rare.

EP18.025 Uncovering the genetic basis of idiopathic intracranial hypertension by SKAT analysis of the ukbiobank data

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Introduction: Idiopathic intracranial hypertension (IIH) is elevated intracranial pressure (ICP) without a known cause. Typically, patients are overweight women of reproductive age who experience symptoms such as headache, transient visual obscurations (TVOs), pulsatile tinnitus, double vision, and neck pain. If left untreated, this condition can lead to significant visual loss.

Aim: This study aimed to 1) identify predisposing genes associated with or protective against the development of IIH, and 2) identify other diseases associated with the genes found in aim 1.

Methods: We used the UK Biobank database to identify individuals with a specific disease code. We employed SKAT, a gene-based analysis method for functional genomic segments. Then, we examined the 15 most associated genes and used this gene set against the entire GWAS catalog and the KEGG knowledge base.

Results: Our findings revealed several novel genetic variants associated with IIH, which could be protective or associated with its development. The NOVA2 gene was found to be highly associated with IIH, although we are still investigating whether this gene is positively or negatively associated with the condition. We also examined the GWAS and KEGG databases to identify other possible disease associations of the 15 most associated genes and found several associations.

Conclusion: This study represents a significant step forward in the understanding of the genetic basis of IIH and its possible associated conditions. The identified genetic variants provide a foundation for further research into the underlying mechanisms of IIH and the development of new, targeted treatments for this debilitating condition.

Conflict of Interest: Ehud Banne Full time, Roei Zucker Full, Idit Maharshak Full, Michal Lineal Full.

EP18.026 MOLGENIS VIP: a flexible phenotype-guided pipeline for integrated variant calling, classification and reporting

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Genome diagnostics aims to find genetic variants causing heritable diseases, providing a prognosis and appropriate treatment. However, for most indications only 20-30% of patients receive genetic diagnoses. While new computational algorithms for variant interpretation are emerging, integrating them remains challenging.

We present the latest release of the MOLGENIS Variant Interpretation Pipeline (VIP), an easy-to-use, flexible and complete diagnostic pipeline to generate manageable shortlists of likely pathogenic variants for expert interpretation. VIP processes short- and long-read data from FASTQ, BAM/CRAM and VCF files, and VIP integrates our powerful CAPICE machine learning based variant predictor with other best in class annotation resources, like ENSEMBL Variant Effect Predictor, SpliceAI, gnomAD, and ClinVar. Clinically relevant filtering can be performed through inheritance matching and Human Phenotype Ontology terms. VIP generates easy-to-use reports, allowing experts to home in on variants, including views of underlying reads, while still being megabytes in size for easy sharing. By default, VIP applies protocols from routine diagnostics and inputs from within VKGL, EU Solve-RD, EJP-RD and CINECA projects. However, VIP is implemented as modular software using NextFlow and portability frameworks to ease addition and modification of all steps. F.e.: we are now piloting non-coding variant annotation using GREEN-VARAN to improve WGS analysis.

We believe that the newest combination of CAPICE with other best in class methods into a free, easy-to-modify open-source platform provides an excellent basis to deliver the best variant interpretation pipelines, empowering laboratory specialists, clinicians and researchers to diagnose patients.

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EP18.027 Bio-Express: Cloud service for high-throughput analysis of genomic big data

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Background: Because of the exponential growth of genomic data since the introduction of next generation sequencing technology, the analysis of large bio-data is becoming more and more complex and difficult problem.

Methods: To overcome this problem, we developed a cloud-based workflow management system, Bio-Express, to provide fast and cost-effective analysis of massive genomic data. We implemented complex workflows making optimal use of high-performance computing clusters. Bio-Express allows users to create multi-step analyses using drag and drop functionality and to modify the parameters of pipeline tools. Bio-Express is a hybrid system that enables users to use both analysis programs providing traditional tools and Apache Spark-based big data analysis programs simultaneously in a single pipeline. We also provide high-speed transmission for transferring of large amounts of data.

Results: To analysis data, about 1200 CPU cores and 2PB storage are dedicated to Bio-Express. Bio-Express provides about 20 analysis pipelines and 100 analysis tools such as whole genome resequencing and single cell analysis tools. After launching of Bio-Express in 2016, about 4000 analysis requests have been completed and provided.

Conclusions: Bio-Express is a scalable and publicly available service for large-scale genomic data analysis. Bio-Express supports the reliable and highly scalable execution of sequencing analysis workflows in a fully automated manner. Bio-Express provides a user-friendly interface to all genomic scientists. The Bio-Express is freely available for use from http://www.kobic.re.kr/bioexpress.

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EP18.028 GeneCaRNA: A gene-centric non-coding RNA compendium for disease decipherment by whole genome sequencing

Shalini Aggarwal[1], *Ruth Barshir*[1], *Simon Fishilevich*[1], *Chana Rosenblum*[1], *Tsippi Iny-Stein*[1], *Ofer Zelig*[2], *Yaron Guan-Golan*[2], *Marilyn Safran*[1], *Shmuel Pietrokovski*[1], *Doron Lancet*[1]

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Background: Interpreting whole genome sequences (WGS) for disease decipherment is a challenge: merely ~2% of variants reside in protein-coding territories. For effective interpretation of functional entities in the remaining "dark matter," we developed GeneCaRNA (PMID:33676929) under the GeneCards Suite, a gene-centric knowledgebase for non-coding RNA (ncRNA) genes, amounting to an additional 5% of exonic genome territory.

Methods: Many ncRNA sources are transcript-centric, and for WGS variant analysis, it is essential to transform this information into a unique and comprehensive genomic ncRNA gene map. Using genomic coordinates, we clustered overlapping transcript entries from all sources (17 transcript sources in RNAcentral plus HGNC, NCBI Gene, and Ensembl), resulting in full coverage of genomic gene positions. Each GeneCaRNA gene is shown on a web card, including affiliated transcripts, affiliation with one of 15 ncRNA category and other annotations.

Results: While only ~32,000 of the ncRNA genes existed in primary gene sources, we defined additional ~188,000 records as novel Transcripts Inferred GeneCaRNA genes (TRIGGs). The GeneCards Suite now benefits from comprehensive ncRNA data access, allowing its variant-disease decipherment tool VarElect (PMID:27357693) to address WGS results.

Conclusion: GeneCaRNA extends beyond WGS interpretation, includes analyzing differentially expressed lncRNA in search of biomarkers for Gastric Cancer (PMID:35571069), miRNA-based biomarker discovery for Alzheimer's disease diagnosis (PMID:35147545), and the creation of a piRNA database and computational model for the relationship between piRNA genes and human diseases (PMID: 35667080). GeneCaRNA's combining extensive ncRNA knowledgebase with gene interpretation algorithms thus allows fathoming the disease-related significance of non-coding variants.

Grant: LifeMap Sciences.

Conflict of Interest: Shalini Aggarwal Full Time, Ruth Barshir Consultant, Simon Fishilevich Part Time, Chana Rosenblum Full Time, Tsippi Iny-Stein Part time, Consultant, Ofer Zelig Full time, Yaron Guan-Golan Part time, Collaborator, Marilyn Safran Part

time, Intellectual property, Consultant, Shmuel Pietrokovski Full time, Principal Investigator, Intellectual property, Doron Lancet Part Time, Principal Investigator, Intellectual property.

EP18.030 Pre-extensively drug-resistant M.tuberculosis detection from TB patients in Kazakhstan by using whole genome sequencing

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Introduction: Regarding to WHO (2022), Kazakhstan is among the thirty countries with high rates of MDR-TB. Pre-extensively drug-resistant TB (pre-XDR-TB) is TB caused by *M. tuberculosis* strains that fulfill the definition of MDR-TB and which are resistant to any fluoroquinolone. The aim of this study was to perform genomic characterization of *Mycobacterium tuberculosis* strains from Kazakhstani patients with pre-XDR-TB.

Materials and methods: Whole-genome sequencing (WGS) was performed for 12 pre-XDR-TB strains by MiSeq platform (Illumina). Data analysis and variant detection were performed using MTBseq pipeline with the genome of MTB H37Rv (NC000962.3) as a reference strain. The genome analysis was performed using TBProfiler, Mykrobe, CASTB, and ResFinder.

Results: We focused on SNPs in genes known to confer resistance to first- and second-line anti-TB. The whole-genome sequencing for pre-XDR *M.tuberculosis* isolates produced 43,752,628 paired reads. The number of SNPs and InDels ranges from 1443 to 1555, and from 349 to 524, respectively. All analyzed clinical isolates were identified as pre-XDR *M.tuberculosis* strains and were resistant to rifampicin, isoniazid and also resistant to one fluoroquinolone anti-TB drug. All strains are related to W-Beijing genotype (East Asian lineage) which associated with a high risk of drug resistance.

Conclusion: This study is to report the use of next-generation sequencing technology for whole-genome annotation of drug-resistant pre-XDR clinical *M.tuberculosis* isolates from Kazakhstan, showing its potential for clinical management and TB control in Kazakhstan. The Beijing genotype is a major cause of tuberculosis in Kazakhstan and possibly contributes to the transmission of pre-XDR-TB.

Grant references: №11022021CRP1511.

Conflict of Interest: None declared.

EP18.033 Sensitive computational workflow for Copy Number Variant detection up to single exon from clinical exomes sequencing

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Background/Objectives: Copy number variants (CNVs) detection is a relevant issue in clinical practice. Genome-wide array-based CNV detection (aCGH) and multiplex ligation-dependent probe amplification (MLPA) are the gold-standards for CNV identification. However, these techniques are expensive and time consuming. Assays based on next-generation sequencing (NGS) data are becoming a standardized way for variants identification, but accurate detection of CNV from clinical exomes is still challenging.

Methods: We set up a computational workflow based on EXCAVATOR2 tool for the detection of CNVs from clinical exome data. An optimized version of EXCAVATOR2 was combined with a Bayesian model for single-exon CNV calling of specific genes. For each exon we exploited the distribution of the ratio between the coverage depth of each sample and the mean coverage of a pool of controls in a Metropolis Hastings algorithm to estimate the parameters of a Bayesian Logistic Regression Model for the posterior probability of correct copy number classification of each single exon.

Results: We analyzed around 3000 clinical exomes and we tested the performances of the method on 80 samples (using the large and well known BRCA genes for single exons test). We obtained a range of sensitivity from 1 (clinical) to 0.66 (technical) and a range of specificity from 0.99 (technical) to 0.90 (clinical).

Conclusion: This new workflow, coupled with prioritization tools, can be used routinely for diagnostic purposes, reducing cost of analysis and increasing detection rate by one step identification of genetic compounds for SNP and CNV.

Conflict of Interest: None declared.

EP18.034 Validation of somatic gene fusion detection by NGS in routine clinical testing

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Background/Objectives: Gene fusions, in which two genes are hybridised by a genomic structural rearrangement, are implicated in the development of haematological and solid cancers. A recent study of 33 cancer types estimated that fusions are drivers in 16.5% of cases. Fusions can be relevant as therapeutic targets, as with the BCR-ABL1 fusion seen in >95% of chronic myeloid leukaemia patients.

Gene fusions are traditionally detected by fluorescence in situ hybridisation (FISH). However, this method only detects one fusion at a time. In contrast, sequencing-based detection is faster, can detect fusions for which FISH probes aren't available, and is able to resolve precise breakpoints. In this project, we aim to validate an RNA-Seq fusion detection pipeline for clinical service.

Methods: The pipeline aligns the RNA-Seq reads and calls fusions using the STAR aligner and STAR-Fusion tools. Called fusions, alongside a 'rescue list' of relevant cancer fusions curated by clinical genomicists, are then in silico validated using FusionInspector. The pipeline was tested on an initial validation set of 15 clinical samples ($n = 15$ fusions) and 2 commercially-available controls ($n = 19$ fusions).

Results: While STAR-Fusion alone finds 85.3% of fusions (29/34), follow-up with FusionInspector finds 91.2% of the expected fusions (31/34), or 96.9% if excluding those fusions which aren't in the rescue list (31/32).

Conclusion: The results indicate that it will be possible for us to offer high-throughput fusion detection for cancer diagnosis in the near future. Our next steps will be to add more fusions to our rescue list, and sequence additional validation samples.

Conflict of Interest: None declared.

EP18.035 Comparing the performance of penalized logistic regression versus other machine learning algorithms in predicting phenotypes of ectodermal dysplasia patients from their genotypes

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Background: Ectodermal dysplasia (ED) is a monogenic genetic disorder characterized by primary defects in two or more structures derived from the ectoderm. These patients have a wide diversity of characteristic phenotypic features that range from mild to severe, consequently affecting their quality of life to varying degrees. Predicting the phenotype severity of ED cases from their genotype profiles is vital for future prognoses and the early planning for future therapies and proper preparation for genetic counseling for parents and patients.

Methods: We applied six machine learning (ML) algorithms (Decision Trees, Random Forests, Extreme Gradient Boosting, K-Nearest Neighbors, Support Vector Machine, and penalized Logistic Regression) to a total of 41 ED patients with 569,650 SNPs, and compared their performances in phenotype prediction. We aimed to optimize the receiver operating characteristics (ROC-AUC) as our primary metric of interest in model evaluation and algorithm choice.

Results: Ensemble techniques (RF and XGboost) together with KNN recorded the highest ROC-AUC values of 0.9 with a 95% confidence interval (0.723-1.077), while penalized LR gave the worst prediction ability to distinguish between different classes (ROC-AUC (95% CI)) = 0.4 (0.11 – 0.69).

Conclusion: Phenotype of ED patients can be predicted with a respectable level of accuracy and that will help them improve their quality of life. Small-sized datasets, obtained from local studies should not hamper researchers from implementing ML algorithms for prognostication purposes. The privilege of using ML in prognostic modeling may be dependent on multiple factors like sample size, selected features, and the underlying disease of interest.

Conflict of Interest: None declared.

EP19 Personalized Medicine and Pharmacogenomics

EP19.001 STrengthening the Reporting Of Pharmacogenetic Studies: Development of the STROPS guideline

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Background: Large sample sizes are often required to detect statistically significant associations between pharmacogenetic markers and treatment response. Meta-analysis may be performed to synthesize data from several studies, increasing sample size and, consequently, power to detect significant pharmacogenetic effects. However, performing robust synthesis of data from pharmacogenetic studies is often challenging because of poor reporting of key data in study reports. No guideline for the reporting of pharmacogenetic studies has previously been developed using a widely accepted robust methodology. The objective of this project was to develop the STrengthening the Reporting Of Pharmacogenetic Studies (STROPS) guideline.

Methods and findings: We established a preliminary checklist of reporting items to be considered for inclusion in the guideline. We invited representatives of key stakeholder groups to participate in a 2-round Delphi survey. A total of 52 individuals participated in both rounds of the survey, scoring items with regards to their importance for inclusion in the STROPS guideline. We then held a consensus meeting, at which 8 individuals considered the results of the Delphi survey and voted on whether each item ought to be included in the final guideline. The STROPS guideline consists of 54 items and is accompanied by an explanation and elaboration document.

Conclusions: Our aim is for the STROPS guideline to improve the transparency of reporting of pharmacogenetic studies and also to facilitate the conduct of high-quality systematic reviews and meta-analyses. We encourage authors to adhere to the STROPS guideline when publishing pharmacogenetic studies.

Conflict of Interest: None declared.

EP19.002 Synthetic adrenocorticotropic ACTH(6-9)PGP peptide involves in the spatial regulation of the ischemic process in the rat brain at the transcriptome level

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Background/Objectives: An effective treatment of ischemic stroke is strongly restricted. Some adrenocorticotropic hormone (ACTH) derivatives are considered as potential drugs[1]. Here, using RNA-Seq, we studied the effect of neuroprotective ACTH(6-9)PGP peptide on gene expression in two rat brain tissues at 24h after transient middle cerebral artery occlusion (tMCAO). Tissues were selected based on magnetic resonance imaging (MRI) and histological examination (HE) data. Thus, the striatum (S) that contained an ischemic focus, and the dorsolateral region of the frontal cortex (DRFC) that contained the penumbra area were studied.

Methods: Wistar rats, tMCAO, MRI, HE, RNA-Seq, real-time RT-PCR, bioinformatics.

Results: We revealed 2066 and 735 differentially expressed genes (DEGs) with cut-off>1.5 and padj<0.05 after triple (100mkg/kg) administration of ACTH(6-9)PGP versus saline at 24h after tMCAO in DRFC and S, respectively. Only 209 DEGs had co-directional expression changed in both tissues. These DEGs (e.g. *P2rx6, Crhr1, Hrh1, Hcrtr1*) were associated with neuroactive interactions, predominantly. Moreover, peptide significantly reduced profile disturbances caused by ischemia for 1743 of 3774 DEGs in DRFC. Concomitantly, this effect was only for 294 of 4409 DEGs, whereas 152 genes (*e.g. Avp, S100a9, Fos, Gabra5*) showed even more disturbing profile in S.

Conclusion: ACTH(6-9)PGP-related spatial regulation of the ischemic process in the rat brain at the transcriptome level was revealed. We believe that genome responses identified can be the key for induction of regeneration processes in brain cells after stroke.

References: Montero-Melendez et al., Semin Immunol. 2022;59:101603.

Grant: The research was funded by the Russian Science Foundation, grant №19-14-00268, https://rscf.ru/project/22-14-35023/.

Conflict of Interest: None declared.

EP19.003 Melanocortin-like peptides and their multiple gene targets in rat brain following early post-stroke period

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Background/Objectives: Stroke therapy is extremely limited by poor drug efficacy and a short therapeutic window. Melanocortin peptides and their derivatives are interest as neuroprotective agents[1]. Here, we used transient middle cerebral artery occlusion (tMCAO) model in rats under magnetic resonance imaging (MRI) control to compare gene expression profiles induced by melanocortin-like ACTH(4-7)PGP (Semax) and ACTH(6-9)PGP peptides at 4.5h after tMCAO.

Methods: Wistar rats, tMCAO, MRI, RNA-Seq, real-time RT-PCR, bioinformatics.

Results: The dozens of differentially expressed genes (DEGs) (cut-off>1.5 and padj<0.05) were revealed after double (100mkg/kg) administration of each peptide compared to saline in penumbra-associated dorsolateral areas of the frontal cortex of rats at 4.5h after tMCAO. Moreover, both peptides significantly reduced expression disturbances caused by ischemia for 52 out of 1281 DEGs (*e.g. RT1-Ba, RT1-Da, Cd74*). However, we identified 315 DEGs associated with ACTH(6-9)PGP versus Semax administration. Most of them (228) were downregulated (*e.g. Il13ra1, Ccl2, Cxcl16*) and inflammatory-related genes. Also, upregulated DEGs (*e.g. Gabrr2, Hcn4, Kcnv1*) were predominantly associated with ion channels.

Conclusion: Both melanocortin-like peptides modulated brain gene expression profile disrupted by ischemia at 4.5h after tMCAO. But their different action to immune-related genes predominantly was revealed. Our study provides correlation between structure of related peptides and their effects at transcriptome level and is valuable in terms of developing interventions for early post-stroke therapy.

References: Montero-Melendez et al., Semin Immunol. 2022;59:101603.

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Conflict of Interest: None declared.

EP19.004 Analysis of the association between MMP-8 gene polymorphisms and the occurrence of adverse effects after acute ischemic stroke treated with recombinant tissue plasminogen activator

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Background/Objectives: Acute ischemic stroke (AIS) is accompanied by a high rate of disability and mortality. Recombinant tissue plasminogen activator (rtPA) can reduce potential incapacities after AIS, but also can cause serious adverse effects (AE). Matrix metalloproteinase 8 (MMP-8) may contribute to cerebral damage after AIS. Polymorphisms rs1940475 and rs11225395 within *MMP-8* gene could potentially affect *MMP-8* gene expression, consequently occurrence of rtPA-induced AE, especially hemorrhagic ones. We aimed to analyze the association between genotypes and haplotypes of the selected *MMP-8* gene polymorphisms and the occurrence of rt-PA-induced AE.

Methods: Study included 164 subsequent patients with AIS treated with rtPA. During hospitalization, occurrence of AE (symptomatic intracranial hemorrhage (sICH) and hemorrhagic transformation (HT)) was followed. Genotypisation was performed using Real-Time PCR. Haplotype analysis was performed by Haploview software.

Results: Patients who had sICH after rtPA were significantly more likely to have the TT genotype of the rs1940475 polymorphism ($p = 0.032$; OR = 5.1048; CI95%:1.27–20.56), meanwhile patients with AA genotype of the rs11225395 polymorphism showed borderline significance for sICH occurrence ($p = 0.051$). Haplotype block was found between *MMP-8* gene rs1940475 and rs11225395 polymorphisms ($D' = 0.98$, $r^2 = 0.64$). Our patients had CG, TA and TG haplotypes (58.5%, 31.7%, 9.5%, respectively). Patients without HT had TG haplotype more often than patients with HT (11.2% vs. 1.7%, $p = 0.023$).

Conclusion: TT genotype of the rs1940475 *MMP-8* gene polymorphism may be associated with sICH after AIS treated with rtPA.

Grant References: Ministry of Education, Science and Technological Development of the Republic of Serbia [grant numbers 175087 and 175091].

Conflict of Interest: None declared.

EP19.005 Role of S100A4, MRC2 and PCA3 as genetic markers of aggressiveness in prostate cancer

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Background/Objectives: Although prostate cancer (PC) is the second most frequent tumour worldwide, its management and screening are still a relevant problem in clinical practice. In this study, we investigated the role of aggressiveness genetic biomarkers for PC stratification.

Methods: We analyzed 12 fresh tissues and 16 plasma samples from patients and controls using RNA-Seq technology and 13 formalin-fixed paraffin-embedded tissues and 178 plasma samples by digital PCR. Candidates biomarkers were validated using Western blot, extracellular vesicles analyses, immunochemistry and immunofluorescence.

Results: We identified three novel non-invasive biomarkers which showed an increased expression pattern in PC patients in comparison with controls (*PCA3*: $p = 0.002$, *S100A4*: $p \leq 0.0001$ and *MRC2*: $p = 0.005$). *S100A4* revealed the most informative AUC (area under the curve, 0.735). Combination of *S100A4*, *MRC2* and *PCA3* increases the discriminatory power between patients and controls and between different more and less aggressive stages (AUC = 0.761, $p \leq 0.0001$).

Conclusion: This is the first time that the role of *S100A4* and *MRC2* has been described in PC aggressiveness. Moreover, the combination of *S100A4*, *MRC2* and *PCA3* has never been described as a non-invasive biomarker for PC screening and aggressiveness.

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Conflict of Interest: Maria Jesus Alvarez Cubero Full part, principal investigator of several research projects, Luis Javier Martínez-González full, principal investigator of several projects, veronica arenas rodriguez full, collaborator, catalina romero full, lucia chica full, collaborator, beatriz alvarez full, collaborator, patricia porras quesada full, collaborator.

EP19.006 Association of AURKA c.169A>G and c.91T>A polymorphisms with higher cancer risk – preliminary results

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Background/Objectives: Aurora kinase A (AURKA) is a serine/threonine kinase that regulates the cell cycle and promotes cell progression. Several studies have investigated the relationship between genetic variations in the AURKA gene and cancer risk, with c.169A>G and c.91T>A being linked to a higher risk of developing breast, prostate, and colorectal cancer. The aim of our study was to examine the frequency of these two variants in patients with cancer.

Methods: The study consisted of 19 patients with colorectal cancer (CRC) and 12 patients with non-small cell lung cancer (NSCLC). Circulating tumor DNA (ctDNA) was extracted from blood plasma and sequenced.

Results: The c.169A>G variant was found in 18 of the 19 CRC patients and in all 12 NSCLC patients tested. The c.91T>A polymorphism was present in 12 of the 19 CRC patients and in 9 of the 12 NSCLC patients.

Conclusion: The results showed a high frequency of the tested genetic variants in both cancer types. Further investigations with a control group of healthy individuals in the same age range are necessary to confirm the association of these variants with a higher risk of malignancies. These findings can inform future studies on the role of AURKA gene variants in cancer development and the possibility of targeted therapies.

References: Wang S, Qi J, Zhu M, et al., AURKA rs2273535 T > A Polymorphism Associated with Cancer Risk: A Systematic Review with Meta-Analysis. Front Oncol. 2020 Jun 30;10:1040. https://doi.org/10.3389/fonc.2020.01040.PMID: 32733797;PMCID: PMC7357424.

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Conflict of Interest: None declared.

EP19.007 Leukocyte telomere length dynamics in breast cancer patients treated with adjuvant radiotherapy

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Background: Ductal carcinoma in situ (DCIS) is non-invasive breast cancer treated with surgery and adjuvant radiotherapy (RT). Even though RT enables good disease control, there is large inter-individual variability in treatment outcome regarding disease recurrence and adverse events. Telomeres are nucleoprotein complexes that protect chromosomes from degradation and studies suggest that cells with shorter telomeres might be more radiosensitive. Our aim was to determine leukocyte telomere length (LTL) dynamics in DCIS patients treated with RT and to evaluate the association of genetic factors with LTL.

Methods: Our study included 89 DCIS patients treated with adjuvant RT. We isolated genomic DNA from blood samples, determined LTL using monochrome multiplex quantitative PCR and genotyped all patients for *TERT* rs2736098, rs2736100, and rs10069690 polymorphisms using competitive allele-specific PCR. LTL was expressed as the ratio between the telomere and housekeeping gene amplicons. Nonparametric tests were used in statistical analysis.

Results: Relative LTL was 0.47 (0.41-0.58) before RT, 0.72 (0.63-0.88) immediately after RT and 0.68 (0.56-0.79) six months after RT. After RT, significant increase in LTL was seen in 91.0% of DCIS patients ($P < 0.001$), followed by a significant decrease in LTL in 58.4% of DCIS patients after six months ($P = 0.043$). Carriers of at least one polymorphic *TERT* rs2736098 T allele had significantly higher LTL before RT than non-carriers (0.51 (0.44-0.60) vs. 0.44 (0.37-0.56), $P = 0.020$).

Conclusion: Relative LTL is altered after RT. *TERT* genetic variability may affect LTL pre-treatment, but not after RT.

Grant References: ARRS J3-1753, J3-2527, and P1-0170.

Conflict of Interest: None declared.

EP19.008 Citywide population genetic screening for BRCA1/BRCA2 founder mutations

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Background: Screening for genetically determined diseases is the basis of future personalized health care. The use of test panels for founder mutations in the *BRCA1* and *BRCA2* genes has proven to be the most effective way to identify individuals at high risk of developing cancer. Here we present the results of the genetic screening of 107300 women and the follow-up monitoring of *BRCA1/2* mutation carriers.

Methods: The study involved women over 18. Genetic analysis of seven *BRCA1* and one *BRCA2* pathogenic mutations was performed by real-time PCR. Identified carriers received in-person genetic counseling and were examined for cancer during clinic visits over the four years.

Results: The median age of participants was 54.3 years. We identified 386 *BRCA1* and 16 *BRCA2* carriers ($n = 402$, 0.374%). Post-test counseling compliance was 81.8%. The relative risk of breast cancer (BC), bilateral BC, and BC under 50 in carriers was 12.7, 175.4, and 21.2 times higher than in founder mutation-free women, respectively ($p < 0.0001$). According to the NCCN Clinical Practice Guidelines in Oncology v.2.2022, genetic testing would only be recommended for 27% of identified carriers, and 6.3% of women without mutations remained underdiagnosed. During the

follow-up monitoring of carriers, 31 malignant neoplasms were found, while the proportion of early-stage cancers increased by 42.9% compared with the cancer cases before testing ($p = 0.0037$).

Conclusion: Broad testing allows for an accurate assessment of the genetic burden of a population and validation of current guidelines, while a mutation carrier surveillance program is an effective tool to improve early cancer diagnosis.

Conflict of Interest: None declared.

EP19.009 NAGENpediatrics: Rapid Whole Genome Sequencing in Neonatal/Pediatric Intensive Care in Navarra, Spain

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Background/Objectives: Whole-genome sequencing (WGS) is a rapid and cost-effective technique that significantly impacts the clinical setting, particularly in the paediatric context. Approximately 2-3% of newborns have a congenital anomaly, and at least 50% of these have a genetic cause. According to the literature, rapid genomic testing (2-3 weeks) allows the diagnosis of around 21-26% of critically ill children, influencing clinical outcomes. This pioneering study at the regional level aimed to evaluate the diagnostic and therapeutic utility of rapid WGS in acutely unwell children.

Methods: This study involved the participation of a multidisciplinary team and the recruitment of 79 families over two years. A specialized paediatric clinical team initially assessed their eligibility and each family received a detailed genetic counselling consultation. WGS was performed on germline DNA, and variants were filtered and reported by extensive bioinformatic analysis.

Results: We identified pathogenic variants in genes in line with the clinical manifestations in 31 families, reaching a diagnostic yield of 39.2%. Genetic results, delivered in an average time of 2-3 weeks, significantly reduced the diagnostic odyssey and influenced clinical decisions.

Conclusion: We demonstrated that rapid WGS in the paediatric context has potential extensive benefits for patients and clinicians. We described new genetic variants associated with rare diseases, not identifiable by other diagnostic methods in rapid turnaround times. This study brings to light the importance of implementing this analysis into routine care within the Regional Health System.

References: Stark, Z et al., 2022

Grants: Proyecto estratégico I + DS3 2020-2022. GEMAIV Gobierno de Navarra

Conflict of Interest: None declared.

EP19.010 TM6SF2-rs58542926 genetic variant modifies the protective effect of a "Prudent" dietary pattern on serum triglyceride levels

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Background/Objectives: The epidemic prevalence of non-alcoholic fatty liver disease (NAFLD), despite extensive research in the field, underlines the importance of focusing on personalized therapeutic approaches. Nutrigenetic effects on NAFLD are poorly investigated. To this end, we aimed to explore potential gene-dietary pattern interactions in a NAFLD case-control study.

Methods: Disease was diagnosed with liver ultrasound and blood collection was performed after an overnight fast. Adherence to four a posteriori dietary patterns was used to investigate interactions with PNPLA3-rs738409, TM6SF2-rs58542926, MBOAT7-rs641738, GCKR-rs738409 in disease and related traits. IBM SPSS Statistics/v21.0 and Plink/v1.07 were used for statistical analyses.

Results: The sample consisted of 351 Caucasian individuals. PNPLA3-rs738409 was positively associated with disease odds ($OR = 1.575$, $p = 0.012$) and GCKR-rs738409 with lnC-reactive protein (CRP) (beta=0.098, $p = 0.003$) and Fatty Liver Index (FLI) levels (beta=5.011, $p = 0.007$). The protective effect of a "Prudent" dietary pattern on serum triglyceride (TG) levels in this sample was significantly modified by TM6SF2-rs58542926 (p-interaction=0.007).

Conclusion: TM6SF2-rs58542926 carriers may not benefit from a diet rich in unsaturated fatty acids and carbohydrates in regard to TG levels, a commonly elevated feature in NAFLD patients.

Grant References: Greek Secretariat for Research and Technology (Cooperation 890/2009); Research Project for Excellence IKY/SIEMENS; Operational Program Competitiveness, Entrepreneurship and Innovation under the call RESEARCH- CREATE-INNOVATE (T2EDK-03044).

Conflict of Interest: None declared.

EP19.011 DPYD variants' frequencies in oncological patients treated with fluoropyrimidines: prevalence in Caucasian population

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Background: *DPYD* gene variants are of particular interest in pharmacogenetics due to the crucial role in the metabolism of fluoropyrimidines. Variations in the *DPYD* gene can result in a reduced ability to break down these drugs, leading to a build-up of toxic levels in the bloodstream and potentially life-threatening side-effects.

Aim: Characterization *DPYD* gene variants is important for personalized medicine, as individuals with certain *DPYD* gene variants may require lower doses of certain medications or alternative treatment options.

Materials and methods: The study analyzed 1'435 oncological patients treated with 5-fluoruracil between 2017 and 2022. *DPYD* variants (DPYD*2A: c.1905+1G > A, rs3918290; DPYD*13: c.1679T>G, rs55886062; D949V: c.2846A>T, rs67376798; HapB3: c.1236G>A, rs56038477; DPYD*6: c.2194G>A, rs1801160) were evaluated by allelic discrimination through Real time-PCR.

Results: The 1'435 analyzed patients comprised 727 women and 696 men. As expected, breast cancer was prevalent in women (34.8%), while gastrointestinal (GI) (90%) and head and neck (H&N) (4.6%) were prevalent in males ($P < 0.001$). The genotypes identified were: 1'220 DPYD*1/*1, 137 DPYD*1/*6, 49 heterozygous for HapB3, 9 DPYD *1/*2A, 3 heterozygous for D949V, 3 DPYD*6/*6, 2 DPYD*2A/*6, 1 compound heterozygous for *2A/HapB3 and 1 compound heterozygous for HapB3/*6. No differences were observed according to sex ($P = 0.280$) or age ($P = 0.234$).

Conclusions: Our findings showed a difference in frequencies compared to the literature, which could indicate a difference in sample population, possibly due to the migration phenomena. This concept highlights the importance of considering population-specific information when interpreting genetic testing results and developing pharmacogenetic guidelines.

Conflict of Interest: None declared.

EP19.012 A polygenic risc score for asthma in individuals from Volga-Ural region

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Background/Objectives: Polygenic score (PGS) is one of the modern bioinformatic approaches which involves combining information about many polymorphisms into a single system of the trait risk assessment. Asthma is a common multifactorial disease with a hereditary component based on polygenic nature. The aim of the study is the development of significant PGS model of asthma for individuals from the Volga-Ural region.

Methods: The selection of polymorphisms for PGS is based on the results of meta-analysis of worldwide asthma genome-wide association studies conducted by the international TAGC consortium (Demenais, 2018). Genotype data for 370 individuals with asthma and 380 controls from the Volga-Ural region were used for PGS. The effects of selected polymorphisms were estimated by logistic regression in studied case/control groups.

Results: A PGS was based on 31 polymorphisms associated with asthma development with the highest level of significance in meta-analysis of asthma GWAS studies by the TAGC consortium. An associative analysis of a given PGS model of asthma for individuals from the Volga-Ural region revealed an odds ratio of 2,07 ($p = 1.43*10^{-15}$; 95%CI 1.74-2.49), the area under the ROC-curve for this model was 0.69 (95%CI 0.65-0.73, sensitivity - 64%; specificity - 66%).

Conclusion: The resulting PGS model exhibits a certain predictive value for assessing the risk of asthma development in individuals from the Volga-Ural region.

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Conflict of Interest: None declared.

EP19.013 Molecular-genetic diagnosis of helicobacter pylori as a factor of gastric malignant neoplasms

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Background/Objectives: Nowadays, Helicobacter pylori (HP) infection is widespread throughout the world. Early detection and treatment of HP is highly important to prevent the development of malignant neoplasms (MN) of the stomach.

Increasing antibiotic resistance of HP is another significant issue. According to the literature, resistance to levofloxacin and clarithromycin is 30%, thus it is extremely important to quickly and accurately identify HP and its antibiotic resistance.

Materials and Methods: A set of primers and a protocol for diagnosing HP and its resistance to clarithromycin and levofloxacin were developed. 10 samples of gastric biopsy with suspected HP were tested and the results were confirmed using a rapid urease test and a serological method.

Results and discussion: Sequencing of HP genome regions containing targets, that allow detection of HP infection and identification of its resistance to clarithromycin and levofloxacin was carried out for 10 samples. In all 10 cases HP infection was detected. The method is highly accurate, since it employs the analysis of the HP nucleotide sequence.

The introduction of molecular-genetic diagnostic testing of HP and its antibiotic resistance into clinical practice will allow the doctor to quickly and accurately identify the infection and choose the right therapy, which ultimately will serve as one of the components of the stomach cancer prevention.

Conflict of Interest: None declared.

EP19.014 Gene editing for inherited retinal dystrophies: precise correction of pathogenic variants and optimization of the assay design

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Background/Objectives: Inherited retinal dystrophies (IRD) are a broad group of genetic eye diseases causing vision loss and other complications that have no treatment. Gene editing is one of the most promising strategies for future personalized and translational medicine, and is suitable for the single-nucleotide substitutions found in many of the IRD cases. This study aims to correct several IRD mutations and analyze different key factors for the successful edition of the pathogenic variants.

Methods: To correct the damaging variants we used CRISPR/Cas9 and TALEN technologies in hiPSCs obtained from IRD patients affected by Retinitis Pigmentosa, Stargardt disease, Best disease or Achromatopsia. We performed in silico design of several CRISPR (sgRNA) and TALEN guides, and repair templates considering some important parameters. Afterwards, we performed CRISPR or TALEN trying different conditions for assay optimization. Additionally, we performed Sanger sequencing for on-target and off-targets screening.

Results: We have repaired several IRD mutations in patient-derived iPSCs with no detected off-target aberrations. We have found that, sgRNAs harboring the pathogenic variant effectively discriminate between the two alleles in heterozygous cases. Regarding repair templates, the introduction of a Cas9-silent mutation was important to ensure a single repair cycle avoiding DNA re-cut by Cas9. Notably, we found that TALEN technology was significantly less efficient than CRISPR/Cas9.

Conclusion: sgRNAs, TALENs and repair templates designs are important to achieve precise and efficient single-nucleotide gene editing in hiPSCs. Research done on these therapeutic approximations is crucial for the development of potential IRDs' treatment through permanent correction of the pathogenic variants.

Conflict of Interest: None declared.

EP19.016 Applicability of breast cancer 313 Polygenic Risk Score (PRS313) in the Spanish region of Navarra

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Background/Objectives: Polygenic risk scores (PRS) have demonstrated their potential to predict the probability of developing cancer. This allows risk stratification and thus improved screening programmes. Among them, PRS_{313} is the best-performing breast cancer PRS for European-ancestry women (Mavaddat et al., 2018). The NAGENMx project aims to provide the necessary evidence to implement a personalized breast cancer-screening program in Navarra based on this PRS. Therefore, prior validation in our population is essential to ensure an accurate stratification.

Methods: We evaluated the application of the PRS_{313} in 803 whole genome sequenced samples from Navarra. The SNP and sample call rates and frequency of each SNP in our population were calculated. In an initial exploratory analysis, we tested the implementation of the PRS_{313} by replacing missing SNPs with previously described methods (MAF substitution, averaging and omission) (Collister et al., 2022).

Results: Each sample had at least 274 available SNPs, but only 211 were evaluable in all samples. Regarding the SNPs allele frequencies, 115 showed a significant difference ($p < 0.05$) in our population compared to that described by Mavaddat (2018). Finally, we showed significant risk distribution deviation shifts between the different approaches.

Conclusion: These results highlight the need to adapt and develop an alternative PRS appropriate to our target population and existing data. Ultimately, applying existing PRSs in different populations is challenging and requires standard guidelines for their evaluation and for dealing with missing data.

Grant References: Proyecto estratégico I + D S3 2021-2023. GEMA V Gobierno de Navarra

Conflict of Interest: None declared.

EP19.018 Association of SLC22A2 c.808T>G (rs316019) in the occurrence of hematological toxicity of Oxaliplatin in patients with colorectal cancer

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Background: Colorectal cancer is a common malignancy and Oxaliplatin is a chemotherapy agent used to treat it. However, Oxaliplatin can cause adverse drug reactions (ADRs), including hematological toxicity. The identification of predictive biomarkers for Oxaliplatin-induced ADRs is crucial to individualize treatment and improve patient outcomes. This study aimed to investigate the association between SCL22A2 rs316019 and hematological toxicity in patients with colorectal cancer treated with Oxaliplatin.

Methods: A total of 16 patients with colorectal cancer were included in the study. Circulating tumor DNA was isolated from blood plasma and targeted sequencing of SCL22A2 was performed with next-generation sequencing (NGS). All 16 patients were treated with Oxaliplatin, and the occurrence of hematological toxicity was studied.

Results: SCL22A2 rs316019 was identified in 12 patients with colorectal cancer. Among these 12 patients, all of them had hematological toxicity following treatment with Oxaliplatin. In contrast, none of the four patients without SCL22A2 rs316019 had hematological toxicity.

Conclusion: Our study suggests that SCL22A2 rs316019 may be a predictive biomarker for hematological toxicity in patients with colorectal cancer treated with Oxaliplatin. However, the sample size of this study is small, and further studies with larger sample sizes are needed to confirm these findings. If confirmed, SCL22A2 rs316019 may be used to identify patients at high risk for hematological toxicity and to adjust the dosage of Oxaliplatin to reduce the incidence of ADRs.

Grants: Project HO 13/2020, funded by Medical University of Plovdiv.

Conflict of Interest: None declared.

EP19.019 An in-vitro primary cell model for the assessment of response to omalizumab therapy in asthma patients

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Paediatric biological therapies are still in early stages and >40% of patients fail the treatment. Thus, there is an urgent need to identify biomarkers to guide therapy selection. We have adopted an in-vitro primary cell model to study the (non)response to omalizumab in asthmatic children and identify relevant biomarkers.

Primary immune blood cells from biological therapy naïve asthmatic children were in vitro treated with omalizumab, then

challenged by patient-specific allergen. Basophil activation test (BAT) was used to estimate omalizumab effect on the basophil activation. In parallel, omalizumab treated basophils were sorted and used for transcriptome analysis (RNA-seq).

Based on the BAT results patients were divided into good and poor/non-responders to omalizumab. RNA-seq identified OAS3, HLA-C, EPSTI1, HERC5, CMPK2, RSAD2, HBB, HBA1, HBA2 as potential biomarkers of the omalizumab (non)response. Further, gene ontology analysis suggested molecular pathways implicated in the omalizumab (non)response: response to virus, regulation of extrinsic apoptotic signalling pathway, cellular response to type I interferon and regulation of cytokine-mediated signalling pathway.

By combining the in vitro cell model and cell-specific transcriptomics we identified potential biomarkers and pathways influencing the differential response to omalizumab, that represent relevant targets for further analysis of omalizumab (non) response in asthmatic children.

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Conflict of Interest: None declared.

EP19.020 Pharmacogenetic testing in Psychiatry – are they useful?

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Background/Objectives: Treatment of mental disorders is often a complex and lengthy process. Based on the principles of personalized medicine, psychiatrists can tailor medications, dosages and combinations of medications according to pharmacogenetic test reports. We present clinical cases that demonstrate benefits and uncertainties of pharmacogenetic testing.

Methods: Blood DNA extraction and pharmacogenetic testing were performed according to the manufacturers` protocols. Genes included in pharmacogenetic analysis: *COMT,CYP2C9, CYP2C19,CYP2D6,MTHFR,CYP3A4,CYP3A5,CYP1A2,SLCO1B1,VKORC1*.

Results: Case no.1: young man with obsessive-compulsive disorder who has been unsuccessfully treated for eight years. The pharmacogenetic testing revealed an unusual phenotype. Treatment based on recommendations of prescribing guidelines was not effective for that patient. The patient is currently living an independent and socially active life while taking a treatment not indicated by the pharmacogenetic testing. Case no.2: young female with schizoaffective disorder, for whom all previously used psychotropic medications were discontinued due to reported adverse effects. After numerous unsuccessful treatment attempts, a pharmacogenetic testing was performed and showed a normal metabolizer phenotype for all psychotropic medications. The pharmacogenetic results led to an open conversation in which the patient admitted to be non adherence.

Conclusion: There is still a lack of knowledge and data on the genes involved in drug metabolism and drug interactions. In the absence of sufficient information in treatment guidelines, the question is what to do when treatment fails? Further research is needed to identify other factors contributing to tolerance when a drug is not recommended on the basis of pharmacogenetic testing, or intolerance when a drug is recommended.

Conflict of Interest: None declared.

EP19.021 1 + MG European Initiative and Italian response to this important challenge

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Launched in April 2018, the '1+MillionGenomes' (1 + MG) initiative aims to enable secure, cross border access to at least 1 million genomic sequences across EU Member states, to foster the application of genomics in clinical practice and empower healthcare and research. Italy was among the signatories of the state of 1 + MG declaration and was included in 'Beyond 1 MillionGenomes' (B1MG) a H2020 RIA action, funded to provide infrastructural and technical support for 1 + MG. In 2023 the "Genomic Data Infrastructure"(GDI) project was funded by the EU to bring the infrastructure developed by B1MG at the operation level. The Italian representatives for the GDI are Graziano Pesole (ELIXIR-IIB) and Serena Battilomo (MoH). To coordinate 1 + MG/ B1MG/GDI activities at a national level, National Mirror Working Groups were established under the Coordination of Stefania Boccia and Bruno Dallapiccola, supported by a national grant by the Centre for Control of Diseases (CCM) of MoH, coordinated by Paolo Villari.

At the same time MoH financed with 55M euros "Health Big Data" (HBD) a 10 years project aiming to implement a national infrastructure for the sharing of clinical and omics data between Italian Research Hospitals (IRCCS). HBD is coordinated by Ruggero De Maria and Pier Giuseppe Pelicci, in collaboration with Politecnico of Milan. Other stakeholders are the Biobanks Network of the European Infrastructure BBMRI-ERIC, with the Italian node coordinated by Marialuisa Lavitrano, and the Italian node of Elixir Infrastructure.

We will present the state of art of ongoing activities for the sharing of clinical and omics data in Italy.

Conflict of Interest: None declared.

EP19.022 Does Cancer Diagnosis Alter the Lifestyle of Individuals with HBOC and Lynch Syndrome? Results from the Swiss CASCADE Cohort

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Introduction: Individuals with pathogenic variants associated with HBOC and Lynch syndrome have significantly increased risk for primary and secondary cancer diagnoses. Studies indicate that lifestyle (i.e., alcohol intake, BMI, physical activity, and smoking) may modify cancer risk (e.g., age of cancer onset). We hypothesized that carriers of pathogenic variants who have already developed cancer may have a healthier lifestyle compared to those without cancer, presumably to avoid a second cancer diagnosis.

Methods: This analysis focuses on HBOC and Lynch syndrome cases (identified as carriers of a pathogenic variant with and without a cancer diagnosis) that participated in the Swiss CASCADE cohort and submitted baseline data and/or 18-month follow-up data between September 2017 and November 2022. Generalized linear models with random effects were run for each lifestyle behavior.

Results: We analyzed 635 observations from 415 individuals (HBOC = 326 and Lynch syndrome = 89). The average age of the sample is 51.5(±13.1) years, 81.7% females, 58.7% had at least one cancer diagnosis. Preliminary analyses show that HBOC and Lynch syndrome cases with cancer diagnosis have lesser odds of being current alcohol consumer than individuals without any cancer diagnosis, but it is not statistically significant (OR:0.18, CI:0.22-1.48). Recent cancer diagnosis (OR:0.12, CI:0.01-1.02) has borderline significant effect on current alcohol consumption.

Conclusion: Analyses are still ongoing. Findings may have implications for personalized interventions.

Conflict of Interest: Mahesh Sarki University of Basel, günther fink Swiss School of Tropical Public Health, EPOCH - Women's political EmPOwerment and Child Health, Souria Aissaoui Hirslanden Clinique de Grangettes, Nicole Bürki University Hospital Basel, Rossella Graffeo-Galbiati Oncology Institute of Southern Switzerland, Karl Heinimann University of Basel, Christian Monnerat Hospital of Jura, Manuela Rabaglio Inselspital, Bern University Hospital, Ursina Zuerrer Cantonal Hospital Winterthur, Pierre O. Chappuis University Hospitals of Geneva, Maria Katapodi University of Basel, The Swiss CASACADE Study.

EP19.023 Assessment of a pharmacogenetic test to aid optimised drug treatment in the use of drugs associated with depression, anxiety and other mental health conditions in an NHS setting

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NHS mental health services are seeing increases in referrals and struggling to meet demand. There is a need to explore how this demand can be met by using more efficient treatment pathways. One issue is identifying the most effective drug for individual patients. Cycling through multiple drugs and doses is frustrating, time consuming and costly. There is evidence that using pharmacogenomic markers to aid treatment decisions can increase adherence, reduce the number of drugs tried before a successful outcome and reduce incidence of adverse drug reactions.

To assess the impact of pharmacogenomics on patient outcomes within NHS mental health services, West Midlands Regional Genetics Laboratory (WMRGL), working with local pharmacy and mental health (Forward Thinking Birmingham) colleagues, have collaborated with Inagene Diagnostics Inc. to incorporate a pharmacogenomic panel into patient management protocols. Inagene Diagnostics Inc. are a Canadian pharmacogenomics company offering a panel of targets in 37 genes to predict responses to >140 drugs used to treat pain and mental health conditions. Agena MassArray data, generated at WMRGL, is interpreted using the Inagene patient portal to generate an online patient report which provides the clinician with easy-to-interpret information that can be incorporated into protocols to support drug treatment decisions.

The aim of this project is to perform a local pilot to identify and overcome obstacles to using pharmacogenomics as part of prescribing pathways within the NHS. Initial outcomes will be presented.

Conflict of Interest: Jessica Woodley: None declared, Ania Skowronska: None declared, Samantha Court: None declared, Alison Tennant: None declared, Gareth Rees: None declared, Jackie O'Brien Inagene Diagnostics UK Ltd, Stephen Abbs Inagene Diagnostics UK Ltd, Ben Pinder Inagene Diagnostics Inc, Jennie Bell: None declared.

EP19.024 Isoform-Level Immune-Cell Deconvoluted Colon Tissue Transcriptome Profiling of Adalimumab response in Crohn's disease patients

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Over the past decade studies have explored the heterogeneity of anti-TNF response in Crohn's patients. Despite numerous studies, there is lack of studies with sufficient thresholds and no reproducibility between genetic and expression data exists. To date, genetic studies of anti-TNF research in Crohn's disease focused merely on genes, with disregard to specific isoforms.

DNA from peripheral blood mononuclear cells was extracted from 84 Slovenian patients with Crohn's disease and genotyped using the genotyping microarray. Additionally, RNA from 22 colon tissue biopsies was obtained and transcript specific tissue RNA-seq with deconvolution analysis was performed independently on healthy and inflamed tissue samples. Gene ontology analysis and functional annotation using the HaploReg database were also carried out. Subsequently, the validation of identified transcripts and variants was estimated using machine learning random forest algorithm.

Our analysis revealed nine genes (*MACF1*, *CTSE*, *HDLBP*, *HSPA9*, *HLA-DMB*, *TAP2*, *LGMN*, *ANAPC11* and *ACP5*) with 15 transcripts involved in adalimumab response. 16 variants in aforementioned gene regions were identified using genomic integration. Variants have shown impact on gene expression through epigenetic modifications, chromatin remodeling or protein/motif binding alternations.

The present study showed the importance of -omics integration and identified new loci involved in adalimumab response. Our results may help to elucidate the insight in molecular pathways of anti-TNF treatment response and contribute to the discovery of

new potential treatment targets for Crohn's disease patients, who lose response to anti-TNF treatment.

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Conflict of Interest: None declared.

EP19.025 Secondary findings: Experience of a clinical genetics unit in Portugal

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Introduction: Clinical exome sequencing (CES) has shown great utility in the diagnosis of genetic disorders. Secondary findings (SFs) are medically actionable variants unrelated to the primary indication for testing, and are a potential consequence of CES with some associated challenges. In order to solve these difficulties, the American College of Medical Genetics and Genomics (ACMG) published the first recommendations for reporting SFs, indicating a list of genes to be evaluated when performing CES in the clinical context. Since then, updates to this list have been published.

Methods: We evaluated the SFs identified in selected patients for CES at our clinical genetics unit, between January 2017 and June 2022. SFs were screened according to ACMG recommendations.

Results: The overall frequency of SF was 2%. 10 unique variants were identified, including 8 pathogenic and 2 likely pathogenic variants, within 8 ACMG-reportable genes. The disease categories of SFs were cardiovascular (60%), cancer (30%) and miscellaneous disease (30%). The SFs results affected the medical management and follow-up strategy in 6 (60%) patients.

Conclusion: To our knowledge, this was the first study investigating SFs in Portugal. We observed that the information of SF from CES could be a valuable and lifesaving effort. On the other hand, it can pose some clinical and ethical difficulties in counselling patients and their families. This study reinforces the need for additional work and consensus in order to support clinical decisions. Additionally, it will be a pioneer for studies in Portuguese population.

Conflict of Interest: None declared.

EP19.026 DPYD haplotype structure for variants *2A, *13, c.2846A>T, c.1236G>A/HapB3, c.496A>G (rs2297595), *6 (rs1801160) and *9A (rs1801265) in the Croatian population

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Background/Objectives: Dihydropyrimidine dehydrogenase, coded by the DPYD gene, is a rate-limiting enzyme in the metabolism of fluoropyrimidines. Data on the frequency of DPYD haplotypes and impact of common DPYD variants on fluoropyrimidine-related toxicity is limited. We analysed genotype and haplotype frequencies of four DPYD variants from pharmacogenomic guidelines and three additional common DPYD polymorphisms in the Croatian population.

Methods: In this study, we analysed data DPYD genotyping from routine pharmacogenetic testing in cancer patients treated with fluoropyrimidine therapy. A total of 998 subjects of Caucasian ancestry were genotyped for DPYD *2A (c.1905+1G > A), *13 (c.1679T>G), c.2846A>T, c.1236G>A/HapB3, c.496A>G (rs2297595), *6 (c.2194G>A, rs1801160) and *9A (c.85T>C, rs1801265) by TaqMan real-time PCR. Genotyping data were analysed to estimate allele frequencies, common haplotypes and haplotype frequencies.

Results: The DPYD variants allele frequency were *2A $q = 0.01653$, *13 $q = 0.0005$, c.2846A>T $q = 0.00401$, c.1236G>A $q = 0.02555$, *6 $q = 0.05962$, *9A $q = 0.23447$ and c.496A>G $q = 0.13176$. 439 subjects (44.0%) were carriers of wt alleles, 141 (14.1%) were carriers of c.496G/*9A genotype, 134 (13.4%) of *1/*9A genotype, 67 (6.7%) of *1/*6 genotype, 36 (3.6%) of *1/ c.496G genotype, 29 (2.9%) of c.1236A/*9A genotype, 6 (0.6%) of *1/ c.1236A genotype. Analysing the combination of DPYD variants revealed that 263 subjects (26.4%) were carriers of one variant allele, while 271 (27.2%) were carriers of two or more different (compound) variant alleles.

Conclusions: This study provides information on DPYD haplotype frequencies in the Caucasian population, which is essential for the design of pharmacogenetic studies investigating the impact of common DPYD variants and haplotypes on fluoropyrimidine-related toxicity.

Conflict of Interest: Lana Ganoci University Hospital Centre Zagreb, Croatian Science Foundation project collaborator, Lucija Lešnjaković Teva, Livija Šimičević University Hospital Centre Zagreb, Croatian Science Foundation project collaborator, Jozefina Palic School of Medicine University of Zagreb, Croatian Science Foundation project collaborator, Iva Mucalo University of Zagreb, Nada Božina: None declared.

EP19.027 Identifying chrono-pharmacogenomic variation to inform chrono-dosing potential of medicines

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Background/Objectives: Pharmacogenomics aims to utilise patients' genomic variation to optimise drug treatment and improve patient outcomes. Circadian and circannual clocks influence the differential and rhythmic expression of genes across time, tissues, and individuals. Genes involved in pharmacological pathways, are chrono-pharmacogenes (CPG), and may associate with disease. Chronotherapy aims to optimise treatment timing, to maximise efficacy and minimise adverse effects. Biological clock mechanisms are affected by a patient's chronotype, core clock gene DNA-binding proteins binding to CPG promoter clock motifs (PCMs). We define personalising chronotherapy and prevention guided by chrono-biomarkers, and pharmacogenomic variation as chrono-pharmacogenomics (CPGx).

Method: Databases of human genes with putative circadian (CircaDB), seasonal (BABYDIET), pharmacological (DrugBank, PharmGKB), and disease (OMIM, PharmGKB) associations were collated and intersected. Variants from dbSNP were mapped in 1000bp PCM regions for each CPG. DrugBank annotations were used to associate common drugs with corresponding CPGs. The

100,000 Genome Project (100kGP) patient data were mined for CPGx variants and enrichment in disease.

Results: We identified 1675 chronopharmacogenes (CPG) and 17,690 unique Single Nucleotide Polymorphisms in PCMs. The CPGs are associated with 918 common drugs. A subset analysis in 100kGP patients identified 2,082 SNPs, some associating with disease.

Conclusion: We computationally identified the existence and utility of three tiers of variant discovery for chrono-pharmacogenomic medicine influencing pharmacological outcomes; variants underlying chronotype, in clock genes, and regulatory elements of rhythmic genes.

Pharmacological and disease pathways are intertwined with clock mechanisms highlighting the chrono-dosing potential of numerous common drugs, to maximise efficacy and minimise adverse effects.

Conflict of Interest: Aris Saoulidis AS also employed by NHS England, Master of studies degree funded by Health Education England Genomics Education Programme, Timothy Hearn: None declared.

EP20 Population Genetics and Evolutionary Genetics

EP20.001 A robust pipeline for ranking carrier frequencies of autosomal recessive and X-linked Mendelian disorders

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Background/Objectives: Single gene disorders are individually rare but collectively common leading causes of neonatal and pediatric morbidity and mortality. Both parents or the mothers of affected individuals with autosomal recessive or X-linked recessive diseases, respectively, are carrier(s). Carrier frequencies of recessive diseases can vary drastically among different ethnicities. This study established a robust pipeline for estimating and ranking carrier frequencies of all known 2,699 recessive genes based on genome-wide sequencing data in healthy individuals.

Methods: The discovery gnomAD cohort contained sequencing data on 76,156 genomes and 125,748 exomes from individuals with seven ethnicity backgrounds. The three validation cohorts composed of the SG10K Project with 4,810 genomes on East Asian and South Asian, the ChinaMAP project with 10,588 Chinese genomes, and the WBBC pilot project with 4,480 Chinese genomes.

Results and Conclusion: Within each cohort, comprehensive selection criteria for various kinds of deleterious variants were instituted, including known pathogenic variants (Type 1), presumably loss-of-function changes (Type 2), predicted deleterious missense variants (Type 3), and potentially harmful in-frame INDELs (Type 4). Subsequently, carrier frequencies of the 2,699 genes were calculated and ranked based on ethnicity-specific carrier rates of Type 1 to Type 4 variants. Comparison of results from different cohorts with similar ethnicity background exhibited high degree of correlation, particularly between the ChinaMAP and the WBBC cohorts (Pearson correlation coefficient $R = 0.92$), confirming the validity of our variant selection criteria and the overall analysis pipeline.

Grant References: HMRF #08191216, GRF ECS #24101921, NSFC #32170583 & #82202045.

Conflict of Interest: None declared.

EP20.002 Single nucleotide variants in microRNA biosynthesis genes in mexican individuals

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Background: MicroRNAs (miRNAs) are important regulators in a variety of biological processes and their dysregulation have been associated with multiple human diseases. Single nucleotide variants (SNVs) in genes involved in the processing of microRNAs may alter miRNAs regulation and could present high allele heterogeneity in populations from different ethnic groups. Thus, the aim of this study was to genotype 15 SNVs in eight genes involved in miRNA processing pathway in Mexican individuals and to compared their frequencies across 21 populations from five continental groups.

Method: Genomic DNA was extracted from 399 healthy Mexican individuals. SNVs in genes AGO2 (rs2293939, and rs4961280) DGCR8 (rs720012), DICER (rs3742330, and rs13078), DROSHA (rs10719, and rs6877842), GEMIN3 (rs197388, and rs197414), GEMIN4 (rs7813, rs2740349, and rs4968104), TNRC6B (rs9611280) and XP05 (rs11077, and rs34324334) were genotyped using TaqMan probes. Minor allele frequency of each SNV was compared using Chi2 test with those reported in the 1,000 genomes database.

Result: All 15 SNVs were found in Hardy-Weinberg equilibrium in Mexican individuals, with prevalence ranging from 0.04 to 0.45. The SNVs rs4961280, rs2740349, rs34324334, and rs720012 in Mexican individuals showed one of the highest minor allele frequencies worldwide, whereas the minor allele frequencies of rs197388, rs10719, rs197414 and rs1107 was among the lowest in Mexican individuals.

Conclusion: Our data suggest that worldwide distribution of the frequency of SNVs located in components of the miRNA processing pathway has been shaped by different adaptive forces, which could modify the genetic susceptibility associated with human diseases in populations with different ancestry.

Conflict of Interest: Jesús Juárez Luis fellowship no. 288762 Conacyt, Moises Alfredo Canseco Ocaña: None declared, Miguel Ángel Cid Soto PhD INMEGEN, Xochitl Castro Martínez Full time PhD INMEGEN, Angélica Graciela Martínez Hernández PhD INMEGEN, Lorena Sofía Orozco Orozco Full time PhD INMEGEN, Araceli Hernández Zavala PhD IPN, Emilio Joaquín Córdova Alarcón Full time PhD INMEGEN.

EP20.004 Breast cancer knowledge and practice among Saudi Arabia women with and without positive family history: a cross sectional study

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Background/Objectives: Knowledge of association between breast cancer and risk factors of family history can change the health behaviour. To reduce risk factors associated with breast cancer among young women in Saudi Arabia, it is necessary to understand the degree of risk perception and socio-economic differences.

Methods: A cross-sectional study of 253 Saudi women aged 25-64 years investigated the awareness of risk factors (positive family history) for breast cancer. Standard self-administered questionnaire, screening practices including breast self-examination (BSE), clinical breast examination (CBE), and mammography were included in the data collection and analysed for individuals with and without a positive family history.

Results: Prevalence of positive family history (FH^+) and non-family history (FH^-) was 75/253 (30%), and 178/253 (70%) respectively. The mean age of women with FH^+ was 47.1 years and FH^- was 43.4 years. The results showed that both groups were low in practicing BSE (FH^+ 23% and FH^- 13%). The BSE knowledge assessment showed 77% in FH^+ women whereas the knowledge of CBE and mammography in FH^+ women were 75 % and 76% respectively, and 83% and 56% respectively in FH^+ women. The women with lower education and low income have less knowledge about breast cancer screening and FH^+ as a risk factor were identified.

Conclusion: Poor knowledge and practice of breast screening can contribute to late-stage breast cancer disease. Understanding the strengths and importance of the relationship between breast screening activity and its risk factors is essential to promote breast health.

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Conflict of Interest: Noura al-dayan Prince Sattam bin Abdulaziz University, PSAU, Grant # 3411/03/2015.

EP20.007 Association between IL10RA gene haplotypes and rheumatoid arthritis susceptibility

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Background/Objectives: Rheumatoid arthritis (RA) is a chronic autoimmune disease in which altered immune system activity leads to chronic synovitis and synovial joint impairment. Interleukin 10 (IL10) receptor is a part of the IL10 immunoregulatory pathway. SNPs in gene coding IL10 receptor subunit A (*IL10RA*) could affect signalling through this pathway and disrupt immune homeostasis. We aimed to analyse associations between *IL10RA* haplotypes and RA susceptibility.

Methods: The study included 132 RA patients diagnosed and treated at the Institute of Rheumatology in Belgrade, and 128 controls without autoimmune diseases. All RA patients met the 2010 ACR/EULAR Classification Criteria for RA. Genotypes for 6 polymorphisms within the *IL10RA* gene (rs10892202, rs4252270, rs3135932, rs2228055, rs2229113, rs9610) were determined using TaqMan assays. Haplotype analysis was performed using Haploview software.

Results: After using the Confidence intervals LD method, the haplotype block was defined between rs10892202 and rs4252270 ($r2 = 0.96$, $D' = 1$) and between rs2229113, rs9610 polymorphisms ($r2 = 0.35$, $D' = 0.95$). RA patients were harbouring *IL10RA* gene GCAAGA, GCGAAG, GCAAAG, CTAAGG, GCAGGG and GCAAGG haplotypes (42.5%, 18.8%, 11.9%, 10.2%, 8.7% and 3.9%, respectively), while controls had GCAAGA, GCAAAG, GCGAAG, CTAAGG, GCAGGG, GCAAGG haplotypes (49.9%, 16.9%, 10.4%, 9.8%, 6.6%, 5.5%, respectively). Haplotype GCGAAG was significantly more frequent in the RA patients group than in the control group ($p = 0.007$).

Conclusion: The findings of this study suggest that *IL10RA* gene haplotypes could be associated with RA susceptibility. Further studies are needed for definitive conclusion.

Grant References: Science Fund of the Republic of Serbia, PROMIS, grant number 6060866, ROLERS.

Conflict of Interest: None declared.

EP20.008 Mendelian randomization study of birth weight and risk of psychiatric disorders later in life

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Background/Objectives: Low birth weight (BW) has been associated with a higher risk of psychiatric disorders (PD) later in life. In this study, we investigate whether variation in fetal growth has a direct causal effect on six PDs.

Methods: We used BW as a proxy measure for fetal growth and first performed logistic regression analyses to assess associations between observed BW and later diagnosis of PD in the iPSYCH and the ANGI-DK studies. Next, we leveraged publicly available GWAS summary statistics to construct polygenic scores reflecting the effect of fetal genetic variation on BW and test for association with PD. Finally, using a set of 87 SNPs with robust fetal genetic association with BW, we assessed the causal relationship of genetically mediated fetal growth and PD later in life by Mendelian randomization.

Results: Higher observed BW was associated with lower risk of several PD. Genetic data for common variants across the genome supported this pattern for attention deficit/hyperactivity disorder (ADHD) where an increase of one standard deviation in polygenic scores for BW corresponded to an odds ratio of 0.86 ($P = 1.08 \times 10^{-4}$). However, one- and two-sample Mendelian randomization analyses did not support a causal relationship between the BW and risk of PD later in life.

Conclusions: Our study did not find evidence for a direct causal effect of fetal growth (as proxied by BW) on the risk of PD later in life.

Grant References: The Lundbeck, the Klarman Family, and the A.P. Møller foundations.

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EP20.009 Assessment of the potential functional role of paralog genes in polyglutamine spinocerebellar ataxias

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Background/Objectives: Polyglutamine (polyQ) spinocerebellar ataxias (SCAs) comprise a group of autosomal dominant neurodegenerative disorders caused by $(CAG/CAA)_n$ polymorphic expansions. These repeats of adjacent glutamines alter the conformation of the native proteins inducing neurotoxicity, and subsequent motor and neurological symptoms. Although the etiology and neuropathology of most polyQ SCAs have been extensively studied, only a limited selection of therapies is available. One previous study on SCA1 demonstrated that ATXN1L, an ATXN1 paralog gene, alleviated the neuropathology in mice models. Therefore, it is of upmost importance to understand whether paralog SCA-related genes can have a functional role in disease pathogenesis.

Methods: In this study, we analysed paralog genes of seven polyQ dominant ataxias by reviewing published literature and available databases to assess their gene and protein homology, potential function, expression pattern, subcellular distribution, and molecular interactors.

Results: Out of the 20 assessed paralog genes - ATXN2L, ATXN3L1, CACNA1B, ATXN7L1, ATXN7L2, TBPL2, and RERE are promising candidates that present the potential to be functionally relevant, contributing to the neuropathology of the respective SCA, along with the parental gene.

Conclusion: Our preliminary results suggested that seven of the assessed paralog SCA-related genes should be further studied through the analysis of intra and interspecific sequence diversity, evolution rates, and selective constraints in the reconstructed phylogenies. In addition, we ought to evaluate the gene expression of these paralog genes in different human tissues.

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Conflict of Interest: None declared.

EP20.010 Comprehensive within-population characterization of the Transylvanian Seklers based on high-resolution autosomal marker data

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The Seklers (in Hungarian: Székelys) are a Hungarian-speaking, Hungarian-derived catholic ethnic group living mainly in Transylvania, Romania. The first written evidence of Seklers originates from the 12th century. Their exact origin is unknown, their within-population structure has not been investigated yet. Here we intended to describe their genetic makeup investigating subpopulations from different areas of Transylvania.

We analyzed 319 Selker samples collected from 10 distinct regions of Transylvania. Based on self-declaration, they did not mingle with other ethnicities for at least 3 succeeding generations. Using Illumina 720K genotype data, we conducted investigations analyzing allele frequency and haplotype data, like principal component analysis, maximum likelihood estimation-based ancestry analysis, formal tests of admixture, and various DNA segment analysis methods including identity-by-descent segment analysis.

Our results show that the Seklers of Transylvania share similar ancestry components in a similar extent. This was strengthened both by allele frequency and by haplotype-based investigations. However, our allele frequency-based investigations pointed out that several individuals from the area of Szentegyháza and Szováta show a much larger share from one of the ancestry components shared with East Europeans.

According to our investigations, the Seklers living in Transylvania are rather homogeneous except of two regions. These two areas possess a significantly higher share from the East European, West Asian ancestry component, which points to the presumed homeland and origin of the Seklers. This might allow us the further study of the origin of the Sekler people.

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Conflict of Interest: None declared.

EP20.012 National Center Biobank Network (NCBN) in Japan

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Six national centers (6NCs) for advanced and specialized medicine in Japan have conducted basic and clinical research on a variety of diseases that have a significant impact on national health. Disease-specific bioresources and information collected by each NC are stored in a separate biobank. The National Center Biobank Network (NCBN) coordinates these biobanks and researchers of the 6NCs via an open access database (Catalogue Database: http://www2.ncbiobank.org/Index_en) for efficient provision of registered biological resources and data for utilization in research communities. The NCBN resources are characterized by their high quality and rich medical information and are available for life science research, through a deeper understanding of disease pathogenesis. A total of 4480,038 specimens from 129,083 patients are stored in 6NCs Biobanks (as of Jan 31, 2023). In addition to the bioresource and medical information, whole genome sequencing analysis for 9,850 NCBN samples has been conducted since 2020 (Kawai et al., bioRxiv 2023. These can be utilized as control subjects in research into cancer and rare diseases in the Japanese population and their variant frequency information will be registered to NBDC Human Database and

Medical Genomics Japan Variant Database (MGeND) after publication. The total number of obtained genome-wide data for NCBN bioresources is increasing for many diseases through whole genome sequencing analysis or genome-wide SNP array analysis and it is now possible to search the Catalogue Database for bioresources with genomic information. NCBN is creating a system that can provide both bioresources and genomic data to users.

Conflict of Interest: None declared.

EP20.013 Genetic predisposition to obesity and subsequent mobility dysfunction; overlap of genetic effects in brain regions

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Background/Objectives: How obesity earlier in life impacts upon mobility dysfunctions in late life is not well understood. Pernicious effects of excess weight on the musculoskeletal system and mobility dysfunctions are well-recognized. However, increasingly more data support the link of obesity to overall motor defects that are regulated in the brain. We assessed the causal relationship between body mass index (BMI) at midlife and performance of the Timed Up-and-Go test (TUG) in late life among a population-based longitudinal cohort.

Methods: We evaluated genetic predispositions for BMI in 8342 participants who were followed up from measurement of BMI at average 53 years, to TUG test (a functional mobility measure) 20 years later.

Results: A robust 75.83% of genetically determined BMI effects on late life TUG scores was mediated through midlife BMI ($P_{indirect\text{-}effect} = 9.24 \times 10^{-21}$). Utilizing Mendelian randomization, we demonstrated a causal effect between BMI and functional mobility ($\beta_{IVW} = 0.180$, $P_{IVW} = 0.001$). Secondary gene enrichment evaluations highlighted down-regulation of genes at BMI risk loci that were correlated with poorer functional mobility in the substantia nigra and amygdala regions as compared to all other tissues. These genes also exhibit differential expression patterns during human brain development.

Conclusions: We report a causal effect of obesity on mobility dysfunction. We highlight potential neuronal dysfunctions in regulating predispositions on the causal pathway from obesity to mobility dysfunction.

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Conflict of Interest: None declared.

EP20.014 Association of PON1 and NOS3 gene polymorphisms with the severity of COVID-19

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Background/objectives: Coronavirus infection (COVID-19) is a highly contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). According to WHO (February 10, 2023), there are about 755 million confirmed cases of COVID-19, including more than 6.8 million deaths.

Several risk factors have been associated with the severity of COVID-19, including patients with diabetes, hypertension, and other comorbidities. An important factor is also the genetic predisposition and the role of genetic variants.

The aim of this study was to investigate the association of polymorphisms in the genes of paraoxonase 1(PON1) and nitric oxide synthase 3(NOS3), which are enzymes with antioxidant activity, with the severity of COVID-19.

Methods: Genotyping of DNA samples isolated from blood cells of 110 COVID-19 patients (55 mild, 55 severe cases) was carried out by allele-specific RT-PCR.

Results: Significant associations of *PON1* (Q192R-rs662) ($p = 0.005$) and *NOS3* (T786C-rs2070744) ($p = 0.035$) polymorphisms with the severity of COVID-19 were found. Carriers of *PON1* rs662 (QQ) genotype have a more severe course of COVID-19 (OR = 2.323; 95% CI [1.095–4.973]). The TT genotype of *NOS3* rs2070744 was associated with severe cases (OR = 2.719; 95% CI [1.238-5.970]), while the TC genotype was more common in mild cases (OR = 0.381; 95% CI [0.176-0.823]).

Conclusion: The obtained data indicates the importance of *PON1* and *NOS3* genetic variants in predicting the severity of COVID-19 symptoms.

Grant References:
This study was funded by the Ministry of Science and Higher Education of the Russian Federation #0852-2020-0028.

Conflict of Interest: None declared.

EP20.015 Rare pathogenic variants in ciliary dyskinesia genes probably associated with severe and critical COVID-19 in Bulgarian patients

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Background/Objectives: Severe COVID-19 in previously healthy children and adults may result from monogenic predisposition. Thus, many efforts are made to determine rare genetic variants which are associated with severity of COVID-19 infection. There are experimental evidences that SARS-CoV-2 could trigger ciliary and flagellar dysfunction but it is not yet known the role of variants in genes involved in the formation of cilia and flagellum in the response to SARS-CoV-2 infection.

Methods: We performed whole exome sequencing (WES) in a group of 233 SARS-CoV-2 infected individuals of which critical and severe cases were 96, moderate – 56 and mild and asymptomatic - 81.

Results: Known rare pathogenic/likely pathogenic variants in the following genes: *CCDC103*, *DNAH5*, *DNAH11*, *STK36* involved in ciliary dyskinesia were found in 6 patients with critical or severe COVID-19. In addition, 2 patients from this group carried the same novel likely pathogenic splice variant in *CENPF*. In the group of patients with moderate illness one known pathogenic variant in *DNAH11* and one new likely pathogenic variant in *NME8* were found. In only one patient with mild disease likely pathogenic variant was found in *ODAD1*. These results show that pathogenic/likely pathogenic variants in ciliary dyskinesia genes are associated with increased risk for severe COVID-19, with OR 4.06 (95% CI – 1.05-15.72), $p = 0.0425$.

Conclusion: Rare pathogenic variants in genes involved in ciliary dyskinesia are probably involved in the response to SARS-CoV-2 and are associated with COVID-19 severity in Bulgarian patients.

Grant References: MES: KP-06-DK1/8, 29.03.2021,D01-278/14.12.2022,D01-395/18.12.2020, D01-302/17.12.2021,D01-165/28.07.2022

Conflict of Interest: None declared.

EP20.016 Evaluating transcriptome variation of single placental cells in Siberian populations

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Gene expression variation at the population level is a phenotypic trait of particular interest for biomedical research that understanding patterns of gene-expression variation within and among human populations may provide important insights into the molecular basis of phenotypic diversity in normal and pathological. Using laser capture microdissection and high-throughput sequencing (RNA-seq) for the first time we studied the transcriptomic profile of single DSCs from cryopreserved placenta of Russian, Buryat, Tatar and Uzbek women with normal pregnancy and assessed transcriptome variability at several levels. Evaluation of the DSCs transcriptome by the inter-individual and inter-population components of variability indicates that only 5% falls on the groups, while the maximum variability (82%) in the inter-individual component. Totally the 581 genes are differentially expressed (DEGs) in all comparisons (FDR < 0.05). The main molecular mechanisms of DEG when comparing Russian-Buryat and Buryat-Tatar pairs are associated with the immune response and inflammation. In pairwise comparisons of Uzbeks with Russians, Tatars, and Buryats, the most represented molecular categories are associated with ion transport, while DEG for the Russian-Tatar pair was associated with transmembrane metal transport and regulation of the immune response. The 183 DEGs were common for four of six comparisons. They are associated with such pathological conditions as anovulation, selenium deficiency, lipid metabolism disorders and dyslipidemia according to the DisGeNET database. Thus, for the first time we assessment of the inter-individual and inter-population variability of transcriptome on single placental cells. These data are importance for studying the mechanisms of healthy and complication placentation.

Conflict of Interest: None declared.

EP20.017 Genetic analysis of the most common ALS variants in the Cypriot population; are C9orf72 repeat expansions the predominant cause?

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Background/Objectives: Amyotrophic lateral sclerosis (ALS) is a devastating, uniformly lethal degenerative disease of motor neurons, presenting with relentlessly progressive muscle atrophy and weakness. Since the identification of the first causative gene SOD1 in the 1990s and with recent advances in genetics, more than 50 potential causative or disease-modifying genes have been identified, with SOD1, TARDBP, FUS and C9orf72 being the most common.

Methods: A total of 82 ALS patients including 21 fALS (26%) and 61 sALS (74%), provided the cohort for the variant screening in the most common causative genes of ALS including C9of72, SOD1, TARDBP, FUS, ATXN2, and SMN1. Southern blot hybridization using a 954-bp non-radioactive probe has been used to characterize the expansion size range of 14 C9orf72-positive cases.

Results: One patient with the pathogenic c.800A>G (p.Asn267-Ser) genetic variant in the TARDBP (1.25%) and 16 additional patients with a pathogenic hexanucleotide repeat expansion in C9orf72 (20%) have been identified. No pathogenic variants have been identified in the remaining genes.

Conclusions: Collectively, findings indicate that C9orf72 repeat expansions are indeed causative for ALS in the Cypriot population, and agree with findings from other European countries. Finally, Southern blot hybridization will allow to investigate any association between repeat sizes and clinical characteristics in carriers of C9orf72-repeat expansions.

Grand References: TelethonCyprus, The Cyprus Institute of Neurology and Genetics, Cyprus.

Conflict of Interest: None declared.

EP20.019 Var gene B of PfEMP1 is associated with early conversion of asymptomatic parasitaemia to symptomatic malaria

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Background/Objectives: *Plasmodium falciparum erythrocyte*

membrane protein 1 (*PfEMP1*) is a family of roughly 60 var genes that play a key role in the pathogenicity. Only one form of this family is expressed on the infected erythrocyte surface and, to escape the host's antibody response, it can switch to a different form. In endemic areas, common infections are asymptomatic and children are most susceptible to the disease. This study aimed to understand the involvement of parasite virulence genes in the establishment of asymptomatic parasitemia.

Methods: A longitudinal study was done in 03 public schools in an area with high malaria transmission in Cameroon on children with asymptomatic infection. Species identification was performed using multiplex nested PCR and the expression level of var gene A, B and C was quantified by qRTPCR.

Results: Of the 134 individuals, we found 131 (97.8%) positive infections of which 119 (88.8%) were asymptomatic and 12 (9%) were symptomatic. During the 3-week follow-up period, a conversion rate from asymptomatic to symptomatic infections of 11% was observed. The conversion from asymptomatic to febrile malaria was associated with high parasitaemia ($p = 0.0123$). The expression level of var A remained stable over time, var B increased 4-fold after conversion, and var C expression decreased 10-fold with conversion. Moreover, in individuals with the persistence of asymptomatic parasitaemia, var B expression significantly increased over time ($p = 0.0407$).

Conclusion: Overall, this study suggests that the outcome of asymptomatic malaria infection is associated with the expression level of the *var B* family.

Conflict of Interest: None declared.

EP20.020 The whole genome analysis of 300 sequences from an underrepresented region of genetic diversity on the border between Ukraine and Romania

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Abstract: People of Ukraine and Romania have unique shared history in the border region of the Carpathian Mountains and Tisza River lowlands, where the two populations have been in direct contact over the last millennia. We present the analysis and annotation of novel WGS data from 300 Ukrainians and Romanians, combined with genomes of 97 Ukrainians from our earlier study. The annotation reveals more than 13 million of genome variants, with a large fraction of unique and novel mutations. This large contribution to European genome diversity is partially due to the "pioneer advantage", as the genome diversity in the region, especially in Romania, has never been studied. In addition, principal component analysis shows a geographical gradient in genomic data, and the analysis of ancestry components point to the significant admixture in these populations consistent with the historical records. As a result, the modern population in this area is characterized by a unique genetic background distinct from its surroundings. For instance, significant divergences in the pathogenic allele frequencies exist in genes responsible for hereditary pancreatitis (*PRSS1)*, C8 deficiency (*C8B*), risk alleles for Alzheimer's disease, lactase persistence, as well as in multiple drug response variants. All of the participants of this study consented to the public release of their WGS data in an effort to bring attention of the research community to this rich but currently underrepresented region of human genomic diversity.

Funding: funded by JOP Romania-Ukraine 2014-2020, ENI CBC-2SOFT/1.2/48, "*Partnership for genomic research in Ukraine and Romania*".

Conflict of Interest: None declared.

EP20.021 End to end sequencing of TLR7 gene among Bulgarians – a pilot study

Adelina Yosifova[1], *Atanas Syarov*[1;2], *Theodor Todorov*[1], *Angelina Trifonova*[3], *Velislava Terzieva*[3], *Radoslava Vazharova*[1;2]

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Introduction: COVID-19 pandemic provoked plenty of studies aiming to reveal the genetic factors implicated in host-viral interplay. As a part of such an effort an end to end long-read sequencing of *TLR7* gene in a Bulgarian cohort was performed. *TLR7* codes for type 7 Tall-like receptor protein, involved in sensing of single stranded viral RNA and playing a role in first-line defence against pathogens.

Materials and methods: The investigated group includes 97 unrelated adult volunteers of Bulgarian descend (43 males/54 females). DNA samples were collected between 05.2020 and 09.2021. 51 individuals not infected by SARS-CoV-2 at that time were considered the control group. 46 volunteers who at least once tested positive for SARS-CoV-2 were referred cases. Targeted end to end sequencing of *TLR7* gene was performed using a strategy for LR-PCR amplification of the target region followed by nanopore sequencing on MinION Mk1C system. Software packages MinKNOW, Guppy v.6.2.1, minimap2 v.2.22, VarAFT, GenomeBrowse v.3.0.0 and Haploview 4.1 were used for data analysis.

Results: A total of 56 variants in *TLR7* were detected: 4 in exons, 52 in untranslated regions, two intronic variants were not reported in public databases. Two variants rs179021(G) and rs179009(G) revealed a possible association to COVID-19 susceptibility. After applying the Bonferonni correction only rs179021 remained significant with P value of 0.01433 (OR = 3.2, 95%CI 1.31-8.28; corrected $P = 0.017$).

Conclusion: This is the first study using long-read sequencing to characterize *TLR7* variation among Bulgarians. Given the small sample size, these encouraging results should be viewed with caution. Funding: BNSF, KP-06-N43/8

Conflict of Interest: Adelina Yosifova: None declared, Atanas Syarov: None declared, Theodor Todorov: None declared, Angelina Trifonova: None declared, Velislava Terzieva: None declared, Radoslava Vazharova This work was supported by BNSF, grant ID: KP-06-N43/8.

EP20.022 Spectrum of germline patogenic variants in Slovak Roma cancer patients - pilot study

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Background: Detailed knowledge of population specific genetic background represents important predictor for estimation of risk related to different diseases with genetic component. Also, in cancer it is known that different populations share different predisposing germline mutations and to know them could have an important impact on management of screening programs and targeted diagnosis of the corresponding cancer types. Aim of this study was to define the mutation pool of cancer associated mutation in Slovak Roma population.

Materials/methods: In the pilot blood originated DNA samples of 20 cancer patients with different types of cancer and of Roma origin were analyzed using targeted approach with SureSelect HS XT2 customized kit. Sequencing on NextSeq 500 platform and home-made bioinformatic pipelines were used for genomic data generation and analysis. Qiagen Clinical Insight tool was used for variant annotation and filtering for detection of pathogenic/likely pathogenic variants with focus on cancer-related germline mutations.

Results: Predominating diagnoses were breast (22.14%), colorectal (20.71%) and lung cancer (19.28%). In analyzed pilot cohort mutations in *PALB2*, *TP53*, *ATM*, *MCPH1*, *ABRAXAS1*, *RAD51*, *NAT1* genes were identified.

Conclusions: The pilot study brings initial information about spectrum of cancer-related germline mutations present in Slovak Roma patients. The spectrum is different from other population databases and publications records. Currently additional analyses of larger sample cohort (totally 140) are ongoing and results will be correlated with clinical data.

Grants: This work was supported by the OPII program as the project - Center for biomedical research – BIOMEDIRES – II. phase, ITMS 313011W428, co-financed by the ERDF.

Conflict of Interest: Nikola Janostiakova part-time employee of Medirex Group Academy, Oliver Petrovic part-time employee of Medirex Group Academy, Dominik Kodada Part-time employee of Medirex Group Academy, Maria Reckova: None declared, Michal Mego: None declared, Vanda Repiska part-time employee of Medirex Group Academy, principal investigator in grants of Slovak grant agencies - SRDA and VEGA, Gabriel Minarik Full time employee of Medirex Group Academy, principal investigator in grants of Slovak grant agencies - SRDA and RA.

EP20.023 Analyzing the effects of ACE1 and ACE2 gene polymorphisms on COVID-19 severity: meta-analysis

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Background/Objectives: The coronavirus disease 2019 (COVID-19) is one of the serious public health threats around the world. The severity of symptoms varies significantly among patients infected with COVID-19, from asymptomatic infection to severe and critical cases. The entry of SARS-CoV-2 to the host cell is mediated by the angiotensin-converting enzyme 2 (ACE2), angiotensin-converting enzyme 1 (ACE1) modulates the ACE2 expression by regulating the levels of angiotensin II. This study aimed to investigate the association between the ACE1 and ACE2 gene polymorphisms with COVID-19 severity.

Methods: Using PRISMA statement, 15 studies were included in meta-analysis of *ACE1* (rs1799752) and 10 articles with 11 datasets *ACE2* (rs2285666) associations with COVID-19 severity. Data were used to perform meta-analysis in Review Manager 5.4 (Revman) in dominant, recessive, and allelic models of inheritance. The study included severe and non-severe patients.

Results: According to meta-analysis, *ACE1* rs1799752 is associated with lower severity of COVID-19 in recessive (OR = 0.73; 95%CI = 0.60-0.89; P = 0.002) and allelic (OR = 0.82; 95% CI = 0.74-0.92; P = 0.0009) models, but not in dominant (OR = 0.81; 95%CI = 0.63-1.03; P = 0.08), models were with low heterogeneity (I2 = 0%; P = 0.57) and (I2 = 14%; P = 0.29), respectively. *ACE2* (rs2285666) is associated with higher level of COVID-19 severity in recessive (OR = 1.84; 95%CI = 1.37-2.49; P < 0.0001) and allelic (OR = 1.28; 95%CI = 1.00-1.63; P = 0.05) models. No significant association was observed in the dominant model of this analysis (P = 0.55). The heterogeneity of studies *ACE2* (rs2285666) was significant in dominant and allelic models (I^2 = 44%; P = 0.07) and (I^2 = 47%; P = 0.04), respectively.

Conclusion: According to this meta-analysis, genetic polymorphisms ACE1 (rs1799752) and ACE2 (rs2285666) plays an important role for COVID-19 severity.

Conflict of Interest: None declared.

EP20.024 Assessing the completeness of immunogenetics databases across diverse populations

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It has been previously disclosed that most of the study participants in the AIRR-Seq studies were of European ancestry. Expanding the knowledge of the underrepresented populations in AIRR-Seq studies will enhance the understanding of the phenotypic differences in immune cell receptor repertoires. In the study, we examined the completeness of the IMGT database for representing diverse populations. By leveraging the bioinformatics software, MiXCR, we will be able to examine the mismatches in different ancestries samples' read in VDJ gene and evaluate the completeness of the IMGT database across diverse ancestries. MiXCR aligns and compares TCR-Seq reads to the IMGT database. In our preliminary results, we analyzed ten European and Asian samples from SRA and counted the number of substitutions, insertions, and deletions in the V/D/J genes of the TCR-Seq. We discovered that the samples of European ancestry had fewer mismatches in the V genes than samples of Asian ancestry and a similar trend of mismatches are observed in the J genes among the European and Asian group. The current result indicates that the IMGT database is more completely representing European ancestry compared to Asian ancestry. We are currently expanding the analysis to the publicly available datasets on the SRA and aim to run MiXCR across all TCR-Seq samples with available ancestry labels from SRA. Unveiling the completeness of the IMGT database representing diverse populations could highlight the need to improve ancestry diversity in those underrepresented populations and guide future immunogenomics studies to improve ancestry availability and distribution.

Conflict of Interest: None declared.

EP20.025 Association of polymorphisms in IL-6 and TNF-α genes with the severity of sickle cell anemia in Colombian patients

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Background/objective: Sickle cell anemia (SCA) is a monogenic disease more common in the world. The inflammation response is related to clinical manifestations in SCA (i.e., pain crisis). This research aims to evaluate the relationship between genetic variants in IL-6 and TNF-α with the severity of SCA.

Methods: We collected blood samples from 111 patients diagnosticated with SCA from Colombia. Based on their symptoms, the patients were classified into a low-severity or high-severity group. Three polymorphisms (rs1800796, rs2069832, and rs2069849) were genotyped in the IL-6 gene, and two (rs1799964, rs1799724) in the TNF-α gene. Allelic frequencies, and Hardy-Weinberg equilibrium (HWE), were calculated. The association between polymorphisms and severity was calculated by the prevalence ratio.

Results: The polymorphisms are in HWE except for rs2069832, and rs1799724. We observed a high frequency of the T allele in rs2069849, and rs1799724, which is higher than reported for other populations. In rs1800796, 93.8% of patients with genotype GG showed a high severity, meanwhile, 82.4% of GC showed low severity (p-value = 0,0138). In the case of rs1799724, 100% of TC and 96.3% of TT present high severity, this proportion was different from observed in the CC genotype (p-value = 0,0094).

Conclusion: The genotype GG in the polymorphism rs1800796 and the allele T in rs1799724 can be associated with high severity of SCA. However, is necessary to evaluate these associations in more patients. The lack of HWE for rs2069849 and rs1799724 could be caused by historical mechanisms like genetic drift, or admixture.

Grant references: CONADI-INV3094.

Conflict of Interest: None declared.

EP20.026 Genomic analysis of an ancient Armenian population from the Artsakh/Nagorno-Karabakh region

Wayne Grody[1], *Sevak Avagyan*[2], *Evgeni Sokurenko*[3], *Salpy Akaragian*[4], *Ida Chen*[5], *Cora Au*[6], *Layal Abi Farraj*[6], *Mihran Nazaretyan*[7], *Levon Yepiskoposyan*[8], *Jerome I. Rotter*[5], *Kent d. Taylor*[5]

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Genomic analysis of an ancient Armenian population from the Artsakh/Nagorno-Karabakh region

Background/Objectives: Studying isolated populations enables discovery of disease-associated genes because some of the rare genomic variation underlying disease may be found at much higher frequency due to founder and genetic drift effects. As part of our ArmGenia Project involving molecular geneticists in Armenia and Los Angeles, we have chosen to characterize genomic variants in an isolated subpopulation of Armenians living in the currently war-torn region of Artsakh by high-density microarray genotyping.

Methods: Blood was collected from 148 healthy adult subjects with longstanding familial residence in the Artsakh region. Genomic DNA was isolated by standard methods, and genotyping was performed using the Illumina Genetic Diversity Array. Genotypes were analyzed for variants of potential clinical importance and comparison with existing ethnic databases.

Results: Genotypes for 146 subjects (903,878 SNPs per subject) passed QC. Of 146 variants with minor allele frequency >1% and listed in ClinGen, variants in 12 genes are actionable per ACMG recommendations. Most striking was a high frequency of variants associated with hypercholesterolemia and hereditary arrhythmias, which could account for the relatively high prevalence of heart disease in the Armenian population.

Conclusions: Our findings of high cardiac disease allele frequencies can inform molecular screening programs in this population. To our knowledge, this is the first genomic study ever conducted in this ancient and genetically isolated region.

Grant References: This work was supported by the ArmGenia Charitable Trust, the UCLA Promise Armenian Institute, and the Zaruhy Sara Chitjian Charitable Foundation.

Conflict of Interest: Wayne Grody Professor, UCLA School of Medicine, NIH, ArmGenia, UCLA Promise Armenian Institute, Various lectures and medicolegal consultations, Sevak Avagyan ArmGenia, Armenian Bone Marrow Registry, Evgeni Sokurenko University of Washington, NIH, ArmGenia, Salpy Akaragian ArmGenia, Ida Chen Lundquist Institute, Cora Au: None declared, Layal Abi Farraj: None declared, Mihran Nazaretyan Armenian Bone Marrow Donor Registry, Levon Yepiskoposyan Institute of Molecular Biology, Armenian Bone Marrow Donor Registry, Jerome I. Rotter Lundquist Institute, NIH, others, Heligenics, Kent d. Taylor Lundquist Institute, NIH.

EP20.027 the significance of demographic history in human leukocyte antigen heterogeneity: the Cretan paradigm

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Background/Objectives: In allogeneic hematopoietic stem cell transplantation the compatibility between recipient and donor depends on the Human Leukocyte Antigen System (HLA). Prompted by our recent study describing a considerable HLA diversity between Cretans and donors from European populations, we present the significance of demographic history in HLA heterogeneity.

Methods: A combined cohort of 1,744 samples typed for HLA Class I (*-A, -C, -B*) and Class II (*-DRB1, -DQB1*) using 2nd field NGS were stratified to the four Prefectures of Crete based on the origin of each sample's grandparents. The HLA allelic and haplotypic architecture were estimated by the EM algorithm, while the genetic diversity measures (Hardy Weinberg Equilibrium, Linkage Disequilibrium) were corroborated by measuring the genetic distances with the Prevosti's metric and fixation index F_{ST}.

Results: Significant diversity was observed among the 1,239 inferred HLA haplotypes with the most common *A*24:02~B*35:02~C*04:01~DRB1*11:04~DQB1*03:01* (1.38%) being mainly attributed to Rethymno (3.35%), which was also significantly different compared to the other Prefectures. Stratification of the Rethymno samples by district of origin revealed that the high frequency of the top Cretan haplotype is due to the Mylopotamos area, which exhibited an elevated local inbreeding coefficient (e.g.

HLA-B, Fs=4.09%). Further hierarchical analyses of variance revealed a low but significant total inbreeding (F_{ST}) due to genetic differentiation between the Cretan subpopulations.

Conclusion: These results outline the HLA genetic landscape of Crete and highlight the importance of including the geographically or genetically isolated population clusters in donor registries.

Grant References: funded Hellenic Foundation of Research and Innovation (44201/2022-2024)

Conflict of Interest: None declared.

EP20.028 Understanding the maternal genetic affinities of Adivasi (Vedda) population in Sri Lanka: exploring the complete mitochondrial DNA

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Introduction: The origin of Adivasi (Indigenous Vedda) population in Sri Lanka has been the subject of debate for many years. They were originally hunter-gatherers who inhabited the Central and North-Eastern highlands of the island. It is believed that Adivasi populations descended from prehistoric humans in Sri Lanka who migrated through the Southern route Out of Africa during Late Pleistocene. Hence, the present study has focused to understand the female-centric migration of Adivasi population compared to indigenous populations in India, Australia, and Papua New Guinea (APG).

Methodology: Mitochondrial genomes of unrelated Adivasi individuals (N = 20) were sequenced. The mitochondrial haplotypes and haplogroups were determined and Neighbor-Joining tree was constructed including published mitochondrial genomes of indigenous populations [India (N = 518), APG (N = 101)] with bootstrap value of 1,000.

Results: There is a significant prevalence of U (35%) and R (40%) haplogroups in Adivasi population, which are observed in South Asia and Near East populations. Those samples clustered together with Urali Kuruman and Melakudiya tribes in South Asia and aboriginal populations in APG. The prevalence of Haplogroup M was low (25%) compared to the macro-haplogroup N and they were closely related to individuals from Northern regions of India.

Conclusion: Two possible migratory routes can be distinguished, either via the Southern coastal route or Northern route. However, our study suggests that people who migrated via the Southern route had significantly contributed to the gene pool of Adivasi population in Sri Lanka.

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Conflict of Interest: Anjana Welikala: None declared, Amali Fernando Partially supported for the sample collection and data generation, Mishal Faizan Partially supported for the data analysis, Niraj Rai Birbal Sahni Institute of Palaeosciences, Lucknow, India, Collaborator- National Research Council (NRC), Sri Lanka, Kumarasamy Thangaraj Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India, Collaborator- National Research Council (NRC), Sri Lanka, Kamani Tennekoon Institute of Biochemistry, Molecular Biology & Biotechnology (IBMBB), University of Colombo, Colombo 03, Sri Lanka, Co- Investigator- National Research Council (NRC), Sri Lanka., Co-Supervisor, Ruwandi Ranasinghe Institute of Biochemistry, Molecular Biology & Biotechnology (IBMBB), University of Colombo, Colombo 03, Sri Lanka, Principal investigator, Supervisor.

EP20.030 Exploring the Impact of Selective Constraints on the Evolution of Genes Linked to Polygenic Traits

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Understanding the evolutionary processes leading to diseases can facilitate the prediction and prioritization of disease genes. Just like other genes, genes associated with diseases are influenced by various evolutionary pressures, including the two main selective forces that act against deleterious protein substitutions and the loss-of-function mutations. In this work, we analyzed the evolutionary constraints of genes linked to two genetically uncorrelated and polygenic diseases, schizophrenia and coronary artery disease. Our results show that genes associated to both diseases evolve at a lower rate (i.e., they are more conserved) compared to non-significant genes ($p < 0.001$, Wilcoxon rank-sum test performed on dN/dS). Furthermore, these genes have a high evolutionary rate heterogeneity which is greater with respect to genes related to monogenic diseases (DG-CST database; $p = 0.003$, Brown-Forsythe test). To unravel this heterogeneity, we selected significantly associated genes in both diseases with high derived allele frequencies (DAF ≥ 60%) that are less likely affected by deleterious amino acid substitutions. The evolutionary rate of such genes is lower in schizophrenia ($p = 0.02$, Wilcoxon rank-sum test), indicating a greater contribution of selection against loss-of-function, compared to coronary artery disease. Indeed, the probability of loss-of-function intolerance is the most important predictor of the significantly associated genes in schizophrenia ($p = 1.12 \times 10^{-10}$; Kolmogorov-Smirnov test) but not for the genes linked to coronary artery disease. Overall, our study showed the differential impact of deleterious amino acid mutations and loss-of-function intolerance on the evolutionary conservation of genes associated with polygenic diseases.

Conflict of Interest: Pouria Dasmeh Full time employment at the center for human genetics, Marburg University, Group leader and principal investigator at the center for human genetics, Marburg University, Peter Krawitz Head of the Institute for Genomic Statistics and Bioinformatics, Johannes Schumacher Director of the center for human genetics, Marburg University, Carlo Maj Head of bioinformatics at the center for human genetics, Marburg University.

EP20.031 The telomere length related genes polymorphisms predict the change of metabolic syndrome components: a population-based follow-up study in Taiwan

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Background/Objectives: Telomeres are the tips of chromosomes and are composed of proteins and several thousand copies of a hexamer repeat sequence $(TTAGGG)_n$. Some studies reported significant associations between shorter TL and deregulated metabolic syndrome (MetS) components. Several genetic loci associated with telomere length have been identified. The study was aimed to evaluate whether the TL related genetic loci could predict the change of MetS components.

Methods: A total of 3441 adults were recruited from the Matsu community-based integrated health screening project and 1291 were followed-up for ten years. Forty-one SNPs on 17 TL related genes were selected for genotyping. Multiple logistic regression, Cox proportional hazard model and generalized estimating equation adjusting for other covariates were used for data analysis.

Results: We found rs62139200 in *ACYP2* gene was associated with higher fasting plasma glucose and rs33945943, rs75877904, rs35052906 in *TNKS* gene were associated with higher levels of HDL-C. We also found that the increase of each standard deviation of polygenic risk score (PRS) was associated with higher levels of HDL-C ($\beta = 0.840$, 95%CI = 0.482, 1.197). For the longitudinal study, we found that rs4841210 in *TNKS*, rs4691896 in *NAF1* gene, rs10792447 in *CDC42BPG* gene were related to the change of blood pressures. We also observed that rs670358 in *CDC42BPG* gene, rs3827026 in *ZBTB46* gene could increase the levels of triglycerides. Moreover, the increase of PRS also increased the triglycerides.

Conclusion: Our results showed that several TL related genetic variants could significantly predict the change of metabolic syndrome components.

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Conflict of Interest: None declared.

EP20.032 The most common pathogenic mutations in Slovakia: a survey of exome data

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Objectives: Exome sequencing has become a common tool for identifying causal mutations in genetics. It can provide data on the population prevalence of variants responsible for diseases that may be underdiagnosed. We found recurrent pathogenic mutations with a frequency much higher than 1% compared with the population prevalence of the European non-Finnish population. The Roma population constitutes 10% of the Slovak population and carries specific mutations that are virtually absent in the majority population.

Methods: We scrutinized 275 probe-based exome analyses for various patients which were annotated via Qiagen IVA and Varsome clinical tools. Paired-end sequencing was performed on NextSeq 500/550. The pathogenicity criterion was established on the basis of ACMG guidelines.

Results: We found that the most frequent pathogenic polymorphisms in the majority Slovak population are HFE (p.H63D) in 15.27%; BTD (p.D444H) in 4.36%; F5 (p.R534Q) in 4.36%; CFTR (p.F508del) in 3.27%, RBM8A (c.67+32G > C) in 2.5% and FLG (p.S761fs*36) in 2.18% of patients. The frequency of these mutations was comparable to or significantly higher than in the databases. The most frequent pathogenic Roma variant was SLC22A1A (p.T246M) in 6%, which was not present in the majority.

Conclusion: Some mutations are significantly more common in the population than others implying a higher incidence of AD and AR-associated diseases. Knowledge of such variants as secondary findings may be important for further management of patients.

Grants: This work was supported by the OPII programme as the project: Serious diseases of civilization and COVID19, co-financed by the European Regional Development Fund (ITMS code:313011AVH7).

Conflict of Interest: Klaudia Babišová Medirex Group Academy full time employee, Andrej Gnip Medirex Group Academy full time employee, Patrik Krumpolec Medirex Group Academy full time employee, Oliver Petrovic Medirex Group Academy full time employee, Gabriel Minarik Medirex Group Academy full time employee, Michaela Hyblova Medirex Group Academy full time employee.

EP20.033 Analysis of genetic variants associated to aspirin resistance in Brazilian stroke patients

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Background/Aims: Stroke is a severe disorder with high morbidity, mortality and disability-adjusted life year rates and considered a major healthcare problem. The adjusted annual incidence rate of Ischemic Stroke (IS) ranges from 62 to 92 per 100,000 Brazilians, with a mixed pattern of cardiovascular risk factors and high recurrence rates. In Brazil, the Unified Health System (SUS) establishes the continuous use of Acetylsalicylic Acid as standard pharmacological treatment for secondary prevention of stroke. However, individual response variations to these agents are still poorly known. We aimed to demonstrate the frequency of genetic variants associated with aspirin resistance in IS Brazilian patients.

Methods: There were included 525 patients from Joinville Stroke Biobank. All DNA samples were processed by classical phenol-chloroform extraction method and genotyped for polymorphisms previously reported in association with aspirin resistance, by Axiom Precision Medicine Research Array (Affymetrix platform). Statistical analyses were performed in Plink software 1.9 Beta version.

Results: We found the following minor allelic frequencies: 36.7% for rs2768759 (A > C), 17.7% for rs566888 (C > T) 34.1% for rs3732765 (G > A), 5.5% for rs10306114 (A > G), 15.9% for rs5918 (T > C), 10.7% for rs6065 (C > G), and 13.9% for rs1613662 (G > A).

Conclusion: Our data highlight the risk of ineffective stroke prevention treatment in a population with a high incidence of IS, as well as may suggest the implementation of genotyping tests to drive personalized treatments.

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Conflict of Interest: None declared.

EP20.034 Genetic aspects of type 1 diabetes in the Qatari population: Protective and risk HLA alleles linked to a higher or lower risk of disease development

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Background: Recent epidemiological data shows that Qatar has the 4th uppermost incidence of T1D globally alongside Finland and Sweden. T1D is linked with considerable heritable risk, particularly from human leukocyte antigen (HLA) alleles. Population of Qatar is distinguished by unique genetic subgroups, high levels of consanguinity, and admixture from early migration. This implies that our population may have a very distinctive HLA landscape, which is relevant for clinical use but has not yet been investigated.

Aim: Fine mapping of the HLA alleles and haplotypes from 15k whole genomes of Qatar Biobank subjects. Assessment of the risk and protective HLA alleles that exist in Qatari population associating with an increased or a lower risk of T1D development.

Methods: HLA type inference was performed using multiple independent typing methods with high accuracy on the WGS data and association with clinical traits of T1D was performed using linear regression models.

Results: We found a high diversity of rare alleles among class II HLA genes in our population. Multiple alleles from genes DRB1, DQA1 and DQB1 which are known to segregate with T1D predisposition showed a significant association with clinical phenotypes of T1D. This suggests a greater genetic susceptibility for T1D in the general population through known and novel alleles and thus provides an opportunity for the discovery of these novel associations.

Conclusion: We present a genetically distinct landscape of the HLA locus for a consanguineous population with enrichment for several potentially protective and risk alleles for the onset of T1D.

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Conflict of Interest: None declared.

EP20.035 Study of the association of mitochondrial DNA variability and severe Covid-19 in the Slovak population

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Background/Objectives: Recent studies have shown that mitochondria play a role during pathogenesis of COVID-19. Mitochondrial production of ROS and induction of innate immune response are important during infection. It is known that even common variability of mitochondrial DNA affects the efficiency of oxidative phosphorylation, likely modifying the risk of associated clinical complication of Covid-19. However specific effect of mtDNA haplogroups or polymorphism remains unclear.

Methods: In the study, we have analysed DNA in a sample of 446 Slovak patients hospitalized due to Covid-19, and a control population group consisting of 1874 individuals. DNA samples have been analysed for variants in the mitochondrial HVR1 region and classified into haplogroups at various levels of phylogenetic resolution. Binary logistic regression, as well as Fisher's exact test and a chi-square test of independence were used to assess the risk of a Covid-19 within various haplogroups or their clusters as well as individual DNA variants comparing cohorts of patients and controls.

Results: Haplogroups J1 and U5b, haplogroup clusters H + U5b and T2b + U5b and the common mtDNA variant 16189T > C have been found in smaller proportion in patients cohort, thus likely decreasing the risk of developing severe Covid-19, while haplogroups T1, H11, K and variants 16256C > T, 16265A > C, 16293A > G, 16311T > C and 16399A > G have been found significantly increase this risk of severe hospitalisation requiring COVID-19.

Conclusion: Common variability in mitochondrial DNA may apparently modify the risk of severe Covid-19, however further more specific confirmation study is necessary.

Conflict of Interest: None declared.

EP20.037 Genomic deserts of our Globe: Underrepresented populations, challenges, and solutions

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Background: Despite the growing contributions of national genome sequencing projects, they cover only a small fraction of the world population, focusing on developed countries and thus contributing to the global genomic data inequality. The current genomics research efforts are mostly conducted in and by high-income countries due to the prohibitive price of sequencing and high expertise requirements. Consequently, the persistence of multiple regions with complete absence of genomic data limits our understanding of our diversity.

Methods: We have surveyed genomic projects across 195 countries in all regions of the world and investigated their data dissemination methods, sample counts, technologies, and analysis approaches. Our findings show inconsistencies in the representation of worldwide populations in genomic research, the locations of numerous "genome deserts" across all parts of the world, and the roadblocks genomic projects face.

Results: The large variation in the availability of genomic data and its sharing models for the existing releases often hinder our ability to integrate and produce additional insight. We discuss the challenges and bottlenecks of bioinformatic analysis, the infrastructure requirements for the success of genomic projects, and the heterogeneity of the data available currently.

Conclusion: Using this information, we propose steps to standardize the data analysis and sharing approaches that would benefit the research efforts in human genomics. In addition, we provide an overview of leveraging effective data-sharing models, and their role in empowering local science to balance the need for scientific advancement while preserving the rights of local communities.

Conflict of Interest: None declared.

EP20.039 Assessment of breast and gynecologic cancer risk loci in Georgian women

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Introduction: The aim of this study was to analyze cancer susceptibility variants in Georgian women with breast and gynecologic cancer.

Materials and Methods: The study was approved by the ethics committee of the Tbilisi State Medical University. 50 patients with breast cancer, 24 patients with gynecologic cancer and 50 healthy controls were enrolled in this study. Genotyping was carried out using a TaqMan assay (Applied Biosystems, Foster City, CA, USA).

Results: In this initial study we tested rs7579014, rs231775, rs7726159, rs635634, rs10103314 in breast cancer cases and rs7579014, rs231775, rs7726159 variants in gynecologic cancer cases (Table 1). The rs7726159 variant demonstrates a statistically significant risk in gynecologic cancer compared to controls.

Table 1. SNP analysis results

Conclusions: The study suggests that the rs7579014 variant of *BCL11A* may play a role in the initiation and progression of gynecologic cancer in Georgian population.

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Conflict of Interest: None declared.

EP20.040 Variants in IL-6 gene and their association with External Root Resorption in orthodontic treatment

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Background/Objectives: External root resorption (ERR) is the reduction of the root structure of the teeth. ERR associated with orthodontic treatment is an undesirable consequence of the inflammatory response during treatment. The aim of this research was to evaluate the association between polymorphisms in the IL-6 gene and ERR in Colombian orthodontic patients.

Methods: 40 orthodontic patients were studied. For each patient periapical radiography was taken before the beginning of the treatment and then 3 and 6 months later. Also, crevicular liquid samples from four incisors teeth were taken for quantification of IL-6 by ELISA. DNA samples were extracted from saliva for genotyping of polymorphisms in IL-6, rs1800796, and rs2069832. The association between ERR and IL-6 expression and the genotypes were evaluated by logistic regression.

Results: The proportion of teeth with some degree of ERR at three and six months was 7% and 17% respectively. For rs1800796, individuals with genotype CC had a 33% of teeth with roots superficial changes. In individuals, CG 13% of teeth had superficial changes, and 3% had a minimal ERR. In individuals, GG showed 3% of roots with superficial changes. In the case of rs2069832, Individuals AA presented 13% of roots with superficial changes and 3% with minimal ERR. For AG individuals was observed only roots with superficial changes. none of the polymorphisms were associated with ERR.

Conclusion: No association was found between ERR and the rs1800796 and rs2069832 variants, however, ERR needs to be assessed in the advanced stages of treatment.

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Conflict of Interest: None declared.

EP21 Functional Genomics and Epigenomics

EP21.001 Tissue-specific cis-regulation of the CFTR gene

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More than 2000 mutations have been identified on the Cystic Fibrosis Transmembrane conductance Regulator (*CFTR*) gene. However, it is still challenging to understand the distinction between cystic fibrosis (CF) and CFTR-Related Disorders such as congenital bilateral agenesis of the vas deferens (CBAVD) or pancreatitis, as well as extreme phenotype in CF. To explain those complex cases, our project aims to identify dysregulation of the *CFTR* gene expression due to alterations of *cis*-regulatory elements (CREs).

Chromatin study techniques are implemented in three models, intestinal, pancreatic and epididymal cells. The 4C (Circular Chromosome Conformation Capture) technique is used to obtain chromatin interaction profiles and define candidate CREs (cCREs) which are then validated by reporter gene tests. CUT &RUN (Cleavage Under Targets And Release Using Nuclease) method is

SNP	Locus	Gene	Alleles	Common Genotype vs. Risk Genotype	Risk Genotype Frequency Cases/controls			
					Breast cancer (n = 50)	p	gynecologic cancer (n = 24)	p
rs7579014	2p16.1	*BCL11A*	G/A	GG/AA	0.2/0.07	0.06	0.3/0.12	0.02
rs231775	2q33	*CTLA4*	A/G	AA/GG	0.15/0.02	0.1	N/A*	-
rs7726159	5p15	*TERT*	C/A	CC/AA	0.24/0.5	0.27	0.08/0.75	0.04**
rs635634	9q34.2	*ABO*	T/A	TT/AA	0.05/0.07	0.45	-	-
rs10103314	8q24.21	*MYC*	C/A	CC/AA	N/A* -		-	-

*Deviation from Hardy-Weinberg Equilibrium
**Risk genotype frequency was more in control group

used to define epigenetic marks. Then, Next Generation Sequencing is applied to detect variants within CREs.

4C analyses indicate multiple regions interacting with the *CFTR* promoter. Many interactions are conserved between cell-type, but specific peaks are also observed. Reporter gene assays performed on cCRES confirmed the presence of enhancers as well as described a newly silencer. Through sequencing of CBAVD patients, eight potential regulatory variants were identified. To validate their impact on the *CFTR* gene expression, functional tests are performed.

This work provide new elements to better understand the three-dimensional organisation of the *CFTR* locus in a cell-type specific manner. This could help, in the future to better understand the physiopathology of *CFTR* diseases in order to improve the management of patients.

Vaincre la mucoviscidose

Conflict of Interest: None declared.

EP21.002 Evaluation of selected HTS assays for targeted analysis of DNA methylation and epigenetic age prediction

Ewelina Pośpiech[1;2], *Aleksandra Pisarek*[3], *Joanna Rudnicka*[1], *Rezvan Noroozi*[1], *Michał Boroń*[4], *Aleksander Masny*[4], *Bożena Wysocka*[4], *Aneta Sitek*[5], *Andrzej Ossowski*[2], *Magdalena Spólnicka*[6], *Wojciech Branicki*[3]

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Background/Objectives: DNA methylation analysis has proven to be a powerful tool in age assessment. However, the implementation of epigenetic age prediction in forensic laboratories and diagnostics requires good laboratory methods. In this study, we aimed to compare the performance of most promising high-throughput sequencing (HTS) protocols for targeted DNA methylation analysis.

Methods: Three methods were selected, including SureSelect^{XT} Methyl-Seq and Bisulfite Padlock Probes protocols followed by sequencing on MiSeq Illumina and amplicon-based Ion AmpliSeq^{TM} method integrated with Ion Torrent S5. Protocols were designed to target a predefined panel of 161 CpG sites from four known estimators of epigenetic age, aging rate and mortality risk, optimized and validated using artificially methylated controls or blood samples.

Results/Conclusions: We successfully targeted >95% of the selected loci with two different protocols. The assays ensured high accuracy and robustness, and the between-assay comparisons yielded Pearson correlations >0.8. The best all-round performance was observed for the amplicon-based Ion AmpliSeq^{TM} protocol with sensitivity down to 25 ng of input DNA, an average marker coverage of ~6.5k reads and methylation analysis accuracy at a mean absolute error of 5.0%. The method was further validated on an independent set of blood samples from Polish individuals, and summary statistics of age-related parameters were collected for the analytic sample.

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Conflict of Interest: None declared.

EP21.004 Genome-wide methylome profile of mesenchymal cells derived from induced pluripotent stem cells for cartilage regeneration

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Introduction: Osteoarthritis (OA) is the most common age-related degenerative joint disease. Patient-specific induced pluripotent stem-cells (iPSCs) from mesenchymal bone marrow stem-cells (BM-MSCs) and mesenchymal stem-cells derived from iPSCs (iMSCs), already evaluated regarding their proliferative capacity and stability, were tested for differentially methylated regions (DMRs) between initial BM-MSCs, iPSCs and iMSCs from OA patients and healthy controls.

Material and Methods: Genome wide DNA methylation profiling was assessed by a custom ChiP methylation array (Agilent Technologies) to determine DMRs. Each array covers 99% of CpG islands of RefSeq genes (hg18), transcription factor binding sites and intergenic CpG islands. Final comparison of DMRs between MSCs, iPSCs and iMSCs was accomplished using multiple linear models for microarray data (*limma* package in *R* environment). Molecular pathways and biological processes (Wikipathways) in which genes with statistically significant DMR regions (p-adj<0.1) were involved, were presented using Cytoscape. ComplexHeatmap and PCAtools *R* packages were used for graphical representation of the results.

Results and Conclusions: Statistically relevant (FDR < 0.1) DMRs for 3,106 genes (6,586 probes) were found only between BM-MSCs and iPSCs, representing regions of developmental biological processes due to cell re-programming. Comparison of BM-MSCs, iPSCs and iMSCs within the OA patient cohort, revealed DMRs in genes involved in differentiation processes to mesoderm, ectoderm, endochondral ossification, spinochordal processes, pluripotency and endoderm. These DMRs revealed that iMSCs exhibited phenotypic characteristics of rejuvenated cells with the ability to give rise to differentiated chondrocytes with potential use for cartilage regeneration in osteoarthritis.

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Conflict of Interest: None declared.

EP21.005 ANKRD11 pathogenic variants and 16q24.3 microdeletions share an altered DNA methylation signature in patients with KBG syndrome

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Background/Objectives: Pathogenic variants in *ANKRD11* and 16q24.3 microdeletions overlapping this gene are causative of KBG syndrome (KBGS), a neurodevelopmental syndrome characterized by developmental delay, intellectual disability, and skeletal anomalies. Our previous work has shown that syndromic conditions caused by pathogenic variants in epigenetic regulatory genes have identifiable patterns of DNA methylation (DNAm) change termed: DNAm signatures. Given the role of ANKRD11 in chromatin modification, we tested whether pathogenic *ANKRD11* variants underlying KBGS are associated with a unique DNAm signature.

Methods: We profiled whole-blood DNAm for 25 individuals with *ANKRD11* variants, two individuals with microdeletions at 16q24.3, and 28 sex- and age-matched typically developing individuals, using Illumina's Infinium EPIC array.

Results: Using a discovery cohort of KBGS cases with *ANKRD11* variants only ($n = 14$), we identified a DNAm signature of 95 differentially methylated CpG sites that clearly distinguish individuals with KBGS from typically developing controls. This KBGS DNAm signature was validated in 7 individuals with pathogenic *ANKRD11* variants and two individuals with 16q24.3 microdeletions. Using a machine learning model trained on the KBGS DNAm signature, we classified the DNAm profiles of 4 individuals with variants of uncertain significance (VUS) in *ANKRD11* and found inconclusive classifications for an inherited variant in a mother-child duo.

Conclusions: The overlap in DNAm changes between intragenic variant and microdeletions indicates single nucleotide variants associated with KBGS cause ANKRD11 haploinsufficiency. The intermediate scores of the inherited *ANKRD11* VUS in the mother-child duo, emphasizes the consideration of inherited variants and intrafamilial phenotypic variability in genetic diagnostics.

Grant Reference: SFARI.

Conflict of Interest: None declared.

EP21.006 Investigating the potential causes underlying aberrant prenyltransferase expression in cancer

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Background/Objectives: Expression of prenyltransferases (PTases) is dysregulated in many cancers, however, little is known about the genetic and epigenetic regulation of PTase expression. Previously, we showed that polymorphisms in PTase promoters correlate with disease progression in breast cancer. Here, we investigated DNA methylation of PTase promoters in different cancer cell lines to elucidate the causes underlying aberrant PTase expression in cancer.

Methods: We used pyrosequencing to study methylation in four out of five PTase genes harbouring CpG islands (*FNTA*, *FNTB*, *PGGT1B*, *RABGGTA*) in eleven cancer cell lines. As controls, we methylated extracted DNA using bacterial methyltransferase prior to methylation analysis, and included loci annotated as natively methylated or unmethylated, respectively, in two different cell lines.

Results: We found no aberrant PTase promoter methylation in any of the investigated cell lines. Unexpectedly, individual CpG sites exhibited the same distinct methylation levels in both natively methylated samples and samples, artificially methylated after DNA extraction from cell lines natively unmethylated at these loci. Apparently, the only other common feature in both cases was their primary sequence. Additionally, we identified microRNA and transcription factor candidates in silico as potential regulators of PTase expression.

Conclusion&Outlook: Our results indicate that aberrant PTase expression in cancer is not caused by aberrant DNA methylation of PTase promoters. We thus plan to use nanopore sequencing to identify prevalent polymorphisms and structural variations, and to validate our previously undescribed findings regarding CpG-specific methylation maxima with an approach complementary to pyrosequencing. Additionally, we intend to analyse microRNA and transcription factor candidates.

Conflict of Interest: None declared.

EP21.007 Common Imprinting Disorders (ID) Summary. Our experience in methylation/imprinting tests over the last five years (2017-2021)

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Background: The epigenetic process is important in evolution and involves less than 1% of the human genes. Imprinting disruption in certain chromosomal regions, are responsible for specific syndromic phenotypes including: (AS) Angelman syndrome, Beckwith–Wiedemann syndrome (BWS), Russell-Silver syndrome (RS), Multilocus imprinting disorders (MLID), Temple syndrome (TS) GNAS locus imprinting. The "genetic imprinting" mechanism is diverse and includes: CNV deletion/duplication, imprinting center defect, UPD and Genetic mutations.

Methods: Methylation pattern tested by using MS-MLPA (MRC Holland) kits and UPD by polymorphic markers.

Results: We tested 494 samples (413 postnatal and 81 prenatal). 22% (80 samples) of postnatal samples and 10% (8 samples) of prenatal samples were pathologic.

Significant abnormal methylation/ imprinting pattern was observed in postnatal group, PWS (22%) and BWS (24%) suspected patients. In the prenatal samples group, significant pathologic mainly found in BWS tested group (16%). Of note, they tested due to abnormal suspicious sonographic findings. We checked 40 prenatal samples with evidence of LOH in the critical region of AS/PWS (15q11.2) from CMA analysis. Two abnormal tests (5%) had ISODISOMIC UPD methylation pattern. 137 prenatal samples were obtained for UPD following a family translocation involving chromosome 15 ($n = 38$) or chromosome 14 ($n = 99$). All were normal (0% UPD).

Conclusions: Imprinting disruption is an important mechanism in the pathogenesis of developmental disorders. It is important to consider and perform these tests in both, parenteral and postnatal, suspected cases. Higher yield retrieved in prenatal cases presenting suspicious sonographic findings.

Grant References: no.

Conflict of Interest: None declared.

EP21.008 The association between two polymorphisms in MicroRNAs and preeclampsia risk

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Background: Preeclampsia is a pregnancy-specific disease related to hypertension and proteinuria, and it is one of the important reasons for the high prevalence and fatality rate of pregnant women and perinatal children. Preeclampsia has genetic

correlation. Many studies have shown that microRNA polymorphism is highly associated with preeclampsia, but the results are inconsistent. The purpose of this study is to evaluate the relationship between miRNA polymorphisms and preeclampsia.

Methods: Using PRISMA statement, 6 studies were included in meta-analysis of (rs2910164) and (rs2292832) associations with preeclampsia. The subjects are case-control studies on the relationship between miRNA polymorphisms and preeclampsia. The data of the included study are extracted and the literature quality is evaluated. The data are statistically analyzed through Revman 5.4.1 software.

Results: The overall analysis showed no association of miR-149 C > T (rs2292832) and the preeclampsia susceptibility under all genetic models, but a strong association of miR-146a2 C > G (rs2910164) polymorphism was found under all genetic model (G vs. C, OR = 1.48, 95% CI = 1.21–1.81; GC vs. GG, OR = 1.37; 95% CI = 1.05-1.80; CC + CG vs. GG: OR = 1.50, 95% CI = 1.16-1.94; CC vs CG + GG: OR = 2.02, 95% CI = 1.26-3.23; CC vs. GG, OR = 2.21; 95% CI = 1.35-3.60).

Conclusion: To our knowledge, this is the first meta-analysis of miRNA polymorphisms and preeclampsia. In this study, we found association between miR-146a C > G (rs2910164) and preeclampsia in all genetic model and this might be risk factors of preeclampsia. No significant association was observed between miR-149 C > T (rs2292832) and risk of preeclampsia.

Conflict of Interest: Nadeim Alayasa Part-time instructor at University of the People, Tatiana Shkurat Professor, Head of the Department of Genetics, Ekaterina Derevyanchuk Assistant professor at southern federal university.

EP21.010 Birth weight and genome-wide placental DNA methylation

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Being born with either low or high birth weight increases the risk of perinatal complications and of developing chronic diseases later in life. The placenta plays a key role in shaping fetal growth, and its function and structure can be indirectly evaluated through its DNA methylation pattern.

The aim of this study is to investigate the association of genome-wide placental DNA methylation with birth weight in >3,000 samples from 13 cohorts of the Pregnancy and Childhood Epigenetics (PACE) consortium.

Placental DNA methylation was assessed with the 450K or EPIC arrays at birth. Birth weight was retrieved from medical records and was sex- and gestational age-standardized using curves from the INTERGROWTH-21(st) project. Main analyses were restricted to children at term without pregnancy complications. Associations were computed using robust linear regression models adjusted for several covariates including placenta cell type proportions, with placental DNA methylation as the outcome. Results from the cohorts were combined through fixed-effects inverse variance weighted meta-analyses.

In preliminary results, birth weight was associated with placental DNA methylation at 289 CpGs (P < 1E-07). Around 70% of the CpGs showed positive associations. Additional models for other anthropometric measurements at birth and for gestational age will be performed. Finally, associations will be analysed stratifying by sex and ancestry.

Funding: NUTRIPROGRAM (JPI HDHL and Instituto de Salud Carlos III - AC18/00006), ATHLETE (H2020-EU.3.1.2. - 874583) and FI-AGAUR Predoctoral contract awarded by the Agència de Gestió d'Ajuts Universitaris i de Recerca (2022 FI_B 00797), Generalitat de Catalunya – Fons Social Europeu.

Conflict of Interest: None declared.

EP21.011 Building capacity for open access DNA methylation signature-based variant classification for rare neurodevelopmental disorders

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Background: DNA methylation (DNAm) signatures have recently emerged at the forefront of the clinical diagnostic arena based on their validated clinical utility in classifying variants of uncertain significance (VUS) in neurodevelopmental disorders (NDD). Classification of VUS using these signatures can reduce uncertainty, increase diagnostic yield, and most importantly end many diagnostic odysseys. In contrast to the situation for epigenetic signatures for brain tumors, there are currently no open access resources of validated signatures for NDD. This comes at a time when the increased use of NGS in the clinical diagnostic arena is identifying many VUSes, creating a barrier to the comprehensive use of sequence data in delivering precision medicine.

Methods: To address this gap, we have developed an open access web portal named "EpigenCentral", a knowledge base hosting a large number of disease and control reference DNAm profiles where users can upload their DNAm datasets to functionally classify variants in genes using validated signatures.

Results: By submitting the dataset into our portal, you will be able to determine the presence/absence of known DNAm patterns of 17 NDD profiles, with planned expansion to >50 NDDs in the near future. This will enable you to define the likelihood of the patient belonging to one of the known disease cohorts and potentially to quantify the degree of severity and pathogenicity based on machine learning models.

Conclusion: EpigenCentral will provide a rich context for exploring the role of epigenetic dysregulation in a growing number of NDDs.

Grant References: Simons Foundation Autism Research Initiative (SFARI).

Conflict of Interest: None declared.

EP21.013 The level of methylation of the LINE-1 retrotransposon subfamilies in the placenta of the first trimester of pregnancy

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Background/Objectives: The origin of aneuploidy coincides with the waves of epigenetic reprogramming, which affects genes and other genome regions. In preliminary studies, the methylation index of the LINE-1 retrotransposon was increased in the chorionic villi of miscarriages with aneuploidy. The aim of this study was to analyze the methylation profile of different LINE-1 subfamilies (L1HS, L1PA2, L1PA3, L1PA4, L1P1) in miscarriages.

Methods: Chorionic villi were sampled from miscarriages with trisomy of autosomes 16 ($n = 47$), monosomy X ($n = 36$), normal karyotype ($n = 121$), and 30 induced abortions. The LINE-1 methylation index was analyzed by targeted bisulfite massive parallel sequencing at 19 CpG sites in LINE-1 promoter on average across the genome and individually in 5 different LINE-1 subfamilies (L1HS, L1PA2, L1PA3, L1PA4, L1P1) using subfamily-specific single nucleotide polymorphisms.

Results: The methylation level of the L1HS subfamily was higher than that of other subfamilies for all samples ($p < 0.05$) and accounted for 59% of all reads. In miscarriages with trisomy compared with induced abortions, increased levels of LINE-1 methylation were found in all subfamilies. The older the subfamily was, the more differences were found between miscarriages with trisomy and induced abortions (L1HS: 1 CpG, L1PA2: 2 CpGs, L1PA3 and L1P1: 5 CpGs, L1PA4: 8 CpGs, $p < 0.05$).

Conclusion: The increase in the LINE-1 methylation index in miscarriages is associated primarily with the evolutionarily ancient LINE-1 elements. This can lead to impaired function of genes adjacent to LINE-1 loci, abnormal development, and embryo death.

Grant References: This study was supported by Russian Science Foundation, grant 19-74-10026.

Conflict of Interest: None declared.

EP21.014 Integration of intron and exon expression QTLs reveals genetic effects on post-transcriptional gene regulation with links to complex traits

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Background/Objectives: Typically, expression quantitative trait loci (eQTL) studies consider exon expression levels and discard intronic RNA sequencing reads despite of their known information on RNA metabolism. Here, we integrate exon and intron expression QTLs to better resolve the gene regulatory processes affected by genetic variants.

Methods: We determined cis-QTLs for exon (exQTL) and intron (inQTL) expression levels of genes and their ratio (ex-inQTL) in lymphoblastoid cell lines from European individuals ($n = 901$).

Results: We identified cis-QTLs for 10721 genes. Genetic variants often affected multiple gene expression measures, but 41% of genetic effects were not detected at exon levels. Consistent with previous observations, exQTLs appeared to primarily capture transcriptional regulation, especially when the effect was shared with inQTLs, while ex-inQTLs appeared more sensitive to post-transcriptional processes. Trans-effects of transcription factors (TFs) and RNA-binding proteins (RBPs) further confirmed this observation, as TFs had significantly more trans-exQTL and RBPs more trans-ex-inQTL associations.

Adding cis-QTLs for intron levels and exon-intron-ratios to cis-exQTLs increased the colocalization with GWAS trait variants substantially (by ~40%), indicating that all QTLs types are similarly functional. Furthermore, combining the information on cis-effects from different QTL types allowed to dissect the gene regulatory processes underlying GWAS associations.

Finally, cis-QTLs of all types with large effect sizes appeared to have selective disadvantages, and their risk alleles were more prevalent among individuals with common diseases and shorter lifespan.

Conclusion: Integrating exQTLs with inQTLs and ex-inQTLs expands the understanding of genetic effects on gene regulatory processes and on human traits.

Grant References:

Conflict of Interest: None declared.

EP21.015 In vivo profiling of glucose-induced chromatin changes to understand the molecular mechanisms of diabetes development

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Diabetes mellitus is predicted to affect 592 million worldwide by 2035, being considered a 21st century's pandemic. Type2 Diabetes (T2D) accounts for approximately 90% of the cases and it has both environmental and heritable origin. This disease is partially caused by pancreatic islet dysfunction, which has been associated with transcriptional dysregulation. Transcriptional regulation is achieved by the action of non-coding sequences of DNA, among them enhancers that activate the transcription of target genes in specific tissues. Recently, it has been shown that T2D associated SNPs can affect endocrine enhancer function, suggesting that changes in the activity of these sequences can contribute to T2D development. Additionally, it is also known that active enhancers can be predicted by chromatin availability detected by techniques such as Assay for Transposase-Accessible Chromatin using sequencing. T2D is a multifactorial disease characterized in part by impaired insulin secretion from the endocrine pancreas. Elevated glucose levels have different effects on insulin secretion, depending on the duration of exposure. Short-term hyperglycaemia stimulates insulin secretion whereas long-term impairs insulin

secretion, causing an irreversible state for glucose response, suggesting that chromatin changes might be induced. We are currently profiling the chromatin state of endocrine pancreatic cells in the context of glucose exposure, using zebrafish as a model-organism. We will present our most recent results describing glucose induced chromatin changes in the zebrafish pancreas. These results will help to understand transcriptional dysregulation in the context of T2D development and will help to formulate new therapeutic strategies to revert T2D.

Grant reference: PTDC/BIA-MOL/3834/2021.

Conflict of Interest: None declared.

EP21.016 Peripheral DNA methylation biomarkers for the identification of early stages Alzheimer's disease patients

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia. Identifying the individuals in the early stages of the disease could greatly improve clinical management allowing interventions to slow down neurodegeneration. The aim of the current study was to search for DNA methylation biomarkers for AD patients in the prodromal/early stages of the disease.

DNA methylation analyses of the *APOE* gene promoter and IV exon, *PM20D1* gene promoter, and mitochondrial D-loop region, were evaluated by means of Methylation-Sensitive High-Resolution Melting in peripheral blood of 32 well-characterized AD patients, including 14 mild cognitive impairment (MCI) and 18 AD subjects at early stages of the disease, and in a group of sex and age-matched control subjects. Patients received a clinical and biomarker-based diagnosis, including brain imaging and/or cerebrospinal fluid investigations.

We observed that *APOE* exon IV methylation levels were lower in female AD patients compared to female control subjects. No differences in promoter *APOE* methylation between patients and controls were detected. *PM20D1* methylation levels were lower in MCI patients when compared to both control subjects and AD patients at the early stages of the disease. On the other hand, we found higher D-loop methylation levels in MCI patients than in control subjects and AD patients at the early stages of the disease.

Current results show that peripheral DNA methylation of specific genomic regions differs between AD and control subjects and in patients at different stages of AD pathology potentially providing early biomarkers of disease.

Grant: Researchers' intramural funds.

Conflict of Interest: None declared.

EP21.017 Epigenome-wide association study reveals six candidate methylation patterns of radiotherapy late severe toxicities in prostate cancer patients

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Objective: To identify DNA methylation patterns associated with the presence of severe toxicities after radiotherapy treatment in prostate cancer patients.

Methodology: Sixty-four prostate cancer patients -32 cases and their matched controls- treated with three-dimensional conformational radiotherapy (3D-CRT) at the Clinical University Hospital of Santiago de Compostela (Spain) between 2014 and 2018, were selected. Cases developed some late toxicity CTCAE grade >=2 after twelve months of starting radiotherapy. Methylation study was performed over 865,918 probes with the chip *MethylationEPIC Bead Chip*. Patients with CpG island detection <96%, low quality probes (<3 beads in > 5% of samples) and low detection (P-value < 0.01) were filtered out. Non-CpG, non-autosomal, multi-hitting or containing polymorphisms probes were eliminated. Batch effect was corrected through a *ComBat* approach. Differentially methylated positions (DMPs) and regions (DMRs) were estimated with *limma* and *DMRcate* packages respectively with age and cell type proportions as covariates. Unadjusted p-values were corrected using the Fisher approach.

Results: We detected 28 DMRs of which 10 were significantly associated with a F < 0.05. Of these, 6 DMRs were located within the upstream or genetic region of 6 genes (*HKR1, ABCA13, STK32C, ZAP70, SOSTDC1* and *SHANK2*).

Conclusions: We identified six genes with DMRs associated with severe toxicities after radiotherapy treatment. The differential expression of two of them -*ABCA13* and *ZAP70*- has been related to the efficacy of radiotherapy in other types of cancer. Our results require validation; however, they suggest that the epigenome plays a key role in the tolerance of patients to radiotherapy.

Conflict of Interest: Carlos López full, Miguel E. Aguado-Barrera: None declared, Carlos Carlos Pérez-Míguez: None declared, Olivia Fuentes-Ríos: None declared, Javier Manuel Galego Carro: None declared, Ana Crujeiras: None declared, Ana Vega: None declared.

EP21.019 Investigation of the methylation profiles of a panel of genes in thymic epithelial tumors

Vanessa Nicoli[1], *Bianca Mihaela Ciurea*[1], *Eleonora Pardini*[1], *Iacopo Petrini*[1], *Diana Bacchin*[2], *Vittorio Aprile*[2], *Franca Melfi*[3], *Marco Lucchi*[2], *Roberta Ricciardi*[4], *michelangelo maestri*[4], *Lucia Migliore*[1], ***Fabio Coppedè***[1]

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Background/object: Thymic Epithelial Tumors (TETs) are heterogeneous malignancies originating from epithelial cells of the thymus. Their complexity is due to the varying histological subtypes, which reflect the severity of the malignancies, as well as the commonly associated paraneoplastic disorders, including myasthenia gravis (MG). The THYMOGENE project is aimed to investigate the specific genetic and epigenetic signatures of TETs and their correlation with clinical features and associated syndromes.

Methods: We collected 65 TET biopsies and blood samples from patients who underwent a surgical resection of the thymus. More than 90% of the recruited patients had thymomas, and the remaining cases manifested a more aggressive thymic carcinoma (TC). MG was the most common paraneoplastic syndrome observed. Some healthy thymic biopsies were also available for

the gene specific methylation analysis of a panel of 15 selected genes, conducted by means of methylation sensitive high-resolution melting technique.

Results: We observed hypermethylation of *IL1RN1*, *GHSR*, and *IFI16* genes in TET biopsies compared whit blood, while *RAG1* and *KSR1* resulted hypomethyled in the tumors. Some of these genes also showed DNA methylation differences in the comparison of TETs and the available healthy thymic tissues, or among TET subtypes. For example, *IL1RN1* showed a significant hypomethylation in healthy thymic tissues compared with TETs, and differences between thymomas and TCs.

Conclusion: We identified potential epigenetic biomarkers of TETs. Next-generation sequencing of a panel of 77 genes is ongoing in the recruited samples to better characterize their genetic and epigenetic landscape.

Grant References: Bando Salute Tuscany Region.

Conflict of Interest: None declared.

EP21.020 Using methylation sequencing to identify an epigenetic mechanism of action for a GWAS-identified prostate-cancer risk allele

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Background/Objectives: Prostate cancer is the second most common cancer in men worldwide. This high burden is not shared evenly, with risk dependent on age, environmental factors, ethnicity, and on genetics more generally.

The rs11568818 SNP, like many others, has an allele that increases prostate cancer risk. This SNP, upstream of the MMP7 gene, has been shown to interfere with the binding of transcription factor FOXA2, which has been suggested as its mechanism of action for prostate cancer risk.

While rs11568818 and some other risk variants are relatively common, prostate cancer is notable for lacking a highly recurrent somatic driver mutation. Some epigenetic changes are highly recurrent and DNA methylation (especially in non-cancerous prostate tissue) is a natural place to look for evidence of drivers of tumorigenesis.

Methods: To understand the risks of prostate cancer, we have sequenced the genomes of >200 cancers and have sequenced also the methylomes of those patients in both their tumour and matched morphologically-normal prostate tissue.

Results/Conclusions: We show that there is an alternative method of action for the rs11568818 risk allele – one using epigenetic regulation – that can explain the increased risk. Through integration with external data we show that this is a more convincing mechanism for influencing the biology of the prostate than is its effect on transcription factor binding. The implications of this result for the biological and clinical understanding of the disease are then discussed.

Grant References: Cancer Research UK Grant Award C5047/A22530.

Conflict of Interest: Andy Lynch: None declared, Radoslaw Lach: None declared, David Wedge: None declared, rosalind eeles Honoraria for speaking from GU-ASCO, The Royal Marsden NHS Foundation Trust/Jannsen, University of Chicago, ESMO educational honorarium paid by Bayer & Ipsen, AstraZeneca UK Limited Prostate Dx Advisory Panel., Colin Cooper: None declared, Dan Brewer: None declared, Charlie Massie: None declared.

EP21.021 Splice variants in stop-codons of the DMD gene: in silico vs. in vitro

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Introduction: One of the mechanisms of the molecular pathogenesis of Duchenne/Becker muscular dystrophy formation are variants that lead to impairment splicing in the *DMD* gene. It is known that not only mutations in canonical regions, but also missense and nonsense mutations and intronic variants can affect splicing.

The aim of the study was to compare in silico predictions and in vitro functional study of the influence of nonsense variants on the mRNA sequence.

Materials and methods: We performed the bioinformatician analysis of 129 nonsense variants of *DMD* gene, that were identified in Russian patients with Duchenne/Becker muscular dystrophy. The splice site prediction programs Splice AI and SPiP were used. Then we perform minigene functional study for 6 nonsense variants with high damaging score: p.Gln1144Ter, p.Tyr1247Ter, p.Glu1425Ter, p.Gln1501Ter, p.Arg1763Ter, p.Arg3370Ter.

Results: Out of 6 nonsense variants, 3 variants were lead to affect splicing: p.Gln1144Ter, p.Gln1501Ter, p.Arg1763Ter. Interesting that in all cases we find 2 isoforms. For variants p.Gln1144Ter and p.Arg1763Ter we found isoforms of WT and skipping a whole exon. For variant p.Gln1501Ter we found WT isoform and shortened by 98 nucleotides isoform. For other 3 nonsense variants were not identified difference from wild type.

The results of the study shows importance of functional confirmation of influence nonsense variants on the molecular mechanism of the pathogenesis of Duchenne/Becker muscular dystrophy.

Conflict of Interest: None declared.

EP21.022 Functional characterization of Cryptochrome 2 gene SNPs

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Background/Objectives: Circadian clock is an innate mechanism that enables organisms to adapt to the rhythmic changes of the environment. In mammalian cells, this mechanism is achieved by the interaction of four core clock proteins (CRYs, PERs, CLOCK, and BMAL1). Human circadian core clock gene SNPs are associated with numerous behavioral and physiological diseases, including cancer, neurological disorders, and endocrine and metabolic disorders. Currently, the functional consequence of these variations and the strength of their association with the disease remains unclear. However, functional analyses of variants help us to understand the role of amino acid residues in the structure and function of proteins.

Methods: We have studied CRY2 gene SNPs which were selected from the Ensembl database. SNPs were filtered based on homology conservation, location of the functional domain, and pathogenicity effect. After the prediction of deleterious SNPs

using in-silico tools, the selected SNPs were further characterized by in-vitro functional analyses.

Results: We have found that the p.Ser420Phe CRY2 variant (the mutation was located in the coil-coil (CC) helix of CRY2) was unable to suppress CLOCK/BMAL1-driven transcription and properly localize in the nucleus using biochemical methods. Further stability results suggested that the p.Ser420Phe CRY2 variant was degraded by a noncanonical mechanism.

Conclusion: Collectively, our results show that the CC helix region plays an important role in the degradation of CRY2.

Grant References: We thank TUBITAK 121Z862 for the financial support.

Conflict of Interest: Gizem Parlak M.S student, M. S student, TUBITAK 121Z862, Şeref Gül Full, PI, TUBITAK 121Z862, Ibrahim Barış Full, PI, TUBITAK 121Z862, İbrahim Halil Kavaklı Full, PI, TUBITAK 121Z862.

EP21.023 DNA methylation-based cell composition and epigenetic age can improve understanding of atherosclerosis

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Background: There are reports that aberrant DNA methylation patterns associate with atherosclerosis, however cellular heterogeneity and methodological diversity remain a challenge. Our aim was to identify the most significant signature of atherosclerotic damage and plaque instability using DNA methylation-based parameters.

Methods: We used our DNA methylation array datasets ($n = 16$) and the GEO database (GSE149759, $n = 18$; GSE46394, $n = 30$; GSE66500, $n = 38$) to reconstruct cell composition (EpiDISH, limma) and epigenetic age (methylclock). To achieve cell composition analysis, we developed a deconvolution library of immune and vascular cells (endothelial, smooth muscle cells (SMCs), and fibroblasts).

Results: The cell type deconvolution and epigenetic age calculation of the 102 vascular samples showed a clear distinction between intact vessels (IVs) and atherosclerotic plaques (APs). IVs had SMCs >0.73% and either had monocyte fraction <5.5% or epigenetic age <52 years. Surprisingly, the predicted cell composition of both symptomatic and histological unstable APs was more similar to IVs than asymptomatic and histological stable APs (GSE149759), but unstable APs were differentiated from IVs after cell composition and epigenetic age adjustment. The most informative CpG sites were cg08079908 (HOXD8), cg17818471 (HOXD4/MIR10B), and cg19373813 (HOXD9) for sample type discrimination (FDR < 0.05). The stable and unstable APs, and IVs were distinguished well by cg08079908 and monocyte and SMCs fractions.

Conclusion: The identified DNA methylation-based parameters such as cell composition and epigenetic age can facilitate assessing the atherosclerotic plaque states.

Conflict of Interest: None declared.

EP22 New Treatments for Genetic Disorders

EP22.001 Small molecules to fix the genetic defects of pathogenic Reelin mutations in ADLTE

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Introduction: Autosomal Dominant Lateral Temporal Epilepsy (ADLTE; OMIM 600512) is a genetically heterogeneous syndrome, clinically characterized by focal seizures with prominent auditory symptoms. It is inherited with autosomal dominant pattern with reduced penetrance (about 70%). One of the causal genes, RELN, encodes Reelin, a secreted protein that is important for brain development and functioning. Recently, we demonstrated that pathogenic mutations in the RELN gene inhibit or significantly reduce Reelin secretion due to misfolded three-dimensional structure of mutated proteins. These data allowed us to design new experimental approaches based on the use of small molecules, developed to rescue ΔF508-CFTR trafficking and known as CFTR correctors.

Materials and Methods: We tested the efficacy of eight CFTR correctors and four chemical correctors approved by the USA Food and Drug Administration for clinical use in other diseases on cells transiently expressing a Reelin mutant (p.G2783C), which completely inhibits its secretion. The cells were incubated for 24h with CFTR correctors at different concentrations known to rescue in vitro CFTR mutants or chemical correctors. As negative controls, cells were incubated with compound vehicle (1‰ DMSO). Reelin expression in total protein lysates and in conditioned media were analysed by western blot.

Results and Conclusions: Preliminary experiments revealed that five CFTR correctors are able to partially restore mutant Reelin secretion. This experimental evidence constitutes an excellent starting point for evaluating the effectiveness of correctors and lays the foundation for a specific therapy for ADLTE patients carrying mutations in the RELN gene.

Financial support: Fondo di Beneficenza Intesa Sanpaolo.

Conflict of Interest: None declared.

EP22.002 Airway basal cells derived from human induced pluripotent stem cells as a model for development gene therapy for cystic fibrosis

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Background: Airway basal cells (BCs) are tissue-specific multipotent epithelial cells capable of self-renewing and differentiating into specialized cells in the airway epithelium. BCs derived from human induced pluripotent stem cells (hiPSCs) have promising applications, such as editing genetic mutation, disease modeling, and regenerative medicine. The purpose was to obtain and to characterize BCs derived from hiPSCs from a healthy donor and a donor with cystic fibrosis (CF, *CFTR* F508del/F508del), as well as to optimize the viral and the non-viral delivery of transgenes into cells.

Methods: BCs were selected for the surface marker CD271 by cell sorting of NKX2.1+ lung progenitors derived from hiPSCs. Immunocytochemical staining was performed to confirm expression of specific markers of basal cells (TP63, CK5, EPCAM). Transfection of the plasmid with *GFP* into BCs was performed by electroporation (Neon). Transduction into BCs was performed by recombinant adeno-associated viruses (rAAVs) serotypes 6 and 9 (*GFP* gene was used as a reporter). Transfection and transduction efficiency were assessed by flow cytometry using GFP fluorescence.

Results: Immunocytochemical analysis confirmed that the BCs derived from hiPSCs expressed markers of basal cells (TP63, CK5, EPCAM). The efficiency of transfection of the plasmid with *GFP* in BCs was 25.1 ± 8.5%. The highest efficiency of transduction of rAAV6 and rAAV9 were 86.4 ± 1.4% and 33.4 ± 2.1%, respectively, at 1E + 06 multiplicity of infection.

Conclusion: The results of the work demonstrate the possibility of obtaining BCs from hiPSCs and the highest transduction efficiency by AAV6, that makes BCs a promising object for editing the F508del mutation in *CFTR* gene.

Conflict of Interest: None declared.

EP22.003 ESALIT Study ((Efficacy- Safety-Lithium-TBR1): an open-label evaluation of lithium carbonate in patients with TBR1-related neurocognitive disorders: a 24-month multi-centre pilot study

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Background: TBR1–related disorder is a syndromic neurocognitive disorder, linked to a reduced Wnt7b cortical expression and synaptic functioning. Lithium, a WNT-signalling agonist, rescued the synaptic function in Tbr1 + /- mutants' mice. We designed a French clinical trial to evaluate the safety and efficacy of lithium in *TBR1* human patients.

Methods: We plan to include 10 patients ≥6 years old with pathogenic/probably pathogenic, *TBR1* variant. Primary objective is to evaluate the effect of lithium carbonate (LC) at 24 months on improvement in social communication, defined as a change ≥4 points in socialization and communication subscale standard scores of the Vineland adaptive Behaviour Scales (VABS). Secondary objectives include the effects at 6 months of LC on social communication and its safety, and the adverse events (CTCAE V5.0). Exploratory objectives include effects on behaviour, Intellectual Quotient, autism spectrum disorders, epileptic symptoms and quality of life. After an observation period of 6 months without treatment, patients will be randomised (1:1) to start LC treatment at randomisation or 6 months later. At the end, all patients will have had 24 months of treatment. LC treatment will be started at a weight-adjusted dose and gradually increased to achieve a target lithiaemia of 0.8-1.2 mEq/L.

Results: The results will allow a first assessment of the benefit-risk balance of LC in patients with *TBR1* variants.

Conclusion: Beyond the studied disease, these results will allow to make progress in the design of therapeutic trials in orphan neurodevelopmental diseases.

Study funded by a national hospital clinical research program.

Conflict of Interest: None declared.

EP22.004 Innovative treatment of arterio-venous malformations associated to PTEN by Alpelisib

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Background: Therapeutic options for arterio-venous malformations (AVMs) of *PTEN* syndrome are limited. The development of PI3K-AKT-MTOR pathway inhibitors in oncology allows us to expect their repositioning in patients with *PTEN* variants. We report the first observation of response to Alpelisib (BYL719), a specific inhibitor of the alpha subunit of PI3-kinase.

Methods: We collected data of 2 patients carrying a truncating germline *PTEN* variant and AVMs treated by Alpelisib.

Results: First patient, a 30-year-old man, presented multiple AVMs, a clear cell renal carcinoma, macrocrania and genital pigmentation. These multiple AVMs were complicated by cardiac hyperflow (cardiac index (CI) of 5.7 L/min/m^2 at 21 year-old), motivating the introduction of Sirolimus 1mg/day, and then at 2mg/day, interrupted in the absence of CI reduction. After obtaining an authorization for compassional use by the ANSM, Alpelisib 150 mg/d was introduced and followed by stability of AVMs, regression of pain, and reduction of CI from 6.6 L/min/m^2 to 5.6 L/min/m^2 in 34 months.

Second patient, a 61 year-old man, presented 2 AVMs and cardiac hyperflow. A first treatment by Sirolimus 1mg/day, was interrupted after 14 months. Alpelisib 250 mg/day is now prescribed since 10 months. Evaluation at 7 months of treatment showed a decrease of the CI from 6.5 to 6.2 L/min/m^2, a stability of AVMs, a decrease of dyspnea and foot's oedema. Both patients reported an improvement of quality of life.

Conclusion: These observations suggest signs of effectiveness, which need to be confirmed on a larger cohort of patients with inactivating *PTEN* variants.

Conflict of Interest: None declared.

EP22.005 iMDK and neratinib are active in in vitro systems against malignant pleural mesothelioma cell lines

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Background/Objectives: Malignant pleural mesothelioma (MPM) is a rare aggressive chemotherapy-resistant tumor with a bad prognosis and still without effective therapies. The goal of this research is to detect novel MPM-driver genes highlighting molecular targets for future therapies.

Methods: Firstly, we analyzed TCGA-database for detecting the overexpressed genes. Then, we evaluated the potential carcinogenic-driver role of selected candidate genes by observing the effect of their functional knock-down following small interfering RNA treatments. MSTO and MeT-5A cell lines were employed as model of malignant and non-malignant cells, respectively. The phenotypic changes following gene silencing were verified by IncuCyte® technology.

Results: The gene silencing of *BAG2, MDK*, and *MAD2L1* caused a reduction of cellular growth of MSTO cells, while MeT-5A was affected to a lesser extent. Moreover, a statistically significant increase in caspase activation was observed in MSTO cells following *BAG2* and *MDK* silencing.

Cellular migration wasn't significantly affected by single gene depletion. Then, we also tested two small molecules with inhibitory activity of MDK (i.e. iMDK) and MAD2L1 (Neratinib). A statistically significant decrease in cellular growth and an increase in cytotoxicity were observed for both drugs in MSTO cells.

Conclusion: iMDK and Neratinib showed promising activity against MPM cells and their testing in in vivo models is fully warranted. These drugs could represent novel therapeutic candidates suitable for delaying the malignant progression of MPM, especially in patients with increased expression of MDK or MAD2L1.

Grant References: AIRC Investigator Grant to Prof. Stefano Landi (IG 2018-project Id. 21853).

Conflict of Interest: None declared.

EP22.006 A Phase II double-blind multi-center, placebo-controlled trial, to assess the efficacy and safety of alpelisib (BYL719) in pediatric and adult patients with Megalencephaly-CApillary malformation Polymicrogyria syndrome (MCAP): the SESAM trial

Maxime Luu[1], *Agnes Maurer*[2], *Guillaume Canaud*[3], *Nathalie Boddaert*[4], *Adelaide Rega*[5], *Laurent Guibaud*[6], *Philippe Khau Van Kien*[7;8], *Florence Petit*[9], *Estelle Colin*[10], *Bénédicte DEMEER*[11], *Christine Francannet*[12], *Olivia Boccara*[13], *Alinoe Lavillaureix*[14], *Annabel Maruani*[15], *Aurélie Espitalier*[2], *camille fleck*[16], *amelie cransac*[17], *maud carpentier*[16], *Julie Charligny*[1], *Nadia Bahi-Buisson*[18], *Marc Bardou*[1], *Laurence Faivre*[2]

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Background/Objectives: The MCAP syndrome (Megalencephaly Capillary malformation Polymicrogyria) results from mosaic gain-of-function *PIK3CA* variants. Main features are macrocephaly, somatic overgrowth, neurodevelopmental delay, and brain anomalies. Alpelisib (VIJOICE®) is a PI3Kα-specific inhibitor recently approved by the FDA in PIK3CA-related overgrowth spectrum (PROS). Neurocognitive endpoints and blood brain barrier crossing were not studied in these patients. We aim to evaluate the efficacy of a 24-month treatment of alpelisib on adaptive behavior and safety in patients with MCAP syndrome.

Methods: SESAM is an academic, national, two periods multi-center phase II trial, with a 6 months double-blind, placebo-controlled period followed by open label period. Primary endpoint is a ≥4 points improvement in the Vineland II Adaptive Behavior Scale (VABS), 24 months after treatment initiation. Secondary objectives are: safety, VABS improvement at 6 months, impact on quality of life, epilepsy and hypotonia. Sixteen patients (3 already included) aged 2 to 40 years old, with a MCAP diagnosis and neurodevelopmental disorders, will be followed monthly in local centers, centrally assessed (clinical, biological, neuropsychological and functional evaluation) at baseline and every 6 months, and they will have a MRI at baseline and 24 months at Dijon or Necker hospitals). An optional lumbar puncture will be performed for pharmacokinetics. Enrollment should be completed in June 2023, and the end of follow-up in December 2025.

Conclusion: Results will provide a first assessment of the benefit-risk ratio of alpelisib in patients with MCAP, and provide insights for future trials in neurodevelopmental disorders.

Grant References: CHU Dijon and Novartis.

Conflict of Interest: Maxime Luu Dr Luu has received consulting fees from Novartis, Agnes Maurer: None declared, Guillaume Canaud A patent application ("BYL719 (alpelisib) for use in the treatment of PIK3CA-related overgrowth spectrum" #WO2017140828A1) has been filed by INSERM (Institut National de la Santé et de la Recherche Médicale), Centre National De La Recherche Scientifique (CNRS), Université Paris Cité, and Assistance Publique-Hôpitaux De Paris (AP-HP) for the use of BYL719 (alpelisib) in the treatment of PIK3CA-related overgrowth spectrum (PROS/CLOVES syndrome). Dr. Canaud is the inventor. This patent is licensed to Novartis., Dr. Canaud receives or has received consulting fees from Novartis, Fresenius Medical Care, Vaderis, Alkermes, IPSEN and BridgeBio., Nathalie Boddaert: None declared, Adelaide Rega: None declared, Laurent Guibaud: None declared, Philippe Khau Van Kien: None declared, Florence Petit: None declared, Estelle Colin: None declared, Bénédicte DEMEER: None declared, Christine Francannet: None declared, Olivia Boccara: None declared, Alinoe Lavillaureix: None declared, Annabel Maruani: None declared, Aurélie Espitalier: None declared, camille fleck: None declared, amelie cransac: None declared, maud carpentier: None declared, Julie Charligny: None declared, Nadia Bahi-Buisson: None declared, Marc Bardou: None declared, Laurence Faivre: None declared.

EP22.007 A novel RHEB germline variant associated with intellectual disability and epilepsy: expanding the spectrum of mTORpathies

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Background/Objectives: Pathogenic variants in genes encoding upstream regulators of the mTOR pathway cause different overgrowth syndromes, epilepsies and neurodevelopmental disorders. Hyperactivation of the mTOR pathway appears to be the essential pathomechanism of these disorders known as mTORpathies. Recently, brain somatic pathogenic variants and germline de novo pathogenic variants in the *RHEB* gene have been described associated with cortical malformation defects, intellectual disability (ID), megalencephaly, and epilepsy. The experimental evidence on these mutations show a gain-of-function effect and hyperactivation of the mTOR pathway. In vivo studies suggest that patients with pathogenic variants in the *RHEB* gene may benefit from therapies with mTOR inhibitors, such as rapamycin.

Methods: Whole exome sequencing was performed on a 19 years old female affected by mild ID, macrocephaly, epilepsy, scoliosis, and attention deficit hyperactivity disorder.

Results: A de novo germline heterozygous missense variant c.71T>C; p.Ile24Thr was identified in the *RHEB* gene. The variant is located in the RAS region and is absent in gnomAD control population database. The variant was classified as likely pathogenic following ACMG guidelines.

Conclusion: We have identified a novel *RHEB* germline variant that expanding the spectrum of mTORpathies. Gain of function variants in the *RHEB* gene have been shown to hyperactivate the mTOR pathway. In order to check if the patient may benefit from therapies with mTOR inhibitors, we are performing functional studies to determine the variant's effect on mTOR activity.

Conflict of Interest: None declared.

EP22.008 G-protein coupled receptors as potential therapeutic targets in movement disorders/epilepsy caused by GNAO1 de novo mutations

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Dominant mutations in the *GNAO1* gene underlie a severe neurological condition characterized by hyperkinetic movement disorders, epilepsy, developmental delay, and cognitive decline, with infantile/childhood onset. *GNAO1* encodes the α-subunit of an inhibitory G-protein regulating ion channel activity and neurotransmitter release. The pathogenic mechanisms underlying *GNAO1*-related disorders remain largely elusive and to date there are no effective therapies. Here, we generated CRISPR-Cas9-engineered *C. elegans* strains harboring four pathogenic variants in *goa-1*, the *C. elegans* orthologue of *GNAO1*, associated with diverse clinical features. Like null mutants, homozygous knock-in animals showed increased egg laying and were hypersensitive to aldicarb, an inhibitor of acetylcholinesterase, suggesting excessive neurotransmitter release by different classes of motor neurons. Automated analysis of *C. elegans* locomotion indicated that *goa-1* mutants move faster than control animals, with more frequent body bends and a higher reversal rate, and display uncoordinated locomotion. Phenotypic profiling of heterozygous nematodes revealed a mutation- and cell-specific dominant-negative behavior of the mutant alleles. In a pilot drug screening performed with compounds targeting G-protein coupled receptors (GPCRs), caffeine and istradefylline, an FDA-approved drug in the treatment of PD, were found to rescue the hyperactive motor behavior of *goa-1* mutants, by blocking, at least in part, a putative adenosine receptor in the nematode. Moreover, knocking-down the expression of GPCRs playing a role upstream to stimulatory G-proteins by RNAi reduced the locomotion defect of *goa-1* mutants. Overall, our findings establish *C. elegans* as an efficient drug-screening platform for *GNAO1*-related disorders and highlight the potential role of GPCRs modulation in controlling dyskinesia.

Conflict of Interest: None declared.

EP22.009 Post-GWAS validation of target genes associated to HbA2 and HbF levels

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Beta-haemoglobinopathies afflict an impressive number of people worldwide. No definitive cure, generally available, exists. Amelioration of these inherited diseases by increasing of HbF

(alpha2,gamma2) has been extensively proved. Less studied is the possibility to increase HbA2 (alpha2,delta2). However, previous results by our group validate HbA2 as a therapeutic target in preclinical disease models (Manchinu et al., 2014; Porcu et al., 2021).

Genome-wide association studies (GWAS) have identified, in the last decades, an impressive number of variants associated with different traits. The aim of this project is to validate in vivo two genes previously identified by a recent GWAS to be associated to the level of HbF and/or HbA2: *Cyclin D3* (*CCND3*) and *Nuclear Factor I X* (*NFIX*) (Danjou et al., 2015). To investigate, in vivo, the feasibility of exploiting these two genes as possible therapeutic targets against beta-haemoglobinopathies, we mated *Ccnd3* KO or *Nfix* KO mice to a transgenic mouse line carrying the entire human beta-globin gene cluster (Ln72). Analysis of Ln72/*Ccnd3* KO mice showed a robust increase of both globin RNA levels. These increases are detectable from intrauterine to adult life in our model. Expression studies showed an increase in gamma-globin levels in ln72/*Nfix* KO mice as well, although to a less extent, starting from intrauterine life and persisting until 14 days after birth.

Our preliminary data validate *CCND3* and *NFIX* as genes involved in HbA2 and HbF levels which could represent new targets for beta-haemoglobinopathies therapy.

This work has been supported by Telethon Grant 20046 to MSR.

Conflict of Interest: None declared.

EP23 Genetic Counselling/Services/Education

EP23.001 Genomics in healthcare: UK Public Attitudes and Understanding

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Background: The UK Government aims to create 'the most advanced genomic healthcare system in the world', but what do the public think?

PET commissioned a piece of wide-ranging, nationally representative research to assess opinions and understanding of genomics in relation to healthcare.

Methods: Ipsos interviewed 2,233 UK adults aged 16-75 in March 2022. Data was weighted to known population proportions for age, working status and social grades within gender and region. Questions developed by PET and its advisers with input from Ipsos experts explored:

- Understanding of the term 'genome'
- Attitudes to storing genetic and medical data for personal healthcare, family healthcare and medical research
- Use of whole genome sequencing in newborns
- Necessity of genetic research in developing new treatments

Results included:

- 43% selected an incorrect definition of a genome or answered 'don't know'
- 57% supported, and 12% opposed genetic data being stored in a national database accessible by the patient or their doctors
- 53% supported, and 16% opposed deidentified genetic data being stored in a national database for medical research
- 57% agreed that more genetics research is needed to develop new treatments, but only 36% were excited about the potential of genome editing

Conclusions Across questions, significant minority of respondents answered 'neither support nor oppose' or 'don't know', indicating that 20-30% of the public do not yet have strong views on these issues, or feel they do not understand them. Our research indicates a need for increased public education, engagement, and outreach.

Grant references: Project sponsored by Ferring Pharmaceuticals.

Conflict of Interest: None declared.

EP23.002 Referral criteria to clinical genetics from Primary Care: Consensus document

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Introduction: Primary care (PC) is the first contact between the patient and the doctor, so it is essential to be clear about the criteria for suspecting a genetic disease and where it should be referred for study.

Material and methods: Four scientific societies: the Spanish Society of Family and Community Medicine (semFYC), the Spanish Association of Human Genetics (AEGH), the Spanish Association of Pediatrics (AEP) and the Spanish Society of Medical Oncology (SEOM), have reviewed the criteria for referral to the clinical genetics services of the different published guidelines with the purpose of define the recommendations for PC.

Conclusions: With this consensus document, the PC doctor and pediatrician will know when, how and where to refer their patients with hereditary and/or genetic pathology to clinical genetics services.

Conflict of Interest: None declared.

EP23.003 A qualitative study of prostate cancer patient experiences with genetic testing results return

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Background/Objectives: Germline genetic testing for prostate cancer patients is increasingly common with the expansion of eligibility due to implications for treatment decision-making. It is important to understand patient comprehension of genetic test results in order to address barriers to follow-up care. This study explores prostate cancer patients' perceptions about genetic testing and post-test results communication.

Methods: Qualitative, semi-structured interviews were conducted with prostate cancer patients at an urban safety-net hospital who received a referral to genetic counseling. Interview questions focused on patient experiences with genetics referrals, genetic counseling, and genetic test result disclosure. Audio recordings were professionally transcribed and analyzed by the study team utilizing an inductive thematic approach in order to generate themes from recurring codes.

Results: Interviews were conducted with 23 English, 6 Spanish, and 3 Haitian-Creole speaking patients, 24 of whom completed genetic testing. Four themes emerged: 1) confusion about implications of negative test results in the context of a personal and family history of cancer; 2) uncertainty regarding the utility of genetic testing when already affected with cancer; 3) limited recollection of the genetic testing process due to prioritization of active cancer treatment; and 4) desire for more extensive discussions surrounding genetic testing results.

Conclusion: Participants revealed several post-test communication gaps that created misconceptions about hereditary cancer risk. Understanding communication barriers in genetic testing results return is imperative in order to provide high quality genetics care.

Grant References: This work was funded by the United States Department of Defense, W81XWH-20-1-0110.

Conflict of Interest: Kimberly Zayhowski United States Department of Defense, W81XWH-20-1-0110, Catharine Wang United States Department of Defense, W81XWH-20-1-0110, Stephanie Loo United States Department of Defense, W81XWH-20-1-0110, Gretchen Gignac United States Department of Defense, W81XWH-20-1-0110, Christine Gunn United States Department of Defense, W81XWH-20-1-0110.

EP23.004 Launching the pilot phase of Hong Kong Genome Project: Implementation of public engagement strategy

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Background/Objectives: Public trust and confidence remain a global challenge in population-based genome projects. This study aims to engage the public and evaluate participants' views, concerns, and aspirations related to genomic studies and the Hong Kong Genome Project (HKGP), to guide the preparation and publicising strategies for the launch of the HKGP pilot phase.

Methods: Three focus group meetings involving 20 rare diseases and hereditary cancers patients, family members and caregivers, and healthcare professionals from Hong Kong were conducted. Data collected were analysed using thematic analysis.

Results: Four major themes were identified. First, "decisional considerations of undertaking genetic testing"; participants' decisional motivators to undertake genetic testing and participate genome projects were identified, including perceived personal and familial benefits, and altruistic motivations to contribute to genomic advancement. Second, "concerns and worries in genomic research", including personal, familial and societal concerns, underscoring the importance of a "transparent" and "fully informed" procedure to tackle public distrust and enhance public engagement. Third, "importance of a patient-oriented, transparent, and decommercialised campaign" to enhance involvement and recruitment. Fourth, "the need to enhance public genomic literacy and awareness" by providing informational support.

Conclusion: This study provides empirical evidence through engaging the public in focus group meetings, which guides the preparation and publicising strategies of the pilot phase of HKGP, and laid an important patient-oriented foundation of the main phase of HKGP.

Grant References: N/A.

Conflict of Interest: None declared.

EP23.005 Midwives' views of parents' questions and expectations on prenatal genetic testing –Identifying informational needs in prenatal genetic counseling

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Background. Autonomy and informed decision making are important aspects for prenatal genetic diagnostics. Midwives' knowledge and skills are essential to provide adequate information about prenatal testing to expecting parents to enable informed decisions. Information from midwives to parents about prenatal genetic testing has been found to not always be adequate, and parents' needs not always understood. As new methods are introduced, the scope of analysis is widening. To achieve informed decision-making, it is important to understand the questions and expectations midwives meet from expecting parents.

Aim: This study explores questions and expectations midwives meet from expecting parents regarding prenatal genetic testing, and how uncertainties are perceived and valued.

Method: A questionnaire was answered by midwives (N = 71) in different health care regions in Sweden, working both in primary maternity healthcare and as ultrasonography specialists.

Results: Midwives were found to perceive an increased number of questions about noninvasive prenatal testing (NIPT) but a proportion of midwives are not completely confident to answer these questions. Midwives get questions about trisomy 21, other trisomies and sex chromosome abnormalities, but also neuropsychiatric conditions. Methods for invasive, diagnostic testing do not seem to be discussed when accepting offer of initial screening. Midwives are aware of uncertain and secondary findings, but fewer have discussed this with parents.

Conclusion: Continuing education and support to midwives should put additional focus on developing understanding of new prenatal methods, including NIPT, as well as tools for explaining to parents and make parents aware of potential outcomes of prenatal genetic testing.

Conflict of Interest: Lisa Åkerman: None declared, Charlotta Ingvoldstad Karolinska university hospital, Department of clinical genetics and center for fetal medicine, Maria Johansson Soller Karolinska University hospital.

EP23.006 Genetic sounds: the European Society of Human Genetics podcast

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Background/objectives: Podcasts are accessible and engaging learning tools, offering broad exposure to core content and personalized learning while simultaneously fostering a sense of connection to diverse communities and allowing for

asynchronous listening. We proposed a podcast with the aims to (a) stimulate new conversations around human genetics that include current social debates, (b) make accessible the biggest stories in human genetics, and (c) expand the Society's educational resources and portfolio.

Methods: We combined expertise in Media, Communications, Psychology, Genetic Counselling, Patient and Public Involvement, Narrative practices, Rare disease and Qualitative research methods and Human Genetics and Genomics. The episodes were delivered ensuring the participation of patients, their representatives and practitioners. All materials and events were disseminated through ESHG communication channels (website, YouTube Channel, Twitter).

Results: We created Series 1: six episodes covering scientific, clinical practice, education and ethical considerations around developments in human genetics and genomic medicine. The last episode was recorded as part of the 2022 ESHG conference with the participation of a live and virtual audience. A film about Series 1 was developed which will be shown during the 2023 ESHG conference. The podcast has been downloaded >2500 times across the globe.

Conclusions: We are currently delivering Series 2. The first episode of Series 2 was developed in collaboration with the African Society of Human Genetics. We invite collaborations for developing Series 3, planned for 2024.

Grant references: None.

Conflict of Interest: None declared.

EP23.007 Mapping service provision of genetics health care in Portugal

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In recent decades, genetics has undergone important technological advances. The rapid shift to genomics has made a strong impact on health systems around the world. In Portugal, this huge increase in consultations and typologies of genetic tests has joined the serious limitations of the few existing genetics services. The following study aims to characterize the current state of the network of genetics services in Portugal regarding its functioning, main challenges, and opportunities. Five semi-structured interviews were conducted, corresponding to 83.33% of the directors of the public genetics services of the National Health Service. Four thematic categories emerged from the analysis: (1) specialty and technical developments, (2) structural difficulties, (3) potentialities, and (4) future directions. The developments are due to the emergence of more comprehensive genetic applications, specific protocols and patient referral standards, and accreditation of services. The main difficulties encountered in the service's' functioning were difficulty in obtaining funding, lack of human resources, service overload, and lack of exclusive time for training and research. The potentialities mentioned were the establishment of multidisciplinary teams and the best articulation with specialists from other areas. Among the various future directions pointed out, better management of patients' waiting lists, the importance of research, the simplification of test request procedures, and the creation of specialized units inside the genetic services, were reported. The results showed several gaps in the practice of medical genetics that should be addressed with the development of public policies for the recognition and restructuring of medical genetics in Portuguese health care.

Conflict of Interest: Catarina Costa This study was carried out with the financing of the PhD scholarship concluded with the Foundation for Science and Technology (Fundação para a Ciência e Tecnologia) with the reference SFRH/BD/145679/2019, attributed to the author Catarina Costa., Luís Azevedo: None declared, Marina Lemos: None declared, Milena Paneque: None declared.

EP23.008 The utilisation of multi-disciplinary team working for high risk breast cancer patients; incorporating healthcare professional views, in an era of mainstream genetic testing

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Multidisciplinary team meetings (MDTs) are well versed in the medical field. Management of patients with cancer predisposition syndromes benefit from the utilisation of MDTs involving various specialities. In Cornwall, women with a high risk of breast cancer are discussed at an MDT; with input from Breast Surgery, Clinical Genetics, Breast Imaging and Health Psychology. This structure allows for collaborative working, creating the opportunity to support patient care in a collegial way. Our pathway clearly guides the process and eligibility of a patient being referred in relation to a high risk of breast cancer to a risk reducing surgery. This includes the requirement of Health Psychology prior to discussions with Breast Surgery.

In the era of transition to mainstream diagnostic genetic testing, the value of MDTs is ever important. This framework creates space for learning and development, particularly with the recent changes to genetic testing for inherited breast cancer syndromes.

We have asked healthcare professionals to provide an insight into their experience of the current patient pathway for patients with a high risk of breast cancer considering risk reducing breast surgery. Through assessing this data and reviewing our pathway we hope to reflect on the impact of this pathway and its potential utilisation in other areas, to provide the best possible service to our patients. We plan to share our pathway for our multidisciplinary framework.

Conflict of Interest: Aamisha Kyada Full.

EP23.009 Genetic counselling supervision: luxury or necessity? A qualitative study with genetic healthcare professionals in Portugal

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Background/Objectives: In Portugal, such like in other European countries, genetic counselling is emerging as an independent scientific field. Genetic Counselling supervision is precisely one of those areas in which there is no structured guiding legislation common to different parts of the globe. The aim of this study is to understand (1) if and how genetic counselling is supervised in Portugal, (2) the perception of genetic healthcare professionals on its relevance and (3) to identify factors relevant to effective counselling supervision.

Methods: Medical geneticists practicing in Portugal were invited to participate in an online focus group Two dates were defined, and all the major genetic services in Portugal were represented. Four major areas were explored: (1) if and how genetic counselling supervision is conducted in Portugal; (2) how genetic counselling processes impact the professionals; (3) moments/challenges/impacts

that should be accessed; (4) how should genetic counselling supervision be implemented in Portugal.

Results: Twelve geneticists from seven services of the Portuguese national health system participated in two sessions. None had access to genetic counselling supervision. Professionals considered that there are major areas that should be part of a supervision routine: difficulties/ barriers in communication; pressure from patients and from peers; situations in which professionals feel despair. Participantes considered that supervision should be mandatory.

Conclusion: Genetic counselling supervison is understood as a necessity and professionals feel it should be an integral part of their practice. Implementation of such service routine is desirable and should be included as part of the reorganization of national genetic services.

Conflict of Interest: None declared.

EP23.011 Longitudinal genetic counselling from fetus to childhood

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Since "Genetics" is more about family rather than single person, it is important to provide genetic counselling and suport as family care for their lives. The timing people need genetic counselling used to be when your child showed specific symptoms, when you are diagnosed as genetic conditions and when you consider family planning. However, due to improvement of prenatal diagnosis with ultrasound and genomic analysis, more fetus and/or neonates can be diagnosed as genetic condition these days.

Most of medical center provide genetic counselling as each stage depending on specialty such as maternity care, paediatrics and adults. This system make patient and their family to visit hospital several times to see different specialties. At our medical center, single genetics team (geneticists, obstetrician, neonatologist, midwife, nurse) could cover maternity care including prenatal diagnosis and through paediatrics genetics as well. This helps patient (mother of child) can be familiar with GC provider and they don't have to explain detailed information of their present medical history. In addition, patient's family can discuss on further family planning and child's condition all together.

In this presentation, we would like to introduce a few cases who have been through this longitudinal genetic counselling for 5-10 years. We hope this could be an opportunity to share good model for prenatal-paediatrics longitudinal genetic care by same GC team for the future.

Conflict of Interest: Akane Kondo part-time, Mikio Morine full, Kazuhisa Maeda full, Nobuhiko Okamoto full, AMED, Setsuko Nakayama full, Michie Kawashima full, Grants-in-aid for Scientific Research, Communication.

EP23.013 International Telehealth for Genetic Counseling: Past, Present and Future

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Background: Telehealth implementation has expanded in recent years and is now the standard of care for many reasons, including improved access to services. The utilization of telehealth for the provision of healthcare services across international borders (termed 'international telehealth') is an emerging service delivery model (SDM) within genetic counseling.

Methods: To evaluate the logistic, demographic, and ethicolegal variables associated with international telehealth, we conducted a landscape analysis by surveying two stakeholder groups: (a) U.S.-based genetic counseling/ testing company representatives and (b) practicing genetic counselors from relevant professional special interest groups.

Results: Over half of the companies that responded reported engaging in international telehealth and had clients and/or genetic counseling providers in different countries. About 75% of the practicing genetic counselors reported receiving requests to provide genetic counseling services to clients outside the U.S. Canada, Mexico, and England were amongst the most common countries where clients were located, and twenty other countries were reported at least once. The biggest concern reported was the interpretation of the available regulations, such as the European Union General Data Protection Regulation (GDPR) and the U.S. Federal Trade Commission (FTC). Additionally, the lack of practice guidelines and limited professional recognition of genetic counselors in many countries confounded the implementation of international telehealth.

Discussion: In this presentation, we will describe the results of our landscape analysis, our subsequent global initiatives to increase communication between genetic counselors, and possible collaborative solutions to improve access to genetic counseling services.

Conflict of Interest: None declared.

EP23.014 Translation and cross-cultural adaptation of the Genetic Counselling Outcome Scale (GCOS-24) for use in Cyprus: A qualitative study

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Background/Objectives: GCOS-24 is a patient-reported outcome measure developed in the UK, for evaluating Clinical Genetics Services (CGS), designed to measure the five dimensions of Empowerment. The objective was to translate and culturally adapt GCOS-24 for use by Greek-Cypriots in Cyprus, following international guidelines for the translation and cross-cultural adaptation of self-reported measurements questionnaires.

Methods: Two independent forward, and two back-translations were carried out by native Greek and English speakers, respectively. An expert comittee resolved any issues identified and a consensus was reached on wording of the Greek-Cypriot GCOS-24 for pre-testing. Participants were recruited from the Cyprus Institute of Neurology and Genetics and data was collected using semi-structured cognitive interviews with open-ended questions, followed by thematic analysis with an inductive approach to coding.

Results: Twelve patients participated in the cognitive interviews. Semantic validation indicated all items within the Cognitive Control and Emotional Regulation dimensions were easy to understand, whereas items within the Decisional Control, Behavioural Control and Hope dimensions presented with semantic difficulties. Participants provided suggestions for how the items could be modified to facilitate understanding, and their feedback

was considered for the formulation of the final version of the Greek-Cypriot GCOS-24.

Conclusion: This is the first study reporting the translation and cross-cultural adaptation of GCOS-24 in Greek for use in Cyprus. Study findings suggest GCOS-24 may be useful to evaluate CGS in Cyprus. Next steps include assessment of reliability and responsiveness of the Greek-Cypriot GCOS-24.

Conflict of Interest: None declared.

EP23.015 Lost in translation: language barriers in genetic counselling

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Background: Often genetic counsellors are faced with patients, whose mother tongue is different than ours. Whether it is because of migrations, globalisation, or improved access to genetic services for minorities, it puts both the patient and the counsellor in a difficult position. Counselling is a mutual process of exchanging information and empowering the patient, however we are sometimes unable to provide it as such. Too many times professional interpreters are unavailable at the time of the consultation, or a person brought in by the patient is inappropriate (a minor, a related dominant figure, an unrelated acquaintance, a person unable to speak both languages fluently). The topics normally addressed in genetic clinic are sensitive and intimate, so it is important to consider to whom the information is disclosed.

Conclusion: Legislature is generally protective of patients' privacy and rights. In practice, however, we are regularly faced with less than ideal – even improvised – options that leave both the patient and the counsellor unsatisfied with the consultation. There are several options to contemplate if a professional interpreter is not available at the time of consultation. While none of them is ideal, they can help us facilitate the process of genetic counselling.

Conflict of Interest: None declared.

EP23.016 Genetic counselling for psychiatric disorders: clinical geneticists' perceptions of needs, effects and possibilities

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Background/Objectives: Psychiatric disorders are prevalent and can have large implications for individuals and their families. Despite knowledge that genetic factors contribute to the development of these disorders and that genetic counselling have beneficial effects, genetic counselling is rarely offered at present. More knowledge is needed regarding why genetic counselling is not accessible to a greater extent in this area. This study explored possible obstacles to offering psychiatric genetic counselling to patients and their families in Sweden.

Methods: A focus group interview was carried out with three clinical geneticists and the transcribed interview was evaluated with qualitative content analysis.

Results: The analysis revealed three main categories: i) perceptions of the individual perspectives of psychiatric disease and genetic counselling for psychiatric disorders, ii) perceptions of the roles of Clinical genetics and Psychiatry, and iii) identified obstacles and challenges. A theme was identified: clinical geneticists' view of the needs, effects, and possibilities for genetic counselling for psychiatric disorders.

Reported obstacles and challenges include uncertain contribution of genetic data in risk assessments, emotional barriers, challenges in taking family histories, lack of knowledge and competence, and resources and priorities. The participants describe that genetic counselling has an important therapeutic purpose and identify potential benefits such as more knowledge about risks and risk factors, help to process emotions such as guilt, and help to adapt.

Conclusion: The results reveal important factors that can be of value for developing a more accessible genetic counselling for psychiatric disorders in a Swedish context.

Conflict of Interest: None declared.

EP23.018 Reflections on an audit of a historical dystrophinopathy family register

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Background/Objectives: The South East Thames Regional Genetics Service, London, UK serves over seven million people. The service routinely provides genetic counselling to families impacted by dystrophinopathy, including Duchenne muscular dystrophy and Becker muscular dystrophy.

An audit of the service's historical dystrophinopathy family register was undertaken. The aim of this was to ensure known carriers of dystrophinopathy and women at risk of being a carrier had access to regular cardiac surveillance, as they are at increased risk of cardiac complications.

For women identified as at risk of being a carrier, the audit also aimed to prompt them to access genetic counselling locally, to facilitate information provision around carrier testing, management of female carriers and family planning options.

Results: The audit reviewed over 8,000 patient records, identifying over 250 confirmed carriers of dystrophinopathy, including obligate carriers. A further 95 individuals were identified to be at risk of being a carrier. The audit also highlighted a group of patients where further genetic testing could be performed. The respective GPs of these patients were subsequently contacted to notify them of our recommendations around cardiac surveillance or genetic counselling.

Reflections/Conclusions: The clinicians involved in the audit anecdotally noticed the challenges of keeping accurate and up-to-date NHS departmental registers, the inconsistency of genetic information sharing in and between families and the benefits of the genetic testing evolution, from the use of linkage to gene sequencing. Lessons learnt and reflections from this audit will continue to influence clinical practice, particularly when coordinating patient follow up care.

Conflict of Interest: None declared.

EP23.019 A proposal of a patient-centred PtDA in genetic counselling for hereditary deafness

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Understanding d/Deaf patients' cultural assimilation and identities, and the social aspects of their personal beliefs about d/Deafness improves the process of shared decision-making and outcomes in genetic counselling for hereditary deafness. However, communication barriers between Deaf patients – members of a Deaf community whose first language is sign language, and a hearing genetic counsellor who primarily uses spoken languages can reduce Deaf patients' autonomy for informed consent and understanding of the risks and benefits of genetic testing. Our attempt to overcome both cultural differences and communication challenges was through a patient decision aid (PtDA) that can be used as an additional tool in genetic counselling. We propose a patient-centred design of a PtDA booklet in genetic counselling of three different patient groups – the Deaf patients who identify themselves with a Deaf community, the deaf patients who identify themselves with a hearing community and hearing patients with a positive family history of hereditary deafness. An emphasis in our PtDA is placed on defining Deafness in a social context as "a common trait of the minority group" rather than in the medical context as "an illness and undesirable trait". Genetic terminology and events related to the inheritance of hereditary deafness were explained using clear and straightforward sentences, along with visual aids such as images and graphics, to enhance the patient's understanding and ability to grasp the information.

Conflict of Interest: Klara Francic Full-time - speciality trainee in clinical genetics, Sarah Swann: None declared, Saeeda Bhatti Full-time, University of Glasgow.

EP23.021 Establishment of a national multidisciplinary team meeting for rare genetic diseases in Luxembourg

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Background/Objectives: The multidisciplinary meeting (MDM) is an effective model of care for rare diseases, allowing a group of experts of different fields to contribute collectively to the care, diagnosis and treatment of patients with rare and complex diseases. In Luxembourg, the first national MDM for rare genetic diseases, hosted by the National Hub for Rare Diseases, was created in 2021. We aim to describe here the process of establishment of this new organization, and the outcome for the patients.

Methods: We supply a description of the Luxembourgish MDM for rare genetic diseases, and a retrospective study evaluating the consequences of the MDM for the patient's care.

Results: A MTM was organized on a monthly basis, involving health professionals from 3 public institutions and from private practices in Luxembourg, taking into account ethical (patient consent) and data protection considerations. During the first two years, specialists from 15 medical specialties were involved, for 96 individual cases. Of these, 53 cases concerned the organization of an initial etiological assessment, 23 the clarification of the interpretation of existing genetic results, and 20 the coordination of a patient's care.

Conclusion: The organization of MDM in the context of rare diseases is in line with internationally recognized good practice, and is of great interest to the patient. The practical organization of these meetings must meet ethical and data protection requirement, in association with a valuation of the working time devoted to this expertise, in order to allow the generalization of this system under satisfactory conditions.

Conflict of Interest: None declared.

EP23.022 Conveying recurrence risk for an apparent de novo pathogenic variant: What should the counseling be?

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Background/Objectives*:* For years, parents with children affected with rare disease due to a de-novo variant (DNV) were counseled that the recurrence risk for the same mutation in a future pregnancy is very low. However, the increasing sensitivity of currently applied genomic technologies is more commonly revealing low-level mosaicism for variants in apparently healthy parents.

Methods*:* Clinical and molecular data were collected for three families in which an apparent DNV recurred. In addition, an anonymous online survey was conducted among residents and specialists in medical genetics, as well as licensed and training genetic counselors, across multiple medical centers in Israel, during April-May 2022. Respondents were asked regarding their views and perceptions of different aspects pertaining to the way in which recurrence risk of DNVs is conveyed.

Results: A total of 51 professionals responded to the questionnaire. When asked what is the recurrence risk they would convey to the parents of a child with a pathogenic DNV, 42% chose '1%', 9% chose '2-3%', 8% replied 'up to 5%' and 40% replied that the recurrence risk would depend on the genetic diagnosis. Diverse responses where also noted when presented with a scenario in which trio exome sequencing would reveal that the DNV was actually detected in a single read in one of the parents, or when asked regarding their recommended testing for DNVs in future pregnancies.

Conclusion*:* Based on these findings, we suggest that the way medical genetics professionals convey the estimated recurrence risk of a pathogenic de novo variant should be reconsidered.

Conflict of Interest: None declared.

EP23.024 Master's in Genomic Medicine framework: a multidisciplinary first in NHS postgraduate education

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Background: Health Education England (HEE) is the education and training arm of the National Health Service (NHS) in England. In 2015, HEE's Genomics Education Programme (GEP) launched the Master's in Genomic Medicine framework. This comprises a GEP-designed curriculum delivered by universities across England. NHS employees can build up from a single module to a postgraduate qualification. The framework is interprofessional by design – a first for HEE.

Methods: The framework was evaluated using a mixed-methods approach to gain data from learners and their managers, including online surveys, interviews and focus groups. The study aimed to survey opinions of the framework, assess whether it is delivering its objectives and whether further development is needed.

Results: In the period studied (2015-21), the framework funded 1,557 NHS professionals, with the largest groups being doctors (41%), healthcare scientists (30%), and nurses and midwives (12%). Among learners who responded to surveys ($n = 212$), 86% said it enhanced their practice and 64% enhanced departmental practice, with managers ($n = 55$) in agreement (69% and 58% respectively). Additionally, 82% of learners said the interprofessional approach was beneficial to their learning; many said it helped them make connections that extended into the workplace. There were also some tensions identified in the study, which informed a set of recommendations for future development.

Conclusions: Overall, the Master's framework has made a positive impact in increasing genomic knowledge of healthcare professionals in the NHS. Its interprofessional approach could offer a model for other countries' healthcare systems to consider as part of their genomics education strategy.

Conflict of Interest: Siobhan Simpson Health Education England, Kathleen Lynch Health Education England, Aine Kelly Health Education England, Michelle Bishop Wellcome Connecting Science, Karl Nightingale King's College London, Kate Tatton-Brown Health Education England; St George's University Hospitals NHS Foundation Trust, St George's, University of London.

EP23.025 Hereditary breast and ovarian cancer – genetic counseling experience report

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Background: Clinical testing for gene variants facilitates precise identification of women at increased risk of inherited breast/ovarian cancer – 5-10% of all affected. Screening and prevention strategies should be instituted for prophylaxis even in countries lacking a national healthcare program. It is important for potential high-risk patients to be able to accurately evaluate themselves and attend genetic counseling for further assessment.

Methods: We provided genetic counseling for a period between January 2021 to December 2022. The consultands were either self-referred due to an online-advertised campaign or specialist-referred. We evaluated their cases based on NCCN guidelines along with risk calculation. We also subjectively summarised breast/ovarian cancer inheritance "myths".

Results: We consulted 60 women, with 50 of them (83.3%) being self-referred. Overall assessment appeared adequate - 50% of consultands were evaluated as high-risk, with the majority being under 50y (75%) and currently unaffected (60%). However, three major misconceptions have been reappearing during counseling sessions:

- Breast/ovarian cancer predisposition cannot be paternally inherited
- Several affected relatives indicate a 100% cancer development risk for the consultand
- Affected mothers can only transmit risk to their daughters

Conclusions: The evaluated group is not representative - being young proactive women with awareness of belonging to a family with certain hereditability. Still, the present misconceptions potentiate false reassurance or excessive anxiety, further affecting proper and timely management. Improved public education regarding hereditary cancer is essential along with genetic testing and further prophylaxis. Self-education is especially invaluable where national healthcare lacks such coverage.

Conflict of Interest: None declared.

EP23.026 e-Genetic counselling in the Cypriot population. What has changed post COVID-19 pandemic?

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Background/Objectives: Genetic counselling is a communication process where individuals are referred to discuss their personal or family history of a genetic condition and help them understand their potential risks. Traditionally, genetic counselling sessions are conducted in person. The COVID-19 pandemic challenged medical genetic services and patient care. A reshape of genetic services delivery included e-genetic counselling (e-GC). The aim of the study was to assess Cypriot patients' and genetics healthcare professionals (GHCPs) experience of e-GC.

Methods: Semi-structured interviews were conducted to explore the experience of 30 patients and the clinical genetics team of Karaiskakio Foundation, regarding e-GC.

Results: All patients had an overall positive experience regarding e-GC. Both parties and especially parents of children with multiple disabilities, found it beneficial to have a first e-GC appointment prior to the physical appointment. In addition, gathering all the information made GHCPs feel better prepared for the physical appointment. GHCPs were able to accommodate a significantly increased number of patients through e-GC without compromising access to genetic testing and clinical volumes. Although, some participants had concerns regarding age, privacy, and potential technical difficulties, while others considered e-GC more convenient due to safety, reduced travel and waiting times.

Conclusion: e-GC was quickly implemented in the context of the COVID-19 pandemic and was viewed positively by patients and the GHCPs. Limited barriers were identified for practice. However, it is anticipated that in the future, genetic services will presumably adopt 'hybrid' models offering both in-person and virtual visit options.

Grant References: No grants.

Conflict of Interest: None declared.

EP23.028 Healthcare Providers Knowledge, Confidence and Practice Regarding Clinical Genomics - The Role of Clinical Informatics and Ancillary Services to Support Implementation

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Background and Purpose: We measured healthcare providers' (HCPs) subjective self-appraisal of their knowledge of clinical genetic tests and techniques, combined with an objective assessment of same. Self-confidence around ordering, interpreting and explaining clinical genetic tests was measured. We also measured HCP's attitudes and opinions regarding clinical informatics tools and ancillary supports for their genetics workflows.

Finally, we enquired as to whether HCPs viewed genetic testing as a mainly research orientated activity or as being clinically essential.

Methods: The survey was conducted via electronic means, utilising Google Forms, which was distributed to a wide variety of HCPs. 30 HCPs from a large academic teaching hospital in Ireland replied, including nurses, doctors in training, qualified consultants and general practitioners.

Results: More than half of respondents (65.6%) thought they knew less than enough about clinical genetics to do their jobs. Regarding confidence in the steps of ordering, interpreting and explaining genetics tests, only a minority were fully or somewhat confident. Most HCPs were positively predisposed to utilising clinical informatics tools, genetic counsellors, second opinions services and molecular tumour boards (MTBs) in their genetics workflows. Finally, the majority (83.9%) were supportive or strongly supportive that genetic testing was important to their clinical practice, rather than research only.

Conclusion: Despite a small subset of respondents having high self-appraisals of their clinical genetics' knowledge, when objectively measured most Irish HCPs lack knowledge and confidence in clinical genetics. This is important, given the growing prominence of clinical genetics in mainstream clinical practice, as reported by respondents.

Conflict of Interest: Stephen Kearney Full Time - Beacon Hospital, Dublin.

EP23.029 Is there an untold story of women in human genetics? An analysis of publications of female scientists

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According to the UNESCO institute for statistics, worldwide there were 29,3% of scientists were female. In the fields of science, technology, engineering, and math (STEM) women are underrepresented. There are several publications on this topic, but those are different.

This investigation is based on search in worldwide catalogues of libraries as well as internet research. The publications are articles as well as information on homepages. The period time lasts from 1900 to 1960.

The analyzes of the literature shows that several scientists were named again and again. Those are the biologist Harriet B. Creighton (1909-2004), botanist Barbara McClintock (1902-1992), mathematician Julia Bell (1879-1979), physicist Rosalind Franklin (1927-1958) and biologist Martha Chase (1927-2003). But there a lot more that are not so well-known in the public. But those are mostly in the second row like laboratories.

On the other hand, there are women like Esther Lederberg (1922-2006), who was a eminent pioneer of bacterial genetics, she discovered the lambda phage. Although she was recognized as outstanding in the laboratory, experimentally and methodologically, she struggled to get a permanent academic position. From 1942 to 1968 she was married with Joshua Lederberg (1925-2008), the Nobel laureate of 1958 together with GW Beadle and E Tatum. He mentioned her in his Nobel lecture, just one under others.

Regarding the Nobel prize, the situation is similar to those in general: less than 30% were awarded to women.

In summary one can state, women should move to the center of attention.

[Literature by the author.]

Conflict of Interest: None declared.

EP23.030 Democratizing Sickle Cell Disease Gene Therapy Knowledge: A Community Based Model for Stakeholder Engagement

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There has been a sharp increase in the number of gene therapy clinical trials for sickle cell disease (SCD). Given the anticipated availability of gene therapy in high income countries for individuals living with SCD, it is paramount that there are understandable, accurate, and actionable patient education materials (PEMs) on gene therapy.

An objective of this project was to examine the process of engaging community stakeholders to develop open-source PEMs that are not sponsored by a specific company or research group. A community-based deliberative engagement approach was used to engage patients, advocacy groups, researchers, clinicians, industry, and government stakeholders ($n = 54$) to work together purposefully and collaboratively to create five PEMs. Quantitative and qualitative survey data was collected after participation. For data analysis, we divided the group into two cohorts based on their roles: 1) Research Community (RC) and 2) Patients and Advocacy Community (PAC).

Results showed that the majority of all participants felt satisfied with their participation in the workshops (93% PAC; 95.1% RC). However, these two groups had differing views on the role of power dynamics in this process. The RC majority felt power dynamics did not influence the workshops (59.3%), versus the PAC majority felt power was an influence (55.2%). Further qualitative results confirmed this finding, revealing how belief of power differential impacted the role of patients and advocacy groups in stakeholder engagement.

We will present data on the stakeholder engagement process highlighting how this project is a model for engagement with genetic disease communities and their stakeholders.

Conflict of Interest: None declared.

EP23.031 Creation of a Turkish PTEN hamartoma tumour syndrome patient registry: ptenturkiye.org

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Introduction: 'PtenTurkiye.org' is a national, web-based registry for PTEN Hamartoma Tumour syndrome (PHTS) established in December 2022. It is designed to increase awareness, gather scientific knowledge by collaboration and increase data accessibility, collect high-quality data on the epidemiology, genetic background and natural history of PHTS especially for pediatric patients.

PTEN Hamartoma Tumour Syndrome (PHTS) is a rare disease, causing tumor predisposition syndrome in adults and macrocephaly autism syndrome in children. For children there is one guideline on follow up by Ciaccio et al (Eur J Med Genet 2019) based on 16 pediatric patients from Italy.

Materials and methods: In 2 months, we included 21 pediatric patients (10 females,11 males) aged 3 to 18 years from 4 centers in Turkey. Our cohort differs from other PTEN registries as it includes patients with Middle Eastern, Asian and Eastern European backgrounds.

Important Findings: There are 8 novel, 11 known and 2 variant of unsignificant *PTEN* variations. One 4 year old patient had solid nodule on thyroid (no biopsy done), one patient

had aort aneurysm which was not reported before in the literature, one patient also had Joubert syndrome (*AHI1* mutation) and one patient had unnecessary colonoscopy with normal result.

Conclusions: Our aim is to increase the awareness of this registry, improve the follow up guidelines for pediatric patients, find genotype-phenotype correlations in a population that was not studied before and as a result provide the best care for these families.

Grant: Boston Children's Hospital, Developmental Synopathies Consortium

Conflict of Interest: None declared.

EP23.32 Differences in the empowerment of couples who decide to terminate the pregnancy based on the communicative process in decision-making

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Objectives: Detect difficulties in decision-making in the termination of pregnancy to facilitate the decision-making of couples.

Methods: Pregnancy losses due to genetic causes or foetal malformation between 2019 and 2020 at the Parc Taulí hospital were included. Two groups confront: Group A includes pregnancy losses with a genetic diagnosis of Down syndrome and group B the rest of pregnancy losses. Group A is informed by the reference obstetrician since it is presumed that there is greater prior knowledge of the pathology, and group B was informed in a genetic counselling consultation. Of the 50 registered gestational losses, 23 couples agreed to participate. The couples participated through a 27-question form and a semi-structured interview. The form is divided into six blocks: demographic data, information, impact of the viability of the foetus, directionality in decision making, counselling consultation and psychological support. The interviews were qualitatively analysed to clarify some of the responses on the form. The forms were analysed by descriptive analysis (Fisher's exact statistical test) of independent groups and qualitative variables.

Results: In group B, 76.9% of the couples consider that they had the necessary time and sufficient information to make the decision, while 50% of group A think that it was a hasty decision. In group B, 84.6% of couples feel secure with the decision they made compared to 42.9% in group A.

Conclusion: In the decision-making process, at least one genetic counselling consultation must be included to facilitate the empowerment of couples regardless of prior knowledge of the pathology.

Conflict of Interest: Núria Capdevila FULLTIME, Neus Baena FULLTIME, Montserrat Comas FULLTIME, cristina lesmes FULLTIME, silvia pina FULLTIME, laura vilajuana FULLTIME.

EP24 Ethical, Legal and Psychosocial Aspects in Genetics

EP24.001 The right to ask, the need to answer. When patients mee t research: how to cope with time

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Background/Objective: Reaching a diagnosis and its communication are two of the most meaningful events in the physician-patient relationship. Rare diseases are a peculiar subset of conditions in which the search of a diagnosis might reveal a long and painful journey requiring, in most cases, long waiting time. For many patients, turning to research might represent their last chance to obtain an answer. Time is the worst enemy, threatening to disrupt the fragile balance among affected individuals, their referring physicians, and researchers. It is consuming at all levels, draining economic, emotional and social sources, and triggering unpredictable reactions in each stakeholders.

Methods: We performed a thorough review of the medical literature searching for draining of economical, social, and emotional sources due to diagnostic delay in RDs, and provide patients, physicians and researchers' perspectives drawing from more than 20-year experience focused on RDs.

Results: Managing with waiting time is one of the most burdensome tasks for all the parties playing a role in the search of a diagnosis. While moving towards the same goal, patients, clinicians and researchers might have different expectations and perceive the same waiting time as differently hard or tolerable. The absence of an effective communication are the most common mechanisms of the failure of the therapeutic alliance.

Conclusions: In the landscape of the modern Medicine that goes faster and claims high standards of cure, rare diseases represent an exception in which the best thing to do to care for patients is to learn to cope with time.

Conflict of Interest: None declared.

EP24.002 Genomic newborn screening in France: from a social acceptability study to a pilot project

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France is one of the European countries with the lowest number of diseases screened at birth. Several metabolic diseases, detected by biochemical techniques, have recently been included. But the development of next-generation sequencing (NGS) technologies, therapeutic progress and recent changes in bioethics laws, imply

that the scope of newborn screening (NBS) could be broadened to include these technologies. The SeDeN Project aims to assess the social acceptability (professionals, parents and decision-makers) of its extension and the specificities due to the use of NGS in first line.

85% of 1,200 health professionals are strongly in favour of screening for variants related to treatable childhood pathology. 77% of geneticists would like to integrate a limited number of genes in first line. In preliminary results of parents' study, a minority group wants to know everything to be as prepared as possible, and another group only wants to know about treatable diseases in childhood or adolescence. All agree that using NGS makes no difference if it is not more invasive for the child.

These results, the strong future cost reduction of GS, and the collaborations with international pilot initiatives allow us to prepare the PERIGENOMED Project, which aims to assess the feasibility of NBS by rapid solo-GS with targeted analysis of a list of genes responsible for treatable rare diseases of early onset, to question the relevance of its deployment on a larger scale and to analyse the associated ethical, regulatory, psychosocial and economic issues.

SeDeN's Grant: Pfizer, Fondation Maladies Rares, Sanofi, PFRSP-BFC.

Conflict of Interest: None declared.

EP24.003 Navigating the ethical, legal, and social/societal implications (ELSI) of the Recall-by-genotype (RbG) design through stakeholder engagement: the case of the Cooperative Health Research in South Tyrol (CHRIS) study on genetic risk factors of Parkinson's disease

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To leverage existing genetic and genomic data, bottom-up approaches such as Recall-by-Genotype (RbG) have been used to conduct follow-up studies in biobanks. Genetic information may be partially disclosed when certain participants are recalled for RbG studies, and information on the study design and eligibility criteria are provided. This and other peculiarities of RbG approaches have ethical, legal, social, and societal implications. We present empirical studies with various stakeholders developed to refine the CHRIS RbG policy.

The Cooperative Health Research in South Tyrol (CHRIS) and ProtectMove study used RbG approaches to investigate genetic risk factors for Parkinson's disease. CHRIS participants were recalled based on the presence of a particular genotypic variant in the Parkin gene, either genotypically inconspicuous or with a single heterozygous variant that may slightly increase the risk for some attenuated clinical symptoms. Individual carrier status was not disclosed, the disease and the variant studied were. Alongside the RbG study, we surveyed participants, and the results display heterogeneity in positions on disclosure. Further studies with larger samples are needed to clarify trends and more nuanced stakeholder views.

In focus group discussions with researchers and CHRIS study personnel, difficulties with communication strategies of RbG eligibility criteria and the family-based approach were discussed. Specifically for cases with studies about genetic variants with unclear significance. Researchers agreed on the value of tailored case-by-case solutions to ease the potential impact of partial disclosure on participants, families, and cohorts. Further research is needed to contextualize these findings into policies for other RbG studies.

Conflict of Interest: Katharina Tschigg Department of Cellular, Computational, and Integrative Biology, University of Trento, Italy Institute for Biomedicine, Eurac Research, Bolzano, Italy & Affiliated Institute of the University of Lübeck, Germany, The study was funded by the Deutsche Forschungsgemeinschaft (FOR2488) and the Department of Innovation, Research and Universities of the Province of South Tyrol., Roberta Biasiotto: None declared, Luca Consoli: None declared, Deborah Mascalzoni: None declared.

EP24.004 Correcting Our Course: Responsive Policy Initiatives to Build Equity in Genomics

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The genomics community is at a pivotal moment at the intersection of its history with its future. Globally, the field of genomics is acknowledging and reckoning with the systemically racist legacy that has plagued the field since its inception. Research organizations, government agencies, and foundations have launched antiracism programs and are evaluating their efforts. Medical and genomics professional societies are publicly apologizing for their historical roles in advancing eugenics and their silence on the matter.

Internationally, the genetics and genomics community has started to take steps to build a more diverse, inclusive, and equitable field. Engagement with low- and middle- income countries and support to improve their healthcare infrastructure and resources is becoming an important focus. Organizations are ensuring collaboration between government, academia, and industry aimed at improving global health. In the United States, the National Institutes of Health (NIH) established a programs and initiatives to address racism in biomedical research.

The session will present initiatives happening at the National Human Genome Research Institute at the NIH in the United States and will examine them in the context of cross-cutting themes emerging in international report findings. It is imperative that the genomics community pair acknowledgment of harms with effective actions that foster advancement in equity, inclusion, and diversity in genomics. We recognize that these efforts require an international level of engagement that extends across borders. Given both the historical context and fastmoving pace of genomic science, our field must lead by example in holding itself accountable.

Conflict of Interest: None declared.

EP24.005 Narrating ataxia: psychosocial impact of an online genetic counselling narrative group intervention for persons with hereditary ataxia

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Persons with hereditary ataxia commonly show high levels of psychological distress, yet support interventions are lacking. Thus, a short-term narrative-based group intervention was adapted and delivered remotely. This study aimed to explore and evaluate the intervention's impact on participants' illness narratives and psychological wellbeing.

A multiple, mixed-method case study was conducted with six participants (three women and three men, age range 39-53). Measures included expressive writing, GAD-7, PHQ-9 (taken at baseline, post-intervention, and follow-up), expressive writing questionnaire and a focus group (at follow-up). Data analysis included statistical analysis of scores' differences and thematic analysis.

Results showed no significant differences in anxious (GAD-7:$\chi2(2) = 4000$, $p = 0.135$) and depressive (PHQ-9:$\chi2(2) = 4364$, $p = 0.113$) symptoms over time. However, symptoms decreased from clinical (pre-intervention) to subclinical scores (post-intervention), with an increase at follow-up. Analysis of expressive writing showed illness-related traumatic and positive experiences including intergenerational trauma, impacts of ataxia at physical, psychological, and social levels, as well as the relevance of social support and a positive outlook on life. All participants reported satisfaction with the intervention, enhanced understanding of their experiences and emotions, and most felt better prepared to cope with similar experiences.

Participants described the intervention as having a positive impact on perceived coping, resilience, self-efficacy, and emotional processing. The intervention impacted participants' well-being positively, promoting empowerment and awareness of one's resources. High satisfaction and adherence to the intervention suggests that it was appropriate for this population and shows potential for inclusion in genetic counselling protocols.

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EP24.006 Assessing how regulations shape the accuracy of clinical genomics tools for diverse populations

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Background/objectives: Emerging clinical genomics tools—which combine the latest sequencing technologies with complex analytical algorithms— have a variety of promising applications from diagnosing rare diseases to generating genetic risk profiles for individual patients. However, there are concerns regarding the accuracy of some clinical genomics tools for racially and ethnically diverse sub-groups because of a lack of diversity in the genomic data used to develop them. Here we investigate how regulations in Europe and the United States (US) address diversity problems in clinical genomics tools.

Methods: We reviewed the relevant regulatory guidance on medical devices and clinical software to determine the performance requirements for clinical genomics tools that relate to data diversity or representativeness. We then considered these requirements in relation to the structural and legal obstacles to sourcing data needed to ensure the accuracy of devices for diverse populations.

Results: Current regulatory requirements for medical devices and clinical software are targeted toward minimizing potential biases in output, but they are agnostic to many of the obstacles to sourcing diverse or representative data. Some challenges include reconciling scientific and social categories, creating proper metrics for representativeness or diversity, and overall data quality. In addition, the transparency and anti-discrimination measures found under recently proposed regulations for clinical algorithms are not necessarily crafted with regard for these challenges either.

Conclusion: Without clearer or at least widely agreed upon standards for diverse data, many laws may serve to inhibit equitable progress in clinical genomics rather than promote it.

References:

Grants: FWO Flanders-Quebec grant.

Conflict of Interest: None declared.

EP25 GWAS

EP25.001 IndCovid project phase I identifies the genetic variants associated with Long COVID among Indians

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Background/Objectives: The coronavirus disease (COVID-19) is characterized by a wide spectrum of clinical phenotypes ranging from asymptomatic to severe disease presentation. Additionally, many suffer from Long-COVID. Genome-wide studies revealed that the host genetic variability can play important role in modulating the clinical outcomes of COVID-19. In the IndCovid Project we aim to identify the genetic variants, associated with the severity of COVID-19 and post COVID complexities in the Indian context.

Methods: In this study, we utilize the genome-wide data of diverse Indian samples with history of COVID-19, genotyped on Illumina Infinium Global Screening Array v3.0. We assess three different case-control models, wherein *cohort 1* compares the genomes of asymptomatic and mildly symptomatic COVID-19 patients (controls) against individuals suffered through severe COVID-19 (cases), *cohort 2* compares non-hospitalized (controls) and hospitalized (cases) individuals, and *cohort 3* compares individuals with post-COVID complexities (cases) versus individuals without any perceivable symptoms of Long COVID (controls).

Results: The Phase I results indicate that the majority of SNPs that show frequency variation between the cases and controls are involved in neurological and olfactory pathways. Among the three GWAS models, the Long COVID model (*cohort 3*) identified the highest number of significant SNPs and revealed striking genomic distinctness between individuals experiencing Long COVID versus who are not.

Conclusions: Our study is one of the first to uncover novel genetic variants associated with the severity of COVID-19 using Indian patient genomic information. This study can further

facilitate the development of personalized vaccines and therapeutics.

Conflict of Interest: Ranajit Das Full, University intra-mural support, pooja umesh shenoy Full Time, University Intramural support, hrushikesh udupa Full Time, University Intramural support.

EP25.002 Going deep into the identification of new genetic modifiers in ATTRV30M amyloidosis

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Background/Objectives: Transthyretin (ATTR) amyloidosis is a severe, fatal, autosomal dominant disease that is clinically heterogeneous. It is caused by variants in the *TTR* gene that lead to extracellular deposition of amyloid fibrils in multiple organs. *TTR* Val30Met (ATTRV30M) is the most common pathogenic variant in Portugal and worldwide and ATTRV30M amyloidosis can affect the peripheral and autonomic nervous systems, heart, kidney, gastrointestinal tract, and eyes. However, phenotype, severity and age-at-onset are highly variable among patients. Our aim is to study genetic variants that might shed light on disease mechanisms and variability of patients carrying ATTRV30M, using a genome wide association study (GWAS).

Methods: We gathered DNA samples from 96 patients with ATTRV30M amyloidosis from the Unidade Corino de Andrade registry and 187 control samples. All samples were ATTRV30M sequenced to confirm case/control status. We screened over 850,000 GWAS markers using the Precision Medicine Diversity Array and analysed the data using Axiom Analysis Suite. Plink was used to perform an association test and, subsequently, a Manhattan plot was assembled using R software to represent the significant genes between cases and controls.

Results: We found 10 variants significantly associated with ATTRV30M amyloidosis whose genes belong to calcium signaling pathways or are implicated in cardiomyopathies and muscular dystrophies.

Conclusion: We uncovered new genetic modifiers associated with ATTRV30M amyloidosis and intend to bioinformatically assess them to predict their potential deleteriousness and select the most promising to study functionally.

Grant References: WI216825 2016 Global ASPIRE TTR Amyloidosis; FCT 2022.01656.PTDC; EC: i3S/16080709/2022.

Conflict of Interest: None declared.

EP25.003 Pathway enrichment analysis of post GWAS data identifies metastasis-relevant genes in sporadic colorectal carcinomas

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Background/Objectives: Colorectal cancer (CRC) is one of the most lethal malignancies worldwide attributable to metastasis to distant organs likely due to genetic predisposition and notoriously challenging to treat. Early diagnosis and further understanding of the biological mechanisms are critical in enhancing prognostication and long-term survivorship.

Methods: We performed case-case whole genome genotyping using the Affymetrix SNP6 human-genechip array on 2,833 CRC patients with clinically defined metastasis status and cllinicopathological features. Patients were sub-categorized into different subgroups such as tumor staging, metastasis site and gender. Correlation trend test was performed on each subgroup independently based on metastasis status with Golden Helix-SVS software. We performed pathway analysis using summarized SNP data with Functional Mapping and Annotation (FUMA) software.

Results: FUMA identifies functional genes that are distinctly different between stage I/II and stage III tumors. Genes enriched in stage I/II tumors are involved in DNA binding and transcription factor activities. These genes are master regulators of the epithelial-mesenchymal transition (EMT) pathways. Genes enriched in Stage III tumors are associated with ephrin receptor signaling. Molecular function identified from combining all stages are relevant to lipases and esterases catalysis suggesting that dysregulation of lipid metabolism is important in metastasis. These results confirmed that CRC is heterogenous and suggest that patients with different staging should be examined independently.

Conclusions: Identifying disease-relevant genes and pathways is critical for understanding the biological mechanisms of metastatic CRC to improve risk assessment and identify novel therapeutic targets to improve clinical management.

Grant References: Singapore National Medical Research Council grant (NMRC/OFIRG/0004/2016).

Conflict of Interest: None declared.

EP25.004 The role of lifestyle and genetic factors in cardiovascular comorbidity in mental disorders

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Patients with mental disorders have higher mortality and somatic comorbidity than the general population. Cardiovascular diseases represent one of the major comorbidities in mental illnesses. In this study, we aim to gain insight into underlying mechanisms of cardiovascular comorbidity in mental disorders. Phenotypic and genetic data in the population based Tromsø Study will be incorporated with the disease phenotypes and medication-use data that are collected from electronic health records. We will perform genome-wide association analyses to determine genetic heritability and susceptibility of the major mental illnesses and cardiovascular diseases. Meta-analysis of our dataset and summary statistics from other large-scale studies will be conducted to reveal genetic effects across datasets. Shared genetic architecture of the mental disorders, cardiovascular diseases, and lifestyle factors, e.g., tobacco use, will be determined to explore common candidate risk variants. Post-genome-wide association study analyses will inform functional mapping and annotations of the genetic associations. Polygenic risk scores and lifestyle factors will be utilized to construct trajectorial analysis of disease causation and to predict disease outcome in the population.

Funding: Northern Norway Regional Health Authority.
Conflict of Interest: None declared.

EP25.005 Exome sequencing association analysis replicated the role of rare variants of SLC13A1 in back pain

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Background: Back pain is the leading cause of disability-related years lived worldwide. Its heritability has been estimated at 40–60%, with half attributed to common genetic variants. Rare and ultra-rare variants are expected to provide additional explanations for missing heritability. In this study, we estimated for the first time the impact of rare and ultra-rare variants on chronic back pain (CBP).

Methods: CBP was defined as back pain lasting more than 3 months. Using UK Biobank exome sequencing data (N = 179,000), we performed a gene-based association study including only variants with MAF ≤ 0.01. Ultra-rare variants with MAC ≤ 10 were collapsed and considered as a single variant, which was then used together with all other variants for gene-based association analysis. Four sets of within-gene variants with specific protein-coding properties were used in the analysis.

Results: We detected a significant association between CBP and *SLC13A1* (p-value = 2.50×10^{-6}). The signal was obtained for LoF +missense variants. A rare LoF variant of *SLC13A1* has recently been identified as being responsible for another back pain trait, intervertebral disc disorder [Bjornsdottir et al., 2022].

Conclusion: We replicated the association between back pain and *SLC13A1* and showed that the gene effect on back pain can be explained by both LoF and rare missense variants.

Grant References: The study was conducted using the UK Biobank resource. The work was supported by the budget project of the Institute of Cytology and Genetics FWNR-2022-0020, Russian Science Foundation (RSF) No.22-15-20037 and Government of the Novosibirsk region, RSF No.23-25-00209.

Conflict of Interest: None declared.

EP25.006 Genome-wide linkage and association study identifies novel genes and pathways implicated in Polycystic ovarian syndrome

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Objective: Polycystic ovarian syndrome (PCOS) is a complex heterogeneous condition affecting women of reproductive age, conferring increased cardiovascular morbidity and mortality. The syndrome is characterized by oligomenorrhea, hyperandrogenism, and/or polycystic ovaries and is often associated with obesity and type 2 diabetes. PCOS is predisposed by environmental factors and risk variants in genes mostly involved in ovarian steroidogenesis and/or insulin resistance. Genetic risk factors have been identified by both familial and genome-wide (GW) association studies. However, most genetic components are still unknown and missing heritability needs to be elucidated. To fill the gap in knowledge about the genetic determinants of PCOS, we performed a GW study in genetically highly homogeneous peninsular families.

Patients and Methods: We conducted the first GW-linkage and linkage disequilibrium (i.e., linkage + association) study in Italian families with PCOS.

Results: We identified several novel risk variants, genes, and pathways potentially implicated in the pathogenesis of PCOS.

Conclusions: This is the first GW-linkage and linkage disequilibrium study performed in peninsular Italian families and reporting novel genes in PCOS.

Conflict of Interest: None declared.

EP25.007 The risk of death conferred by aquaporin 3 genotype is modulated by plasma osmolality in hospitalized COVID-19 patients

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Severe corona virus infectious disease 2019 (COVID-19) has been associated with dehydration, and early dehydration has been proposed as a mechanism of more severe disease. Recently the COVID-19 Host Genetics Initiative (https://www.covid19hg.org) release data freeze 7 that included 49,033 COVID-19 cases and 3,393,109 controls and identified a genome wide significant variant in the water channel aquaporin-3 (AQP3) promotor (rs60840586, Odds Ratio: 1.068, P = $2.5*10^{-9}$). The variant is an expression Quantitative Trait Locus (eQTL) for AQP3 (NES: 0.507, P = $2.9*10^{-9}$ in GTEx). We used 1073 patients from Biobanque Qubecoise de la COVID-19 (BQC19) to investigate the relationship between genotype and dehydration estimated using plasma osmolality (eOSM = [$2Na^+ + 2K^+$ + Urea + Glucose]). The allelic dose of AQP3 SNP rs60840586:G was calculated as: GTAAC:GTAAC = 0, GTAAC:G = 1, G:G = 2. rs60840586:G was not associated with maximal estimated osmolality (P = 0.12) using linear regression, nor mortality (OR = 0.61 [95% CI = 0.3-1.12], P = 0.13) in a multivariable logistic regression adjusting for sex and age, hospital and the top ten principal components with an interaction term rs60840586*eOSM. Interestingly, the interaction term identifies a strong effect on mortality in dehydrated patients (OR: 1.56, P = 0.00935) but shows no effect in normoosmotic indicating that AQP3 expression may be important for the cellular compensatory response to dehydration in COVID-19. In conclusion, dehydration is strongly associated with mortality in COVID-19, and the relationship is exacerbated by a genetically determined variation of AQP3 expression.

Conflict of Interest: Michael Hultstrom: None declared, Hugo Zeberg: None declared, Brent Richards Founder and CEO of 5 Prime Sciences., Advisor to GlaxoSmithKline, and Deerfield Capital.

EP25.008 Bidirectional Mendelian randomization analysis of back pain and 22 associated factors

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Background/Objectives: Epidemiological observations established associations between back pain (BP) and multiple factors. However, it is unknown if these associations are causal. To address this issue, we performed a bidirectional Mendelian randomization (MR) study to examine causal relationships between BP and 22 putative risk factors.

Methods: We utilized public data for BP and 22 risk factors from the largest GWAS performed in Europeans to conduct MR analysis using inverse-variance weighted meta-analysis (IVW), Causal Analysis Using Summary Effect (CAUSE) and sensitivity analyses. We considered the findings concordant with a hypothesis of causality if results of IVW or CAUSE were statistically significant ($p < 0.0017$), and the effect was consistent between all analyses.

Results: We found statistically significant causal relationships between education (OR = 0.54 [0.51,0.58] per ~4 years of schooling), smoking (OR = 1.20 [1.15,1.26]), alcohol consumption (OR = 1.28 [1.19,1.39]), sleep duration (OR = 0.75 [0.63,0.89]), depression (OR = 1.37 [1.25,1.50]), diastolic (OR = 1.10 [1.04,1.17]) and systolic blood pressure (OR = 1.09 [1.04,1.15]), neuroticism (OR = 1.51 [1.37,1.67]), BMI (OR = 1.14 [1.05,1.23]) and BP risk. We also observed a significant causal influence of BP on depression (OR = 1.28 [1.12,1.47]), neuroticism (beta MR = 0.12) and type 2 diabetes (OR = 1.40 [1.13,1.73]).

Conclusion: We clarified causal relationships between BP and associated factors. Fewer years of schooling, smoking, increased alcohol intake, less sleep, higher blood pressure and greater BMI increase BP risk. Conversely, BP causes type 2 diabetes, depression and neuroticism. These findings may facilitate BP management improvement.

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EP25.009 The impact of human genetics on Mycobacterium tuberculosis intra-host evolution

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Background/Objectives: Tuberculosis (TB) is an infectious disease that is predominantly caused by the *Mycobacterium tuberculosis* (M.tb) bacteria. The outcome of *M.tb* infection is highly variable, driven by both human and bacterial genetic factors in addition to risk factors such as HIV co-infection, alcohol abuse, and malnutrition. In this study, paired human and bacterial genomes from 1000 Tanzanian patients were leveraged to identify the impact of human genetics on *M.tb* intra-host evolution.

Methods: Bacterial isolates were whole-genome sequenced and human blood samples were either genotyped and/or whole-genome sequenced. We used a genome-to-genome approach to test for associations between human and bacterial genetic variants. Specifically, we conducted a case-control genome-wide-association study (GWAS) for each of 519 *M.tb* amino acid variants present in at least 15 samples. To correct for multiple testing, a modified GWAS threshold of 9.6e-11 was applied.

Results: We identified two significant associations. The first one between *M.tb* variant Rv2348c I101M and human variant rs12151990 ($p = 4.7e\text{-}11$, OR = 5.6); rs12151990 is an intronic variant in *PRDM15*, a gene involved in apoptosis regulation and *M.tb* clearance. The second one between *M.tb* variant fixA T67M and human variant rs75769176 ($p = 6.3e\text{-}11$, OR = 6.7); rs75769176 maps to an intergenic region close to *FBXO15* and *TIMM21*.

Conclusion: The results suggest that specific *M.tb* variants may be selected in response to host genetic pressure. Our genome-to-genome analysis highlights host-pathogen interaction loci that might play a role in pathogenicity.

Grant References: Swiss National Science Foundation #177163.

Conflict of Interest: None declared.

EP25.012 Biobanking as a tool for genomic research: applications for identification of robust genetic associations for complex traits

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Background/Objectives: Over the recent years, considerable efforts have been made in biobanking, which boosts the progress of medical genomics, from providing reference allele frequency data to enabling large-scale cross-ancestry meta-analyses. Application of biobank data for the analysis of genetic associations is useful for identification of robust genetic associations for complex traits.

Methods: We have reviewed potential benefits and challenges of biobank and trans-biobank data application in medical genetics

and genomics. Furthermore, we used genome-wide association statistics from UK Biobank and FinnGen to identify loci that are reproducibly associated with such pregnancy complications as pregnancy hypertension (HP), gestational diabetes (GD), and preterm birth (PTB).

Results: Trans-biobank integration empowers genome-wide association studies (GWAS) of individual and multiple traits, helps to explain the common genetic architecture of diseases and discover main biomarkers. We highlight that different sample handling and phenotype encoding procedures, custom data preprocessing pipelines could affect the meta-analysis results. Despite these challenges, our analysis of GWAS summary statistics for 24 pregnancy complications allowed us to identify 6 loci associated with HP, GD, and PTB in the UKB + FG meta-analysis. We also managed to replicate 14 out of 40 known genetic markers of pregnancy pathologies.

Conclusion: Overall, our study suggests that the enlargement of biobanks collection with high-throughput data and further trans-biobanks integration with consideration of aforementioned caveats provide ample opportunities for the investigation and implementation of the results into clinical practice.

Grant References: This work was supported by grant 075-15-2021-1058 from the Ministry of Science and Higher Education of Russia.

Conflict of Interest: None declared.

EP25.013 Frequency of factor V Leiden mutation and prothrombin 20210 G/A mutation in patients with venous thromboembolism from west Ukraine

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Venous thromboembolism (VTE), including pulmonary thromboembolism (PTE) and deep venous thrombosis (DVT), has become a global health problem. VTE is a multifactorial disorder, resulting from the complex interaction between genetic and environmental factors. Factor V Leiden mutation (FV1691G/A,) and prothrombin 20210 G/A mutation (FII 20210 G/A) is the most well-known genetic factors associated with VTE. The aim of this study was to determine the frequency of FV1691G/A and FII20210 G/A mutations in patients with VTE.

Our study included 255 patients with VTE (155 male and 100 female) with deep-vein thrombosis (212 persons) and pulmonary embolism (43 persons). Median age was 37.5 years for patients and 40 years for controls. Polymerase chain reaction (PCR-RFLP) was used to determine the distributions of the factor V Leiden and prothrombin FII20210 G/A mutations.

The frequency of FV1691G/A mutation was 26% in VTE group compare to 3% in control group. FII 20210 G/A mutation was detected in 9% of VTE group and 1.6% in control group. We found 5 cases of VTE group were homozygous for Leiden mutation and 5 cases were carriers for FV1691G/A and FII 20210 G/A mutations (2%). We found that factor V Leiden mutation was associated with a 12-fold (95% CI = 5.02-27.9, OR = 11.88, $p < 0.001$) and FII 20210 GA mutation with 6-fold (95% CI = 1.9-21.7, OR = 6.4, $p < 0.001$) increased risk of VTE.

It was found a statistically significant higher frequency of FV1691G/A and FII20210 G/A mutations in patients with VTE. We have reported that both mutations are risk factors for VTE.

Conflict of Interest: None declared.

EP25.014 Deciphering genetic aetiology of short uterine cervix during pregnancy: a replication study

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Background: Pathologic shortening of the uterine cervix is one of the common pathways to preterm birth associated with its own complex aetiology. Previously our group demonstrated that the development of cervical insufficiency is influenced by the variants in genes involved in extracellular matrix (ECM) production, thus proposing an idea of cervical shortening as a subtle form of collagenopathy.

Methods: We developed assessment form (which includes Brighton-Beighton criteria) to identify subtle connective tissue-related phenotypes including obstetric outcomes. We sequenced exomes of 93 women with cervical shortening during pregnancy and performed gene pathway enrichment, rare variant analysis, and genotype-phenotype correlation analysis.

Results: We were able to replicate the finding of increased rare deleterious variant burden in ECM-associated pathways (ECM organization, ECM-receptor interaction, integrins, laminin interactions, collagen formation, as well as identified previously unnoticed pathways of RAC1/RHOA GTPase cycles known to control cytoskeleton organization, adhesion, and cell immune responses).

Fifty rare variants of unknown significance and likely pathogenic variants in cervical insufficiency-associated and connective tissue disorder-related genes (including *PLOD1*, *COL1A1*, *COL6A1*, and *B4GALT7* causing Ehlers-Danlos syndrome) were classified as potential to increase susceptibility to the development of cervical shortening in 40 patients. Collagenopathy assessment score reached the threshold indicating subtle collagenopathy in 18.6% of patients. No correlation between gene variants and the expressivity of collagenopathy features across the patients was found.

Conclusions: The discovery of a link between genetically determined collagenopathy and shortening of the cervix may open up new opportunities for early diagnosis of this condition and the prevention of preterm birth.

Conflict of Interest: None declared.

EP25.015 Multi-trait genome-wide association study in 34,394 Chinese women reveals the genetic architecture of plasma metabolites during pregnancy

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Metabolite levels during pregnancy are indicative of maternal health and have been associated with adverse birth outcomes. While most of the genome-wide association studies (GWAS) conducted to date have focused on ordinary European populations, the genetic basis of maternal metabolites during pregnancy remains largely unexplored. To address this knowledge gap, we leveraged the genetic information obtained from non-invasive prenatal testing and conducted a genetic analysis of 84 metabolites, including amino acids, vitamins, trace elements, and hormones, among 34,394 pregnant women in China. Of these metabolites, 52 had not been studied in East Asians before, and

we identified 53 independent signals, including 26 novel ones, associated with the metabolites. Our study revealed that genetic effects on metabolites during pregnancy may differ from those during non-pregnancy periods. We also observed pervasive pleiotropic effects, with 43.6% of significant genetic loci for one metabolite influencing multiple other metabolites. Using Mendelian randomization, we established a causal relationship between maternal metabolic levels and complex diseases, including ischemic stroke, Graves' disease, open-angle glaucoma, fractional shortening, and rheumatoid arthritis, in the East Asian population. This study represents the largest scale metabolite GWAS among the East Asian population. Our findings offer valuable genetic insights into human metabolism and support the development of improved clinical trials and public health strategies.

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EP25.016 Genetic-by-age interaction analyses in UK Biobank and their potential to identify genetic effects on longitudinal biomarker change

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GWAS have identified thousands of loci for disease-related biomarkers in cross-sectional data. However, identifying genetic effects on longitudinal biomarker change has been hampered by relatively small sample sizes for longitudinal measurements. Such effects, indicating genetic susceptibility to disease progression, can be highly clinically relevant. Under the assumption of no cohort effect, we hypothesize that observed genetic-by-age interaction in cross-sectional data can be indicative of a genetic effect on longitudinal change that would be observed if the same individuals attended multiple visits.

We thus conducted genome-wide genetic-by-age interaction analyses in UK Biobank (European ancestry, n~370,000, excluding individuals with multiple visits) for eight biomarkers: body mass index (BMI), estimated glomerular filtration rate (eGFR), HDL-cholesterol, LDL-cholesterol, triglycerides, systolic and diastolic blood pressure, and pulse pressure. We identified 79 significant genetic-by-age interaction loci ($P_{GxAge}<5\times10^{-8}$; or by a 2-step approach incorporating marginal effects) including 48 for pulse pressure, 9 for BMI and 8 for eGFR. We followed these loci in independent individuals (with longitudinal measurements available, n~52,000 in UK Biobank, n~340,000 for eGFR in CKDGen) and observed: (i) significant effects on BMI-change (near *APOE*, *PVRL2* and *TMEM18*), eGFR-change (near *PDILT* and *FGF5*) and pulse pressure change (near *FBN1*; all at trait-level Bonferroni-corrected significance; all missed by a genome-wide screen on longitudinal change in UK Biobank); (ii) enrichment of nominally significant and directionally consistent effects on BMI-change, eGFR-change and pulse pressure change ($P_{enrich}<0.05/8$).

In conclusion, cross-sectional genetic-by-age interaction can help pinpoint longitudinal effects, when the cross-sectional sample size and thus power outnumbers the longitudinal sample size.

Conflict of Interest: None declared.

EP25.017 Dissecting etiology of clinical features in multiple sclerosis patients using a genome wide approach

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Background/Objectives: Multiple Sclerosis (MS) is a complex autoimmune disease of the central nervous system. Over 230 independent loci have been associated with MS susceptibility. Conversely, the influence of genetic variants in modifying the various clinical features has not been explored deeply. To fill this gap, we studied the association of genetic variants with two clinical features (relapses rate and sequelae after the first relapse).

Methods: We performed the first genome-wide association study (GWAS) with the presence of sequelae and analyzed 10 SNPs associated with relapse rate in a recent study (Vandebergh 2021).

Results: One variant (rs79719335 in DMXL2 gene) shows a significant association ($p = 0.034$, Wilcoxon test) with relapse rate: 80% of carriers of the rare allele show a shorter time to second relapse compared to non-carriers ($p = 0.003$ log rank test). The GWAS identified 4 SNPs that show suggestive association ($p < 1*10\text{-}5$) with the sequela event. Interestingly, two of these SNPs map in the intron of SORCS2 gene, encoding a receptor for the precursor forms of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). The replication of these results is ongoing on 850 MS patients.

Conclusions: The replication of the association with the relapse rate in our population strengths the role of DMXL2 locus with this clinical feature. The preliminary results on the sequelae highlight to an interesting biological path for these events, as they are a consequence of an impaired regenerative process of myelin damaging during the relapse. These results could have an important translational consequence.

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Conflict of Interest: None declared.