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P3-33

Missense variants in *MAGEL2* as a potential cause of Schaaf-Yang syndrome related phenotypes

S. Unser[1], F. Marbach[1], P. Caro[1], C.P. Schaaf[1]

[1]*Institut für Humangenetik, Heidelberg, Deutschland*

Introduction: *MAGEL2* is a maternally imprinted gene in the Prader-Willi syndrome locus on chromosome 15. Pathogenic variants of the non-silenced paternal allele are associated with the neurodevelopmental disorder Schaaf-Yang syndrome (SYS). Practically all SYS patients carry loss-of-function variants of *MAGEL2*, and it is currently unknown whether missense variants in *MAGEL2* can cause a recognizable human phenotype (only one individual with a potentially pathogenic *MAGEL2* missense variant has been previously described in the literature). In order to address this question, our study aims to identify further individuals with missense variants in *MAGEL2* and SYS-like phenotypes.

Methods: We gathered a database of 1499 individuals with 327 distinct missense and in-frame indel variants in *MAGEL2* from exome sequencing data (Baylor College of Medicine, Houston, USA; GeneDX, Maryland, USA) and personal communications. We selected candidates for further investigation based on allele frequency, inheritance pattern, clinical information, and *in silico* predictions. The candidates' families and/or physicians were contacted and those willing to participate received a clinical questionnaire. Additional investigations included a structured family history, as well as collection and analysis of DNA samples.

Results: We identified 15 patients from 11 families with missense variants in *MAGEL2*, including a family with four and another family with two affected siblings. *De novo* variants are present in one patient and paternal variants in twelve patients, with two patients undetermined. The patients' symptoms largely agreed with the established phenotype of SYS. However, growth retardation, motor and speech delay as well as manifestations of neonatal hypotonia were overall less frequent compared to the classic SYS phenotype, and the typical feature of contractures at birth was almost absent. The identified variants do not cluster in particular domains along *MAGEL2*, arguing against a singular molecular cause of pathogenicity.

Conclusion: We present a set of patients with SYS-like phenotypes and missense variants in *MAGEL2*. We suspect that at least some of the described variants are in fact pathogenic, and propose that missense variants in *MAGEL2* are a rare cause of SYS-like phenotypes.

Abstract ID: 10

Mono- and biallelic variant effects on disease at biobank scale

H. Heyne[1,2,3,4], J. Karjalainen[2,3], K. Karczewski[3], S. Lemmelä[2], W. Zhou[3], A. Havulinna[2], M. Kurki[2,3], H. Rehm[3], A. Palotie[2,3], M. Daly[2,3], FinnGen

[1]Digital Health Center, Hasso Plattner Institut, Universitaet Potsdam, Personalized Medicine, Potsdam, Deutschland, [2]Finnish Institute for Molecular Medicine (FIMM), University of Helsinki, Helsinki, Finnland, [3]Broad Institute of Harvard and MIT, Cambridge, Vereinigte Staaten, [4]Hasso Plattner Institute Mount Sinai School of Medicine, New York, Vereinigte Staaten

Introduction: Identifying causal factors for Mendelian and common diseases is an ongoing challenge in medical genetics. Population bottleneck events, such as those that occurred in the history of the Finnish population, enrich some homozygous variants to higher frequencies, which facilitates the identification of variants that cause diseases with recessive inheritance.
Methods: Here we examine the homozygous and heterozygous effects of 44,370 coding variants on 2,444 disease phenotypes using data from the nationwide electronic health records of 176,899 Finnish individuals.
Results: We find associations for homozygous genotypes across a broad spectrum of phenotypes, including known associations with retinal dystrophy and novel associations with adult-onset cataract and female infertility. Of the recessive disease associations that we identify, 13 out of 20 would have been missed by the additive model that is typically used in genome-wide association studies. We use these results to find many known Mendelian variants whose inheritance cannot be adequately described by a conventional definition of dominant or recessive. In particular, we find variants that are known to cause diseases with recessive inheritance with significant heterozygous phenotypic effects. Similarly, we find presumed benign variants with disease effects.
Conclusion: Our results show how biobanks, particularly in founder populations, can broaden our understanding of complex dosage effects of Mendelian variants on disease.

Abstract ID: 13

P5-1

Mosaic chromosomal alterations in non-neoplastic cells of children with Burkitt lymphoma

A. Fischer[1], S. Glaser[1], M. Hummel[2], M. Schlesner[3], S.M. Mbulaiteye[4], R. Siebert[1]

[1]Institut für Humangenetik, Universitätsklinikum Ulm, Ulm, Deutschland, [2]Institute of Pathology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Deutschland, [3]Biomedical Informatics, Data Mining and Data Analytics, University of Augsburg, Augsburg, Deutschland, [4]Division of Cancer Epidemiology & Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Vereinigte Staaten

Burkitt lymphoma (BL) is the most common pediatric cancer in Sub-Saharan Africa (SSA), particularly in countries where *P falciparum* malaria occurs year-round and Epstein-Barr virus (EBV) infection occurs during infancy. We have recently observed a significantly increased burden of clonal mosaic chromosomal alterations (mCAs) >2 Mb in peripheral blood of 931 BL cases as comparted to 3,822 BL-free control children in 4 SSA countries using genotyping array data (Zhou et al., submitted). As these findings suggest a form of chromosomal instability in normal cells from patients with endemic BL, we here sought to analyze whether mCAs can also be detected in lymphoblastic cell lines (LCLs) of patients with BL. In particular, we studied paired LCL and lymphoma cell lines from two well-characterized patients with BL.
We used genome-wide single nucleotide polymorphism (SNP) array and whole-genome sequencing (WGS) from paired BL- and LCL cell lines whose relatedness was confirmed using DNA fingerprinting. Resulting copy number changes were validated using FISH.
While no mCAs were found in the IARC-304/BL-2 pair, we detected one mCA in the WW1-LCL/BL cell line. For the WW1 cell lines, the published t(8;14)/*IGH*::*MYC* fusion was verified in the analyzed WW1-BL and confirmed to be detected only in the tumor cell line but not in the corresponding normal WW1-LCLs. Copy-number changes comprising gains in 3q (harboring the *BCL6* locus), 8p, 14q and losses of 8q, 16p and 20q were identified in the WW1-BL cell line. Of these, a gain in 3q was also identified in two WW1-LCLs. The 3q gain identified in the tumor cell line was not identical to the gain identified in the LCLs indicating that the gains originated independently in different cells. Additionally, the WGS data revealed a uniparental disomy (UPD) of the q arm of chromosome 16 in both the WW1-BL and the LCLs. Interestingly, the tumor sample has lost the other parental allele than the normal sample. This pattern was confirmed by DNA fingerprinting with markers for chromosome 16.
Our findings suggest that unique mCAs can be detected in some EBV-transformed LCLs of patients with BL, including those shared with tumor cell lines and those unique to the LCLs. These results highlight the potential for some well-characterized paired LCL/BL cell lines to serve as models to investigate and elucidate the contribution of pre-BL mCAs to the pathogenesis of eBL.

Abstract ID: 14

P3-4

Parallel deletion and duplication at 7q11.23 in a silent carrier for two reciprocal syndromic disorders

J.L. Lühmann[1], G. Schmidt[1], B. Auber[1], A.K. Bergmann[1], O. Brandau[2], A. Louis[3], S. Hentze[3], B. Schlegelberger[1], R. Kläs[2], D. Steinemann[1]

[1]Hannover Medical School, Department of Human Genetics, Hannover, Deutschland, [2]SYNLAB MVZ Humangenetik GmbH, Mannheim, Deutschland, [3]Labor für Humangenetische Diagnostik, Mannheim, Deutschland

The chromosome 7q11.23 region is susceptible to chromosomal rearrangements, due to flanking segmental duplications (SD) and long repetitive DNA segments. Deletions at 7q11.23 are causative for Williams-Beuren syndrome (WBS), whereas duplication of this region leads to the 7q11.23 duplication syndrome. Both syndromes occur mostly *de novo* with a frequency of 1:10,000-20,000. Depending on gene dosage effects, these disorders display similar and syndrome-specific phenotypic features. Both disorders are inherited in an autosomal-dominant manner with full penetrance. The diagnostic standard is fluorescence *in situ* hybridization (FISH) with probes targeting the *ELN* locus. We identified a family with two children diagnosed with WBS, while both parents were clinically unaffected.

For further characterization of the father, intensive genetic testing using inter- and metaphase FISH, short-read whole genome sequencing (WGS) and optical genome mapping (OGM) were performed.

Metaphase FISH showed one single *ELN*-signal on one chromosome 7 and no signal on the other chromosome 7 in the non-syndromic father. In the interphase FISH two signals in very close proximity were observed. Using OGM a 1.5 Mb deletion in 7q11.23 on one allele as well as a corresponding duplication of 1.8 Mb on the other allele were identified. Since the breakpoints of these copy number variants were located within the SDs flanking the region, their exact positions could not be determined. However, manual assessment allowed to narrow down the breakpoints to an 80 kb window. DNA molecules of >400 kb spanning the breakpoints unambiguously confirmed the presence of these variants. WGS data did not show a breakpoint-spanning read, but, the presence of heterozygous SNPs gave no indication for a deletion in the region, confirming the presence of at least two alleles. The deletion and duplication were finally validated with a three-color FISH using custom probes. Taken together, the data revealed that the father carried a 1.5 Mb deletion on one allele and a reciprocal 1.8 Mb duplication on the other allele.

The unsuspicious phenotype in the father is very likely due to a balancing of the two alterations to a normal copy number. This result has a huge implication regarding further family planning and genetic counselling, since the risk to transmit a disease allele is 100%. This is the first case reported where the phenotype of a microdeletion/-duplication syndrome was rescued by its reciprocal partner.

Abstract ID: 16

Single-nucleus RNA sequencing in a fetal lamb model of hypoplastic left heart syndrome

M. Reuter[1], D. Sokolowski[1], J. Diaz-Mejia[2], J. Keunen[1], B. de Vrijer[3], C. Chan[1], L. Wang[1], G. Ryan[4], D. Chiasson[1], T. Ketela[2], S. Scherer[1,5], M. Wilson[1], E. Jaeggi[1], R. Chaturvedi[1]

[1]The Hospital for Sick Children, Toronto, Canada, [2]University Health Network, Toronto, Canada, [3]Western University, London, Canada, [4]Mount Sinai Hospital, Toronto, Canada, [5]University of Toronto, Toronto, Canada

Introduction: Hypoplastic left heart syndrome (HLHS) describes a complex cardiac defect, characterized by absent or small mitral and aortic valves, with a small left ventricle and ascending aorta. Infant mortality is high, and those who survive face multiple challenges, including heart failure and neurodevelopmental delays. Reduced blood flow through the fetal left heart has been proposed as an etiology for HLHS. The development of novel therapies requires a better mechanistic and molecular understanding of the left heart hypoplasia.
Methods/Results: We generated a (non-genetic) fetal lamb model of left ventricular inflow obstruction, which resulted in a growth failure of the left heart, and recapitulated important clinical features of HLHS. miRNA-seq and mRNA-seq of the hypoplastic tissue found inversely differentially expressed miRNA-gene pairs annotating to cell growth and proliferation. To evaluate changes in the cellular composition and cell type specific differential gene expression, we performed single-nucleus RNA-seq of the hypoplastic myocardium (17,245 nuclei, 4 samples) compared to controls (12,992 nuclei, 3 samples). Multinomial logistic regression analyses found an increase in fibroblasts with a reciprocal decrease in cardiomyocyte nuclei proportions. Fibroblasts, cardiomyocytes and endothelial cells from hypoplastic myocardium had increased expression of genes associated with extracellular matrix components, fibrosis, and there was evidence of dysregulated fibroblast growth factor signaling. In line with those cellular and molecular changes, hypoplastic myocardia had a thickened endocardial layer of connective tissue in histology.
Conclusion: A sustained reduction of blood flow in fetal left hearts was sufficient to cause left heart hypoplasia. Hypoplastic ventricles presented with changes in the cellular composition and gene expression, consistent with a pro-fibrotic environment and aberrant induction of mesenchymal programs. Even subtle enhancements of collagen deposition could affect myocardial stiffness and further growth, including the outcome of fetal and early postnatal interventions aimed at restoring blood flow.

Abstract ID: 17

P2-8

Identification of human papillomavirus 16 genomic integration sites in oropharynx squamous cell carcinoma and their impact on host gene expression

M. Gregersen[1], T. Abou Kors[2], M. Bens[3], O. Ammerpohl[1], C. Brunner[2], T.K. Hoffmann[2], S. Laban[2], R. Siebert[1], E. Chteinberg[1]

[1]Ulm University and Ulm University Medical Center, Institute of Human Genetics, Ulm, Deutschland, [2]University of Ulm and Ulm University Medical Center,, Department of Otorhinolaryngology, Head & Neck Surgery, Ulm, Deutschland, [3]Fritz Lipmann Institute, Institute of Aging, Jena, Deutschland

Introduction: Head and neck cancers are the sixth most common cancer for men with estimated 17840 cases for 2022 in Germany. Most cancers of this group constitute oropharyngeal squamous cell carcinoma (OPSCC) which are located in the oropharynx. Of those, 30-50% are associated with human papillomaviruses (HPV). HPV integration into the host genome is a crucial step in carcinogenesis. Here we developed a capture sequencing assay to identify and map genomic viral integration sites (IS) and investigated the influence of these IS on targeted gene expression and DNA methylation.

Methods: Genomic DNA of 18 OPSCC tissues was PCR-screened for HPV16 positivity and subjected to capture-based next generation sequencing using HPV16 specific baids. The obtained fastq-files were aligned against the viral and human genomes using either STAR aligner or BWA/ Segemehl. After filtering the viral sequences using the BWA aligner, Segemehl automatically delivered a list of chimeric paired end reads. This list was used to identify possible IS. The IS prediction of STAR aligned chimeric reads was achieved by using Arriba with a custom blacklist and by manually filtering. Credibility of resulting IS was graphically assessed in the Intergenomic Gene Viewer and verified using specific PCR amplification of viral IS. Furthermore, the total RNA of 17 of the OPSCC samples was also sequenced with Illumina HiSeq2500/NovaSeq 6000 and aligned using STAR aligner.

Results: Of all 18 patients, eight were tested positive for HPV16. In total, only in the eight positive cases we could identify 60 and 25 breakpoints using Arriba and Segemehl, from which 20 and 8 were double sided IS. The highest number of breakpoints for both bioinformatic methods was detected in the HPV16 E1 gene. In the human genome, 38 and 9 breakpoints were intergenic, 19 and 5 intronic and 4 and 2 exonic. From the IS, 58% identified using Arriba and 50% using Segemehl could be confirmed by PCR. In 3 out of 7 HPV positive OPSCC the verified viral integration in exonic regions led to a outlying higher RNA expression level and altered exon usage of GREB1L, LMCD1-AS1 and METAP1, whereas DNA methylation of these genes was not significantly affected.

Conclusion: In total, we have established capture-based NGS pipeline for the rapid identification and characterization of HPV16 IS in the human genome. Moreover, we could show novel insights into dysregulation of host genes in the pathogenesis of OPSCC.

Abstract ID: 18

P7-4

Development of a quantitative splice assay for the characterization of variants of uncertain significance using long-read sequencing

A.-L. Katzke[1], M. Kayali[2], J. Hauke[2], G. Schmidt[1]

[1]Institut für Humangenetik, Hannover, Deutschland, [2]Zentrum Familiärer Brust- und Eierstockkrebs, Köln, Deutschland

Introduction: A major challenge in the genetic diagnosis of hereditary breast and ovarian cancer (HBOC) is the occurrence of variants of uncertain significance (VUS). To date, the central database of the German consortium for HBOC alone contains more than 700 VUS which are predicted to be potentially spliceogenic. Due to the presence of natural occurring splice isoforms the precise characterization and quantification of the effect of spliceogenic variants is very difficult.
Currently, most laboratories are using qualitative or semi-quantitative methods like RT-PCR, Sanger or Short-read sequencing for the analysis of such variants. Experience shows that these methods require many control samples due to the high inter-sample variability. Often no quantitative statements regarding the observed effect can be made.
Through the utilization of Oxford Nanopore long-read sequencing technology aberrant splicing events can be differentiated from naturally occurring alternative splicing events, as full isoforms can be reconstructed from the sequencing data. This could improve the characterization and quantification of splicing events, leading to a better classification of spliceogenic VUS.
Methods: RNA was isolated from blood samples of patients with known spliceogenic mutations and control individuals. Samples were converted into cDNA and amplification by long-range PCR using gene specific primers located each in the first and last exon was performed, followed by sequencing on the MinIon. Basecalling was performed using Guppy (v. 5.0.11) and reads were mapped to GRCh38 reference genome using minimap2 (v. 2.23). Transcripts were identified using the FLAIR package. Quantification was performed using salmon (v. 1.6.0).
Results: In first experiments using Oxford Nanopore long-read sequencing for the classification of splicing events in HBOC risk genes, we were able to reconstruct complete isoforms and identify previously described naturally occurring alternative splicing events in negative controls. Additionally, aberrant splicing events that were previously characterized using Sanger sequencing in positive controls could be detected.
Conclusion: Our first results show that Oxford Nanopore long-read sequencing using RNA from blood is suitable for characterizing the effect of potentially spliceogenic VUS. In the future phasing of variants could further improve the quantification of aberrant splicing events and additionally enable the detection of allele specific effects.

Abstract ID: 20

P4-5

Analyses of bipolar disorder polygenic risk in iPSC-derived neural progenitors from genetically characterized patients and controls

K. Hünten[1], T. Krutenko[2], F.S. David[1], E.C. Beins[1], S. Sivalingam[3], B. Burger[4], L. Sirignano[5], F. Streit[5], F. Stein[6], P.I. Pfefferle[7], S. Herms[1,4], P. Hoffmann[1,4], T. Kircher[6], U. Dannlowski[8], M. Rietschel[5], M. Peitz[2], S. Cichon[4], M.M. Nöthen[1], O. Brüstle[2], A.J. Forstner[1,9]

[1]Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Deutschland, [2]Institute of Reconstructive Neurobiology, University of Bonn Medical Faculty & University Hospital Bonn, Bonn, Deutschland, [3]Core Unit for Bioinformatics Data Analysis, Medical Faculty, University of Bonn, Bonn, Deutschland, [4]University of Basel and University Hospital Basel, Department of Biomedicine, Basel, Schweiz, [5]Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Department of Genetic Epidemiology in Psychiatry, Mannheim, Deutschland, [6]University Hospital for Psychiatry and Psychotherapy, UKGM, Philipps-Universität Marburg, Marburg, Deutschland, [7]Medical Faculty, Comprehensive Biobank Marburg (CBBMR), Member of the German Biobank Alliance (GBA) and the German Center for Lung Research (DZL), Philipps University of Marburg, Marburg, Deutschland, [8]Institute for Translational Psychiatry, University of Münster, Münster, Deutschland, [9]Institute of Neuroscience and Medicine (INM-1), Research Center Jülich, Jülich, Deutschland

Bipolar disorder (BD) is a common psychiatric disorder characterized by recurrent episodes of (hypo)mania and depression. Recent genome-wide association studies (GWAS) support a strong polygenic influence, but its functional impact on early neuronal development is largely unknown. Therefore, we generated induced pluripotent stem cells (iPSCs) from patients with BD type 1 (BD1) and healthy controls (HC) with a high or low polygenic risk score (PRS) for BD1, respectively, to study the polygenic influence during differentiation into neural progenitor cells.

The selection process of individuals has been presented at the last GfH conference (Hünten et al., poster presentation). Briefly, three unrelated male BD1 patients and three HCs from the FOR2107 cohort (https://for2107.de/) were selected for iPSC generation based on the following criteria: 1) high or low BD1 PRS, 2) absence of copy number variants associated with BD or schizophrenia and 3) absence of rare variants associated with BD. In a pilot experiment, generated iPSC lines were differentiated into cortical neural progenitors (Rehbach et al., 2019) and cells were harvested at day 0, 12, 22 and 32 for transcriptome analysis (n=3 per donor and timepoint). 3’mRNA-sequencing was performed at the NGS Core Facility of the University of Bonn and data was analysed using Partek Flow Genomic Analysis Software. Reads were aligned to GRCh38 using STAR-2.7.8a and to genes using Partek E/M. Differentially expressed genes (DEGs) found with DESeq2 were filtered for the Gene set enrichment analysis (GSEA) using a p-value ≤0.05 and a fold change <-1.5 or >1.5.

Data of two iPSC lines (BD1 n=1, HC n=1) had to be excluded from the analysis due to aberrant gene expression profiles, consistent with observations of unsuccessful differentiation in cell culture. Analysis of the remaining samples identified several significantly DEGs between BD1 and HC cells at the different timepoints. Most DEGs were found at day 12 after neural induction, representing early cortical progenitors. Significant DEGs included *WNT5B*, *BCORL1*, *IFITM2*, *SLITRK4*, *SLIT1* and *FOXO3B*, all of which have been associated with cell development and/or psychiatric disorders. GSEA revealed pathways such as “DNA binding” and “response to calcium ions”.

In summary, these preliminary findings suggest that polygenic risk for BD1 alone influences expression of genes related to neuronal development and cellular functions, especially at the early stages of differentiation.

Abstract ID: 21

P3-9

Exome sequencing individuals and families with suspected monogenic primary immunodeficiencies – experiences of a single center

U. Hüffmeier[1], C. Kraus[1], M. Rieger[1], K. Blum[1], A. Wilms[1], A. Hueber[2,3], N. Naumann-Bartsch[4], A. Karow[4], G. Fecker[4], A. Hoerning[5], M. Galiano[6], J. Rech[2,7], T. Krickau[6], A. Reis[1,8]

[1]Institute of Human Genetics, Universitätsklinikum Erlangen, FAU Erlangen-Nürnberg, Erlangen, Deutschland, [2]Friedrich-Alexander-University Erlangen-Nürnberg and Universitätsklinikum Erlangen, Department of Internal Medicine 3 - Rheumatology and Immunology, Erlangen, Deutschland, [3]Division of Rheumatology, Klinikum Nürnberg, Paracelsus Medical University, Nürnberg, Deutschland, [4]Comprehensive Cancer Center Erlangen-EMN (CCC ER-EMN), University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg, Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Erlangen, Deutschland, [5]Comprehensive Cancer Center Erlangen-EMN (CCC ER-EMN), University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Pediatric Astroenterology, Department of Pediatrics and Adolescent Medicine, Erlangen, Deutschland, [6]University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nuremberg, Pediatric Rheumatology, Department of Pediatrics and Adolescent Medicine, Erlangen, Deutschland, [7]Deutsches Zentrum Immuntherapie, Friedrich-Alexander-Universität Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Deutschland, [8]Center for Rare Diseases Erlangen (ZSEER), University Hospital Erlangen, Erlangen, Deutschland

Primary immunodeficiencies comprise a group of immunologic disease categories caused by single gene defects. In order to assess the contribution of known genetic diseases in this field, we performed ES in 34 affected individuals. Diagnoses were autoimmunity (n=24), bone-marrow failure (n=5), increased susceptibility to infections (n=4), suspicion of a specific immunologic diagnosis (n=4) and/ or early-onset inflammatory bowel disease (IBD) (n=2). We assessed the role of variants in a panel of previously published genes for human inborn errors of immunity (hIEI) (Tangye et al. 2022), supplemented by single genes based on new findings/ authors' experience [n=449], and/ or gene panels based on human phenotype ontology terms.

The average age at genetic testing was 5.9 +/- 4.1 years in 20 children and 45.9 +/- 21.3 years in 14 adults; the group included 14 female and 20 male individuals. The most common molecularly confirmed diagnosis was VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome, caused by *UBA1* missense variants affecting p.Met41 in four single males with tentative clinical diagnosis/ unspecific autoimmune symptoms. Infantile lymphopenia was related to an inherited *TBX1* stop variant for which loss of the nucleosome-localization signal was predicted. This variant was not fully penetrant in the parent, but potentially related to symptoms typical for 22q11.2 microdeletion syndrome in other family members. In two patients with increased susceptibility to infections, we identified compound heterozygosity for *loss-of-function* variants in *RAG2* and *POLE*, permitting diagnoses of atypical severe combined immunodeficiency or FILS syndrome, respectively. In total, the diagnostic yield was 29%. It was highest in increased susceptibility to infections/ suspicion of a specific immunologic diagnosis (50%), lower in bone-marrow failure (20%) and autoimmunity (17%) and 0% in IBD. We detected variants of unknown significance in eight individuals (24%). Systematic assessments of all rare variants suggested variants in new candidate genes in five further individuals (15%).

Molecularly confirmed diagnoses allowed specific recommendations for therapy, prevention and an individual prognosis. In addition, in two children, they lead to informed decision pro/ contra bone-marrow-transplantation. Our data suggest an increasing diagnostic yield in primary immunodeficiencies, although genetic defects in many individuals remain to be identified.

Abstract ID: 22

P6-3

Variants in *FGF10*: a cause of isolated neonatal lung developmental disorder?

S. v. Hardenberg[1], A. Schmidt[2], K. Schütz[3], B. Gerard[4], E. Schaefer[4], M.C. Antal[5], S. Peters[2], M. Griese[6], C. Rapp[6], A.K. Bergmann[1], G. Schmidt[1], B. Auber[1], N. Schwerk[3,7], J.C. Kamp[8,7], F. Länger[9]

[1]Hannover Medical School, Institute of Human Genetics, Hannover, Deutschland, [2]University of Bonn, Institute of Human Genetics, Bonn, Deutschland, [3]Hannover Medical School, Department of Pediatric Pneumology, Allergology and Neonatology, Hannover, Deutschland, [4]Unité de Génétique Moléculaire, Nouvel Hôpital Civil, Laboratoires de Diagnostic Génétique, Strasbourg, Frankreich, [5]Hôpitaux Universitaires Strasbourg, UF 6349 Fœtopathologie, Département de Pathologie, Strasbourg, Frankreich, [6]University Hospital, LMU Munich, Dr. von Hauner Children's Hospital, Department of Pediatrics, München, Deutschland, [7]German Center of Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Hannover, Deutschland, [8]Hannover Medical School, Department of Respiratory Medicine, Hannover, Deutschland, [9]Hannover Medical School, Institute of Pathology, Hannover, Deutschland

Introduction: Rare heterozygous and deleterious variants in the fibroblast growth factor 10 (*FGF10*) gene are known causes of both, the lacrimo-auriculo-dento-digital (LADD) syndrome as well as aplasia of lacrimal and salivary glands. Interestingly, variants in *FGF10* have been only once reported in lethal human developmental disorders of the lung, although its role in lung branching morphogenesis is known since late ‘90s. However, there are still no entries in public databases regarding the association to an isolated neonatal lung developmental disorder.
Methods: International variant matchmaking via GeneMatcher identified four patients with different heterozygous variants in *FGF10*, each detected by exome or genome sequencing. Re-evaluation of the clinical course of all patients as well as morphological analysis of the lung biopsy samples of three diseased patients was performed.
Results: Our cohort consists of four children (three female, one male), two of whom carry missense variants and two of whom carry a frameshift or splice variant, respectively. None of our patients showed distinct syndromic LADD-features but all children presented with postnatal respiratory distress followed by persistent oxygen support and revealed pulmonary hypertension. The children carrying the frameshift or splice variant died in the postnatal period. Lung tissue specimen from the autopsy revealed significant architectural disruption with a widened, cellular interstitium, pulmonary interstitial glycogenosis (PIG) and vascular misalignment. The children with missense variants showed a milder clinical phenotype. One child is now 5 years old, chest CT revealed bipulmonary groundglass opacities and interlobar septal compaction along with subpleurally accentuated flaps and bullae. The other child died at 12 years of age due to terminal respiratory failure. Two lung tissue samples of this child (including one follow up sample) revealed alveolar simplification with progressive interstitial fibrosis. These findings suggest that *FGF10* variants may be associated with isolated neonatal lung developmental disorders.
Conclusion: Genotypes as well as clinical and histological phenotypes of four patients with severe lung phenotype and *FGF10* variants demonstrate that it is essential to consider an *FGF10*-mediated pathology, even in the absence of the previously described syndromic stigmata in children with postnatal respiratory distress, especially when leading to death in the neonatal period.

Abstract ID: 23

De novo variants in *DLGAP1* cause a variable neurodevelopmental disorder

H. Lyubenova[1,2], H. Kemmer[1], A. Brady[3], J. Cobben[3], J. Coursimault[4], M. Dasouki[5], B. de Vries[6], M. Holtgrewe[7], D. Horn[1], R. Ibitoye[3], R.A. Jamra[8], F. Lecoquierre[4], R. Pfundt[6], K. Platzer[8], S. Redler[9], M. Ross[10], P. Saugier-Veber[4], E. Torti[11], B. Fischer-Zirnsak[1,2], N. Ehmke[1,2,7]

[1]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Medical Genetics and Human Genetics, Berlin, Deutschland, [2]Max Planck Institute for Molecular Genetics, FG Development and Disease, Berlin, Deutschland, [3]North West Thames Regional Genetic Service, Northwick Park and St Mark's Hospitals, London, Vereinigtes Königreich, [4]Normandie Univ, UNIROUEN, CHU Rouen, Inserm U 1245, FHU G4 Génomique, Department of Genetics and Reference Center for Developmental Disorders, Rouen, Frankreich, [5]AdventHealth Research Institute, Department of Medical Genetics & Genomics, Orlando, Vereinigte Staaten, [6]Radboud University Medical Center, Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Nijmegen, Niederlande, [7]Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Berlin, Deutschland, [8]University of Leipzig Medical Center, Institute of Human Genetics, Leipzig, Deutschland, [9]Heinrich-Heine-University Düsseldorf, Institute of Human Genetics, Medical Faculty and University Hospital Düsseldorf, Düsseldorf, Deutschland, [10]New York Presbyterian Hospital, Columbia University, Division of Clinical Genetics, Department of Pediatrics, New York, Vereinigte Staaten, [11]GeneDx, Gaithersburg, Vereinigte Staaten

In the mammalian brain, distinct scaffold proteins such as SAPAP/DAP and PSD-95 family members establish the postsynaptic density (PSD) of glutamatergic synapses, a dense protein network generated by multiple molecular interactions. The PSD is thought to concentrate and organise neurotransmitter receptors in the postsynaptic membrane and regulate signalling of excitatory synapses. Mutations in genes encoding various postsynaptic scaffold proteins have been associated with neurodevelopmental disorders such as autism spectrum disorder (ASD) and intellectual disability (ID), including *DLG4* which encodes PSD-95. DAP1/SAPAP1, encoded by *DLGAP1*, links PSD-95 to Shank, which is crucial for the PSD architecture, and indirectly controls proteasomal degradation of PSD-95 upon dissociation. *DLGAP1* has been suggested as a candidate for ASD and ID in a single large cohort sequencing study.

Here, we present seven unrelated individuals with variable phenotype, including global developmental delay, ID, ASD, seizures, muscular hypotonia and hearing loss, harbouring heterozygous *de novo* variants in *DLGAP1*. We identified five missense and two nonsense variants in *DLGAP1* by exome sequencing and established the cohort using GeneMatcher. Four of the five missense variants locate to the 14-amino acid (a.a.) repeat domain of DAP1 which interacts with the guanylate kinase (GK) domain of PSD-95. We transiently overexpressed DAP1 wildtype (WT) and two missense variants in HeLa cells. Western blot analysis of crude cell lysates revealed a similar abundance of WT and mutant proteins. Moreover, WT and mutant DAP1 co-immunoprecipitated with recombinant PSD-95. In the immunoprecipitates, a reduced amount of WT-DAP1-PSD-95 complex was detected compared to mutant DAP1-PSD-95. This finding may indicate reduced turnover of mutant complexes.

Our data suggest haploinsufficiency of *DLGAP1* as well as disturbed DAP1-PSD-95-interation as a disease mechanism. The *DLG4*-associated phenotype is comparable to that of individuals in the here described cohort and can also be due to missense variants in the DAP1-interacting domain of PSD-95, greatly supporting our findings. Our results add a novel disorder to the known causes of neurodevelopmental phenotypes.

Abstract ID: 24

P3-23

Infantile acute liver failure in *EFL1*-associated Shwachman-diamond syndrome

A. van der Ven[1], T. Bierhals[1], F. Brinkert[2], M. Jankofsky[2], T. Clauditz[3], M. Hempel[1,4]

[1]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland, [2]Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland, [3]Department of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland, [4]Institute of Human Genetics, University Heidelberg, Heidelberg, Deutschland

Introduction: Shwachman-diamond syndrome (SDS) is a genetic disease condition characterized by the triad of pancytopenia, exocrine pancreatic insufficiency and short stature. A disease-associated involvement of liver tissue predominantly presenting as hepatomegaly and mild-moderately increased transaminases has repeatedly been observed in patients with SDS. Up until recently, the *SBDS*-gene was the only established genetic cause of SDS, accounting for >90% of clinical cases. Meanwhile, additional genetic causes have been described. Since 2017, biallelic (mostly) missense variants in the *EFL1*-gene were identified to account for a fraction of *SBDS*-negative clinical cases of SDS. *EFL1*-associated SDS is referred to as SDS2 or SD-like syndrome in the corresponding literature.
Methods and Results: We present a dystrophic premature newborn (weight: <1P. -2.6z; length: 1P., -2.5z) delivered at 34+3 weeks of gestation via cesarean section due to pathologic CTG. Additional symptoms included pancytopenia, acute liver failure, and (later) exocrine pancreatic insufficiency. Liver transplantation was conducted at the age of one month. Histopathologic examination of explanted tissue demonstrated extensive micronodular cirrhosis with marked cholestasis and siderosis. Trio-exome sequencing revealed a homozygous missense variant in *EFL1* (NM_024580, c.904T>C, p.(Trp302Arg)) in our patient. The variant was absent from gnomAD, and resulted in pathogenic scores in multiple prediction programs. Family history included multiple lines of consanguinity across generations. Our patient was the 4th born child of healthy 2nd degree cousins of Afghan descent. Two siblings were reported healthy. One sister was born prematurely with severe dystrophy. She developed acute liver failure early postnatally and deceased at the age of 4 months post liver-transplantation. Histopathologic examination of explanted liver tissue demonstrated micronodular cirrhosis, intrahepatic cholestasis and extensive siderosis. The *EFL1*-variant was shown to co-segregate with hepatic disease in nine family members tested.
Conclusion: Sofar, less than 10 patients have been described with *EFL1*-associated SDS (SDS2) in the literature. The understanding of the clinical presentation of SDS2 therefore remains limited. We here present a novel homozygous missense *EFL1*-variant as molecular cause of SDS2. We further propose the *EFL1*-variant as origin of the severe hepatic phenotype observed in our patient and his sister, thereby expanding the phenotypic spectrum of SDS2.

Abstract ID: 25

1 Mutation 1 Medicine: a European platform for ASO development and treatments for individuals with nano-rare neurological diseases

H. Graessner[1,2], M. Synofzik[3,2], A. Aartsma-Rus[4,2], W. van Roon-Mom[4,2], M. Lauffer[4,2], R. Schüle[5,3,2]

[1]University Hospital Tübingen, Centre for Rare Diseases and Institute for Medical Genetics and Applied Genomics, Tübingen, Deutschland, [2]1M1M, Tübingen, Heidelberg, Leiden, Deutschland, [3]University of Tübingen, Hertie Institute for Clinical Brain Research, Tübingen, Deutschland, [4]Leiden University Medical Center, Department of Human Genetics, Leiden, Deutschland, [5]University Hospital Heidelberg, Department of Neurology, Heidelberg, Deutschland

Introduction: The last decade has seen a breakthrough in genetic therapies which has opened up the novel field of personalised genetic medicine, allowing to develop targeted mechanistic therapies for thus far untreatable diseases. The goal of the academically driven 1 Mutation 1 Medicine (1M1M) initiative is to develop single patient tailored antisense oligonucleotide (ASO)-mediated exon skipping treatments for individuals with nano-rare mutations. 1M1M focuses on patients with severely debilitating or life threatening (SDLT) nano-rare (<1:1.000.000) neurological diseases (RND) that are in danger of not being able to benefit from the advances of genetic treatments.
Methods: Using the recently provided proof-of-concept that development of a mutation-specific, intrathecally delivered splice-switching ASO treatment is feasible within 18 months (PMID: 31597037) as a blueprint, 1M1M is currently building a European platform for the development of single patient tailored ASO treatments. Respective European Medicine Agency (EMA) advice processes and standardised infrastructure from patient identification to treatment logistics are being established, validated and improved.
Results: 1M1M has been developing standardised workflows for: potential splice-altering variant interpretation; ASO design and testing; patient identification including variant, disease and patient perspective; regulatory-like process towards start/stop of development and treatment decision including patient dossier, gene group meetings and treatment board.
Fit for purpose and sustainable infrastructures such as a 1M1M registry are currently being set-up. A European 1M1M network of >20 hospitals (mainly ERN-RND sites) has been formed.
The following numbers and facts reflect the 1M1M achievement by date: >2.000 RND genes of interest identified; >40 potential cryptic splice mutations of RND patients investigated; ASO development programs underway for six genes; full patient dossiers have been used to organise *ATM*, *POLR3A* and *PLP1* gene group meetings; EMA advice received in March 2022; first treatment of a patient with an individualised ASO in Europe has started in September 2022 at the University Hospital Tübingen.
Conclusion: 1M1M has successfully started to build a European platform for ASO development and treatments for individuals with nano-rare SDLT RNDs. Open issues such as robust quality management, reimbursement by healthcare insurances, suitable regulatory oversight and up-scaling on European level remain to be addressed.

Abstract ID: 26

Novel pharmacological approaches for non-alcoholic steatohepatitis based on a single patient with a de novo variant in FASN

M. Musfeldt[1], A. Worthmann[2], J. Ridder[1], S.Y. Piel[1], I. Evangelakos[1], H. Voß[3], M. O'Farell[4], A.W. Fischer[2,5,6], S. Adak[7], M. Sundd[8], H. Siffeti[1], J.-J. Scholz[1], K. Kloth[1], T. Bierhals[1], M. Heine[2], P. Pertzborn[2], M. Pauly[2], F. Haumann[1], S. Kundu[9], M.M. Fuh[2] A. Neu[10], C. Beysen[11], C. Tödter[2], M. Hempel[1], U. Knippschild[12], C.F. Semenkovich[7], H. Schlüter[3], J. Heeren[2], L. Scheja[2], C. Kubisch[1], C. Schlein[1]

[1]Institute of Human Genetics, University Medical Center, Hamburg- Eppendorf, Deutschland, [2]University Medical Center, Department of Biochemistry and Molecular Cell Biology, Hamburg-Eppendorf, Deutschland, [3]Institute of Clinical Chemistry and Laboratory Medicine, Section/Core Facility Mass Spectrometry and Proteomics, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland, [4]Sagimet Biosciences Inc., California, Vereinigte Staaten, [5]Harvard Medical School, Department of Cell Biology, Boston, Vereinigte Staaten, [6]Harvard T. H. Chan School of Public Health, Harvard University, Department of Molecular Metabolism, Boston, Vereinigte Staaten, [7]Division of Endocrinology, Metabolism & Lipid Research, Department of Medicine, Washington University, St. Louis, Vereinigte Staaten, [8]National Institute of Immunology, New Delhi, Indien, [9]University of Delhi South Campus, Department of Biochemistry, New Delhi, Indien, [10]University Medical Center, Department of Pediatrics, Hamburg-Eppendorf, Deutschland, [11]FluxBio, San Mateo, California, Vereinigte Staaten, [12]University Hospital, Department of General and Visceral Surgery, Ulm, Deutschland

Background: Non-alcoholic steatohepatitis (NASH) is closely related to the triple epidemic of obesity, dyslipidemia and diabetes and reflects a metabolic dysfunction ultimately leading to excessive fat storage in the liver. Moreover, it is characterized by inflammation and liver damage which can leads to fibrosis and cirrhosis. So far, no medication for NASH has been clinically approved. The development of NASH is closely linked to aberrant synthesis of endogenous fatty acids in the liver, called *de novo* lipogenesis, which is mediated by the enzyme fatty acid synthase (FASN). Saturated or monosaturated fatty acids are produced by de novo lipogenesis, whereas polyunsaturated fatty acids (PUFAs) are strictly dietary cannot be synthesized. Saturated fatty acids are discussed to cause cell stress and inflammation which link lipogenesis to metabolic disease whereas PUFA directly inhibit lipogenesis and thus reduce plasma triglycerides, cholesterol and liver steatosis. The aim of the study was to investigate the systemic metabolism with genetically or pharmacologically altered *de novo* lipogenesis.
Methods: By trio whole exome sequencing of a 5-year-old patient, we identified a putatively pathogenic *de novo* missense variant in *FASN*. The plasma samples of controls and the patient were analyzed with liquid chromatography mass spectrometry to study functional consequences of the variant *in vivo* and in liver biopsies of individuals with exceptionally high hepatic *FASN* expression. Further we examined the effect of FASN in a clinical trial with a human FASN inhibitor, as well as in mouse and cell culture models with genetically or pharmacologically induced low lipogenesis.
Results: Exome analysis revealed a putative pathogenic de novo missense variant in *FASN*. This variant leads to reduction of the *FASN* activity and thus fatty acid biosynthesis in fibroblasts. Interestingly we could show that the reduced *FASN* activity leads to an increase of essential PUFAs, which cannot be produced by the body and need to be taken up by food. In further mechanistic studies we established PUFA storage as an active, enzymatic process controlled by *FASN* in a complex relationship with *DGAT2* and *MFSD2A*.
Conclusion: These findings provide evidence that the success of PUFA supplementation may depend on the endogenous lipogenesis rate by FASN and that a combination of PUFA supplementation and inhibition of *de novo* lipogenesis may be a novel pharmacological approach for the treatment of NASH.

Abstract ID: 27

Patient and variant stratification for individualized genetic therapies for ultra-rare diseases

M. Lauffer[1], B. Zardetto[1], I. Huitink[1], S. Kumar[1], I. Fokkema[2], J. Laros[2,3], W. van Roon-Mom[1], A. Aartsma-Rus[1]

[1]Leiden University Medical Center, Dutch Center for RNA Therapeutics - Department of Human Genetics, Leiden, Niederlande, [2]Leiden University Medical Center, Department of Human Genetics, Leiden, Niederlande, [3]National Institute for Public Health and the Environment, Department of Bioinformatics and Computational Services, Bilthoven, Niederlande

Up to 6% of the world's population is affected by at least one of the 7,000+ known rare diseases, 80% of which are of genetic origin. For over 90% of these disorders, no disease-altering therapy is yet available, and new treatment strategies are urgently needed. In recent years, it has been shown that antisense oligonucleotides (ASOs) are one option to treat inherited diseases, and splice-modulating ASOs have been approved for disorders like spinal muscular atrophy and Duchenne muscular dystrophy. Notably, individual patients with nano-rare mutations are now being treated with mutation-specific ASOs. These efforts are coordinated by national (Dutch Center for RNA Therapeutics), European (1Mutation1Medicine), and global (N=1 Collaborative) networks and consortia.

One aim of these consortia is to offer guidance and streamline the development of tailor-made ASOs. For instance, selecting which patients and genetic variants are eligible for personalized ASO treatment is challenging. At the same time, prioritization of patients and genetic variants is important to facilitate treating those that benefit most and not treating those unlikely to benefit.

Therefore, we have developed a set of practical guidelines for patient stratification and prioritization of genetic variants. The guidelines take the clinical presentation as well as the variant itself into account and can be used as an aid for clinicians and researchers alike.

To accelerate variant evaluation, the Dutch Center for RNA Therapeutics has built a computational tool that automates variant assessment and predicts how likely it is that a variant is suitable for an individualized ASO treatment. We aim to provide a publicly accessible web application for the variant evaluation as well as the possibility to integrate the tool directly into diagnostic pipelines. Especially the latter will aid in identifying suitable patients faster, directly within the diagnostic process. Furthermore, we want to provide users of the tool with the possibility to connect and share knowledge and data for personalized genetic treatments. This will aid in aligning compound development, especially once multiple patients within the same gene or disease group are identified.

We additionally discuss that this tool will eventually include the selection for other personalized approaches like base- and prime-editing and aid in pre-designing ASOs and gRNAs for said approaches to further accelerate treatment development.

Abstract ID: 29

P6-17

Novel variant in Kozak sequence of *KCNC3* might affect ion channel expression

M.C. Reis[1], A.M. Richter[2], M. Weiß[1], R. Dammann[2], D. Nolte[1]

[1]Justus-Liebig-Universität Gießen, Institut für Humangenetik, Gießen, Deutschland, [2]Justus-Liebig-Universität Gießen, Institut für Genetik, Gießen, Deutschland

Autosomal-dominant spinocerebellar ataxias (SCAs) are a genetically heterogeneous group of neurodegenerative diseases. Key features are ataxia, dysarthria and abnormal eye movements. Additional symptoms such as tremor, dystonia, myoclonus, and cognitive impairment may occur (1).

To date, 49 subtypes of SCA have been described, some of which are caused by trinucleotide repeat expansions in the corresponding genes. In rarer cases, missense mutations are disease-causing (1). Of these, SCA13 is associated with point mutations in the *KCNC3* gene, which encodes the voltage-gated potassium channel Kv3.3 expressed mainly in the Purkinje cells of the cerebellum (2). Until now, nine amino acid substitutions are known in Kv3.3, which influence channel expression and function and are associated with variable disease onset (2,3). To date, no non-coding variant in *KCNC3* is known to be associated with SCA13.

A novel variant in the Kozak sequence of *KCNC3* (c.-6C>A) was identified in a patient with ataxia symptoms. The Kozak sequence is a conserved motif of nucleotides encompassing the start codon AUG. Interestingly, the Kozak sequence of *KCNC3* does not correspond to the canonical sequence (GCC RCC AUG G) typically present in vertebrates, but is a less common variant (CGT CCA AUG C). The c.-6C>A variant could affect and compromise the efficiency of translation initiation due to its localization in the extended Kozak sequence.

To determine whether c.-6C>A is pathogenic, the effects of base change on transcription and translation of the *KCNC3* gene were analyzed by RT-qPCR and luciferase reporter assay, respectively. For this purpose, constructs were created containing the *KCNC3* 5’untranslated region and Kozak sequence upstream of a luciferase gene. Expression in HEK cell culture was used to examine the differences in expression of wild type and mutant sequence at the transcriptional and translational level. Interestingly, the mutant sequence enhances the luciferase activity compared with wild type. Although experiments are ongoing, these preliminary results suggest that the variant might affect the expression of Kv3.3.

1. Bird TD, 2019. Hereditary Ataxia Overview. *GeneReviews* (1993-2021)
2. Waters et al., 2006 (doi: 10.1038/ng1758)
3. Duarri et al., 2015 (doi: 10.1371/journal.pone.0116599)

Abstract ID: 30

P6-32

MECR is essential for coordinated energy transformation

I. Evangelakos[1], F. Haumann[1], A. Worthmann[2], S. Kotschi[3], A. Turid Bischoff[4], S. Yun-Lan Piel[1], M. Heine[2], M. Pauly[2], U. Knippschild[5], T. Klopstock[4], J. Heeren[2], L. Scheja[2], A. Bartelt[3], C. Kubisch[1], C. Schlein[1]

[1]University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Deutschland, [2]University Medical Center Hamburg-Eppendorf, Department of Biochemistry and Molecular Cell Biology, Hamburg, Deutschland, [3]Ludwig-Maximilians-University of Munich, Institute for Cardiovascular Prevention (IPEK), Munich, Deutschland, [4]University Hospital LMU Munich, Department of Neurology, Friedrich-Baur-Institute, Munich, Deutschland, [5]University Hospital Ulm, Department of General and Visceral Surgery, Ulm, Deutschland

Introduction: Mitochondria are often seen as the cell's powerhouse that mediate catabolic processes, such as beta-oxidation, but they are also important for anabolic processes, such as hormone production or fatty acid synthesis. Next to the canonical cytosolic fatty acid synthesis (FAS I), mitochondria can newly synthesize lipids via mitochondrial fatty acid synthesis (FAS II or mtFAS). Mitochondrial enoyl-CoA-reductase (MECR) is catalyzing the last step of mtFAS and serves as a key mediator for lipoic acid production and acetyl-CoA-dependent cellular metabolic pathways. In humans, genetic mutations in MECR disturb the mtFAS pathway and cause MEPAN syndrome, a neurometabolic disorder. However, how mtFAS affects systemic lipid and glucose metabolism has not been investigated.

Methods: Whole exome sequencing and lipidomic analysis was performed in a 20-year-old male patient with muscle weakness and optic atrophy. To study the role of *Mecr* in systemic metabolism, mice lacking *Mecr* in liver ($Mecr^{fl/fl}$-Alb^{cre}) and adipose tissues ($Mecr^{fl/fl}$-$Adipoq^{cre}$) were subjected to oral glucose and fat tolerance test combined with radioactive tracer uptake and indirect calorimetry, respectively. Electron microscopy, western blotting, gene expression and *ex vivo* measurement of enzymatic activities as well as metabolite levels were determined.

Results: We identified a patient with compound-heterozygous loss-of-function and missense variants in MECR associated with lower levels of specific plasma lipid species while variant overexpression in HEK cells led to an opposite lipidomic fingerprint. In $Mecr^{fl/fl}$-Alb^{cre+} and $Mecr^{fl/fl}$-$Adipoq^{cre+}$ mice, electron microscopy revealed that hepatic and adipose tissue mitochondria were irregularly shaped with critically affected cristae structures. Hepatic *Mecr* deletion led to profound insulin resistance, reduced lipoic acid synthesis as well as PDH activity. Adipose tissue *Mecr* ablation exacerbated tissue inflammation, attenuated brown fat thermogenic capacity and critically affected survival upon cold exposure.

Conclusions: We show that tissue specific *Mecr* loss in liver or adipose tissue is required to maintain energy breakdown by regulating the PDH complex activity, which is important for glucose and lipid use from the circulation. Here we introduce mtFAS as a critical regulator of metabolic health, by determining systemic and intracellular glucose and lipid metabolism in liver as well as thermogenic responses in adipose tissues.

Abstract ID: 31

A novel intronic GAA repeat expansion in *FGF14* causes autosomal dominant adult-onset ataxia

K. Lohmann[1], C. Helmchen[2], M. Thomsen[1], H. Rafehi[3,4], M. Borsche[1,2], J. Read[5,6], D.J. Szmulewicz[7,8], M. Bahlo[3,4], P.J. Lockhart[5,6], N. Brüggemann[1,2]

[1]Universität zu Lübeck, Institut für Neurogenetik, Lübeck, Deutschland, [2]Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Klinik für Neurologie, Lübeck, Deutschland, [3]The Walter and Eliza Hall Institute of Medical Research, Parkville, Australien, [4]University of Melbourne, Parkville, Australien, [5]Murdoch Children's Research Institute, Parkville, Australien, [6]Royal Children's Hospital, Parkville, Australien, [7]Cerebellar Ataxia Clinic, Eye and Ear Hospital, Melbourne, Australien, [8]The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Australien

Introduction: Cerebellar ataxia (CA) is defined as a disturbance of the normal coordination of movements and has been linked to cerebellar dysfunction. The underlying genetic causes are highly heterogeneous and often involve expansions of coding or non-coding short tandem repeats (STR). Based on dedicated bioinformatics analysis of short-read genome data, we have identified a novel genetic cause in ataxia patients, i.e., the expansion of an intronic, non-coding GAA repeat in the *FGF14* (Fibroblast Growth Factor 14) gene.
Methods: We designed a long-range PCR assay to test the size of the *FGF14* STR in a group of 104 German *RFC1*-negative, late-onset ataxia patients or exome-negative unsolved ataxia families. The latter also included a parent-offspring trio. We also tested 190 healthy controls. The patients were recruited from the ataxia and vertigo outpatient clinic. Vestibulo-oculography, calorimetry, NCS, and MRI were performed in a clinical setup. Further, we applied a linear regression model to determine the relationship between FGF14 repeat length and age at onset (AAO).
Results: This analysis identified 15 affected individuals (14.4%) with $(GAA)_{>250}$, compared to ten in controls (5.3%) [Fisher's Exact Test P-value 0.014, Odds Ratio=3.03 (95% CI=1.3-7.02)]. The largest observed allele in a control individual was $(GAA)_{330}$, while the repeat number in patients was up to 400, with nine patients (8.7%) having ≥330 repeats. Within the trio, the expanded allele increased from app. 280 alleles in the unaffected mother to about 315 repeats in the affected child with a complex neurodevelopmental disorder, including ataxia.
All other affected individuals had adult-onset, slowly progressive cerebellar ataxia with variable features, including vestibular impairment, hyperreflexia, and autonomic dysfunction. A negative correlation between AAO and repeat number was observed (R^2=0.55, p=0.0058, slope=-0.14). The regression suggests that for every $(GAA)_{10}$ increase >250 repeats, the AAO is reduced by ~1.35 years.
Conclusion: This study validates GAA repeat expansions in *FGF14* as a frequent cause of adult-onset ataxia. The results suggest that expanded alleles $(GAA)_{>330}$ are pathogenic and fully penetrant [Fisher's Exact Test P-value 0.0001)], while alleles with >250 but <330 repeats show reduced penetrance. ATX-FGF14 seems to be a very frequent cause (~10%) in late-onset ataxia, and its role in neurodevelopmental disorders needs to be further evaluated.

Abstract ID: 32

P6-9

Non-5q-SMAs: a conundrum with lessons for 5q-SMA or an overdiagnosed clinical picture?

M. Karakaya[1], N. Keller[1], J. Gorenaia[1], N. Fuhrmann[1], K. Becker[2], H. Thiele[2], G. Wunderlich[3], J. Kirschner[4], B. Schrank[5], R. Maroofian[6], C. Paketci[7], U. Yis[7], P. Nürnberg[2], B. Wirth[1]

[1]Institute of Human Genetics, Center for Molecular Medicine Cologne, Institute of Genetics, and Center for Rare Diseases Cologne, University of Cologne,, Köln, Deutschland, [2]Cologne Center for Genomics (CCG), University of Cologne, Köln, Deutschland, [3]University Hospital Cologne, Department of Neurology, Köln, Deutschland, [4]University Hospital Freiburg, Albert-Ludwigs-University, Department of Neuropediatrics, Freiburg, Deutschland, [5]DKD HELIOS Kliniken Wiesbaden, Department of Neurology, Wiesbaden, Deutschland, [6]Molecular and Clinical Sciences Institute, St. George's University of London, Cranmer Terrace, London, Vereinigtes Königreich, [7]Dokuz Eylül University, Department of Pediatric Neurology, Izmir, Türkei

Introduction: Spinal muscular atrophy without deletions/mutations in *SMN1* (non-5q-SMA) is a heterogeneous group of disorders and hard to distinguish from 5q-SMA. The new treatment options for 5q-SMA have necessitated extensive genetic testing of SMA, which in turn has revealed a large group of patients without 5q-SMA.
Methods: We used NGS methods to uncover the genetic defects in patients with non-5q-SMA. Since 2012, 195 families with SMA-related phenotype have been tested via gene panels, exome sequencing (ES) and/or genome sequencing (GS).
Results: The neuromuscular disease gene panel enabled a genetic diagnosis in 43% of tested probands. In only 1/3 of cases, a causal mutation was found in genes associated with SMA/lower motor neuron disease. Furthermore, ES/GS in panel-negative patients yielded an extra diagnosis in 42% of the cases. Both findings highlight the large genetic heterogeneity of patients with non-5q-SMA. Despite diverse causes of non-5q-SMA, we observed a strong overlap of altered cellular processes. Thus, defects in mitochondrial bioenergetics, endocytosis, axonal RNA transport and local translation were the leading compromised cellular functions, as predicted by the identified pathogenic mutation.
Conclusion: The causes of non-5q-SMAs are diverse, which poses a challenge for classification. However, the strong coherence of the cellular defects in both 5q- and non-5q-SMA groups might suggest new therapy indicators and underlines combinatorial treatment approaches in SMA.

Abstract ID: 33

P3-34

Male patient with a novel likely pathogenic variant in *HDAC4* causing neurodevelopmental disorder with central hypotonia and dysmorphic facies

J. Köhler[1], M. Spielmann[1,2], N. Hornig[1]

[1]University of Kiel, Institut of Human Genetics, Kiel, Deutschland, [2]University of Lübeck, Institut of Human Genetics, Lübeck, Deutschland

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*. The prevalence of this disorder is not known and likely to be very low. So far there are 7 patients described. Here we report a 6 year old patient with NEDCHF and a stop gain variant in *HDAC4*. *HDAC4* encodes for a protein of the histone deacetylase family (HDACs) and is a regulator of the chromatin structure and gene expression throughout the lifetime of the eukaryotic cell. An enhanced brain and thymus expression of HDAC4 has been seen during human embryonic development.

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. He also showed a frontal hair upsweep, long palpebral fissures and a full lower lip. 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.

Abstract ID: 34

Intra-operative prediction of brain tumor classes from nanopore data

B. Brändl[1], M. Steiger[2], C. Rohrandt[3], C. Kubelt[4], O. Ammerpohl[5], M. Synowitz[4], A. van Boemmel[6], H. Kretzmer[2], F.-J. Müller[1,2]

[1]Christian-Albrechts-Universität zu Kiel, Kiel, Deutschland, [2]Max-Planck-Institut für molekulare Genetik, Abteilung für Genomregulation, Berlin, Deutschland, [3]University of Applied Sciences Kiel, Kiel, Deutschland, [4]University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Deutschland, [5]Ulm University and Ulm University Medical Center, Ulm, Deutschland, [6]Max-Planck-Institut für molekulare Genetik, Berlin, Deutschland

Several diseases are associated with abnormalities in the genome as well as with differential DNA methylation. Thus, molecular classifiers can be utilized to identify conditions associated with specific epigenetic patterns, such as congenital or imprinting disorders. Furthermore, genome-wide methylation profiles even allow the differentiation of particular tumor entities. Nanopore sequencing is an emerging technology offering the opportunity to examine DNA sequence and methylation information only milliseconds after a DNA segment passes through a nanopore. Yet, the clinical interpretation of nanopore sequencing datasets in real-time has not been utilized so far. We conducted a study to demonstrate that an optimized nanopore-sequencing workflow combined with a novel statistical approach enables accurate and clinically relevant diagnosis of various CNS neoplasms in less than an hour. Notably, our statistical method does not require an explicit feature selection step, thus relying only on matching nanopore reads to empirically observed genome-wide CpG methylation patterns. Our algorithm can be trained on existing, publicly available array and second-generation sequencing datasets providing methylation rates for an arbitrary number of methylated CpGs. These methylation rates are utilized to interpret sparse, binary methylation events at any given number of the same CpGs present in Nanopore sequencing reads. The resulting tumor class models can be matched in real-time with methylation information from unbiasedly sequenced CpGs and thus provide a diagnostic assessment within one hour from biopsy to result. Benchmarking on Illumina Infinium 450k array data from CNS tumors (Capper et al., 2018) exemplifies that more than 95% of all samples can be classified correctly based on 1,000 randomly selected CpGs using our algorithm.

In summary, our results suggest that Nanopore sequencing could deliver a reliable molecular diagnosis of a set of predefined, distinct malignancies solely based on CpG methylation readouts. The development of robust machine learning models suitable for data streams offers the unique opportunity to obtain diagnostic information within the timeframe of an oncologic operation. We conclude that such tools will enable - among other applications - intraoperative sequencing for the diagnosis of brain tumors in neuro-oncology.

Abstract ID: 35

P3-35

When you hear hoofbeats, think zebra, not horse

J. Tecklenburg[1], M.K. Moddemann[1], J. Martinez[1], M. Drasdo[1], R. König[1]

[1]*Bioscientia Institut für Medizinische Diagnostik, Humangenetik, Ingelheim, Deutschland*

Introduction: Learning disability (LD) is defined as limitations in intellectual functioning and adaptive behaviour and occurs with a prevalence of 2-9.7%. Risk factors are prenatal and perinatal morbidity, psychological and physical trauma, exogenous factors and a family history of LD. About 50% of the cases have a genetic cause. First line investigations include history, physical examination and metabolic and radiological investigations. Genetic testing is recommended for confirming a suspected diagnosis or when no exogenous cause is established. Patients with LD face difficult access to the health care system and significant inequity in wellbeing compared to the average population resulting in a 20-year lower life expectancy.

Case Presentation:

Patient 1 was an 8 y/o girl presenting with LD, speech delay, mild muscular hypotonia and mild dysmorphic features. She had been born full term after a pregnancy complicated by maternal alcohol abuse. Both parents were affected by LD. She lived in foster care. Chromosome analysis and repeat-analysis of the *FMR1*-gene were unremarkable. Array CGH revealed a 1,4Mb duplication on chromosome 7q11.23 establishing the diagnosis of a 7q11.23 duplication syndrome that fully explained the patient’s phenotype. The same duplication was detected in the mother.

Patient 2 was an 11 y/o boy presenting with LD, speech delay and mild dysmorphic features. He was born full term after a pregnancy complicated by maternal alcohol abuse and had a healthy twin sister. Both parents were affected by LD and the twins lived in foster care. Chromosome analysis and repeat-analysis of the *FMR1*-gene were unremarkable. Array CGH revealed a 1,6Mb duplication on chromosome 3q29 establishing the diagnosis of a 3q29 duplication syndrome that fully explained the patient’s phenotype. The parents were not available for segregation analysis.

Conclusion: Identification of a genetic cause of LD helps to improve care and overcome health related inequities, especially if associated health risks are known. However, genetic testing may not be considered if other risk factors for LD are present. In both our cases fetal alcohol syndrome was suspected and the genetic cause for the phenotype would have been missed, if the foster parents had not pursued genetic examination. The cases highlight that the presence of exogenous factors for LD should not lead to the exclusion of a genetic diagnosis since they might rather be the result of than the cause for LD.

Abstract ID: 36

P3-36

COL4A1/*2*-related disorders - a report of two affected children with rare multisystem diseases

M.K. Moddemann[1], M. Drasdo[1], B. Oehl-Jaschkowitz[2], Z. Yüksel[1], R. König[1], J. Martinez Sena[1], I. Schwaab[1], J. Tecklenburg[1]

[1]Bioscientia Institut für medizinische Diagnostik GmbH, Humangenetik, Ingelheim, Deutschland, [2]Bioscientia MVZ Labor Saar GmbH, Humangenetik, Homburg, Deutschland

Introduction: The *COL4A1* and *COL4A2* gene encode the alpha-1 and alpha-2 chains of the connective tissue structural protein collagen type IV, which forms reticular structures and is found in the basement membrane of many organ systems. Pathogenic variants in the genes *COL4A1* and *COL4A2* cause a spectrum of rare autosomal-dominant multisystem diseases. These primarily include cerebral vascular defects, ocular abnormalities (i.e. microphthalmia, cataract, and refractive error), myopathy, and renal abnormalities. Imaging typically reveals diffuse periventricular leukoencephalopathy, porencephaly, lacunar infarcts, dilated perivascular spaces, aneurysms, and/or intracerebral hemorrhages. Life expectancy can be limited, especially due to hemorrhagic strokes. To the best of our knowledge, only about 100 families with *COL4A1*-related disorders have been described in the literature so far.

Case Report: Here, we report the clinical phenotype of two unrelated children (4 y/o girl and 1 y/o boy) with a pathogenic *de novo COL4A1*- and *COL4A2*-variant respectively, presenting with porencephaly/schizencephaly as well as periventricular leukomalacia and global developmental delay. The girl also showed spastic cerebral palsy and severe hypermetropia, whereas microcephaly was present in the boy. Whole exome sequencing was done at the Bioscientia Institute of Medical Diagnostics in Ingelheim.

Conclusion: *COL4A1/2*-related disorders are rare but due to the advancements in genetic analyses, especially using WES, there may be more patients diagnosed in the future. In approximately 50% of patients, the *COL4A1/2* variant was inherited from an affected parent. Cerebral palsy is caused by abnormal development or damage of the brain, only about 2% of cases are due to a genetic cause. To establish the diagnosis of a *COL4A1/2*-related disorder is particularly important to ensure regular and close clinical surveillance as well as genetic counselling.

Abstract ID: 37

P2-25

TP53 single hit and double hit events and their impact on prognosis in hematological malignancies

A. Stengel[1], M. Meggendorfer[1], W. Walter[1], C. Baer[1], S. Hutter[1], W. Kern[1], T. Haferlach[1], C. Haferlach[1]

[1]*MLL Munich Leukemia Laboratory, Munich, Deutschland*

TP53 is altered in ~50% of human cancers. Alterations (alt) mainly include mutations (mut) and deletions (del), but also copy-neutral loss of heterozygosity (cnLOH) was reported. Frequently, both *TP53* alleles are altered, leading to a “double hit” event. We analyzed *TP53* alt and their impact on overall survival (OS) using whole-genome sequencing (median coverage 100x) in 4646 cases with 29 different hematological malignancies. In the total cohort, in 582/4646 (13%) cases at least one alt involving *TP53* was detected (comprising mut, del and cnLOH). Cases were categorized into: (1) 1 *TP53* mut only (n=166), (2) del only (n=100), (3) cnLOH only (n=15), constituting the single hit (sh) events. Further, (4) cases with mut+del (might include 1 or more mut, n=211), cases with mut+cnLOH (≥1 mut, n=41), cases with ≥2 mut only (without del or cnLOH, n=49), resulting in double hit (dh) events. Thus, in 7% (41/582) of cases with *TP53* alt, cnLOH transforms a sh into a dh. Frequency of *TP53* alt and of sh vs. dh differs markedly between entities, as high frequencies of *TP53* alt were mainly detected in lymphoid malignancies (e.g. high grade B-cell lymphoma (HGBL), marginal zone lymphoma (MZL)), whereas they were infrequent or absent in many myeloid malignancies (e.g. chronic myeloid leukemia, myeloproliferative neoplasms (MPN)). Comparison of sh vs. dh revealed that MPN, myelodysplastic syndrome, multiple myeloma and T-cell neoplasms (T-NHL) predominantly showed a sh, whereas the dh was frequent in e.g. MZL, mixed phenotype acute leukemia (MPAL), chronic lymphocytic leukemia and mantle cell lymphoma (MCL) cases. However, the type of dh and thus the kind of *TP53* complexity differed between both lineages, as myeloid neoplasms showed a high frequency of cases with ≥2 mut only, whereas in many lymphoid malignancies the dh was predominantly generated by mut+del. All *TP53*-associated events were found to be associated with a complex karyotype in the total cohort and also in most entities (exceptions: MZL, T-NHL). Regarding OS, in the total cohort, all events involving *TP53* impact on OS. Moreover, although the sh already impacts on OS, the dh leads to an even inferior outcome (no hit vs. sh vs. dh: 84 vs. 39 vs. 14 months, $p<0.001$). The respective entities can be divided into three categories: (i) no significant influence of *TP53* alt on OS (HGBL, MZL, T-NHL), (ii) dh required for impact on OS (MCL, MPAL),(iii) influence of both sh and dh with inferior outcome of dh (all other).

Abstract ID: 38

P6-1

Altered Notch signaling in Dowling-Degos disease - a transcriptomic insight into disease pathogenesis

S. Kumar[1], J. Hausen[2], S. Sivalingam[2], A. Humbatova[1], A. Buness[2], J. Frank[3], D.J. Ralser[1], R.C. Betz[1]

[1]Institut für Humangenetik, Universitätsklinikum Bonn, Bonn, Deutschland, [2]Institut für Genomische Statistik und Bioinformatik, Universitätsklinikum Bonn, Bonn, Deutschland, [3]Klinik für Dermatologie, Venerologie und Allergologie, Universitätsmedizin Göttingen, Göttingen, Deutschland

Introduction: Dowling-Degos disease (DDD) is a rare autosomal-dominant hyperpigmentation disorder caused by mutations in *KRT5*, *POFUT1*, *POGLUT1* and *PSENEN*. There is growing scientific evidence that altered Notch signaling is implicated in DDD pathogenesis. The latter three causative genes encode for Notch pathway proteins while loss of *KRT5* expression is linked to altered Notch signaling. We examined transcriptional changes caused by *POGLUT1*/*PSENEN* siRNA-mediated knockdown applying unbiased transcriptome-sequencing to decipher a possibly common pathomechanism underlying DDD.
Methods: After transfecting distinct siRNAs targeting *POGLUT1* and *PSENEN* into a keratinocyte (HaCaT) - and melanocyte-derived (MZ7-mel) cell line, RT-PCR, RNAseq, pathway analysis and ELISA-based functional assessment of Notch activity were performed.
Results: In MZ7-mel cells, knockdown of either *POGLUT1* or *PSENEN,* leads to a pronounced decrease in Notch signaling activity. Comparison of the respective transcriptomic datasets revealed subtle differences across profiles; in particular, critical members of the Notch pathway were differentially expressed across all knockdown MZ7-mel cells. On the contrary, in HaCaT cells, no Notch activity was detectable, and pathway analysis revealed no enrichment of Notch signaling, although we demonstrated substantial reduction in *POGLUT1* and *PSENEN* expression, and the altered expression of other Notch pathway genes in HaCaT cells in our RNAseq data.
Our enrichment analysis based on a combined data set of POGLUT1- and PEN2-deficient cells yielded in four overrepresented pathways in MZ7-mel cells and six in HaCaT cells. Notch signaling was the most enriched pathway in MZ7-mel cells, while not being enriched at all in HaCaT cells. Furthermore, genes involved in estrogen signaling receptor-mediated signaling, receptor tyrosine kinase signaling and membrane trafficking were among highly overrepresented pathways in MZ7-mel cells only.
Conclusion: Our data show that only the melanocyte-derived cell line but not the keratinocyte-derived cell line exhibits decreased Notch signaling and relevant changes in transcriptional activity mediated by *POGLUT1/PSENEN* knockdown. Furthermore, a common downstream pathomechanism for both, *POGLUT1* and *PSENEN* mutation carriers can be assumed. Additionally, we identified three promising downstream pathways through which decreased Notch signaling may lead to the phenotypic manifestations in DDD.

Abstract ID: 39

P4-1

A transgenerational mutational signature from ionizing radiation exposure

F. Brand[1], H. Klinkhammer[1,2], M. Holtgrewe[3], A. Knaus[1], D. Beule[3], P. Krawitz[1]

[1]Institut für Genomische Statistik und Bioinformatik, Bonn, Deutschland, [2]Institut für Medizinische Biometrie, Informatik und Epidemiologie, Bonn, Deutschland, [3]Berlin Institute of Health, Core Unit Bioinformatics, Berlin, Deutschland

Effects of accidental and prolonged radiation exposure on the human germline remain a topic of medical interest. Transgenerational signatures of ionizing radiation exposure are primarily implicated to be clustered *de-novo* mutations (cDNMs, multiple *de-novo* mutations within 20bp).

We sequenced the whole genome of 110 children from 70 families (Radar cohort). In addition, we analyzed 130 children who were born to cleanup workers of the Chernobyl nuclear accident for a total of 240 children and their likely exposed parents (Chernobyl cohort, CRU). 1275 offspring from unexposed families serve as population reference. Dosage estimations have been obtained for all exposed persons in both exposed cohorts. The "*Strahlenmessstelle der Bundeswehr*" estimates 0-353 mSv of exposure for radar soldiers, while exposures range from 0-4,080 mSv in the Chernobyl cohort. All genome data was processed using identical bioinformatics pipelines and joint-called using GLNexus, to ensure consistent data quality.

In agreement with previous works, we found no change in the number of isolated DNMs between the exposed and control groups ($p > 0.4$), when accounting for age differences between the groups. Also, in line with other literature, we found a paternal age effect with an increase of roughly 2% of DNMs per year of age of the father.

In total, we observed 1,475 cDNMs with a median of two clustered mutations per offspring of Radar soldiers and of Chernobyl cleanup workers. This is a significant increase ($p < 0.005$) in the number of cDNMs compared to a set of age matched controls. Furthermore, we show that the number of cDNMs increases with increasing paternal exposure to ionizing radiation ($b = 0.0005$, $p < 0.001$). Since error rates for cDNMs are expected to be high in contrast to isolated DNMs, we performed an iterative validation and assessment of cDNMs using short read alignments and Sanger and PacBio sequencing data for selected mutations. The positive predictive value (PPV) for cDNMs is estimated at 0.24. In simulations accounting for the PPV, the difference in cDNM rates remains significant. Using read-based phasing methods, we could phase 25 cDNMs of the radar cohort with a paternal to maternal ratio of 17:8 and 46 cDNMs in the CRU cohort with a ratio of 25:21.

Although the impact on health for subsequent generations is likely minimal, our findings confirm cDNMs as transgenerational biomarker for paternal exposure to ionizing radiation.

Abstract ID: 40

P3-37

Establishment of a Genetic Counsellor association for the German-speaking countries

G. Schwaninger[1], S. Heidemann[2]

[1]Medizinische Universität Innsbruck, Institut für Humangenetik, Innsbruck, Österreich, [2]Institut für Tumorgenetik Nord, Kiel, Deutschland

Since the start of an MSc programme for genetic counselling in Innsbruck in 2019, the Genetic Counsellor (GC) profession is established in Germany (D), Austria (A), and Switzerland (CH). While the French-speaking areas of Switzerland have employed GCs for several years, recently also the remaining cantons are moving forward with their implementation. In Germany and Austria, regulation is still missing but efforts to create a sound basis for the profession are being undertaken. The vision of the Genetic Counsellor Association D-A-CH (GCA D-A-CH) is the successful introduction and support of the genetic counsellor profession in all German-speaking countries.

Mission:

1. Establishment of a professional association for German-speaking GCs affiliated with the German Society for Human Genetics (GfH). Through participation in the GfH and its committees, interprofessional cooperation in human genetics is strengthened.
2. Recognition and implementation of the profession of GCs in Germany, Austria and Switzerland.
3. Definition of a "Scope of Practice" for GCs in the German-speaking region, to facilitate collaboration in interprofessional teams and the development of financing models for GCs.
4. Cooperation with international academic societies and interest groups for GCs, such as the Association Suisse des Conseillers en Génétique, and recognition of the qualification of German-speaking GCs in the international realm. Support of registration as European Certified Genetic Counsellors with the European Board of Medical Genetics.
5. Development and support of continuing education and supervision in the field of genetic counselling as an integrated component of genetic services. Benefiting from synergy effects, in particular with the GfH and the Human Genetics Academy.
6. Development and support of academic teaching and research in the field of genetic counselling.

Internationally, GCs increase access to genetic counselling for patients, expand counselling capacity, and promote genetic competency in the healthcare systems. They work in close cooperation with medical specialists in human genetics/medical genetics, to facilitate prompt and competent genetic services. GCs have a unique set of skills that they add to the interprofessional human genetic teams, especially in the support of patients and their families in complex decision-making and psychosocial distress. GCs can help guarantee the broad accessibility of human genetic services for the population in the D-A-CH region.

Abstract ID: 41

P7-9

Inferring gene-level expression levels from mid-to-low coverage cell-free DNA shotgun sequencing

K. Köhler[1], S. Röner[1], F. Kapidzic[2], M. Karic[2], M.R. Speicher[2], M. Kircher[1,3]

[1]Berlin Institute of Health (BIH) at Charité- Universitätsmedizin Berlin, Berlin, Deutschland, [2]Institute of Human Genetics, Diagnostic and Research Center for Molecular BioMedicine, Medical University of Graz, Graz, Österreich, [3]Institute of Human Genetics, University Medical Center Schleswig-Holstein, University of Lübeck, Lübeck, Deutschland

Analyzing cell-free DNA (cfDNA) from liquid biopsies can facilitate the diagnosis and monitoring of diverse physiological conditions like pregnancies, donor organ rejection, or cancer progression. Prior studies highlighted that DNA fragmentation in these samples is linked to nucleosome occupancy and binding of transcription factors, suggesting that fragment positioning and resulting sequence coverage around a gene's transcription start sites (TSS) carries information on its transcriptional activity. Here, we present a machine learning model that leverages various features around TSSs to predict gene-level expression, opening another window into capturing the tissue-specific, transcriptional landscapes of contributing cells. Therefore, a random forest regression model was trained on >19,000 genes measured by proteinatlas.org with cfDNA feature values (e.g., coverage, windowed protection and Fourier Transform intensities) derived from healthy donor samples and sample-independent features (e.g., promoter GC, average expression across tissues). As reference, protein atlas log-expression values (RPM) from peripheral blood mononuclear cells (PBMCs) were used, as these are expected to have the largest contribution to the plasma DNA pool of healthy individuals. After hyper-parameter optimization, a feature-trimmed model (20 most important SHAP features) was used to infer gene expression of 14 independent samples (8 healthy, 4 prostate cancer, 2 colon cancer). Resulting gene expression predictions were compared using Wilcoxon rank sum (WRS) tests and Pearson correlations and tissue-specific expression patterns were analyzed.
On the hold-out set, the model showed a high Pearson correlation of 0.83 between PBMC and predicted expression confirming the large PBMC contribution to the cfDNA and excellent training performance. Comparing inferred expression patterns between unseen healthy and cancer samples showed a clear separation in WRS p-value distributions between both conditions. Moreover, we observed a significant decrease in correlation between blood-associated tissues (e.g., PBMCs, granulocytes) and cancer samples in comparison to healthy donor samples, likely reflecting the increased proportion of epithelial DNA in the circulation of cancer samples. Thus, we demonstrate a new avenue into capturing the transcriptional landscapes of contributing cells and the potential for applying expression-derived deconvolution approaches to liquid biopsy data.

Abstract ID: 42

TMCO3, a putative K^+:proton antiporter at the Golgi apparatus, is important for longitudinal growth in mice and humans

T. Holling[1], T. Scholz[1], T. Bierhals[1], T. Herget[1], P. Meinecke[1], T. Schinke[2], R. Oheim[2], K. Kutsche[1]

[1]University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Deutschland, [2]University Medical Center Hamburg-Eppendorf, Department of Osteology and Biomechanics, Hamburg, Deutschland

Isolated short stature is a common genetically heterogeneous condition. Family-based whole-exome sequencing is a useful tool to identify the underlying genetic defect in known and novel causative genes. We report two sisters with isolated short stature. Radiological studies, biochemical measurements, assessment of the skeletal status, and 3D bone microarchitecture revealed no clinically relevant skeletal and bone abnormalities in both affected individuals. By whole-exome sequencing in the two affected individuals and their normal height parents, we identified the homozygous nonsense variant c.1832G>A/p.(Trp611*) in the disease gene candidate *TMCO3* in both sisters. Sanger sequencing confirmed segregation of the *TMCO3* variant with short stature in the large family. RT-qPCR revealed reduced *TMCO3* mRNA levels by ~50% in leukocyte-derived cDNA of both affected sisters compared with controls, likely due to nonsense-mediated mRNA decay. *TMCO3* encodes a functionally uncharacterized protein. Coordinated co-expression of C-terminally HA-tagged TMCO3 wildtype or $TMCO3^{W611*}$ mutant with EGFP in HeLa cells followed by immunoblotting revealed a drastically reduced $TMCO3^{W611*}$ level compared with TMCO3 wildtype. Subcellular localization analysis by immunofluorescence of ectopically expressed C-terminally EGFP-tagged TMCO3 in HeLa cells identified wild-type TMCO3 predominantly at the Golgi apparatus and in cytoplasmic vesicles, while $TMCO3^{W611*}$ did not reach the Golgi. By *in silico* tools and homology modeling, TMCO3 is predicted to have an N-terminal secretory signal peptide, forms a dimer localized to the membrane, is organized in a dimerization and a core domain, and contains a cation:proton antiporter 2 (CPA2) motif essential for K^+ binding and selectivity. Thus, TMCO3 is a potential member of the monovalent CPA2 family mediating the exchange of K^+ for one or two protons across the membrane. Monitoring of *Tmco3* expression in different mouse tissues and cultured skeletal cells confirmed *Tmco3* expression in all relevant skeletal cell types. Measurement of long bones in publicly available X-ray images of *Tmco3* knockout mice revealed significant shortening of fore- and hindlimbs. Combined data from human and mouse with biallelic loss-of-function *TMCO3* variants as well as from a thale cress triple mutant of the *TMCO3* homologs *kea4-6* suggest that the maintenance of cellular and organelle ion and pH homeostasis by cation:proton antiporters seems to be important for growth.

Abstract ID: 43

P5-14

Cas9-mediated nanopore sequencing enables precise characterization of structural variants in *CCM* genes

D. Skowronek[1], R.A. Pilz[1], L. Bonde[1], O.J. Schamuhn[1], J.L. Feldmann[1], S. Hoffjan[2], C.D. Much[1], U. Felbor[1], M. Rath[1,3]

[1]University Medicine Greifswald, and Interfaculty Institute of Genetics and Functional Genomics, Department of Human Genetics, Greifswald, Deutschland, [2]Ruhr-University, Department of Human Genetics, Bochum, Deutschland, [3]MSH Medical School Hamburg, Department of Human Medicine and Institute for Molecular Medicine, Hamburg, Deutschland

Introduction: Familial cerebral cavernous malformations (CCMs) which are caused by heterozygous germline variants in the *CCM1*, *CCM2*, or *CCM3* gene can cause severe neurological complications. Deletions in the *CCM* genes are a common cause of this neurovascular disease. In current molecular genetic laboratories, targeted next-generation sequencing or multiplex ligation-dependent probe amplification are mostly used to identify copy number variants (CNVs). However, both techniques are limited in their ability to specify the breakpoints of CNVs and identify complex structural variants (SVs).
Material and Methods: To overcome these constraints, we established a targeted Cas9-mediated nanopore sequencing approach for CNV detection with single nucleotide resolution. The libraries were sequenced on an Oxford Nanopore MinION device. Breakpoint information was used to perform variant-specific PCR analyses and Sanger sequencing.
Results: We achieved complete coverage for the *CCM* genes and determined the exact size of CNVs in all positive controls. Long-read sequencing for a *CCM1* and *CCM2* CNV revealed that the adjacent *ANKIB1* and *NACAD* genes were also partially or completely deleted. In addition, an interchromosomal insertion and an inversion in *CCM2* were reliably re-identified by long-read sequencing. The refinement of CNV breakpoints by long-read sequencing enabled fast and inexpensive PCR-based variant confirmation, which is highly desirable to reduce costs in subsequent family analyses.
Conclusion: Used as a complementary approach to short-read sequencing, Cas9-mediated nanopore sequencing is a cost-effective and flexible tool for molecular genetic diagnostics and can be easily adapted to various target regions.

Abstract ID: 44

P3-38

Clinical exome analysis revealed a mosaic *KMT2A* deletion in a patient with unexplained developmental delay

S. Döhnert[1], H. Reichenbach[2], B. Eichhorn[1]

[1]MVZ Mitteldeutscher Praxisverbund Humangenetik GmbH, Dresden, Dresden, Deutschland, [2]MVZ Mitteldeutsche Praxisverbund Humangenetik GmbH, Leipzig, Leipzig, Deutschland

Introduction: Clinical exome sequencing (SNV and CNV) has a high benefit for genetically unsolved cases, especially for children with developmental disorders. In contrast, targeted analysis for few genes is an expensive and time-consuming challenge for these patients. Furthermore, the diagnostic yield of exome sequencing is about 30% for patients with rare diseases and is further increased up to 41% if focused on children with developmental delay. Latter includes Wiedemann-Steiner syndrome (WSS) and associated phenotypes, which are caused by pathogenic variants in *KMT2A* gene. WSS is characterized by facial features, developmental delay and intellectual disability and inherited in an autosomal dominant manner.

Material and Methods: Here, we report a 21-year-old male with the following clinical symptoms: short stature, microcephaly, facial dysmorphic features (flat forehead; vertically narrow palpepral fissures; alternating strabismus; small, low-set and retroverted ears; broad thumbs and clinodactyly), hypertrichosis of the arms and legs, vertebrostenosis of the craniocervical junction, mental retardation and fine motor skill deficits. A trio clinical exome diagnostic via sequencing by synthesis was conducted. Our result was verified by a targeted gene panel. Beside analysis of DNA from peripheral blood, we also examined DNA from buccal mucosa.

Results and Discussion: The CNV analyses of our patient showed reduced signals for *KMT2A* exons 17 to 26, confirmed by targeted gene panel analysis in DNA from blood as well as from buccal swab. About 30% of the alleles carry this *de novo* deletion. This intragenic deletion is previously undescribed, but Mendelsohn *et al.* 2014 (Am J Med Genet A. 164:2079) have detected another intragenic deletion in a patient with WSS. Regarding mosaic *KMT2A* mutations, a splice site variant is described in a patient with a mild Cornelia de Lange syndrome (CdLS) like phenotype. The variant was heterozygous detected in DNA from buccal swab, but not in DNA from blood (Krawczynska *et al.* 2019 Front. Pediatr. 7:203). Additional, Li *et al.* 2018 (Orphanet J R Dis 13:173) identified a mosaic (23%) *KMT2A* nonsense variant in DNA from blood of a patient with severe form of WSS.

Conclusions: In summary, this mosaic deletion im *KMT2A* is causative for the phenotype of our patient. Pathogenic variants in *KMT2A* gene are associated with the WSS and syndromic intellectual disability respectively, for example CdLS-like phenotype.

Abstract ID: 45

Elucidating the genetic network in SHOX2-dependent atrial arrhythmias using human iPSC-derived cardiomyocytes

K. Rädecke[1,2], S. Hoffmann[1,2], A. Löwen[3], S.A. Sumer[3], S. Hücker[4], F. Zhang[5,6], B. Campbell[5], A. Paasche[7,2], A. Chan[8,2], V. Eckstein[9], T. Dorn[5,6], C. Dieterich[8,2], C. Schmidt[7,2], S. Kirsch[4], A. Moretti[5,6], G.A. Rappold[1,2]

[1]Institute of Human Genetics, Heidelberg, Deutschland, [2]DZHK, German Center for Cardiovascular Research, Partner Site, Heidelberg/Mannheim, Deutschland, [3]Institute of Human Genetics, AG Kardiogenetics, Heidelberg, Deutschland, [4]Fraunhofer-Institute for Toxicology and Experimental Medicine, Division of Personalized Tumor Therapy, Regensburg, Deutschland, [5]Klinikum Rechts der Isar - Technical University of Munich, First Department of Medicine, Munich, Deutschland, [6]DZHK, German Center for Cardiovascular Research, Partner Site, Munich, Deutschland, [7]Heidelberg Center for Heart Rhythm Disorders, Heidelberg, Deutschland, [8]Klaus Tschira Institute for Integrative Computational Cardiology, Heidelberg, Deutschland, [9]University Hospital Heidelberg, FACS Core Facility, Heidelberg, Deutschland

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia, and there is a strong genetic component that contributes to the disease. The coordinated contraction of atrial muscle depends on regulatory networks, in which transcription factors drive downstream effectors to maintain proper cardiac conduction. The transcription factor SHOX2 is involved in the development and function of the sinoatrial node (SAN), the primary pacemaker of the heart, and is one of the main pacemaker genes associated with conduction-related diseases. *SHOX2* variants within the coding region and the 3´UTR have been identified in early-onset AF patients and functionally characterised in different model systems.
To model SHOX2-related conduction dysfunction in a human system, iPSCs have been generated from an AF patient carrying a functional non-coding *SHOX2* variant in the 3´UTR (c.*28T/C; rs138912749), together with isogenic control lines. By differentiating these cells to nodal and atrial cardiomyocytes, SHOX2-related atrial fibrillation and its associated genetic networks can be analysed. First, we showed that *SHOX2* expression and function are significantly impaired in patient-derived SAN cells compared with isogenic control SAN cells. Furthermore, action potential characteristics and expression of genes involved in cardiac electrophysiology and development were significantly altered in patient-derived SAN cells, providing a mechanistic understanding of this disease.
To generate single SAN cells from iPSCs, we have isolated SHOX2 positive nodal cardiomyocytes from a mixed population. The workflow included a differentiation and maturation period, sorting of cardiomyocytes from heterogenous cell pools, labelling of SHOX2 positive cells via viral transduction of lineage-specific promoter-driven fluorescent reporter gene constructs, and subsequent FACS sorting. Single-cell RNA sequencing of SHOX2 pacemaker cells was performed to dissect transcriptional profiles of patient- and isogenic control cell lines, to assign specific alterations and uncover drivers of dysfunctional signalling pathways.
Our data show that this human iPSC model of SHOX2-related AF reflects both the phenotype seen in early-onset AF patients and Shox2 animal models, and thus is well suited to understand how *SHOX2* variants influence the occurrence and progression of atrial arrhythmias.

Abstract ID: 46

P3-77

The phenotype in adulthood of rare genetic diseases: Nicolaides-Baraitser, Coffin-Siris, Costello and cardiofaciocutaneous syndrome

A. Schmetz[1], N. Bramswig[1], M. Zenker[2], D. Wieczorek[1,3]

[1]Institute of Human Genetics, Medical Faculty and University Hospital Düsseldorf, Düsseldorf, Deutschland, [2]Institute of Human Genetics, University Hospital Magdeburg, Magdeburg, Deutschland, [3]Center for Rare Diseases, Medical Faculty and University Hospital Düsseldorf, Düsseldorf, Deutschland

Introduction: In the past decades, the widespread use of next generation sequencing technologies has enabled the identification of the genetic basis for many rare diseases such as Nicolaides-Baraitser (*SMARCA2),* Coffin-Siris (*ARID1B, ARID1A, SMARCB1, SMARCA4, SMARCE1, ARID2, DPF2, SMARCC2, SOX11, SOX4, SMARCD1, BICRA*), Costello (*HRAS*) and cardiofaciocutaneous syndromes (*BRAF, KRAS, MAP2K1, MAP2K2*).
However, there is very little information on the clinical phenotype of these rare syndromes in adulthood. In particular, the long-term outcomes and associated risks are not well known.
Methods: Through an ERN-ITHACA call for collaboration, social media, collaboration with syndrome-specific foundations and collaborating colleagues, we are recruiting adult patients with one of the above mentioned rare genetic syndromes. Participants' phenotype will be assessed using a comprehensive questionnaire.
Results: Recruitment was ongoing at the time of abstract submission. However, we have already had feedback from physicians or families from 10 patients with Nicolaides-Baraitser, 20 patients with Coffin-Siris, 11 patients with cardiofaciocutaneous and three patients with Costello syndrome. Preliminary analyses of the phenotype of selected patients indicate interesting new features of these syndromes in adulthood. In particular, there is evidence of dental and ocular problems in Nicolaides-Baraitser syndrome and Coffin-Siris syndrome that need further investigation.
Conclusion: In order to fill the knowledge gap on adult phenotype in these rare genetic syndromes, we initiated our study as a collaborative project. The phenotypic data from our adult-only cohort of patients will allow a more accurate prediction of the relative risks in these syndromes and will be of great value to affected patients and their caregivers in making preventive and therapeutic decisions.

Abstract ID: 47

Disruption of *zfhx4* leads to defects in zebrafish craniofacial development matching features of human nonsyndromic cleft lip with/without cleft palate

N. Ishorst[1,2], S. Hölzel[1,2], C. Greve[1,3], F. Degenhardt[1,4], Ö. Yilmaz[2], T. Lindenberg[2], J. Lambertz[2], D. Drichel[5], C. Maj[6], M. Nothnagel[5,7], J.Y. Hehir-Kwa[8], J.A. Veltman[9], B. Zametica[1], T. Kruse[10], S. Nowak[1], C. Carels[11], I. van Rooij[12], K.U. Ludwig[1], B. Odermatt[2], E. Mangold[1]

[1]Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Deutschland, [2]Institute of Anatomy, Division of Neuroanatomy, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Deutschland, [3]current address: LOEWE Centre for Translational Biodiversity Genomics, Frankfurt am Main, Deutschland, [4]current address: Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Hospital Essen, University of Duisburg-Essen, Duisburg, Deutschland, [5]Cologne Center for Genomics, University of Cologne, Köln, Deutschland, [6]Institute for Genomic Statistics and Bioinformatics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Deutschland, [7]University Hospital Cologne, Köln, Deutschland, [8]Donders Institute, Radboud University Medical Center, Department of Human Genetics, Nijmegen, Niederlande, [9]Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle-upon-Tyne, Vereinigtes Königreich, [10]University of Cologne, Department of Orthodontics, Köln, Deutschland, [11]KU Leuven, Department of Human Genetics, Leuven, Belgien, [12]Radboud Institute for Health Sciences, Radboud University Medical Center, Department for Health Evidence, Nijmegen, Niederlande

Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is a frequent congenital malformation with a multifactorial background and high heritability estimates. Although >40 common nsCL/P risk loci have been identified so far, major fractions of its complex genetic background yet remain unknown. Recent studies have shown that rare highly penetrant variants also contribute to nsCL/P risk. The aim of the present study was to identify novel candidate genes for nsCL/P by detecting rare copy number variants (CNVs).
We reanalyzed an exome sequencing dataset comprising 50 European nsCL/P trios with an affected index patient and unaffected parents by combining CNV-calling algorithms that were based on depth-of-coverage (XHMM, CoNIFER, EXCAVATOR2). After stringent quality control, we identified a heterozygous 86 kb *de novo* deletion that affects exons 3 to 11 of 11 of *ZFHX4* and that could be validated using quantitative PCR. Targeted sequencing of *ZFHX4* in 711 independent nsCL/P patients identified two novel truncating *ZFHX4* mutations in two patients. *ZFHX4* encodes the transcription factor Zinc Finger Homeobox 4 which recently has been shown to be enriched for *de novo* mutations in patients with nonsyndromic orofacial clefting.
To functionally characterize the role of *ZFHX4* in zebrafish craniofacial development, we knocked down/out the zebrafish orthologue *zfhx4* in wild type zebrafish larvae (zfl) by a translational blocking morpholino (KD) and transient CRISPR/Cas9 F_0-generation knock-out (KO). Cartilage staining of KD and KO zfl at 4 days post-fertilization (dpf) showed abnormally shaped anterior neuro- and viscerocranium, including the Meckel´s cartilage (homolog to the human lower jaw) and the ethmoid plate (homolog to the human hard palate). To systematically quantify these changes we measured the Meckel's-palatoquadrate angle in 4 dpf cartilage stained zfl and could show a statistically significant difference between KD/KO zfl in comparison to controls. The Meckel's-palatoquadrate angle is a parameter for the assessment of craniofacial defects in zfl.
In conclusion our human genetic findings and subsequent functional studies in zfl further emphasize the importance of *ZFHX4* in the process of craniofacial development and further confirm its role as susceptibility gene for nsCL/P.

Abstract ID: 48

Epigenetically regulated microRNA-449a inhibits triple negative breast cancer by inducing chromosomal instability

B. Vajen[1], R. Bhowmick[2], V. Schäffer[1], M. Eilers[1], T. Reinkens[1], A. Stalke[1], G. Schmidt[1], J. Fiedler[3], T. Thum[3], D. DeLuca[4], I.D. Hickson[2], B. Schlegelberger[1], T. Illig[1], B. Skawran[1]

[1]Medizinische Hochschule Hannover, Institut für Humangenetik, Hannover, Deutschland, [2]University of Copenhagen, Center for Chromosome Stability, Copenhagen, Dänemark, [3]Medizinische Hochschule Hannover, Institute of Molecular and Translational Therapeutic Strategies, Hannover, Deutschland, [4]Medizinische Hochschule Hannover, German Center for Lung Research, Hannover, Deutschland

Introduction: Chromosomal instability (CIN) can be a driver of tumorigenesis, but is also a promising therapeutic target for cancer associated with poor prognosis such as triple negative breast cancer (TNBC). Treatment of TNBC cells with defects in DNA repair genes with poly(ADP-ribose) polymerase inhibitor (PARPi) massively increases CIN, resulting in apoptosis. Here, we identified a previously unknown role of microRNA-449a in CIN.
Methods: Newly identified targets of microRNA miR-449a were identified by microarray analyses followed by RNA-immunoprecipitation and luciferase assays in the TNBC cell lines (HCC38, HCC1395, HCC1937). Meta-analyses on gene expression were performed using published annotated breast cancer transcriptomic data. Proliferation and apoptosis were analyzed after ectopic expression of miR-449a or silencing gene expression by si-RNA. Chromosomal instability was detected by immunofluorescence analyses of ultrafine bridges, 53BP1 nuclear bodies and yH2AX signals.
Results: Here, we identified a previously unknown role of microRNA-449a in CIN. Ectopic expression of miR-449a led to induced apoptosis, reduced cell proliferation, and reduced expression of genes in homology directed repair (HDR) in TNBC cell lines. *EME1* was identified as a new target gene by immunoprecipitation and luciferase assays. The reduced expression of *EME1* led to an increased frequency of ultrafine bridges, 53BP1 foci, and micronuclei.
Induced expression of microRNA-449a elevated CIN beyond tolerable levels and induced apoptosis in TNBC cell lines by two different mechanisms: (I) promoting chromatid mis-segregation by targeting endonuclease *EME1* and (II) inhibiting HDR by downregulating key players of the HDR network such as *E2F3, BIRC5, BRCA2* and *RAD51*. Ectopic expression of miR-449a enhanced the toxic effect of PARPi in cells with pathogenic germline *BRCA1* variants.
Conclusion: The newly identified role makes microRNA-449a an interesting therapeutic target for TNBC.

Abstract ID: 49

P5-5

Rare prenatal case with trisomy 22 and triploidy mosaicism – need for cytogenetic expertise

A.-C. Teichmann[1], J. Hentschel[1], H. Oppermann[1]

[1]*Institute of Human Genetics, University of Leipzig Hospitals and Clinics Leipzig, Leipzig, Deutschland*

Genetic testing was initiated in the 27th pregnancy week due to intrauterine growth retardation, hypoplastic nasal bone, less mobile fetus in flexion, oligohydramnion, Blake´s pouch cyst, hypoplastic corpus callosum and vitium cordis. The pregnant woman had previously a miscarriage at 20 weeks of gestation probably caused by an anhydramnion.
We performed Short Tandem Repeat (STR) analysis of native amniotic fluid as first tier to screen for numeric aberration of chromosomes 13, 18, 21, X and Y, and we cultured amniocytes for standard chromosome analysis. Due to the multiple ultrasound abnormalities, we initiated in parallel exome sequencing.
The STR analysis revealed an unremarkable result for numeric aberration of chromosomes 13, 18, 21 at female sex. However, there was evidence for maternal contamination of 15-30 %. Exome sequencing including copy number variation (CNV) analysis revealed no pathogenic variants. Surprisingly, the chromosome analysis showed a mosaic of cells with trisomy 22 and triploidy. There were no normal cells visible. We performed re-sequencing of the initial material, as the quality of the first exome sequencing did not fulfil our standards regarding coverage and uniformity. After re-sequencing, data merging and with the knowledge of the mosaic, the trisomy 22 was now detectable by CNV analysis. Despite the triploidy still wasn´t detectable by coverage based CNV-calling algorithms, analysing the alternative allele frequency of parental inherited variants could also detect the triploidy by exome sequencing.
Diploid/triploid mosaicism is a very rare condition. The triploid line typically reflects digyny. It is suspected that the inclusion of the second polar body at a very early stage after conception of a diploid zygote may the pathomechanism. In this special case, first a nondisjunction leads to an oocyte with 24,X,+22 and a polar body with 22,X,-22. After conception with a 23,X sperm, the primary karyotype was 47,XX,+22 and finally the inclusion of the polar body with 22,X,-22 leads to the 69,XXX karyotype.
Despite all the benefits of next-generation-sequencing, single cell based cytogenetics can reliably identify chromosome abbreviations, which can be missed by exome sequencing. Although diploid/triploid mosaicism is a rare condition, in a state of the art diagnostic setting, it underlines that labs and geneticists should also be able to analyse, detect and understand chromosomal abnormalities using cytogenetic methods.

Abstract ID: 51

P3-39

Siblings with two different molecular diagnoses – a challenge for a family-based exome sequencing

B. Eichhorn[1], S. Riedel[1], M. Klaus[1], S. Demuth[2]

[1]MVZ Mitteldeutscher Praxisverbund Humangenetik GmbH, Dresden, Deutschland, [2]MVZ Mitteldeutscher Praxisverbund Humangenetik GmbH, Erfurt, Deutschland

Exome sequencing is becoming more important as a first-tier analysis for children with neurodevelopmental disorders. Particularly, the involvement of parents and equally affected sibling in this complex examination represents an effective tool. However, you should be aware about mistakes in data interpretation, especially concerning the family constellation.
Here, we report a family with two affected children and healthy parents. Both siblings (7 $^{2}/_{12}$ year-old girl, 5 $^{9}/_{12}$ year-old boy) showed intellectual disability, language delay and behavioural psychiatric manifestations. Certainly, the boy additionally had facial dysmorphism (deep front hairline, deep-set eyes, down slanted palpebral fissures, long and narrow nasal bridge, mild retrognathia, small and triangular ears with preauricular fistula). Since the quatro exome analysis (sequencing by synthesis) was unremarkable, the evaluation was separately repeated for each child with the parents.
The girl carried the heterozygous variant c.2579C>G / p.(Ser860*) in the *EHMT*1 gene (NM_024757.5), which is previously undescribed in mutation and population databases. The *de novo* occurrence of the *EHMT1* variant and the clinical characteristics (intellectual disability, autistic-like features) confirms a Kleefstra syndrome for the child.
Surprisingly, the boy harbour another *de novo* heterozygous variant c.6001C>T / p.(Arg2001*) in the *ZNF292* gene (NM_015021.3), which is also previously undescribed. Pathogenic variants in *ZNF299* gene cause an intellectual developmental disorder with autosomal dominant inheritance (MRD64). The typical facial dysmorphism, impaired intellectual development, speech delay and behaviour disorders is in accordance with the symptoms of this patient.
Family-based exome sequencing poses a great challenge for data analysis and represents an appreciable opportunity to establish a diagnosis for children with neurodevelopmental disorders. Here, two different neurodevelopmental disorders in siblings were identified.

Abstract ID: 52

P3-40

Case report: rapid progressive ataxia and mild cognitive impairment due to digenic inheritance of pathogenic variants in *TGM6* and *PRNP*?

M. Neidhardt[1], P. Winter[1], A. Weber[1], D. Nolte[1]

[1]*Justus-Liebig-Universität Gießen, Institut für Humangenetik, Giessen, Deutschland*

The index patient came to clinical attention at age 54. She exhibited a variety of symptoms, including rapidly progressive gait disturbance with trunk, stance, and gait ataxia. Reduced bilaterally reflexes with positive Babinski's sign were noted. In addition, a resting tremor, dysarthric speech, and motor impairments in writing were prominent. Mild cognitive impairment was also observed. However, EEG, cMRI, and CSF analyses revealed no pathologic findings. Family history was positive for a movement disorder combined with mild cognitive decline, so molecular ataxia screening was initially initiated.
The heterozygous variant c.688G>A (p.Asp230Asn, rs147591485) was detected in gene *TGM6*, which is associated with spinocerebellar ataxia type 35 (SCA35) (Wang et al., 2010). The variant segregates with the disease, as it was also detected in the affected father, who has since died. Entries in ClinVar and bioinformatics prediction programs resulted in an inconsistent classification of the variant, which was therefore classified as a variant of uncertain significance.
Reevaluation of the father's clinical findings revealed evidence for the possible presence of a prion disease in the sense of a Gerstmann-Sträußler-Scheinker syndrome (GSS) (Hsiao et al., 1989). A genetic analysis of the father's DNA was inconclusive at this time. However, in the daughter, the heterozygous pathogenic variant c.305C>T (p.Pro102Leu; rs74315401) was detected in the *PRNP* gene. Therefore, a diagnosis of GSS should also be considered in ataxia patients with cognitive impairment and positive family history. It is possible that in the case presented here, the clinical picture is exacerbated by the additional variant in *TGM6*.

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2. Hsiao K, Baker HF, Crow TJ, et al. (1989) Linkage of a prion protein missense variant to Gerstmann-Straussler syndrome. Nature 338: 342-345.

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P3-41

CYLD cutaneous syndrome. A case presentation

D. González Fassrainer[1], T. Schulz[2], A. Wernstedt[1], C. Nevinny-Stickel-Hinzpeter[1]

[1]Synlab Humane Genetik München, Genetik, München, Deutschland, [2]Helios Klinikum München West, Klinik für Hals-Nasen-Ohren-Heilkunde, München, Deutschland

CYLD cutaneous syndrome (OMIM #605018) is a rare genetic autosomal dominant disorder with variable penetrance. Its main feature consists of the development of multiple adnexal tumors that originate from the follicular-sebaceous-apocrine unit, most commonly: cylindromas, trichoepitheliomas and spiradenomas. Although the tumors are usually benign, malignant transformation is recognized. We report a 67-year old male who presents to the dermatologist with lesions in the left earlobe. Anamnestic he had a wound that wouldn't heal, his father had similar lesions around the nose with a history of "bad skin". After examination and skin biopsy a multicentric basalioma was diagnosed. Few months later same lesions appeared on the right earlobe. After generous resection of the affected area, the histological analysis was unclear (solid carcinoma, Trichoblastoma). Due to the results of the histological findings and the rapid growth of the lesions, it was recommended to pursue the correct course of the therapy. Suspected Diagnosis based on clinical findings: Brooke -Spiegler syndrome.
Method: After isolation of the patient´s genomic DNA, the coding regions including the adjacent intronic sequences were enriched with the TWIST Human Core Exome Kit (Twist Bioscience). Massive parallel sequencing was then performed on the NextSeq 550 instrument (Illumina). The alignment and the analysis of deviations (variant calling (SNV) and copy number variations (CNV)) of the sequence data were carried out using the software Varvis - version 1.19.1 - (Limbus Medical Technologies GmbH) against the human reference genome GRCh37-1kG. NGS-Panel (*CYLD, PLCD1, PTCH1, SMO, SUFU, TP53*)
Results: *CYLD* gene pathogenic variant c.2290A>T, p.(Lys764*) heterozygous.
Conclusion: CYLD cutaneous syndrome or Brooke-Spiegler Syndrome is a rare disorder. Although the majority of neoplasms associated with Brooke–Spiegler syndrome are benign, malignant transformation does occur in 5 to 10 % of cases. The finding draws attention to the importance of lifetime monitoring. Radiotherapy should be avoided as it causes DNA damage and may result in further tumor formation or malignant transformation of existing lesions. Physical examination, histological findings and genetic analysis are important for establishing the right diagnosis and treatment. The treatment remains a challenge and must be individualized based on the type, location and number of the lesions.

Abstract ID: 54

P5-6

Case report of two patients carrying a partial trisomy 16q

C. Wilmsen[1], E. Lausch[2], U. Matysiak[2], K. Komlosi[1], J. Fischer[1], B. Gläser[1]

[1]Department of Human Genetics, Freiburg, Deutschland, [2]Department of Pediatrics, Freiburg, Deutschland

Introduction: We report two female patients with a partial trisomy 16q, a very rarely described chromosomal imbalance. The clinical manifestation depends on the translocation partners involved, as partial trisomy 16q often derives from an unbalanced translocation between chromosome 16 and another chromosome. However, some overlapping clinical features are low birth weight, growth retardation, intellectual disability, muscular hypotonia, facial dysmorphia and urogenital malformations.

Clinical report: In the first case, polyhydramnion, growth retardation, complex cardiac anomaly and anal atresia were noted prenatally. After birth, hypoplastic pulmonary arteries and aortic arch, ventricular septal defect and laryngeal anomaly were confirmed. Additionally, facial dysmorphia, duplex kidney and anal atresia with vestibular and perineal fistula were diagnosed. At the time of the report, the patient was 4,5 years old.

The second female patient presented with intrauterine retardation and oligohydramnion. After birth, total anomalous pulmonary venous connection, atrial septal defect and mild respiratory adaptation difficulties were noted. The patient had facial dysmorphia with low set ears and left facial skin tag, further, an imperforate anus with anterior anal fistula was diagnosed. The patient deceased at the age of five month due to untreatable cardiac conditions.

Methods: Conventional karyotyping was performed in the patients and their parents. From the patients, additionally aCGH and F*is*H analysis was done to determine the location of the translocation partners.

Results:

Case 1 had the following karyotype: 46,XX,der(22),t(16;22)(q21;p11.2), carrying a *de novo* translocation between the long arm of chromosome 16 and the short arm of chromosome 22, resulting in a duplication of around 23 Mb at 16q21q24.3.

Case 2 showed a marker chromosome. The child’s mother had the karyotype 46,XX,t(16;22)(q22.1;q11.21). The child inherited the two normal chromosomes 16 and 22 in addition to the derivate chromosome 22 of the mother. Incorrect 3:1 distribution must have occurred, resulting in the child carrying a duplication of around 22 Mb at 16q22.1q24.3 and a duplication of around 3.5 Mb at 22q11.1q11.21.

Conclusion: Descriptions of partial trisomy 16q are very rare. Our cases show the importance of the combined conventional and molecular cytogenetic methods in the elucidation of rare chromosomal abnormalities and add details to the phenotypic spectrum of partial trisomy 16q.

Abstract ID: 55

P6-18

Affection of the catalytic triad of UFSP2 leads to a severe phenotype of spondyloepimetaphyseal dysplasia type Di Rocco

L. Mattern[1], M. Begemann[1], H. Delbrück[2], P. Holschbach[3], S. Schröder[2], S.M Schacht[4], I. Kurth[1], M. Elbracht[1]

[1]Institute for Human Genetics and Genomic Medicine, Medical Faculty, RWTH Aachen University, Aachen, Deutschland, [2]Medical Faculty, RWTH Aachen University, Department of Orthopaedic, Trauma and Reconstructive Surgery, Aachen, Deutschland, [3]Medical Faculty, RWTH Aachen University, Department of Pediatrics, Division of Neuropediatrics and Social Pediatrics, Aachen, Deutschland, [4]Medical Faculty, RWTH Aachen University, Department of Diagnostic and Interventional Radiology, Aachen, Deutschland

Introduction: Skeletal dysplasia comprise a heterogeneous group of distinct entities caused by alterations in more than 400 different genes. Amongst those, variants in the *Ufm1-specific protease 2* (*UFSP2*) gene are related to spondyloepimetaphyseal dysplasia Type Di Rocco (SEMDDR), a rare autosomal dominant hereditary disease characterized by disproportionate short stature combined with vertebral, epiphyseal, and metaphyseal abnormalities. UFSP2 is a cysteine protease involved in the maturation of Ubiquitin-fold modifier 1 (Ufm1). The active site of UFSP2 is located in its C-terminal catalytic domain consisting of the catalytic triad Cys302, Asp426, and His428, as well as Tyr290 participating in the formation of the oxyanion hole. Variants in Tyr290 lead to isolated premature degenerative osteoarthritis of the hip joints (Beukes hip dysplasia, BHD), while variants in Asp426 and His428 are known to cause the more severe phenotype SEMDDR. This is the first description of a variant in the catalytic Cys302 in a two-year-old boy with SEMDDR.
Methods: Whole exome sequencing was performed on a child of non-consanguineous parents with bland family history.
Results: Our patient presented at birth with shortened proximal extremities and a bell-shaped thorax. His motor development was delayed, while speech and mental development were normal. Further clinical evaluation revealed genua vara, muscular hypotonia, lumbar hyperlordosis, and limitation of elbow extension (5°). He has significant growth retardation; the last documented body length was 74 cm at 23 months (<1st percentile, Z = -3.89). Radiological examinations (X-ray and MRI) showed deformities of the femoral epiphyses and metaphyses in the sense of coxae varae. The genetic diagnosis was confirmed at 16 months of age when a *de novo* heterozygous missense variant in the *UFSP2* gene (NM_018359.3:c.905G>C, p.(Cys302Ser)) was detected via whole exome sequencing.
Conclusion: Alterations in the components of the catalytic triad (Cys302, Asp426, and His428) of UFSP2 result in a more severe phenotype of skeletal dysplasia (SEMDDR) than variants in other parts of its active site (i.e., Tyr290 in BHD). Variants in Asp426 and His428 are already known to cause SEMDDR, while this is the first report of a variant in Cys302. The skeletal features of our patient underline the importance of an intact catalytic triad of UFSP2 for physiological bone development. Comprehensive genetic, biochemical, and clinical characterization of rare skeletal disorders are needed to improve the medical support of our patients.

Abstract ID: 60

P6-19

A *TMC8* splice variant causes epidermodysplasia verruciformis in a Pakistani family

X. Xiong[1], S.A. Uddin[2], S. Munir[3], N. Cesarato[1], H. Thiele[4], N. Hassan[2], S. Kumar[5], F.U. Rehman[6], M. Naeem[7], A. Wali[8], S. Basit[9], F.B. Basmanav[1], M. Ayub[2], R.C. Betz[1]

[1]Institute of Human Genetics, University of Bonn, Medical Faculty & University Hospital Bonn, Bonn, Deutschland, [2]Institute of Biochemistry, Faculty of Life Sciences, University of Balochistan, Quetta, Pakistan, [3]Faculty of Basic Sciences, Sardar Bahadur khan women's University, Department of Biochemistry, Quetta, Pakistan, [4]Cologne Center for Genomics, University of Cologne, Cologne, Deutschland, [5]Sheik Zayed Hospital Quetta, Department of Dermatology, Quetta, Pakistan, [6]Faculty of Life Sciences, University of Balochistan, Department of Microbiology, Quetta, Pakistan, [7]Pakistan Naval Services, Sick Bay, Karsaz, Karachi, Karsaz, Pakistan, [8]Department of Biotechnology, Faculty of Life Sciences, BUITEMS, Quetta, Pakistan, [9]Taibah University Almadinah, Department of Biochemistry and Molecular Medicine & Center for Genetics and Inherited Diseases, Medina, Saudi-Arabien

Epidermodysplasia Verruciformis (EV) a rare autosomal recessive skin disease, which is characterized by persistent disseminated flat warts and pityriasis versicolor-like lesions associated with abnormal susceptibility to the human beta papillomavirus. The risk for carcinoma is quite high for these patients, especially in sun-exposed areas. To date, more than half of typical EV cases show pathogenic variants in either *TMC6* or *TMC8*.
To investigate the genetic basis of this disease in a consanguineous Pakistani family, we used whole exome sequencing (WES). A homozygous single base exchange c.668+5G>A in intron 6 of the *TMC8* gene was identified. With Sanger sequencing, we confirmed the variant, and it was co-segregateing with the phenotype in the family. In silico analysis via the HSF Pro tool suggested that the variant c.668+5G>A leads to the impairment of the 5' donor splice site. By use of the exon trap vector in HEK293T cells, we detected that a splice transcript that does not contain exon 6 is generated in the presence of this variant. We also observed that c.668+5G>A leads to an alteration in the relative abundances of the canonical transcript and other splice transcripts of *TMC8*. Interestingly, we saw an additional transcript that retains an intronic fragment suggesting the activation of a cryptic donor site in intron 7, which had not been previously described. We therefore concluded that this might be a biological artefact or a technical artefact arising from the introduction of only a partial TMC8 sequence in the plasmid. Of note, since the variant c.668+5G>A does not lead to a complete abolishment of the correct splicing and rather to a reduction in its extent, we compared whether our patients presented with a milder phenotype than the ones described in the literature. However, we could not find any significant differences. In conclusion, the identified variant, which is apparently associated with an exon-skipping event, broadens the mutation spectrum of EV.

Abstract ID: 61

P3-19

Whole exome sequencing identifies a heterozygous *TRPC3* missense variant in a patient with adult onset spinocerebellar ataxia

T. Bender[1,2], A. Schmidt[1], K. Cremer[1], C. Perne[1], H. Engels[1], S. Heilmann-Heimbach[1], P. Incardona[3], S. Peters[1]

[1]University of Bonn - University Hospital Bonn, Institute of Human Genetics, Bonn, Deutschland, [2]University of Bonn - University Hospital Bonn, Center for Rare Diseases Bonn, Bonn, Deutschland, [3]University of Bonn - Medical Faculty, Core Unit for Bioinformatics Data Analysis, Bonn, Deutschland

Introduction: Spinocerebellar ataxias (SCA) are a group of genetically heterogeneous neurological disorders with the main symptom gait ataxia. Other symptoms, age of onset, mode of inheritance, as well as the underlying gene and mutation type differ between the respective types of SCA. To date, more than 40 genetically distinct types of spinocerebellar ataxias have been reported.

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 (gait ataxia) 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. The patient's father also had had gait ataxia and cerebellar atrophy, suggesting cerebellar ataxia, but without clinical confirmation of the diagnosis.

Methods and Results: Whole exome sequencing and variant prioritization using phenotype data 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 missense variant. The gnomAD missense constraints indicate a high intolerance for missense variants in this gene (Z-score: 3.84).

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 spinocerebellar ataxia 41 (SCA41) (Fogel et al., 2015; PMID: 25477146). 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) and the absence of ataxia symptoms in individuals with heterozygous 4q27-q28.1 microdeletions affecting *TRPC3* (Hickey et al., 2013). The gene-disease validity is still provisional according to OMIM.

Through an ongoing GeneMatcher search, we have so far identified one additional patient with cerebellar ataxia and a heterozygous missense variant in *TRPC3*.

Our findings support the hypothesis that heterozygous pathogenic missense variants in the *TRPC3* gene are a possible rare cause of adult onset spinocerebellar ataxia.

Abstract ID: 62

Systematic analysis and prediction of genes associated with monogenic disorders on human chromosome X

E. Leitão[1], C. Schröder[1], I. Parenti[1], C. Dalle[2], A. Rastetter[2], T. Kühnel[1], A. Kuechler[1], S. Kaya[1], B. Gérard[3], E. Schaefer[4], C. Nava[2], N. Drouot[5,6,7,8], C. Engel[5,6,7,8], J. Piard[9,10], B. Duban-Bedu[11], L. Villard[12,13], A.P.A. Stegmann[14,15], E.K. Vanhoutte[15], J.A.J. Verdonschot[15,16], F.J. Kaiser[1], F. Tran Mau-Them[10,17], M. Scala[18,19], P. Striano[18,19], S.G.M. Frints[15,20], E. Argilli[21,22], E.H. Sherr[21,22], F. Elder[23], J. Buratti[23], B. Keren[23], C. Mignot[2,24], D. Héron[24], J.-L. Mandel[3,5,6,7,8], J. Gecz[25,26,27], V.M. Kalscheuer[28], B. Horsthemke[1], A. Piton[3,5,6,7,8], C. Depienne[1]

[1]Institute of Human Genetics, University Hospital Essen, University Duisburg-Essen, Essen, Deutschland, [2]Institut du Cerveau et de la Moelle Épinière (ICM), Sorbonne Université, UMR S 1127, Inserm U 1127, CNRS UMR 7225, Paris, Frankreich, [3]Unité de Génétique Moléculaire, IGMA, Hôpitaux Universitaire de Strasbourg, Strasbourg, Frankreich, [4]Service de Génétique Médicale, IGMA, Hôpitaux Universitaires de Strasbourg, Strasbourg, Frankreich, [5]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, Frankreich, [6]Centre National de la Recherche Scientifique, UMR 7104, Illkirch, Frankreich, [7]Institut National de la Santé et de la Recherche Médicale, U 964, Illkirch, Frankreich, [8]Université de Strasbourg, Illkirch, Frankreich, [9]Centre de Génétique Humaine, CHU Besançon, Besançon, Frankreich, [10]INSERM UMR 1231, Equipe Génétique des Anomalies du Développement, Université de Bourgogne-Franche-Comté, Dijon, Frankreich, [11]Centre de Génétique Chromosomique, Hôpital Saint-Vincent de Paul, Lille, Frankreich, [12]Aix-Marseille University, INSERM, MMG, UMR-S 1251, Faculté de Médecine, Marseille, Frankreich, [13]APHM, Hôpital d'Enfants de La Timone, Département de Génétique Médicale, Marseille, Frankreich, [14]Radboud University Medical Center, Department of Human Genetics, Nijmegen, Niederlande, [15]Maastricht University Medical Center+, Department of Clinical Genetics, Maastricht, Niederlande, [16]Cardiovascular Research Institute (CARIM), Department of Cardiology, Maastricht University Medical Center, Maastricht, Niederlande, [17]Unité Fonctionnelle Innovation en Diagnostic Génomique des Maladies Rares, CHU Dijon Bourgogne, Dijon, Frankreich, [18]University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, Genoa, Italien, [19]Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italien, [20]Faculty of Health Medicine Life Sciences, Maastricht University Medical Center+, Maastricht University, Department of Genetics and Cell Biology, Maastricht, Niederlande, [21]University of California, Department of Neurology, San Francisco, Vereinigte Staaten, [22]Institute of Human Genetics and Weill Institute for Neurosciences, University of California, San Francisco, Vereinigte Staaten, [23]UF de Génomique du Développement, Département de Génétique, Groupe Hospitalier Pitié-Salpêtrière, APHP-Sorbonne Université, Paris, Frankreich, [24]APHP, Sorbonne Université, Département de Génétique, Centre de Référence Déficiences Intellectuelles de Causes Rares, Groupe Hospitalier Pitié-Salpêtrière and Hôpital Trousseau, Paris, Frankreich, [25]School of Medicine, The University of Adelaide, Adelaide, Australien, [26]Robinson Research Institute, The University of Adelaide, Adelaide, Australien, [27]South Australian Health and Medical Research Institute, The University of Adelaide, Adelaide, Australien, [28]Research Group Development and Disease, Max Planck Institute for Molecular Genetics, Berlin, Deutschland

Disease gene discovery on chromosome X (chrX) is associated with greater challenges compared to autosomes because X-linked variants can be inherited according to different modes and penetrance varies according to sex and chromosome X inactivation in females. We undertook a systematic analysis of all coding genes on human chrX with the aim of predicting gene-disease associations remaining to be discovered on this chromosome. We used OMIM to compare the proportion and characteristics of disease genes and associated disorders on all chromosomes. We observed a higher proportion of disorder-associated genes and an enrichment of genes involved in cognition, language, and seizures on chrX compared to autosomes. We used a threshold approach to analyze gene constraints, exon and promoter conservation, expression, and paralogues, and report 127 genes not yet associated with a disorder sharing one or more attributes with the 205 disorder genes known on chrX. We then collected 83 variables from genes on all chromosomes, including gene constraints, nucleotide conservation metrics, expression data stratified by sex, gene structure attributes, relative position of the gene on the chromosome and data on paralogues, and used machine learning to predict remaining disorder genes in a more systematic and unbiased fashion. We trained and compared different machine learning classifiers to distinguish disease-associated from dispensable genes. We classify 247 genes, including 115 of the 127 identified with the threshold approach, as having high probability of being disease-associated. We provide evidence of an excess of variants in predicted genes in existing databases. Finally, we report damaging variants in *CDK16* and *TRPC5* in patients with intellectual disability or autism spectrum disorders. This study predicts large-scale gene-disease associations that could be used for prioritization of X-linked pathogenic variants.
This study was recently published in Nature Communications [1].

[1] Leitão et al. (2022) Systematic analysis and prediction of genes associated with monogenic disorders on human chromosome X. Nature Communications. 13, 6570 (PMID: 36323681).

Abstract ID: 63

Investigation of optical genome mapping capabilities and its potential to be implemented as a routine diagnostic test

U. Koehler[1], H. Barseghyan[1,2], D. Eisenreich[1], E. Lindt[1], K. Sendelbach[1], H. Erdmann[1], T. Neuhann[1], A. Abicht[1], E. Holinski-Feder[1]

[1]MGZ - Medizinisch Genetisches Zentrum, München, Deutschland, [2]Children's National Hospital, Children's National Research Institute, Center for Genetic Medicine Research, Washington, DC, Vereinigte Staaten

Genomic technologies have helped to identify genetic diagnoses in millions of patient. However, despite this success, many still remain undiagnosed. Each of the current cytogenetic methods – karyotyping, chromosomal microarrays (CMA) and fluorescence in situ hybridization (FISH), have limitations such as resolution, inability to identify balanced events, or being targeted, respectively. On the other hand, affordable genome sequencing, which reliably identifies single nucleotide variants (SNVs) and small insertions and deletions (INDELs), cannot sensitively identify many of the different types of structural variants (SVs) due to its methodological limitations (i.e. utilization of short reads). A promising technology, *Optical Genome Mapping* (OGM), promises to address some of the limitations of the current diagnostic methods with a streamlined approach. OGM captures a pattern of fluorescent labels within long DNA molecules (>150 kbp) in nanochannel arrays for de novo genome assembly and SV calling.
We have performed validation of OGM using 55 samples for which diagnoses had been achieved with karyotype or CMA. The dataset contained aneuploidies, duplications, deletions and translocations. In total, OGM was 98% concordant with previous diagnoses only missing one translocation, breakpoints of which were located within centromeres, a known limitation of OGM. We have also performed OGM testing on 44 samples with gene panels and 17 samples with facioscapulohumeral muscular dystrophy. We have now applied OGM to 20 undiagnosed cases for which CMA and exome sequencing were uninformative. On average, OGM identified ~4000 insertions/duplications, ~2000 deletions, ~100 inversions and 0-1 translocations per individual. To perform analysis, we implemented SV filtration criteria (including quality, size, frequency and gene overlap) to select for potential clinically significant variants. After the filters, there were between 40-70 variants for which classification was performed. We identified several potentially interesting insertions in disease causing genes for which further sequencing was needed for appropriate classification. Additionally, we identified a translocation and 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 either diagnosis in undiagnosed cases.

Abstract ID: 64

P3-7

Biallelic *KITLG* variants lead to a distinct spectrum of hypomelanosis and sensorineural hearing loss

B. Vona[1,2], D.A. Schwartzbaum[3], A.A. Rodriguez[3], S.S. Lewis[4], M.B. Toosi[5], P. Radhakrishnan[6], N. Bozan[7], R. Akın[7], M. Doosti[8], R. Manju[9], D. Duman[10], C.J. Sineni[3], S. Nampoothiri[11], E.G. Karimiani[8,12,13], H. Houlden[14], G. Bademci[3,15], M. Tekin[3,15], K.M. Girisha[4,6], R. Maroofian[14], S. Douzgou[16,17]

[1]University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Deutschland, [2]University Medical Center Göttingen, Institute for Auditory Neuroscience and InnerEarLab, Göttingen, Deutschland, [3]University of Miami Miller School of Medicine, John P. Hussman Institute for Human Genomics, Miami, Vereinigte Staaten, [4]Manipal Academy of Higher Education, Department of Medical Genetics, Kasturba Medical College, Manipal, Indien, [5]Mashhad University of Medical Science, Pediatric Neurology Department, Ghaem Hospital, Mashhad, Iran, [6]Manipal Universal Technology Business Incubator Manipal, Suma Genomics Private Limited, Karnataka, Indien, [7]Yuzuncu Yıl University Faculty of Medicine, Department of Otolaryngology, Van, Türkei, [8]Next Generation Genetic Polyclinic, Department of Medical Genetics, Mashhad, Iran, [9]Renai Medicity, Cochin, Indien, [10]Ankara University Faculty of Health Sciences, Department of Audiology, Ankara, Türkei, [11]Amrita Institute of Medical Sciences and Research Centre, Department of Paediatric Genetics, Kochi, Indien, [12]St. George's, University of London, Molecular and Clinical Sciences Institute, London, Vereinigtes Königreich, [13]Islamic Azad University, Innovative Medical Research Center, Mashhad, Iran, [14]UCL Queen Square Institute of Neurology, Department of Neuromuscular Disorders, London, Vereinigtes Königreich, [15]University of Miami Miller School of Medicine, Dr. John T. Macdonald Foundation Department of Human Genetics, Miami, Vereinigte Staaten, [16]Haukeland University Hospital, Department of Medical Genetics, Bergen, Norwegen, [17]University of Manchester, Division of Evolution, Infection and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester, Vereinigtes Königreich

Pathogenic variants in *KITLG* (OMIM: *184745), a key player in melanocyte proliferation and pigment production, are well-established causes of autosomal dominant non-syndromic hearing loss, Waardenburg syndrome type 2, familial progressive hyperpigmentation as well as familial progressive hyper- and hypopigmentation. Untangling the all-encompassing possibilities with respect to inheritance patterns is critical work to aid a rapid and accurate molecular genetic diagnosis. This work expands understanding of *KITLG* to include a recessive disorder characterized as a hypomelanosis spectrum with or without hearing impairment.

Through multiple sequencing approaches and extensive networking, we characterize the largest case series with biallelic *KITLG* variants comprised of six unrelated individuals. We greatly expand the hypomelanosis spectrum to include distal hypopigmentation, partial depigmentation resembling Tietz albinism-deafness syndrome and complete depigmentation reminiscent of oculocutaneous albinism. Sensorineural hearing impairment was identified congenitally or in the neonatal period and exhibited a unilateral (n=1), asymmetrical (n=2), or bilateral (n=2) hearing impairment in individuals from whom this information was available. We speculate variants with loss-of-function cause oculocutaneous albinism while those with residual function cause Waardenburg syndrome type 2 or albinism-deafness syndrome.

This work broadens the understanding of the mode of inheritance of Waardenburg syndrome type 2 to include autosomal recessive transmission and defines a new sub-type, namely, Waardenburg syndrome type 2F (OMIM: #619947). We emphasize the importance of genetic testing in diagnosing patients with unilateral or asymmetrical hearing impairment. This work defines *KITLG* as a new molecular cause of autosomal recessive albinism-deafness syndrome and cutaneous albinism with deafness.

Abstract ID: 65

Human models for White Sutton syndrome: *POGZ* mutations change the transcriptome and induce defects in neural progenitor cell biology

A. Soliman[1], D. Hartwich[1], S. Schweiger[1], H. Todorov*[1], J. Winter*[1]

[1]*Institut für Humangenetik, Mainz, Deutschland*

Pogo transposable element derived with ZNF domain (*POGZ*) has been identified as one of the most recurrently mutated genes in patients with neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD), intellectual disability, and White-Sutton syndrome; however, the underlying disease-causing cellular and molecular mechanisms are still unclear. Here, we generated several induced pluripotent stem cell lines (iPSCs) with heterozygous *POGZ* mutations, either derived from patient fibroblasts or introduced by CRISPR/Cas9 genomic editing, and differentiated them into neural progenitor cells (NPCs). We demonstrate that frameshift mutations, either in the N-terminus or in the HP1-binding zinc finger-like (HPZ) domain, decrease POGZ protein expression but do not impair its nuclear localization. By using a 3D neurosphere model, we show that POGZ deficiency impairs self-renewal activity and enhances NPC differentiation and neuronal migration.
POGZ binds to chromatin and acts as a transcriptional regulator. RNA sequencing of the iPSC-derived NPCs revealed widespread transcriptome changes in the NPCs carrying POGZ mutations. The differentially expressed genes were significantly enriched in the GO terms of chromosome and mitotic chromatid segregation, DNA repair by homologous recombination, and alternative splicing, among others. Of note, the transcriptomes of different cell lines all carrying POGZ mutations were more similar to each other than were the transcriptomes of different wild-type cell lines. This indicates that POGZ mutations strongly affect cell expression levels and give rise to a POGZ-specific transcriptome. To identify direct POGZ targets, we are now carrying out CUT& RUN sequencing of the iPSC-derived NPCs. At the GfH congress, we will report more on the results of these experiments.

Abstract ID: 66

P3-42

PRNP octapeptide insertion in a familial case of neurodegenerative disease elusive in routine neuropathological examination

V.G. Paul[1], O. Klaas[1], J. Horváth[1]

[1]*Institut für Humangenetik, Universitätsklinikum Münster, Münster, Deutschland*

Introduction: Approximately 15% of cases of human prion diseases are inherited (inherited prion diseases, IPD) and associated with heterozygous mutations in *PRNP* (Prion Protein, OMIM*176640). Major IPD phenotypes include Creutzfeldt-Jakob disease, fatal familial insomnia and Gerstmann-Straussler disease, all of which are caused by truncating or missense mutations. In rare cases insertions of 4 to 12 additional octapeptide repeats (OPRI) lead to heterogeneous phenotypes including early-onset cognitive decline, dementia, movement disorders, ataxia, aphasia and psychiatric symptoms or behavioral disturbances.

Case report: We report on a female patient referred to our clinic at age 25 and unaffected. She asked for carrier testing for a familial form of a neurodegenerative disease affecting her father and deceased paternal grandmother. The causative variant of the disease had not been identified in the family. We offered genetic testing for the affected father, but never received a sample. Our patient returned to our clinic several years after her father's death, when she was 38 years of age and was experiencing symptoms of neurodegenerative disease identical to her father's course of disease. She presented with dysarthria, tremor, personality changes and cognitive decline. According to medical records, her father was suspected of having Gerstmann-Straussler disease, but neuropathological examination failed to prove signs of prion disease.

Methods and Results: We performed whole-exome based analysis of *PRNP* and found a causative mutation, consistent with IPD with 8-OPRI. We will provide detailed clinical and genetic information on our patient and her father, and explore why neuropathological examination failed to observe signs of prion disease in the father.

Abstract ID: 69

LHX2 loss of function causes neurodevelopmental deficits in humans and flies

A. Gregor[1,2,3], C.M. Schmid[1,2], G. Costain[4], C. Morel[5], L. Massingham[6], J. Schwab[6], C. Quelin[7], M. Faoucher[8,9], J. Kaplan[10], R. Procopio[10], C.J. Saunders[11], A.S. Cohen[11], G. Lemire[12,13], S. Sacharow[13], A. O'Donnell-Luria[12,13], R.J. Segal[14], J. Kianmahd Shamonshi[15], D. Schweitzer[15], D.E.-F. Ebrahimi-Fakhari[16], K. Monaghan[17], T.B. Palculict[17], M.P. Napier[17], A. Tao[18], B. Isidor[19], K. Moradkhani[19], A. Reis[20,21], H. Sticht[22], W.K. Chung[23], C. Zweier[24,2,3]

[1]Inselspital Bern, University of Bern, Department of Human Genetics, Bern, Schweiz, [2]University of Bern, Department for Biomedical Research (DBMR), Bern, Schweiz, [3]Bern Center for Precision Medicine (BCPM), University of Bern, Bern, Schweiz, [4]Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Toronto, Kanada, [5]The Fred A. Litwin Family Centre in Genetic Medicine, University Health Network and Mount Sinai Hospital, Toronto, Toronto, Kanada, [6]Division of Human Genetics, Department of Pediatrics, Warren Alpert Medical School of Brown University, Hasbro Children's Hospital/Rhode Island Hospital, Providence, Vereinigte Staaten, [7]Clinical Genetics Department, CHU Hôspital Sud, Rennes, Frankreich, [8]Service de Génétique Moléculaire et Génomique, CHU, Rennes, Frankreich, [9]Univ Rennes, CNRS, IGDR, UMR 6290, Rennes, Frankreich, [10]Division of Genetics, Department of Pediatrics, Nemours/Alfred I. DuPont Hospital for Children, Wilmington, Vereinigte Staaten, [11]Genomic Medicine Center, Department of Pathology and Laboratory Medicine, Children's Mercy Kansas City, Kansas City, Vereinigte Staaten, [12]Broad Center for Mendelian Genomics, Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, Vereinigte Staaten, [13]Division of Genetics and Genomics, Boston Children's Hospital, Harvard Medical School, Boston, Vereinigte Staaten, [14]Schneider Children's Medical Center of Israel, Petach Tikvah, Israel, [15]Division of Medical Genetics, Department of Pediatrics, David Geffen School of Medicine, UCLA, Los Angeles, Vereinigte Staaten, [16]Movement Disorders Program, Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, Vereinigte Staaten, [17]GeneDx, LLC, Gaithersburg, Vereinigte Staaten, [18]Columbia University Vagelos College of Physicians and Surgeons, New York, Vereinigte Staaten, [19]CHU Nantes, Department of Medical Genetics, Nantes, Frankreich, [20]Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Deutschland, [21]Centre for Rare Diseases Erlangen (ZSEER), University Hospital Erlangen, Friedrich-Alexander University of Erlangen-Nürnberg (FAU), Erlangen, Deutschland, [22]Institut für Biochemie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Deutschland, [23]Columbia University, Departments of Pediatrics and Medicine, New York, Vereinigte Staaten, [24]Inselspital Bern, University of Bern, Department of Medical Genetics, Bern, Schweiz

LHX2 encodes the LIM homeobox 2 transcription factor (LHX2), which is highly expressed in brain and well conserved with similar functions across species.
Through international collaboration, we identified 19 individuals from 18 families with variable neurodevelopmental phenotypes, carrying a small chromosomal deletion, likely gene-disrupting or missense variants in *LHX2*. Thirteen variants were shown to be de novo, and one occurred in an affected mother-daughter duo. The affected individuals presented with developmental and/or behavioral abnormalities, autism-spectrum disorder, variable intellectual disability, and microcephaly. A loss-of-function mechanism is likely for the deletion and the ten likely gene-disrupting variants, and we further investigated the functional consequences of six of the seven missense variants by overexpressing mutant *LHX2* in cellular systems. We observed nucleolar accumulation for two variants located within the DNA-binding HOX domain and impaired interaction with co-factor LDB1 for another variant located in the protein-protein interaction mediating LIM domain. Furthermore, we confirmed impaired transcriptional activation by luciferase assay for four tested variants. These results suggest a loss-of-function mechanism also for *LHX2* missense variants. To model the impact of LHX2 loss on neuronal function and behavior, we investigated effects of pan-neuronal knockdown of *LHX2* ortholog *apterous* in *Drosophila melanogaster*. We observed impaired basic locomotor ability as well as reduced daily activity.
We therefore implicate *LHX2* haploinsufficiency as causative for a variable NDD. Together, our observations underscore the importance of LHX2 in nervous system and for variable neurodevelopmental phenotypes.

Abstract ID: 70

DEGS1-associated leukodystrophy: using patient-derived iPSCs to unravel neuro- and gliopathology

L. Buschmann[1], G. Karsai[2], T. Hornemann[2], I. Kurth[1], N. Haag[1]

[1]Uniklinik RWTH Aachen, Institut für Humangenetik und Genommedizin, Aachen, Deutschland, [2]University Hospital Zürich, Institute of Clinical Chemistry, Zürich, Schweiz

Dysfunction of sphingolipid (SL) metabolizing enzymes is linked to diverse pathological conditions, and pathogenic variants in *DEGS1* (Δ4-Dihydroceramide Desaturase 1) have recently been shown by us and others to cause a rare human monogenic neurological disease affecting SL homeostasis. DEGS1 catalyzes the conversion of Dihydroceramide (dhCer) to Ceramide (Cer) leading to a disbalance in the intracellular dhCer/Cer ratio. Elevated Cer levels are known to cause neurodegenerative diseases, but the neuronal and glial activity of its direct precursor, dhCer, is poorly understood.
To study the human condition, we generated patient-derived human induced pluripotent stem cells (hiPSCs) harboring the biallelic pathogenic DEGS1 c.839C>T; p.Ala280Val missense variant. We differentiated the hiPSCs into central nervous system neurons and oligodendrocytes (OLs) and analyzed how DEGS1 dysfunction affects their differentiation and maturation capacities by immunostaining for lineage- and stage-specific markers and by transcriptomics and lipidomics.
Patient-derived DEGS1^{A280V}-hiPSCs show the characteristic patient-specific SL profiles with high levels of dhSLs. Upon differentiation, especially dihydro-Sphingomyelin levels strongly increase in DEGS1^{A280V}-neurons and even more in DEGS1^{A280V}-OLs. DEGS1^{A280V}-neurons are less complex with reduced numbers of neurites and branches implying an impaired differentiation capacity. Upon glial induction, DEGS1^{A280V} neural precursor cells (NPCs) gave rise to only 20% the number of O4+ oligodendrocyte precursor cells (OPCs) differentiated from control NPCs. In addition, the maturation capacity of DEGS1^{A280V}-OPCs towards myelinating oligodendrocytes (MBP+ OLs) was reduced to only 55% compared to control OPCs. The observed differences in the differentiation capability of DEGS1^{A280V}-hiPSCs and -NPCs are well reflected by whole transcriptome RNAseq data.
In conclusion, DEGS1^{A280V} impairs both neuron and oligodendrocyte lineage development and particularly disrupts the maturation of OPCs towards myelination-competent OLs *in vitro*. The findings provide evidence for a developmental defect of both neuronal and glial cells in the *DEGS1*-associated neurodegeneration.

Abstract ID: 71

P3-43

Atypical or particularly mild Rett syndrome? A family with a novel frameshift mutation in *MECP2*

S. Riedel[1], S. Demuth[2], B. Eichhorn[1]

[1]MVZ Mitteldeutscher Praxisverbund Humangenetik GmbH, Dresden, Deutschland, [2]MVZ Mitteldeutscher Praxisverbund Humangenetik GmbH, Erfurt, Deutschland

The classic Rett syndrome (RTT) is a rare progressive neurodevelopmental disorder. It primarily affects girls and is most often caused by mutations of the methyl-CpG binding protein 2 (*MECP2*) gene. The clinical findings are characterized by normal development during the age of 6-18 months, a subsequently short period of developmental stagnation, followed by a regression of motor and speech skills. Additionally, stereotypic hand movements, autistic features, seizures and an acquired microcephaly are also typical. Furthermore different atypical forms of Rett syndrome are described, e.g. Rolando variant (congenital), Zappella variant (preserved speech) and Hanefeld variant (early-onset seizure), and caused by mutations in the genes *MECP2, CDKL5* and *FOXG1*. The most patients (about 70%) also harbour a *MECP*2 mutation.
Here, we present a family with two young maternal half-sisters (8 and 13 year-old) and their mother. Both girls have delayed motor and speech development, learning difficulties, behavioural disorder and showed early adolescence. The older one also developed an epilepsy at the age of 12 years. The mother and maternal grandmother developed early adolescence, mental disorder and learning difficulties as well. All our patients have congenital developmental delay without regression and stereotypic hand movements.
We performed a clinical exome diagnostic via sequencing by synthesis for the two half-sisters and their mother.
Detected variant was confirmed via Sanger sequencing.
We revealed a novel heterozygous frameshift variation c.1040dupA / p.(Glu348Glyfs*45) in exon 4 of the *MECP2* (NM_004992.3) gene. The one-base duplication occurred in all three patients (two half-sisters and their mother). It has not been previously annotated in population databases (e.g. gnomAD or dbSNP) or listed in mutation and disease associated (e.g. Rettbase) databases respectively. The truncating variant is located in the C-terminal domain of the *MECP2* gene, rather an unusual position, but might be the reason for this familiar phenotype.
In summary, there is a strong evidence for the disease-causing effect of the detected *MECP2* variation. Nevertheless, it needs to be discussed, whether our case can be classified as atypical or particularly mild Rett syndrome.–

Abstract ID: 72

P4-11

Epigenetic age acceleration in major psychiatric disorders

F. David[1], L. Sindermann[1], F. Streit[2], J. Frank[2], L. Zillich[2], L. Sirignano[2], S. Herms[1,3], P. Hoffmann[1,3], T. Kircher[4,5], U. Dannlowski[6], S. Witt[2,7], M. Rietschel[2], M. Nöthen[1], A. Forstner[1,8]

[1]Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Deutschland, [2]Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Department of Genetic Epidemiology in Psychiatry, Mannheim, Deutschland, [3]University of Basel, Department of Biomedicine, Basel, Schweiz, [4]University of Marburg, Department of Psychiatry and Psychotherapy, Marburg, Deutschland, [5]Center for Mind, Brain and Behavior, University of Marburg, Marburg, Deutschland, [6]Institute for Translational Psychiatry, University of Münster, Münster, Deutschland, [7]Center for Innovative Psychiatry and Psychotherapy Research, Central Institute of Mental Health, Medical Faculty Mannheim / Heidelberg University, Mannheim, Deutschland, [8]Institute of Neuroscience and Medicine (INM-1), Research Center Jülich, Jülich, Deutschland

Psychiatric disorders, such as major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia spectrum disorder (SSD), are linked to an increase in mortality compared to the general population. This excess mortality is not fully explained by suicide and other external causes of death, and previous studies have implicated accelerated biological aging reflecting comorbid medical conditions that increase mortality, e.g. cardiovascular disease. As a proxy for biological aging, different epigenetic clocks have been developed, allowing for the estimation of epigenetic age acceleration based on DNA methylation data. We here investigate potential epigenetic age acceleration in the German FOR2107 cohort, comprising healthy controls (HC) as well as individuals diagnosed with MDD, BD, or SSD.

In 886 individuals, DNA methylation was profiled in peripheral blood using the Illumina MethylationEPIC array. Quality control and preprocessing of intensity data was performed in R using minfi and ewastools, resulting in the exclusion of seven individuals due to a call rate <98%, a mismatch between estimated and reported sex, or a mismatch in genetic fingerprints. A sample of 337 HC, 343 MDD cases, 99 BD cases and 100 SSD cases remained for analysis. Epigenetic age acceleration based on the DNAm GrimAge and DNAm PhenoAge models was then estimated from methylation beta values using the DNA Methylation Age Calculator (https://dnamage.genetics.ucla.edu). The effect of diagnostic groups on epigenetic age acceleration was examined via linear regression. To account for lifestyle-related factors, additional linear models were fitted including body mass index (BMI) and a DNAm-based proxy for smoking (cg05575921).

For both DNAm GrimAge and DNAm PhenoAge, significant effects of diagnostic groups on epigenetic age acceleration were observed, with accelerated aging in SSD, BD, and MDD compared to HC. When adjusting for BMI and smoking behavior, which were both significantly associated with epigenetic age acceleration, the associations with diagnostic groups were no longer visible.

The epigenetic age acceleration in individuals diagnosed with major psychiatric disorders is consistent with prior research and may contribute to the excess mortality. BMI and smoking appear to play a significant role in this context. For a more comprehensive understanding of the excess mortality, additional factors such as nutrition and physical exercise should be considered in future studies.

Abstract ID: 74

Assessment of the genetic spectrum of uncombable hair syndrome in a cohort of 107 individuals

N. Cesarato[1], F.B. Basmanav[1], S. Kumar[1], O. Borisov[2], P. Kokordelis[1], D.J. Ralser[1], M. Wehner[1], D. Axt[1], X. Xiong[1], H. Thiele[3], V. Dolgin[4,5,6], Y. Gossmann[1], N. Fricker[1], M.K. Dewenter[7], K. Weller[8], M. Suri[9], H. Reichenbach[10], V. Oji[11], M.-C. Addor[12], K. Ramirez[13], H. Stewart[14], N. Garcia Bartels[8], L. Weibel[15,16], N. Wagner[17], S. George[18], A. Kilic[19], I. Tantcheva-Poor[20], A. Stewart[21], N. Dikow[22], B. Blaumeiser[23], M. Medvecz[24], U. Blume-Peytavi[8], P. Farrant[18], R. Grimalt[25], S. Bertok[26], L. Bradley[27], M. Eskin-Schwartz[28], O.S. Birk[28], A. Bygum[29,30], M. Simon[31,32], P. Krawitz[2], C. Fischer[22], H. Hamm[33], G. Fritz[34], R.C Betz[1]

[1]Institute of Human Genetics, Medical Faculty & University Hospital Bonn, University of Bonn, Bonn, Deutschland, [2]Institute for Genomic Statistics and Bioinformatics, Medical Faculty & University Hospital Bonn, University of Bonn, Bonn, Deutschland, [3]Cologne Center for Genomics, University of Cologne, Cologne, Deutschland, [4]Genetics Institute at Soroka University Medical Center, Beer Sheva, Israel, [5]Morris Kahn Laboratory of Human Genetics, National Center for Rare Diseases at the Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel, [6]National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer Sheva, Israel, [7]Institute of Human Genetics, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Deutschland, [8]Charité-Universitätsmedizin Berlin, Department of Dermatology, Venereology and Allergology, Berlin, Deutschland, [9]Nottingham Clinical Genetics Service, Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham, Vereinigtes Königreich, [10]MVZ Mitteldeutscher Praxisverbund Humangenetik, Praxis Leipzig, Leipzig, Deutschland, [11]University of Münster, Department of Dermatology, Münster, Deutschland, [12]Department of Woman-Mother-Child, University Hospital Center CHUV CH 1011, Lausanne, Schweiz, [13]Neurología Pediátrica, Division de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile, [14]Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Foundation Trust, Oxford, Vereinigtes Königreich, [15]University Children's Hospital Zurich, University Hospital Zurich, Pediatric Dermatology Department, Zurich, Schweiz, [16]University Hospital Zurich, Dermatology Department, Zurich, Schweiz, [17]Universitätsklinikum Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Dermatology Department, Erlangen, Deutschland, [18]Brighton General Hospital, University Hospitals Sussex NHS Foundation Trust, Dermatology Department, Brighton, Vereinigtes Königreich, [19]Faculty of Medicine, Balıkesir University, Dermatology Department, Balıkesir, Türkei, [20]University Hospital of Cologne, Department of Dermatology and Venereology, Cologne, Deutschland, [21]Sheffield Clinical Genetics Service, Sheffield Children's Hospital, South Yorkshire, Vereinigtes Königreich, [22]Institute of Human Genetics, Heidelberg University, Heidelberg, Deutschland, [23]University of Antwerp, Department of Medical Genetics, Antwerp, Belgien, [24]Semmelweis University, Department of Dermatology, Venereology, and Dermatooncology, Budapest, Ungarn, [25]Universitat Internacional de Catalunya, Barcelona, Spanien, [26]University Children's Hospital, University Medical Centre, Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, Ljubljana, Slowenien, [27]Children's Health Ireland (CHI) at Crumlin, Department of Clinical Genetics, Dublin, Irland, [28]Genetics Institute at Soroka University Medical Center, Beer-Sheva, Israel, [29]Odense University Hospital, Department of Clinical Genetics, Odense, Dänemark, [30]Clinical Institute, University of Southern Denmark, Odense, Dänemark, [31]Toulouse Institute for Infectious and Inflammatory Diseases, Toulouse University, Toulouse, Frankreich, [32]CNRS, Inserm, Paul Sabatier Toulouse III University, Toulouse, Frankreich, [33]University Hospital Würzburg, Department of Dermatology, Venereology, and Allergology, Würzburg, Deutschland, [34]University of Hohenheim, Department of Cellular Microbiology, Stuttgart, Deutschland

Introduction: Uncombable hair syndrome (UHS) is a rare hair shaft anomaly that manifests during infancy and is characterized by dry, frizzy, and wiry hair that cannot be combed flat. In 2016, our group identified three genes involved in the pathogenesis of UHS: *PADI3*, *TGM3*, and *TCHH*. All three genes encode proteins whose sequential interactions play a role in the formation of the hair shaft. As a result of the publication, we were contacted by clinicians and private persons from all over the world and could collect the largest cohort comprising 107 individuals with UHS. With this cohort, we aimed to elucidate the genetic spectrum of UHS.

Methods: For this cohort, we collected patients with a suspected diagnosis of UHS and - if possible - their parents. Participants of all ages, races, and ethnicities were collected at referral centers or were enrolled on their own initiative. Genetic analyses of DNA extracted from blood or saliva samples were conducted via Sanger or whole-exome sequencing, and array-based genotyping. 3-dimensional protein modeling was used to study the identified variants. Additionally, clinical photographs were collected.

Results: We identified biallelic pathogenic variants in the known UHS genes (*PADI3*, *TGM3*, or *TCHH*) in 80 out of 107 individuals (75%). In most of the affected individuals (71%), the UHS phenotype was associated with pathogenic variants in *PADI3*. In *PADI3,* the two most frequent variants together account for more than 85% of the alleles, while altogether 12 different pathogenic variants were identified, and their consequences could be shown by 3-dimensional protein modeling. *TGM3* and *TCHH* pathogenic variants instead explain only a small fraction of cases (4%). 25% of the cohort remains unexplained. Haplotype analyses for the 4 most commonly observed pathogenic *PADI3* variants suggest the presence of a founder effect.

Conclusion: This cohort study extends and gives an overview of the genetic variant spectrum of UHS based on molecular genetic analyses of the worldwide largest collective, pointing at *PADI3* as the main responsible gene for the phenotype. Our analysis shows that it is unlikely that UHS might be related to a fourth major gene, but rather to the presence of some UHS-

associated genes, each explaining few families. The photographs are showing a quite unique phenotype with blonde, frizzy hair and blue eyes. Right now, we use artificial intelligence to analyze these imaging data and expect the first results.

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P3-14

Austrian Exome meetings – a nationwide competence sharing initiative

S. Verheyen[1], J. Blatterer[1], K. Wagner[1], H. Tichy[1], S. Hammer[1], E. Schreiner[1], B. Spiegl[1], S. Bierbaumer[1], M. Smogavec[2], R. Lehner[2], S. Madritsch[2], V. Stadlmann[2], M. Hofer[2], M. Gerykova Bujalkova[2], N. Riepl[2], E. Umlauf[2], B. Mühlegger[3], S. Burkhard[3], E. Maurer[3], F. Schneebauer[3], R. Gröbner[3], M. Witsch-Baumgartner[3], K. Rötzer-Londgin[4], G. Uyanik[4], D. Nagy[5], M. Maurer[5], I. Bader[6,7], J. Mayr[8], S. Ebner[6], B. Bogner[6], A. Pleyers[6], W. Kranewitter[9], T. Malli[9], G. Webersinke[9], J. Zschocke[3], F. Laccone[2], M. Speicher[1]

[1]Institute of Human Genetics, Diagnostic and Research Center for Molecular BioMedicine, Medical University of Graz, Graz, Österreich, [2]Institute of Medical Genetics, Medical University of Vienna, Wien, Österreich, [3]Institute of Human Genetics, Medical University Innsbruck, Innsbruck, Österreich, [4]Hanusch Hospital Vienna, Department of Medical Genetics, Wien, Österreich, [5]Institute of Medical Genetics, Johannes Kepler University Linz, Linz, Österreich, [6]Institute of Human Genetics, Paracelsus Medical University, Salzburg, Österreich, [7]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Deutschland, [8]University Children's Hospital, Salzburger Landeskliniken and Paracelsus Medical University, Salzburg, Österreich, [9]Labor für Molekulargenetische Diagnostik, Ordensklinikum Linz Barmherzige Schwestern, Linz, Österreich

Introduction: Phenotype-driven exome analysis in Austria is performed at six centers for medical genetics, which are associated with universities or hospitals. Since training measures are of utmost importance in this rapidly moving and complex field, Austrian Exome meetings (AEM) were initiated in 2018. The aims of the AEMs include fostering cooperation, pool resources, and exploiting the differently used diagnostic strategies for exome evaluation. Persons involved in analysis and reporting, i.e., medical geneticists, biologists, bioinformaticians, and technical assistants, can participate. Here, we describe this unique nationwide competence-sharing initiative.

Methods: Since 2018, 12 AEMs have been held. A total of 115 challenging exome datasets (FASTQ files), along with the respective clinical information, were sent to the other participating centers for analysis. The discussion of the collected findings is the centerpiece of each AEM. Furthermore, we discuss exome-related topics and invite colleagues from abroad with long-standing experience in the NGS field.

Results: Regarding the blinded analyses of the exome datasets, we request a clinical report according to the current institute practices to facilitate comparing the results. In general, we observe a very high concordance among results; however, on several occasions, variants received a different classification. Hence, to standardize exome analysis, the classification of variants strictly based on ACMG criteria was agreed on (2nd AEM). Furthermore, we agreed on the design and use of an Austrian-wide patient consent form for genetic analysis (3rd AEM) and to establish a shared Austrian variant database (2nd AEM), the latter is still in progress. Results and discussion points are documented and included in the quality management of the institutes as an interlaboratory comparison. Besides technical questions, we discussed issues such as HPO terms for variant filtering, secondary findings, carrier status, trio exome, reevaluation of datasets, interdisciplinary boards, and whole genome sequencing. As expected, the number of participants steadily increased from the first to the 12th meeting (n=12-28).

Conclusion: The exchange of exome raw data with a blind evaluation by other institutes requires a high degree of trust and the willingness to invest resources to realize a common goal. With the AEMs, we have succeeded in establishing a unique communication platform in Austria to improve the care of our patients.

Abstract ID: 77

P3-80

Delineation of the DLL1-associated phenotype

L.A Koch[1], M. Zhao[1,2], G. Bhat[3], S. Bianconi[4], E. Boltshauser[5], A. Brusco[6,7], S. Carestiato[6], D. Carli[6], A. Cooper[4], C. Coubes[8], M. Demos[9], A. Dressler[10], J. Gburek-Augustat[11], I. Gill[9], H. Goel[12], A.-M. Guerrot[13], M. Hebbar[9], C.L. Hornbuckle[14], L. Huh[9], L. Kalsner[15], H. Kiep[11], C. Knopp[16], E. Lausberg[16], F. Lecoquierre[13], C. Manso Bazus[17], A. Mussa[18], L. Pavinato[6], C. Philippe[19,20], A. Ruiz[21], A.E. Scheuerle[22], W.M. Schmidt[23], S. Spinette[24], A. Vitobello[19,20], A. Zalan[3], B. Fischer-Zirnsak[1,25], N. Ehmke[1,26]

[1]Institute of Medical Genetics and Human Genetics, Charité - Universitätsmedizin Berlin, Berlin, Deutschland, [2]Labor Berlin - Charité Vivantes GmbH, Department of Human Genetics, Berlin, Deutschland, [3]University of Illinois at Chicago, Department of Pediatrics, Section of Genetics, Chicago, Illinois, Vereinigte Staaten, [4]Southern California Permanente Medical Group, Kaiser Permanente, Department of Genetics, San Diego, California, Vereinigte Staaten, [5]University Children's Hospital Zurich, Department of Pediatric Neurology (Emeritus), Zürich, Schweiz, [6]University of Turin, Department of Medical Sciences, Turin, Italien, [7]Medical Genetics Unit, Città della Salute e della Scienza University Hospital, Turin, Italien, [8]Hôpital ADV, Département de Génétique Médicale, Centre Hospitalier Universitaire de Montpellier, Montpellier, Frankreich, [9]Division of Neurology, Department of Paediatrics, BC Children's Hospital, University of British Columbia, Vancouver, Kanada, [10]Member of the ERN EpiCARE, Medical University Vienna, Department of Pediatrics and Adolescent Medicine, Vienna, Österreich, [11]Division of Neuropaediatrics, Hospital for Children and Adolescents, University Hospital Leipzig, Leipzig, Deutschland, [12]Hunter Genetics, Hunter New England Local Health District, Waratah, University of Newcastle, Callaghan, Australien, [13]Univ Rouen Normandie, Inserm U 1245 and CHU Rouen, Department of Genetics and Reference Center for Developmental Disorders, Rouen, Frankreich, [14]Children's Medical Center Dallas, Department of Genetics, Dallas, Texas, Vereinigte Staaten, [15]Divisions of Genetics and Neurology, Connecticut Children's Medical Center and University of Connecticut School of Medicine, Farmington, Connecticut, Vereinigte Staaten, [16]Institute for Human Genetics and Genomic Medicine, Medical Faculty, RWTH Aachen University, Aachen, Deutschland, [17]Center for Genomic Medicine, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Universitat Autònoma de Barcelona, Sabadell, Spanien, [18]Pediatric Clinical Genetics Unit, Department of Public Health and Pediatric Sciences, Regina Margherita Children's Hospital, University of Torino, Turin, Italien, [19]Université Bourgogne-Franche Comté, UMR 1231 GAD, Inserm, Dijon, Frankreich, [20]Unité Fonctionnelle Innovation en Diagnostic Génomique des Maladies Rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, Frankreich, [21]Genetics Laboratory, Center for Genomic Medicine. Parc Taulí Hosspital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Universitat Autònoma de Barcelona, Sabadell, Spanien, [22]UT Southwestern Medical Center, Department of Pediatrics, Division of Genetics and Metabolism, Dallas, Texas, Vereinigte Staaten, [23]Center for Anatomy and Cell Biology, Medical University of Vienna, Neuromuscular Research Department, Vienna, Österreich, [24]Rhode Island Hospital, Hasbro Children's Hospital Genetic Counseling Center, Providence, Rhode Island, Vereinigte Staaten, [25]Max Planck Institute for Molecular Genetics, FG Development and Disease, Berlin, Deutschland, [26]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Deutschland

Delta-like 1 (DLL1) is one of the Notch receptor ligands, which form an important pathway for brain morphogenesis. Heterozygous pathogenic DLL1 variants cause neurodevelopmental disorder with nonspecific brain abnormalities and with or without seizures (NEDBAS; MIM 618709). So far, detailed clinical and molecular data of 15 individuals from 12 unrelated families have been published.

Herein, we report the clinical and molecular features of 17 unpublished individuals from 16 unrelated families with 14 different de novo or inherited (likely) pathogenic DLL1 variants (7 frameshift, 3 splice site, 2 nonsense, 2 missense). The cohort was identified using GeneMatcher and ClinVar, and increases the number of reported individuals to 32. The most common clinical features in all 32 individuals were developmental delay (DD) and/or intellectual disability (ID) (88%), brain abnormalities (66%) and autism spectrum disorder (45%). Attention deficit and hyperactivity disorder (ADHD) occurred in 43% of cases and seizures in 39%. For the current cohort we designed a questionnaire systematically evaluating clinical symptoms only described in single cases before, which revealed common comorbidities, including aggressive and/or self-injurious behavior (59%), sleeping problems (41%), anxiety (33%) and affective disorders (19%). All 17 of the affected individuals receive help with developmental issues, physical therapy and/or psycho/-pharmacological therapy. We performed in-silico modeling on the 3 missense variants using Deep Learning based protein structure prediction using ESM or AlphaFold with subsequent binding site prediction with MaSIF, showing changes on protein structure and protein binding properties. These data are preliminary, but suggest effects on binding of DLL1 to the Notch receptor.

This work further accentuates the complexity of the DLL1-associated phenotype and indicates that an early interdisciplinary approach is important for clinical management, due to the heterogeneous spectrum of symptoms. Functional in vivo and in vitro studies are needed to fully investigate the DLL1-related pathomechanism.

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P3-44

One year of genome sequencing in diagnostic routine

C. Du[1], S. von Hardenberg[1], R. Buurman[1], A. Förster[1], A. Schöner-Heinisch[1], B. Pabst[1], M. Losch[1], B. Haermeyer[1], B. Schnur[1], W. Hofmann[1], F. Kaisen[1], G. Schmidt[1], B. Auber[1]

[1]Medizinische Hochschule Hannover, Institut für Humangenetik, Hannover, Deutschland

Introduction: We performed genome sequencing on samples submitted consecutively to our laboratory. Here we report our preliminary experience on more than 700 independent index cases sequenced in the first year after implementation of diagnostic genome sequencing.

Methods: Following library preparation using IDT Lotus DNA Library Prep Kit, we performed 160 bp paired-end genome sequencing with an insert size of 350-400 bp on an Illumina NovaSeq 6000 instrument, generating 70-90 Gb per sample with a mean sequencing depth of 37x. We performed data analysis inhouse on a high-performance computing cluster using the megSAP pipeline, including ClinCNV, ExpansionHunter and manta packages.

Results: Of all analyses, 87% were performed as singleton analysis, 11% as trio analysis and <1% as duo and quartet analyses, respectively. 58% of index cases were pediatric, 41% were adult. Median turnaround time from receipt of sample to return of result was 38 days. Most prevalent requests regarded hereditary liver diseases, immune disorders and neurological diseases. The overall rate of positive results was 19%. Positive results were most prevalent in patients with dermatologic diseases, epilepsies and hearing disorders. Duo/trio/quartet analyses were ordered mostly for syndromic diseases, developmental delay and epilepsies. Singleton analysis resulted in a higher diagnostic rate of truncating variants and underrepresentation of missense variants compared to duo/trio/quartet analysis. In four cases, genome sequencing revealed cytogenetic diagnoses such as microduplication and microdeletion syndromes. In three cases, genome sequencing was ordered specifically to characterize a previously known structural variant of unknown significance. In all samples, the breakpoints were identified successfully. Additionally, in a family with a history of neuroblastoma and central hypoventilation, that was previously analyzed by Sanger sequencing as well as exome sequencing with a negative result, genome data revealed a pathogenic repeat expansion in exon 3 of the *PHOX2B* gene.

Conclusion: The advantage of genome sequencing is certainly the ability to characterize structural variants by breakpoint sequencing and its potential as a one-fits-all method for detection of structural variants and single nucleotide variants. However, handling of raw genome data is not straightforward due to the sheer size and requires professional (bio-)informatics resources if data analysis is performed inhouse.

Abstract ID: 81

P6-5

Loss of function variants affecting the STAGA complex component *SUPT7L* cause a developmental disorder with generalized lipodystrophy

J. Kopp[1,2,3], L. Koch[1], H. Lyubenova[1,2], O. Küchler[4], M. Holtgrewe[5], A. Ivanov[5], S. Mundlos[1,2], N. Ehmke[1], D. Seelow[1,4], M. Fradin[6], U. Kornak[1,2,7], B. Fischer-Zirnsak[1,2]

[1]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Medical Genetics and Human Genetics, Berlin, Deutschland, [2]Max Planck Institute for Molecular Genetics, FG Development and Disease, Berlin, Deutschland, [3]Institute of Chemistry and Biochemistry, Department of Biology, Chemistry and Pharmacy, Freie Universität Berlin, Berlin, Deutschland, [4]Exploratory Diagnostic Sciences, Berlin Institute of Health Charité - Universitätsmedizin Berlin, Berlin, Deutschland, [5]Core Unit Bioinformatics (CUBI), Berlin Institute of Health Charité - Universitätsmedizin Berlin, Berlin, Deutschland, [6]Service de Génétique Clinique, Centre Référence Déficiences Intellectuelles CRDI, Hôpital Sud - CHU Rennes, Rennes, Frankreich, [7]Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Deutschland

Generalized or partial lipodystrophy is a feature of various hereditary disorders, often leading to a progeroid appearance of the affected individuals. The hitherto described disease genes play a role in a wide range of pathways affecting mitochondrial and nuclear function, lipid biogenesis, DNA repair, and transcription. In the present study we identified the molecular cause of a multisystem condition with generalized lipodystrophy. The proband presented with severe global developmental delay with absent speech, short stature, failure to thrive, neonatal teeth, congenital cataract and reduced subcutaneous fat tissue, leading to an overall progeroid appearance. We performed trio genome sequencing and identified a missense and a frameshift variant in a compound heterozygous state in *SUPT7L*, which encodes a component of the transcriptional coactivator complex STAGA. By transcriptome sequencing, we discovered that the predicted missense variant causes alternative splicing, leading to a frameshift. As a consequence, the SUPT7L protein was absent in dermal fibroblasts and we also found altered expression of genes involved in DNA repair. Using immunofluorescence, we investigated this process and could show an increased rate of γ-H2AX foci in the proband's fibroblasts. Finally, we performed transient overexpression of wildtype *SUPT7L* in the proband’s fibroblasts rescuing the elevated number of γ-H2AX foci. In conclusion, we describe an individual with a severe multisystem condition caused by pathogenic *SUPT7L* variants. We therefore suggest *SUPT7L* as a novel candidate gene for progeroid phenotypes with generalized lipodystrophy.

Abstract ID: 83

P3-45

Only one beer can be mortal: a case report of two sisters with cardiac arrest due to a homozygous *PPA2* mutation

H.H. Manzanilla Romero[1], S. Rudnik-Schöneborn[1]

[1]*Institute of Human Genetics, Medical University of Innsbruck, Innsbruck, Österreich*

Introduction: First described in 2016, the deficiency of mitochondrial inorganic pyrophosphate PPA2 is a rare autosomal recessive disease associated with sudden cardiac death in young patients followed by neurological signs (cerebellar ataxia, peripheral neuropathy). Most children die in early infancy, but for those who survive into their teenage years, intolerance to small amounts of alcohol was shown. Cardiac features are heart failure, dilated cardiomyopathy with fibrosis, and arrhythmias.[1]

Case Report: Here, we briefly describe two teenage sisters who debuted with cardiac arrest after consuming minimal amounts of alcohol. They are the daughters of two healthy Caucasian parents coming from the same region in Tyrol. Patient 1 dramatically survived two cardiac arrests, the first at age 14 after a New Year´s Eve celebration, and the second a year later. Screening for drug intoxications in plasma was negative, including ethyl alcohol (NAD/ADH) <0,1 g/l. Cardiac MRI revealed reduced left ventricular function, and chronic myocarditis was suspected by endomyocardial biopsy. There was no evidence of an inherited cardiac arrhythmia. After the second cardiac arrest, an implantable cardiac defibrillator was provided. A multigene-panel analysis in 2015 (48 arrhythmia-related genes, Amsterdam) showed two heterozygous variants of uncertain significance in *SCN5A* (c.647C>T; p.Ser216Leu) and *CACNA1D* (c.3374C>T; p.Ala1125Val). The younger sister, patient 2, died suddenly in 2019 at age 15 after drinking 1-2 beers with friends. Blood alcohol level was 0.13 permille. The cardiac autopsy did not show structural defects but gave evidence of myocarditis. Segregation studies of the *SCN5A* and *CACNA1D* variants remained inconclusive, as both variants were present in the patients and their unaffected mother. Since patient 1 had a slow progressive cardiomyopathy, a new attempt for genetic diagnosis was initiated in 2021. Duo exome sequencing revealed PPA2-related mitochondriopathy due to a homozygous mutation c.683C>T, p.Pro228Leu in *PPA2* gene in both sisters. At the time of the last examination at age 21, patient 1 did not show neurological abnormalities.

Conclusion: Our case report demonstrates how important it is to question inconclusive genetic results. At the time of the first multigene-panel analysis, the *PPA2* gene had not been discovered. It was finally exome sequencing, which diagnosed the only recently described PPA2-related mitochondriopathy. The correct diagnosis is important for medical care (cardiac and neurological surveillance) and for everyday life. Alcohol intake can trigger cardiac arrest and should be strictly avoided.

1Guimier A, et al. PPA2-associated sudden cardiac death: extending the clinical and allelic spectrum in 20 new families. Genet Med. 2021;23:2415-2425.

Abstract ID: 86

Parallel in-depth analysis of repeat expansions in ataxia patients by long-read sequencing

H. Erdmann[1,2], F. Schöberl[3], M. Giurgiu[1,4], R.M. Leal Silva[1], V. Scholz[1], F. Scharf[1], M. Wendlandt[1], S. Kleinle[1], M. Deschauer[5], G. Nübling[3], W. Heide[6], S.S. Babacan[7], C. Schneider[8], T. Neuhann[1], K. Hahn[9], B. Schoser[2], E. Holinski-Feder[1,10], D.A. Wolf[1,11], A. Abicht[1,2]

[1]Medizinisch Genetisches Zentrum München, München, Deutschland, [2]Friedrich-Baur-Institut an der neurologischen Klinik und Poliklinik des Klinikums der LMU München, München, Deutschland, [3]Neurologische Klinik und Poliklinik des Klinikums der LMU München, München, Deutschland, [4]Experimental and Clinical Research Center des Max-Delbrück-Centrum für Molekulare Medizin und der Charité Berlin, Berlin, Deutschland, [5]Klinik und Poliklinik für Neurologie, Klinikum Rechts der Isar (Technische Universität München), München, Deutschland, [6]Klinik für Neurologie, Allgemeines Krankenhaus Celle, Celle, Deutschland, [7]Klinik für Neurologie und Neurointensivmedizin, Klinikum Darmstadt, Darmstadt, Deutschland, [8]Klinik für Neurologie und klinische Neurophysiologie, Universitätsklinikum Augsburg, Augsburg, Deutschland, [9]Klinik für Neurologie mit Experimenteller Neurologie, Charité Berlin, Berlin, Deutschland, [10]Medizinische Klinik und Poliklinik IV des Klinikums der LMU München, München, Deutschland, [11]II. Medizinische Klinik und Poliklinik, Klinikum Rechts der Isar (Technische Universität München), München, Deutschland

Introduction: Instability of simple DNA repeats has been known as a common cause of hereditary ataxias for over 20 years. Routine genetic diagnostics of these phenotypically similar diseases still rely on an iterative workflow for quantification of repeat units by PCR-based methods of limited precision. It only partly covers complex repeat disorders such as *RFC1* spectrum disorder, SCA31 and SCA37 in which both, repeat motive and repeat length, determines pathogenicity.
Method: We established and validated clinical nanopore Cas9-targeted sequencing (Clin-CATS), an amplification-free method for simultaneous analysis of 15 repeat loci associated with hereditary ataxias including the complex ataxias SCA31, SCA37 and *RFC1* spectrum disorder. The method combines target enrichment by CRISPR/Cas9, Oxford Nanopore long-read sequencing, and a bioinformatics pipeline for parallel detection of length, methylation, and sequence of the repeat loci.
Results: Clin-CATS allowed for the precise and parallel analysis of 15 repeat loci associated with adult-onset ataxia and revealed additional parameter such as *FMR1* promotor methylation and repeat sequence. We analyzed 125 clinical samples of undiagnosed ataxia patients and identified causative repeat expansions in 32 patients including rare conditions such as a very-late onset Friedreich's ataxia and or a Fragile-X-associated-tremor-and-ataxia syndrome (FXTAS) caused by a full *FMR1* repeat expansions with non-methylated *FMR1* promotor. Biallelic expansions within *RFC1* were identified as the most frequent cause of ataxia highlighting the importance of considering this locus in patients with adult-onset ataxia.
Conclusion: Our results highlight the power of Clin-CATS as a readily expandable workflow for the in-depth analysis and diagnosis of phenotypically overlapping repeat expansion disorders that enables a molecular diagnosis of ataxias independent of preconceptions based on clinical presentation.

Abstract ID: 87

P5-2

When should we perform prenatal testing for uniparental disomies and imprinting disorders?

T. Eggermann[1], E. Schlaich[1]

[1]*Institut für Humangenetik und Genommedizin, Aachen, Deutschland*

Introduction: According to current guidelines (S2k-Leitlinie, Reg. No. 078-015: 5.2.1.5), prenatal testing for imprinting disorders (ImpDis) should be performed in case of structural variants prone to formation of uniparental disomies (UPD) affecting imprinted chromosomal regions. These include (Robertsonian) translocations and trisomies of chromosomes 6, 7, 11, 14, 15, 16, and 20, as well as clinical features consistent with ImpDis. Previous studies have shown that the risk for UPD of chromosomes 14 or 15 in fetuses of parents with a Robertsonian translocation is estimated to be 0.06%. Based on own data from routine diagnostics we evaluated under which circumstances prenatal testing for ImpDis should be indicated in the future.

Methods: We ascertained 308 cases which were referred for prenatal molecular testing due to suspicion of UPD or the ImpDis Silver-Russel-syndrome (SRS) and Beckwith-Wiedemann-syndrome (BWS) with respect to the frequency of positive results.

Results: In 96 families with Robertsonian and in 91 other translocations cases UPD was not detected. In 29 pregnancies with trisomies 7 (n=12) and 16 (n=11) we identified three upd(16)mat. In pregnancies referred due to ultrasound features compatible with SRS or BWS the diagnosis could be confirmed in four cases (SRS: n=1/26; BWS: n=3/66).

Conclusion: Our data and those of the literature show that a positive testing result for UPD in case of (Robertsonian) translocation is rare. Therefore it should be asked whether prenatal testing should still be carried out in the future for this indication. In contrast, prenatally detected trisomies and ImpDis specific prenatal features depict a considerable indication for molecular testing. However, prior to invasive prenatal molecular testing, its benefit with regard to the result’s impact on the management of the pregnancy has to be considered.

Abstract ID: 88

P7-8

Navigating the uncharted landscape of RNA modifications by leveraging Oxford Nanopore Technology's direct sequencing and machine learning algorithms

A. Wierczeiko[1], S. Mündnich[2], N. Alagna[1], M. Helm[2], S. Gerber[1]

[1]Institute of Human Genetics, University Medical Center Mainz, Mainz, Deutschland, [2]Institute of Pharmaceutical and Biomedical Sciences, Johannes Gutenberg-University Mainz, Mainz, Deutschland

Post-transcriptional modifications are essential for the structure and stability of RNA molecules and, when aberrant, can cause serious pathologies, such as mitochondrial diseases. The mechanisms underlying the abnormal modification patterns and the role of their writers, readers and erasers in this context are still unclear. Direct sequencing of whole RNA molecules by Oxford Nanopore Technologies (dRNA-seq) provides the ability to simultaneously analyze and integrate transcriptome-wide expression and biochemical modification. In recent years, several tools have been published that are able to detect distinct RNA modification patterns directly in the raw Nanopore signal and/or systematic basecalling errors. These methods have achieved relatively high accuracy for a handful of modifications, focusing on the most popular modification, m6A. However, to date, there is no method capable of quantitatively and qualitatively identifying different modifications in a single molecule simultaneously. This is mainly due to missing training data for most modification types. In our project, we are compiling an extensive database of artificially synthesized dRNA-seq datasets covering multiple modifications that will be utilized to train existing or newly developed machine learning models.

Abstract ID: 89

P6-10

Unmasking splicing effects of Otoferlin missense variants

Z. Zhang[1,2], D. Owrang[1,2], B. Vona[1,2]

[1]University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Deutschland, [2]University Medical Center Göttingen, Institute for Auditory Neuroscience and InnerEarLab, Göttingen, Deutschland

Otoferlin (*OTOF*, OMIM: 603681) is an essential protein with at least three non-mutually exclusive roles in synaptic signaling of the inner hair cells that reside in the inner ear. Genetic variation of otoferlin is responsible for a clinically distinct autosomal recessive auditory neuropathy/auditory synaptopathy (DFNB9, OMIM: 601071). Missense variants are the most common variant type reported in otoferlin patients. An important and often underestimated effect of subtle perturbations of the nucleotide sequence concerns the potentially drastic impact on the contextual landscape of splice signals used for orchestrating proper splicing by the spliceosome. In the literature, RNA studies of missense variants are not commonly performed and RNA-sequencing approaches to characterize putative splicing of *OTOF* variants in patients is not possible due to expression of the functionally-relevant transcript limited to the inner ear. Therefore, in vitro cell assays are required to uncover the mutational signatures of otoferlin on a variant-by-variant basis. A comprehensive understanding of molecular disease mechanisms may uncover a potential source of variability in gene therapy clinical trials and are prerequisite for successful genotype-phenotype correlation studies.

To unravel splicing effects of missense variants, we curated all reported *OTOF* variants from the literature and clinical databases for bioinformatics analysis using multiple splice prediction tools. These tools deliver evidence for possible cryptic splice activation, exon skipping, intron retention, as well as estimating effects on exonic splice enhancers/silencers and branchpoint signals. A ranked list of missense variants according to possible splice effects was generated. To investigate the functional consequence of missense variants on RNA-level, minigene assays were performed using site-directed mutagenesis and transfection into cell lines. cDNAs of each variant and control were visualized on agarose gels and characterized by Sanger sequencing. This work functionally characterizes splicing effects of missense variants that would otherwise be erroneously classified as pure non-synonymous substitutions and is an important step for a global understanding of the molecular mechanisms of missense variants in *OTOF*.

Abstract ID: 91

P2-11

Gene expression profile of eccrine porocarcinoma point to biologically heterogeneous diseases and gene expression signatures of general downregulation

S. Redler[1], S. Holst[1], A. Giesen[1], F. Meier[2], J. Otte[3], P. Petzsch[4], J. Reifenberger[5], D. Westphal[2], M. Ziemer[6], W. Wruck[7], R. Betz[8], J. Adjaye[7], A. Ruetten[9], H. Surowy[10]

[1]Institute of Human Genetics, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Deutschland, [2]Carl Gustav Carus Medical Centre, TU Dresden, Department of Dermatology, Dresden, Deutschland, [3]Institute for Stem Cell Research and Regenerative Medicine, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Deutschland, [4]Biological and Medical Research Centre (BMFZ), Düsseldorf, Deutschland, [5]Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Department of Dermatology, Düsseldorf, Deutschland, [6]University of Leipzig, Department of Dermatology, Leipzig, Deutschland, [7]Institute for Stem Cell Research and Regenerative Medicine, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Düsseldorf, Deutschland, [8]Institute of Human Genetics, University of Bonn, Medical Faculty and University Hospital Bonn, Bonn, Deutschland, [9]Dermatopathology Bodensee, Friedrichshafen, Deutschland, [10]Institute of Human Genetics, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Deutschland

Introduction: Malignant sweat gland tumours are rare, with the most common form being Eccrine porocarcinoma (EP). Eccrine poroma (EPO), the benign counterpart of EP, in turn, is the most common entity and accounts for 10 % of sweat glands tumors. Transition of EPO into EP is described in up to 18% of cases.

Methods: To investigate the mutational landscape of EP, we performed transcriptome profiling in a large and clinically well-characterized cohort of n=23 FFPE EP tumor samples and matched surrounding healthy tissue. Transcriptome analysis (Affymetrix PrimeView Human Gene Expression Microarrays) were carried out in an exploration cohort of 10 EPs and findings were validated in an independent sample of 13 additional primary EPs. In addition, findings from EPs were tested in a cohort of 17 EPOs to gain insights into the potential drivers of the malignant transformation from EPO to EP.

Results: Our data revealed diversity of EPs as a whole and point to biologically heterogeneous diseases. Hierarchical clustering identified two key matrisomal clusters likely representing two major EP subtypes with marked transcription alterations and crucial oncogenic pathways suggesting new mechanisms of tumorigenesis. E.g. VEGF/VEGFR (vascular endothelial growth factor) axis, Endothelin pathway, EGFR pathway, Hair follicle development: cytodifferentiation- part 3 of 3-pathway. The explorative transcriptome analyses by microarrays and subsequently validation not only showed tumor heterogeneity but also, surprisingly, gene expression signatures of general downregulation. In addition, our results provide evidence of possible malignant transformation of EPOs to EPs and reinforce the importance of using integrative research strategies that shall also consider poromas.

Conclusion: Detailed results of the analyses will be presented at the congress.

Abstract ID: 93

P3-46

Progressive congenital microcephaly, growth retardation and dysmorphisms in a male infant with an intronic splice variant in *GPKOW*

J. Lisfeld[1], M. Jankofsky[2], M. Kutsche[1], T. Herget[1], F. Kortüm[1]

[1]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland, [2]University Medical Center Hamburg-Eppendorf, Department of Pediatrics, Hamburg, Deutschland

Background: To date a splice variant is the solely published pathogenic variant in the X-chromosomal gene *GPKOW* which has been shown to segregate in a family manifesting a perinatal lethal condition characterized by severe microcephaly and intrauterine growth retardation (IUGR) in male. We report on an alive born male with progressive microcephaly found to have a *de novo* intronic variant in *GPKOW* also affecting splicing by whole exome sequencing (WES) and subsequent transcript analysis. *GPKOW* encodes the G-patch domain and KOW motifs-containing protein which is essential for pre-mRNA splicing. Thus, GPKOW is one further addition to the group of proteins crucial for key cellular processes being involved in the pathogenesis of congenital microcephaly.

Case report: The boy was born as second child to healthy non-consanguineous parents with unremarkable family history at 38+1 weeks of gestation. He was small for gestational age and presented with congenital microcephaly, bilateral ear tags, congenital cataracts, microphthalmia and suspected hearing impairment. Brain MRI showed hypoplastic corpus callosum. Cardiac workup revealed a hyperplastic thoracic aorta and hypoplastic pulmonary arteries. Microcephaly and severe feeding problems were progressive over time. The patient died due to unexplained cerebellar herniation shortly after percutaneous jejunostomy at 11 months of age.

Methods and results: Trio WES revealed a *de novo* hemizygous intronic variant in *GPKOW* (c.176+42G>A) predicted to impact splicing. By analysing blood-derived RNA, we found an insertion of 39 intronic nucleotides in the majority of *GPKOW* transcripts which introduces an in-frame premature stop codon. Eventually, the *GPKOW*-variant was classified as probably pathogenic and therefore disease-causing in our patient.

Conclusion: Severe microcephaly as leading symptom in pre- and postnatal settings is a frequent reason for performing WES. In a diagnostic setting variants in genes not yet annotated as disease genes in OMIM like *GPKOW* as well as intronic variants can be overlooked by this analysis. Here we present the second pathogenic variant in the *GPKOW* gene in a patient whose phenotype is in line with that of the reported family, however, a survival beyond the perinatal period has not been described yet. We propose that genetic diagnostics in male infants with progressive congenital microcephaly, IUGR, hearing and vision impairment and dysmorphisms should include analysis of the *GPKOW* gene.

Abstract ID: 94

P5-7

Class I and class II analphoid supernumerary marker chromosomes: pitfalls in prenatal diagnostic and parental transmission

Y. Stratis[1], M. Kuschmann[1], C. Müller-Hofstede[1], A. Busche[1], J. Horvath[1], A. Bohring[1], A. Röpke[1]

[1]Institut für Humangenetik, Universitätsklinikum Münster, Münster, Deutschland

Neocentromere formation is a rare event reported in more than 100 cases. Here we report two cases of a small supernumerary marker chromosome with neocentromere formation (analphoid marker chromosome). In the first case, prenatal and postnatal diagnostic was performed. The fetus presented with an omphalocele, an increased nuchal translucency, a hygroma colli, lateral neck cysts and an anomaly of the cerebellum. Direct and long-term culture of chorionic villi revealed a normal male karyotype. SNP array and symptom-based panel analysis on long-term CVS culture showed no pathogenic variant. Postnatally, the one year old boy presented with global developmentally delay, facial dysmorphism, hirsutism, soft cleft palate, cardiomyopathy, and omphalocele. Chromosomal and FISH analysis of peripheral lymphocytes showed a de novo analphoid class I marker which is formed by an inverted duplication of the distal part of the long arm of chromosome 3 resulting in a partial tetrasomy of the chromosomal region 3q26.2 – 3qter. This marker chromosome was present in 94% of the analysed metaphases. Thus, in this case a discrepancy between the analysed chorionic villi sample and the fetus was observed (fetoplacental discordancy). In the second case an analphoid class II marker was found, which was meiotically transmitted from the carrier mother to her son. The five year old son presented with global developmental delay, microcephaly and hexadactyly. The healthy mother shows an insertion of chromosome 4 specific material into the distal part of the long arm of chromosome 12 (ins(12;4)(q24.33;q24q28)) and a simultaneous resection of 12q2?3-12q24.3 material, which generates an analphoid ring chromosome. During meiosis, an imbalanced form of the mother’s karyotype was transmitted to the son. The son’s karyotype showed a normal chromosome 4, the derivative chromosome 12 with additional chromosome 4 specific material (ins(12;4)(q24.33;q24q28)) and the analphoid ring chromosome with material of the region 12q2?3-12q24.3. In case 1 and case 2, FISH analysis with a human pan centromeric probe revealed the absence of alpha-satellite DNA, confirming the existence of a neocentromere.

Abstract ID: 95

P3-47

Hydatidiform mole with coexistent fetus

K. Bahlke[1], Y. Stratis[1], A. Röpke[1], C. Müller-Hofstede[1], A. Bohring[1]

[1]Institut für Humangenetik, Universitätsklinikum Münster, Münster, Deutschland

Hydatidiform moles are characterized by placental pathologies such as hyperplasia of chorionic villi and trophoblastic atypia, which usually don't allow any regular fetal development. Two types of hydatidiform moles can be distinguished due to different formation mechanisms: 1. Complete hydatidiform moles (CHM) result from fertilization of an anuclear, empty ovum either by two sperms or by one sperm, which then reduplicates, both leading to a diploid set of paternally inherited chromosomes. 2. In contrast, partial hydatidiform moles (PHM) result from fertilization of a normal, haploid ovum by two sperms, leading to a triploid karyotype. Additionally, rare cases of focal hydatidiform moles with a coexistent fetus have been reported.

Here we report on a 38-year-old G2 PI AI whose second pregnancy was terminated by sectio parva at week 19+0 due to severe preeclampsia, highly elevated β-hCG and a morphologically altered placenta praevia totalis, suspicious of a hydatidiform mole in ultrasound examination. For that reason, amniocentesis had been performed at week 17+0 with the following chromosome analysis revealing a 46,XY karyotype. SNP-Array on amniotic cells was unremarkable in respect of explaining the placental anomalies. To investigate a possible genetic predisposition for recurrent hydatidiform moles, NLRP7 and KHDC3L were sequenced in maternal blood without pathological findings.

Since fetal autopsy and pathologic examination had shown a normotrophic placental part as well as an altered placental part with trophoblastic hyperplasia possibly suggestive of a twin pregnancy, further genetic diagnostics were performed on cells originating from the hyperplastic placental part. SNP-Array now revealed a genome-wide paternal uniparental isodisomy (arr(X,1-22)x2 hmz pat.) with a 46,XX (androgenetic) karyotype, altogether suggestive of a CHM.

In a synopsis of clinical and genetic findings, the initial existence of a twin pregnancy consisting of a regular pregnancy with a male karyotype and a CHM with a female karyotype seems now very likely.

Clarifying the placental anomaly's cause was a great reassurance for our patient with respect to her future family planning. The case underlines the importance of interdisciplinary teamwork and supports the clinical utility of additional genetic testing in accordance with obstetrical and pathological findings.

Abstract ID: 96

P3-24

Heterozygous *ADAR* pathogenic variant associated with variable neurological symptoms and incomplete penetrance in one family

A.-K. Bauer[1], G.-C. Korenke[2], I Marquardt[2], L. Gieldon[1], T Haack[3], K. Klein[3], K Grundmann-Hauser[3], M. Lee-Kirsch[4], C. Wolf[4], M.-P. Hitz[1]

[1]Institute of Human Genetics, University Hospital Klinikum Oldenburg, Oldenburg, Deutschland, [2]University Children's Hospital, Klinikum Oldenburg, Department of Neuropediatrics, Oldenburg, Deutschland, [3]Institute of Human Genetics, University Hospital Tübingen, Tübingen, Deutschland, [4]University Hospital Dresden, Department of Pediatrics, Dresden, Deutschland

Background: Heterozygous pathogenic variants in *ADAR* are associated with dyschromatosis symmetrica hereditaria and biallelic pathogenic variants are associated with Aicardi-Goutières syndrome 6 (AGS6). However, the heterozygous variant c.3019G>A, has been described to cause neurological manifestations, which resemble AGS6 and are associated with an upregulation of interferon-stimulated genes. Symptoms are thought to be triggered by an infection and fever in previously normally developing children.

Case Summary: We report one family with the heterozygous *ADAR* pathogenic variant c.3019G>A with two symptomatic family members and three unaffected carriers. The index patient (patient 1) normally developed until his 3rd year of life. He then developed a gait disorder with weakness in his legs, a tendency to fall, hyperreflexia, dyslalia and mild cognitive developmental delay. The MRI showed unspecific signal elevation, possibly consistent with myelination defects, and leukodystrophy. A blood test showed a strong interferon signature with activation of type 1- interferon. At 4 years of age his symptoms showed no progression. He inherited the variant from his asymptomatic father (patient 2). The variant was also detected in his 10-month-old sister. So far, she has shown no symptoms (patient 3). A half-brother (patient 4) to the father (patient 2) developed a cerebral movement disorder with spasticity and regression of previous skills at the age of 6 months after a fever reaction (possibly in the context of a vaccination) at the age of 4 months. MRI showed a generalized signal elevation of white matter and progressive brain atrophy. At 19 years of age, the patient was wheelchair-bound and had severe global development delay. Blood tests also showed a strong activation of type 1 interferon. The father of patient 2 and 4 (patient 5) also carries the same pathogenic *ADAR* variant and shows strong activation of type 1 interferon but is asymptomatic at age 58.

Conclusion: The heterozygous pathogenic variant *ADAR* c.3019G>A shows incomplete penetrance in this family and might be causative for variable neurological symptoms in two affected family members. A treatment option to halt disease progression with prednisolone or selective Janus kinase inhibitors has been suggested. This makes the identification of affected children even more important.

Abstract ID: 97

P2-9

CRISPR/Cas9-mediated generation of cell lines with one and two defined fusion transcripts for Next Generation Sequencing based molecular analyses

M.F. Hossain[1], L. Hagenau[1], M. Franz[1], B. Nowack[2], L.R. Jensen[1], A.W. Kuss[1]

[1]Universitätsmedizin Greifswald, Human Molecular Genetics Group, Department of Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, Greifswald, Deutschland, [2]SensID GmbH, Rostock, Deutschland

Next Generation Sequencing (NGS) applications, including RNA-sequencing (RNAseq), have become established tools not only for a still increasing number of research applications but also for a variety of diagnostics purposes. In both research and diagnostics, reference materials are needed to ensure the greatest possible comparability and reliability of the results. Fusion transcripts contain a hybrid sequence derived from two independent genes and can be detected at the RNA level. For RNA-based analyses such as RNAseq, whole cells with known fusion transcripts but also isolated RNA can be used as reference material. In this project, we aim to develop and research fusion transcript-based reference materials for RNAseq applications. First, we wanted to create a reference cell line that expresses a known fusion transcript such as the BCR-ABL1 transcript. This fusion is caused by a t(9;22)(q34;q11) chromosomal translocation of the proto-oncogenes ABL (Chr 9) and BCR (Chr22). It is also known as the “Philadelphia chromosome”, which is well-researched and occurs frequently in acute lymphoblastic leukemia (ALL) and chronic myelogenous leukemia (CML). Using the CRISPR/Cas9 technology, we have developed a protocol to create a fusion gene in different cell types i.e. suspension and adherent cells. In this method, we target the breakpoint region of the two introns of the genes involved in the rearrangement. Co-transfection of CRISPR guide RNAs and Cas9 induces double-strand breaks in the introns of both genes simultaneously, which can result in DNA misrepair by fusing the truncated genes together. First, we selected Jurkat cells. These are human cancer cells derived from the peripheral blood of an acute T lymphocytic leukemia patient, which does not contain the BCR-ABL1 fusion. We successfully introduced this fusion in these cells and confirmed it by sequencing. To understand what happens to a cell in the presence of multiple chromosomal translocations, we then expanded our study by editing cells from a human bladder cancer cell line, which is known to have a fusion of FGFR3-TACC3 at chromosome 4. Using the same CRISPR-Cas9 method, we introduced a BCR-ABL1 fusion in these cells, thus generating a new line with two different fusion transcripts per cell. Having two cell lines with at least one or multiple known fusion transcripts will allow us to use them as reference materials for NGS-based RNA analysis and also to further study the molecular basis of ALL and CML.

Abstract ID: 98

P3-78

Molecular autopsy – proclamation for an undervalued procedure with preventive power for relatives at risk

A.-C. Berking[1], A. Reitz[2], B. Auber[1], M. Klintschar[3], T. Ripperger[1], E. Gradhand[2], A.K. Bergmann[1]

[1]Hannover Medical School, Department of Human Genetics, Hannover, Deutschland, [2]Dr. Senckenberg Institute of Pathology, Goethe University, University Hospital Frankfurt, Frankfurt, Deutschland, [3]Hannover Medical School, Institute of Legal Medicine, Hannover, Deutschland

Introduction: A molecular autopsy can clarify the cause of death in unexplained cases by means of molecular genetic analyses (e.g. immunodeficiencies, neurological-, syndromic- or cardiovascular disorders), which cannot be determined by clinical autopsy only.
According to the German Federal Statistical Office, in 2020 2,081 persons under the age of 45 and 84 children died from unknown causes in Germany. The number of unreported cases is probably much higher.
If an unknown cause of death is due to a genetic disease, the medical consequences and clinical implications are significant for biological relatives. Molecular autopsy is able to provide valuable results in numerous cases of these unexplained deaths.
Methods: We examined 16 recent cases of unexplained death in children via array-CGH/next generation sequencing. Practical workflows and efficiency were reviewed in terms of tissue preservation, cost coverage, genetic analyses and counselling. We systematically screened the literature concerning molecular autopsies.
Results: We identified a (likely) pathogenic variant in five patients (30 %) explaining their death. The variants were associated with a wide range of genetic disorders: Ehlers-Danlos syndrome - vascular type, acute necrotizing encephalopathy, familial hemophagocytic lymphohistiocytosis, duplication 8q syndrome, epileptic encephalopathy. A variant of unknown significance was found in six additional patients.
In the present cases, workflow and efficiency differed greatly. Proposals for improving molecular autopsy workflows were found in the literature, but no general recommendations, incorporating different disease entities and multiple clinical disciplines are established.
Conclusion: Our series and international studies (primarily cardiac/neurological entities) demonstrated that molecular autopsies are able to identify causal genetic variants in 25 - 30 % of unexplained deaths. Yet our study and systematic literature review indicated that an interdisciplinary guideline is needed, addressing issues such as indication, relative`s consent, post mortem tissue preservation, genetic counselling, cost coverage.
Therefore, we developed an interdisciplinary workflow to facilitate and ensure the performance of molecular autopsies – from the relative`s consent to post-mortem tissue preservation and genetic counselling. This workflow offers not only improvements in patient care but also the opportunity to expand clinical and genetic disease knowledge.

Abstract ID: 99

P1-8

Altered DNA methylation profiles in *SF3B1* mutated CLL patients

A. Pacholewska[1,2], C. Grimm[1,2], C.D. Herling[3], M. Lienhard[4], A. Königs[1,2], B. Timmermann[5], J. Altmüller[6], O. Mücke[7], H.C. Reinhardt[3,8,9], C. Plass[7,8], R. Herwig[4], M. Hallek[3], M.R. Schweiger[1,2]

[1]Institute for Translational Epigenetics, Faculty of Medicine, University Hospital Cologne, University of Cologne, Cologne, Deutschland, [2]Center for Molecular Medicine Cologne, University of Cologne, Cologne, Deutschland, [3]Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, German CLL Study Group, Department I of Internal Medicine, Faculty of Medicine, University Hospital Cologne, University of Cologne, Cologne, Deutschland, [4]Max Planck Institute for Molecular Genetics, Department of Computational Molecular Biology, Berlin, Deutschland, [5]Sequencing Core Facility, Max Planck Institute for Molecular Genetics, Berlin, Deutschland, [6]Cologne Center for Genomics, University of Cologne, Cologne, Deutschland, [7]German Cancer Research Center, Cancer Epigenomics, Heidelberg, Deutschland, [8]German Cancer Consortium, Heidelberg, Deutschland, [9]West German Cancer Center Essen, Department of Hematology and Stem Cell Transplantation, University Hospital Essen, Essen, Deutschland

Survival of cancer patients has been shown to be often affected by the mutations in splicing factor genes. Chronic lymphocytic leukemia (CLL) patients with a mutation in splicing factor 3b subunit 1 (*SF3B1*) have worse prognosis than patients without *SF3B1* mutations. Because of the close interconnection between splicing machinery and epigenome, we investigated if the *SF3B1* mutations affect the genome methylation of the CLL patients. Whereas an overall decrease in methylation was observed in CLL carcinogenesis, the interaction between the *SF3B1* mutational stage and the originating B cells epigenetic stage has not been described.

We profiled genome-wide the DNA methylation status of 27 CLL patients with and without *SF3B1* mutations using MEDIP-seq. We identified a local hypomethylation in CLL patients with mutations in *SF3B1* at 67 250bp regions, from which most was proximal to telomeric regions. These differentially methylated regions (DMRs) showed an enrichment in signaling genes known to be important in cancer e.g., *NOTCH1*, *HTRA3*, and *BCL9L*. Moreover, although the patients with *SF3B1* mutations exclusively clustered in two out of three epigenetic stages of the originating B cells, not all the DMRs could be explained by the different methylation programming (maturation) status of the B cells. This suggested that mutations in *SF3B1* cause additional epigenetic aberrations during carcinogenesis. In addition, 12 DMRs contained a binding site for an already implicated in CLL transcription factor, which is required for a proper differentiation of B cells - IKAROS.

We identified regions specifically hypomethylated in CLL patients with *SF3B1* mutations by investigating the genome-wide methylation profiles. In agreement with previous study, some of the changes identified overlapped changes reported as differentially methylated during physiological B cell maturation. Despite the fact that this indicates *SF3B1* mutations are, at least in part, associated with B cell developmental stages, a large fraction of DMRs identified in this study has not been related to B cell development. This indicates that the methylation at these regions is associated with *SF3B1* specific functions. A better understanding of the interplay between DNA methylation and splicing may pave the way for new treatment options for CLL patients with *SF3B1* mutations.

Abstract ID: 100

P6-16

Detection of circular RNAs in a mouse model for intellectual disability

J.-W. Schreier[1], S. Simm[2], L. Hagenau[1], A.W. Kuß[1], L.R. Jensen[1]

[1]Universitätsmedizin Greifswald, Interfakultäres Institut für Genetik und Funktionelle Genomforschung, Greifswald, Deutschland, [2]Universitätsmedizin Greifswald, Institut für Bioinformatik, Greifswald, Deutschland

FTSJ1 is an evolutionarily conserved and ubiquitously expressed protein. Loss of FTSJ1 function causes X-linked non-syndromic intellectual disability and in mouse models for Ftsj1 deficiency cognitive functions are also affected. However, the mice also present with additional features including reduced body mass, disturbed hormone and energy metabolism and hypoalgesia, suggesting that the entire organism is affected.
FTSJ1 encodes a tRNA 2’-O-methyltransferase that targets a subset of tRNAs in the anti-codon loop. These modifications stabilize the tRNAs and ensures efficient translation. Ftsj1 deficiency seems to have a general negative effect on protein synthesis as no single protein or annotated pathway has been reported to be affected. Ftsj1 methylation targets are determined through interaction with protein partners, but tRNAs are the only known FTSJ1 targets.
Non-coding RNAs receive more and more attention as these RNAs can be investigated using novel sequencing technologies. Non-coding RNAs are mainly known to function as regulators of gene or protein expression, but have recently also been found to interact with lipids and to form sub-organelles through protein interactions, which suggest novel roles for non-coding RNAs in cellular function.
As part of establishing a mouse non-coding RNA expression atlas, we analyze an RNA specie known as circular RNA. Circular RNAs are found in all investigated eukaryotes and some of them have been shown to function in gene regulation through binding of specific microRNAs, thereby preventing these from targeting mRNAs. However, circRNAs are not highly expressed making it necessary to enrich RNA samples for circRNAs prior to sequencing and subsequent detection through bioinformatics approaches.
From three male mouse littermates, each consisting of one wild-type and one Ftsj1 -/y mouse, we have extracted RNA from 5 tissues (brain, liver, heart kidney and spleen). After cicrRNA enrichment by RNase R digestion of linear RNAs and sequencing using an Illumina platform we identified candidate circRNAs in all 30 samples. Analysis of the sequences and expression patterns will be used to gain insight into the involvement of Ftsj1 in the expression and stability of this novel RNA specie.

Abstract ID: 103

P7-1

Whole-exome sequencing as a method for identification of uniparental disomies

J. Moch[1], M. Radtke[1], S.H. Drukewitz[1], I. Schumann[1], J. Hentschel[1]

[1]Universitätsklinikum Leipzig, Institut für Humangenetik, Leipzig, Deutschland

Uniparental disomy (UPD) is the inheritance of both homologs of a chromosome only from one parent. Mechanisms leading to UPD can be somatic recombination, gametic complementation, compensatory UPD, monosomy or trisomy rescue. Depending on the causing mechanism, UPDs can be differentiated in three types: isodisomy, heterodisomy and mixed UPDs. UPDs can lead to a wide phenotypic spectrum including obesity, short stature and/or intellectual disability which often occur in a syndromal context, e.g., Angelman-, Prader-Willi- or Beckwith-Wiedemann syndrome due to imprinting effects.
So far, the detection of UPDs in exome sequencing data is not well established. Therefore, the aim of our project is the identification of in silico parameters of different types of UPDs in clinical exomes and to further develop a detection tool with the goal to publicly provide it. For this purpose, we used a custom-made tool based on the allele frequency of single nucleotide variants (SNV) to find parameters for the characterization of UPDs focusing on heterodisomies and mixed UPDs, e.g. an inheritance ratio of maternal and paternal variants. For the first validation, we used 15 parent-child trios with different known UPDs as positive controls to show regions of uniparental inheritance and compare them with SNP array data.
For the verification of our defined parameters, we will use a cohort of unsolved cases with UPD-specific phenotypes and validate the results with SNP arrays and/or STR analysis. As the origin of the SNVs is essential for the analysis of UPDs, a trio analyses might be problematic in older patients or older cases. Likewise, consanguinity can be mixed up with different UPD events due to a similiar distribution of allele frequencies and thus, the occurrence of ROH´s.

Abstract ID: 105

P7-2

Lipidomics as a functional analysis tool of genetic variants in (un-)known hereditary metabolic disease

I. Evangelakos[1], J. Ridder[1], M. Musfeldt[1], S.Y. Piel[1], F. Haumann[1], J.-J. Scholz[1], A. Bischoff[2], V. Reichenbach[1], B. Schoser[2], L. Scheja[3], T. Klopstock[2], H. Prokisch[4], J. Heeren[3], C. Kubisch[1], A. Worthmann[3], C. Schlein[1], the mitoNET Consortium

[1]Institut für Humangenetik, Hamburg, Deutschland, [2]Friedrich-Baur-Institut, München, Deutschland, [3]Institut für Biochemie und Molekulare Zellbiologie, Hamburg, Deutschland, [4]Helmholtz Zentrum, München, Deutschland

Introduction: Many hereditary diseases cause direct or indirect changes in cellular lipid classes, such as storage lipids, membrane lipids, lipid intermediate metabolites or lipid signaling molecules. Subsequently, a broad variety of genetic variants might also show affected lipidomic signatures in distinct pathways. The analysis of cellular and/or systemic lipid metabolism can support the characterization of genetic variants and moreover, can give insights into basic pathomechanisms of genetic disease.

Methods: We conducted lipidomics and metabolomics of human plasma, fibroblasts or organ biopsies of patients of the human genetics, internal medicine or from multicentric studies mostly with confirmed monogenic disease, or undiagnosed disease, via LC/MS-based SCIEX lipidyzer platform, GC/MS and GC/FiD-based fatty acid profiling or commercially available untargeted metabolomic services. Further, these results were compared to different murine knockout or cellular knockdown models with disturbances in lipid metabolism.

Results: We analyzed plasma, organ, cell line and fibroblast samples and discovered both unspecific and specific lipidomic signatures. We could identify specific signatures in triglycerides, phospholipids, bile acids in known disease, such as lipodystrophy, different types of dyslipidemia, obesity, glycogenosis, and mitochondrial disorders, but also pave the way to characterize candidate gene dysfunction. For instance, we were able to characterize variants of unknown significance, such as a putatively pathogenic variants in genes related to lipid biosynthesis and bile acid metabolism with a distinct lipidomic fingerprints in complex lipids. Further we compared several hundred samples of patients with genetically confirmed mitochondriopathies of the mitoNET consortium regarding overlapping lipid species phenotypes to establish mitochondriopathy-associated lipidomic signatures.

Conclusion: In sum, we show that lipidomics are a powerful tool to generate pathomechanistic hypotheses for basic mechanisms of (un)known disorders and might become a useful to characterize samples from patients with monogenic candidate and disease gene variants of unknown significance in the future.

Abstract ID: 106

de novo PHF5A variants cause craniofacial abnormalities and developmental delay

F.L. Harms[1], A.J.M. Dingemans[2], M. Hempel[1,3], T. Bierhals[1], J.-M.M.F. Niermeijer[4], J. Fischer[5], A. Jahn[5], C. Hübner[6], S. Majore[7], E. Agolini[8], A. Novelli[8], J. van der Smagt[9], R. Ernst[9], E. van Bisbergen[9], L. Downie[10,11], L. Pais[12], S.M. White[10,11], B.B.A. de Vries[2], K. Kutsche[1]

[1]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland, [2]Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Department of Human Genetics, Nijmegen, Niederlande, [3]Institute of Human Genetics, University Hospital Heidelberg, Heidelberg, Deutschland, [4]Elisabeth-TweeSteden Hospital, Department of Neurology, Tilburg, Niederlande, [5]Institute for Clinical Genetics, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Deutschland, [6]University Hospital Carl Gustav Carus at the Technische Universität Dresden, Department of Neuropaediatrics, Dresden, Deutschland, [7]Division of Medical Genetics, Department of Experimental Medicine, San Camillo-Forlanini Hospital, Sapienza University, Rome, Italien, [8]Laboratory of Medical Genetics, Translational Cytogenomics Research Unit, Bambino Gesù Children Hospital, IRCCS, Rome, Italien, [9]University Medical Center Utrecht, Utrecht University, Department of Genetics, Utrecht, Niederlande, [10]Victorian Clinical Genetics Service, Murdoch Children's Research Institute, Victoria, Australien, [11]University of Melbourne, Department of Paediatrics, Melbourne, Australien, [12]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, Vereinigte Staaten

The spliceosomal complex is a macromolecular machine comprising multiple uridine-rich (U1, U2, U4/U6, and U5) small nuclear RNAs and associated ribonucleoproteins (snRNPs) as well as associated proteins. The U2 snRNP complex assembles at the 3' acceptor site of the intron and contains stable core subunits and the dynamically associated sub-complexes SF3A and SF3B. The SF3B complex is composed of SF3B1-6 and PHF5A and recognizes the branch point adenosine. PHF5A is a ubiquitously expressed and evolutionarily highly conserved nuclear protein. The absence of any close homolog of *PHF5A* in the human genome suggests an important biological role of PHF5A. Through GeneMatcher, we recruited six individuals with variable congenital malformations, consisting of ear abnormalities and hypospadias, pre- and postnatal growth abnormalities, and developmental delay who all carried a *de novo* heterozygous variant in *PHF5A*. Five individuals had a likely loss-of-function (LOF) variant, including the start-loss variant c.2T>C/p.?, the 2-bp deletion c.69_70del/p.(Cys23*) in two unrelated subjects, the nonsense variant c.70G>T/p.(Glu24*), and the 1 bp-duplication c.276dup/p.(Ser93Glufs*3). One subject carried the missense variant c.185C>A/p.(Gly62Glu). In fibroblasts from four subjects with *PHF5A* LOF variants, we identified wild-type and mutant *PHF5A* mRNAs at a ratio of ~1:1, suggesting escape of nonsense-mediated mRNA decay that was confirmed by similar *PHF5A* mRNA levels in subject and control cells. On protein level, we detected similar amounts of PHF5A with the predicted wild-type molecular weight as well as of SF3B1-3, and SF3B6 in almost all subject and control fibroblasts. SF3B complex formation was unaffected in cells with the p.(Glu24*) or the p.(Gly62Glu) variant. Our data suggest the existence of feedback mechanisms in subject cells with heterozygous *PHF5A* LOF variants to maintain normal levels of PHF5A and SF3B components. Several genes encoding splicing factors, like *SF3B2* and *SF3B4*, the causative genes for craniofacial macrosomia and Nager syndrome, respectively, negatively autoregulate their own expression to control and maintain homeostatic levels of spliceosomal proteins required for effective pre-mRNA splicing. The observed compensatory mechanisms in certain types of subject cells call into question haploinsufficiency as pathomechanism and suggest disturbed autoregulation of mutated splicing factor genes in specific cell types during embryonic development.

Abstract ID: 107

Impact of hypermannosylation on the structure and functionality of the ER and the Golgi complex in AAMR syndrome

P. Franzka[1], S.C. Schüler[2], T. Kentache[3], R. Storm[4], A. Bock[1], I. Katona[5], J. Weiß[5], S. Hoffmann[2], C. Kaether[2], C. Hübner[1]

[1]Institut für Humangenetik, Funktionelle Genetik, Jena, Deutschland, [2]Leibniz-Institute on Aging, Jena, Deutschland, [3]de Duve Institute, Brüssel, Belgien, [4]Molecular Devices, München, Deutschland, [5]Institut für Neuropathologie, Aachen, Deutschland

Glycosylation, the most common post-translational modification, takes place in the endoplasmic reticulum (ER) and the Golgi apparatus. Altered protein glycosylation can manifest in serious, sometimes fatal malfunctions. We recently showed that mutations in the GDP-mannose pyrophosphorylase A (GMPPA) can cause a syndrome characterized by alacrima, achalasia, mental retardation and myopathic alterations (AAMR syndrome). GMPPA acts as feedback inhibitor of GDP-mannose pyrophosphorylase B (GMPPB), which provides GDP-mannose as a substrate for protein glycosylation. Loss of GMPPA enhances incorporation of mannose into glycochains of various proteins, including α-dystroglycan (α-DG), a protein that links the extracellular matrix with the cytoskeleton. Therefore, we used our GMPPA KO mice and mouse embryonic fibroblasts (MEFs) to study the consequences of increased mannosylation for the ER and the Golgi apparatus. Notably, we detected a fragmentation of the Golgi apparatus in GMPPA KO mouse tissues and in GMPPA KO MEFs. Glycoprotein pulldown from skeletal muscle and brain tissue in combination with quantitative mass spectrometry identified alterations of several ER- and Golgi-resident proteins in GMPPA KO mice, which were mostly involved in organelle stabilization, protein folding and trafficking, glycosylation and protein phosphorylation. Moreover, we found that the activity of the Golgi-associated endoprotease furin is decreased in KO MEFs. Moreover, the ratio of α-DG, which is retained in the ER, is increased. Notably, mannose supplementation mimicked these changes in ER and Golgi complex structure and function in WT MEFs. Our data show that protein hypermannosylation does not only result in alterations of the structure of the ER and the Golgi apparatus but also affects their functionality, which underlines the importance of a balanced mannose homeostasis for the secretory pathway.

Abstract ID: 108

Clinical and molecular characterization of *MAU2* variants uncover an essential role of MAU2 in transcriptional regulation

I. Parenti[1], J. Kerkhof[2], L. Alabdi[3,4], F.S. Alkuraya[4], A.O. Khan[5,6], H. Aldhalaan[7], S. Starling[8], P. Jayakar[9], K. Kato[10,11,12], S. Mizuno[13], Y. Nishio[11,12,13], T. Ogi[12], S. Saitoh[14], J. Pié[15], B. Puisac[15], F. Ramos[16], G. Lyon[17,18,19], E. Rush[8], B. Sadikovic[2,20], F.J. Kaiser[1,21]

[1]Institut für Humangenetik Essen, Universitätsklinikum Essen, Universität Duisburg-Essen, Essen, Deutschland, [2]Verspeeten Clinical Genome Centre, London Health Science Centre, London, Kanada, [3]King Saud University, College of Science, Department of Zoology, Riyadh, Saudi-Arabien, [4]Center for Genomic Medicine, King Faisal Specialist Hospital and Research Center, Department of Translational Genomics, Riyadh, Saudi-Arabien, [5]Eye Institute, Cleveland Clinic Abu Dhabi, Abu Dhabi, Vereinigte Arabische Emirate, [6]Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Department of Ophthalmology, Cleveland, Vereinigte Staaten, [7]Neuroscience Centre, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi-Arabien, [8]Division of Clinical Genetics, Children's Mercy Kansas City, University of Missouri Kansas City School of Medicine, Kansas City, Vereinigte Staaten, [9]Nicklaus Children's Hospital, Miami, Vereinigte Staaten, [10]School of Biochemistry, Faculty of Life Sciences, University of Bristol, Bristol, Vereinigtes Königreich, [11]Nagoya University Graduate School of Medicine, Department of Pediatrics, Nagoya, Japan, [12]Research Institute of Environmental Medicine, Nagoya University, Department of Genetics, Nagoya, Japan, [13]Aichi Developmental Disability Center, Department of Pediatrics, Kasugai, Japan, [14]Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Pediatrics and Neonatology, Nagoya, Japan, [15]Unit of Clinical Genetics and Functional Genomics, Department of Pharmacology-Physiology- Legal Medicine, School of Medicine, University of Zaragoza, CIBERER-GCV02 and IIS-Aragon, Zaragoza, Spanien, [16]Clinical Genetics Unit, Service of Pediatrics, University Hospital 'Lozano Blesa', University of Zaragoza School of Medicine, CIBERER-GV02, Zaragoza, Spanien, [17]NYS Institute for Basic Research in Developmental Disabilities, Department of Human Genetics, Staten Island, Vereinigte Staaten, [18]George A. Jervis Clinic, NYS Institute for Basic Research in Developmental Disabilities, Staten Island, Vereinigte Staaten, [19]The City University of New York, Biology PhD Program, The Graduate Center, New York, Vereinigte Staaten, [20]Western University, Department of Pathology and Laboratory Medicine, London, Kanada, [21]Essener Zentrum für Seltene Erkrankungen (EZSE), Universitätsklinikum Essen, Essen, Deutschland

Cornelia de Lange syndrome (CdLS) is a rare disorder characterized by typical facial dysmorphism, intellectual disability (ID), and developmental delay (DD). CdLS is caused by variants in cohesin subunits or subunits of the complex guiding its loading onto the DNA.
The cohesin loader is a heterodimer composed of NIPBL and MAU2. MAU2 enwraps the N-terminus of NIPBL, thereby aiding its proper folding. In the absence of MAU2, the N-terminus of NIPBL is disordered and the full-length protein unstable. Whereas *NIPBL* haploinsufficiency represents the major cause of CdLS, only a single disease-causing variant affecting *MAU2* has been described.
We report on the clinical and molecular characterization of newly identified patients with variants in *MAU2*. The current cohort comprises five patients, harboring one in-frame deletion and four missense substitutions. The most frequently observed clinical features are ID, DD, microcephaly, synophrys, depressed nasal bridge with anteverted nares, long philtrum, thin upper lip with downturned corners of the mouth, and hypertrichosis.
Through an *in vitro* assay, we observed that the majority of *MAU2* variants tested so far display a dramatic reduction of MAU2´s ability to interact with NIPBL. We hypothesized a pathogenic mechanism by which *MAU2* variants result in the instability of NIPBL, with consequent reduced NIPBL protein dosage and haploinsufficiency. Accordingly, the differentially expressed genes (DEGs) in fibroblasts of a *MAU2* patient correlate with the DEGs of a patient with a nonsense *NIPBL* variant. Similarly, through episignature analysis, we could determine that the tested *MAU2* patients display a methylation profile on blood DNA that results in the same CdLS episignature as *NIPBL* variants.
However, additional experiments indicate that MAU2 is not only a mere chaperone for NIPBL: Genome-edited cells expressing an N-terminally truncated form of NIPBL that does not require MAU2 for its stability still display global transcriptional dysregulation. *In vitro* assays also show that transcription of a reporter gene takes place only in the presence of a functional MAU2.
These results confirm the clinical relevance of *MAU2* variants and strongly suggest that MAU2 is essential for gene expression regulation, likely by modulating interactions between the cohesin loader and additional transcriptional factors. Its interaction with distinct disease-associated proteins is currently being investigated.

Abstract ID: 109

P2-22

DDX41-associated donor cell leukemia

R. Meyer[1,2], B. Rolles[2,3,4], M. Begemann[1,2], E. Jost[2,3], M. Stelljes[5], I. Kurth[1,2], M. Elbracht[1,2], T.H. Brümmendorf[2,3], G. Silling[2,3]

[1]Institute for Human Genetics and Genomic Medicine, Medical Faculty, RWTH Aachen University, Aachen, Deutschland, [2]Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf (CIO ABCD), Aachen, Deutschland, [3]Medical Faculty, RWTH Aachen University, Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, Aachen, Deutschland, [4]Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Vereinigte Staaten, [5]University of Muenster, Department of Medicine A, Hematology and Oncology, Münster, Deutschland

Acute myeloid leukemia (AML) is the most frequent acute leukemia in adults and despite new therapeutic options, patients with high/intermediate risk AML, disease relapse as well as high risk myelodysplastic syndrome (MDS) will commonly be treated by allogeneic hematopoietic stem cell transplantation (HSCT). Disease relapse after HSCT occurs in 30-50% in AML and MDS, mainly arising from the original leukemic cell clone. Only rarely a leukemic disease is reported as donor cell leukemia (DCL) arising from donor derived stem cells.

We report a Caucasian patient who developed DCL ten years after the diagnosis of high-risk MDS and a subsequent HSCT from his HLA-identical brother. DCL was proofed by the persistence of a full donor chimerism when relapse occurred with a leukemic blast cell count of 22% in the bone marrow. Whole exome sequencing revealed a heterozygous 3-bp deletion (NM_016222.4:c.992_994; p.(Lys331del)) in the Dead-Box Helicase 41 (*DDX41*) gene in the germline of the patient which has been previously observed as predisposing factor in MDS/AML patients. Analysis in the brother's hair roots and blood using targeted Sanger sequencing identified the *DDX41* variant in heterozygous state, as well.

The molecular genetic workup of the DCL bone marrow sample revealed the somatic appearance of a second variant of the *DDX41* gene (NM_016222.4:c.1574G>A, p.(Arg525His)) with a variant allele frequency of 7.2%. This variant has been described as a recurrent somatic "second hit" in the pathogenesis of *DDX41*-associated MDS/AML and three times in the pathogenesis of DCL. Furthermore a *DNMT3A* variant with a variant allele frequency (VAF) of 12% (NM_022552.5:c.2249C>G, p.(Pro750Arg)) was identified.

There is increasing evidence that inherited variants of *DDX41* predispose to late-onset MDS, AML and DCL. To the best of our knowledge, there are three publications showing DCL in the context of a causative *DDX41* germline variant so far. The case report highlights the importance of analysis for genetic predisposition in myeloid malignant and non-malignant diseases including screening for detected pathogenic germline variants in related potential stem cell donors.

Abstract ID: 110

Meta-analysis of multiomics-neurodegeneration data

S. Klingenberg[1], N. Ruffini[1]

[1]Humangenetik, AG Gerber, Mainz, Deutschland

Research in the past decades has shown, that human diseases like diabetes, cardiovascular diseases, and neurodegenerative diseases are highly complex, as they are caused by a combination of genetic, environmental, and lifestyle factors, which are difficult to evaluate.
Due to the enormous methodological advances in the multi-omics research, the generation of "big data" on different omics-layers has been vastly facilitated and the analysis of single omics-layers already lead to interesting findings of potential risk factors for these diseases.
As each level is unique but complementary to the other levels, a comprehensive analysis of the different levels would be much more informative, but there are still no reliable tools for real data integration that also allow for good biological interpretability of the results.
In our metastudy we used a network-based approach to analyse the genomic, transcriptomic, proteomic, and epigenomic layers of four neurodegenerative diseases, i.e. Alzheimer's disease, Parkinson's disease, Huntington's disease and Amyotrophic Lateral Sclerosis in order to identify common molecular patterns but also significant differences in the development of these diseases.
We constructed protein-protein interaction graphs for each omics-layer of each disease and isolated communities with high modularity and their hubgenes which were then used for the functional analysis with the database StringDB. Finally the data of all omics-layers were manually integrated by classifying the distinct biological processes according to the six main hallmarks of neurodegeneration and their sub-items, that we had defined based on literature research. These biological processes showed a significantly different distribution across the four omics-layers but not across the four diseases.
Therefore, with our work we provide an overview of the features of neurodegeneration, but also many new starting points for further research focussing on specific processes or genes that contribute to the development of these devastating diseases.

Common Factors in Neurodegeneration: A Meta-Study Revealing Shared Patterns on a Multi-Omics Scale
by N.Ruffini, S.Klingenberg, S. Schweiger and S. Gerber
Cells 2020
The Big Picture of Neurodegeneration: A Meta Study to Extract the Essential Evidence on Neurodegenerative Diseases in a Network-Based Approach
N. Ruffini, S. Klingenberg, R. Heese, S. Schweiger, and S. Gerber
Front Aging Neurosci. 2022

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P1-11

Huntington's disease-like phenotype and dystonia: are pathogenic variants in *STUB1* and *TUBB4A* a common cause?

P.C. Panitz[1], A.I. Heidemeyer[1], P. Winter[1], D. Nolte[1]

[1]*Justus-Liebig-Universität Gießen, Institut für Humangenetik, Giessen, Deutschland*

Not all patients clinically diagnosed with Huntington's disease have pathogenic repeat expansions in the *HTT* gene on chromosome 4. If an autosomal dominant inheritance can be concluded from the family history, established differential diagnoses for these patients include variants in *PRNP* (Huntington-like disease 1, HDL1), *JPH3* (HDL2), *ATN1* (DRPLA), and *TBP* (HDL4/ SCA17). Pathogenic changes can also be detected in HDL patients in genes primarily associated with dementia syndromes, such as *c9orf72* and *MAPT*.

Recently, phenotypic overlaps of movement disorders and cognitive-affective impairments have been described in patients with spinocerebellar ataxia type 48 (SCA48), in which mutations in the *STUB1* gene are present, and in patients with Huntington-like disease (HDL). Furthermore, in "whispering dystonia" (DYT4, *TUBB4A*), in which laryngeal dysphonia is primarily present, additional generalized dystonia and choreiform movements may occur.

A collective of 73 DNA samples from HDL patients was initially analyzed for pathogenic variants in *STUB1*. Repeat expansions in the *HTT* gene (Huntington's disease) and in the *TBP* gene (SCA17) were excluded previously. In a subgroup of 53 DNA-samples, there was evidence for the presence of dystonia. These samples were additionally tested for variants in *TUBB4A*. Two likely pathogenic variants have been detected in *STUB1*, one of which has not been described previously. The *STUB1* variant c.689_692delACCT (p.Tyr230Cysfs*9) results in a frameshift and leads to a truncation of the deduced CHIP protein. The novel variant c.520G>C (p.Glu174Gln) is located within the central helical hairpin region of CHIP. However, only a rare homozygous variant was detected in *TUBB4A*, which is currently being investigated as a candidate indicator of a possible major deletion.

In conclusion, at least screening of *STUB1* should be considered as a differential diagnosis for HDL phenotypes.

Abstract ID: 112

P3-48

Clinical phenotype in a male patient with biallelic pathogenic deletions in *PTEN,* a case report

A. Möllring[1], A. van der Ven[1], L. von Elsner[1], S. Fuchs[1], I. Hüning[2], U. Kordes[3], A. Volk[1]

[1]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland, [2]Institute of Human Genetics, University of Lübeck, Lübeck, Deutschland, [3]University Medical Center Hamburg-Eppendorf, Department of Pediatric Hematology and Oncology, Hamburg, Deutschland

PTEN Hamartoma Tumor Syndrome (PHTS) encompasses a set of related syndromes, each of which is known to be caused by heterozygous pathogenic variants in *PTEN*. Besides congenital Bannayan-Riley-Ruvalcaba syndrome (BRRS) and *PTEN*-related Proteus syndrome (PS) as wells as Proteus-like syndrome, Cowden syndrome (CS) is also summarized under PHTS. CS is a multiple hamartoma syndrome and associated with an increased risk for malignant and benign tumors in different organs. Affected individuals usually have pathognomonic mucocutaneous lesions and macrocephaly. Further symptoms are hamartomatous gastrointestinal polyps, psychiatric disorders and vascular features.

Here, we report a 69-year-old man with multiple cutaneous features (facial trichilemmomas and papillomatous lesions), gastrointestinal polyps, recurrent depressive episodes, coronary heart disease as well as Parkinson´s disease. At the age of 44 he underwent a partial thyroidectomy to remove a thyroid nodule which turned out to be benign. The patient was clinically diagnosed with CS by his dermatologist at the age of 54. However, a genetic examination of *PTEN* was not carried out until recently, when his children were clinically and genetically diagnosed with CS, too. Genetic testing in one child revealed a deletion of exon 9 in *PTEN* as well as a deletion of exon 7 and 8 in *PTEN* in the other child. Neither of the two deletions was detected by segregation analysis in the healthy mother of these two siblings.

Genetic testing in the father revealed a deletion of exons 7-9 of the *PTEN* gene. To the best of our knowledge, this patient is the first described case with biallelic pathogenic variants in *PTEN*. Although harboring biallelic *PTEN* deletions, his symptoms do not seem to differ from patients with heterozygous pathogenic variants in the *PTEN* gene. Interestingly, he suffers from severe Parkinson´s disease, whose connection to the biallelic *PTEN* deletions remains unclear.

Abstract ID: 113

TSHZ3 is mutated in human CAKUT

E. Kesdiren[1], H. Martens[1], F. Brand[1], L. Werfel[2], L. Wedekind[3], M.-O. Trowe[3], J. Schmitz[4], I. Hennies[2], R. Geffers[5], Z. Gucev[6], T Seeman[7], S. Schmidt[8], V. Tasic[6], J.H. Bräsen[4], A. Kispert[3], A. Christians[1], D. Haffner[2], R.G. Weber[1]

[1]Hannover Medical School, Department of Human Genetics, Hannover, Deutschland, [2]Hannover Medical School, Department of Pediatric Kidney, Liver and Metabolic Diseases, Hannover, Deutschland, [3]Hannover Medical School, Institute of Molecular Biology, Hannover, Deutschland, [4]Hannover Medical School, Department of Pathology, Hannover, Deutschland, [5]Helmholtz Centre for Infection Research, Genome Analytics Research Group, Braunschweig, Deutschland, [6]University Children's Hospital, Pediatric Nephrology, Skopje, Mazedonien, [7]University Hospital Motol, Department of Pediatrics and Transplantation Center, Prague, Tschechische Republik, [8]University Medical Center Göttingen, Department of General-, Visceral- and Pediatric Surgery, Göttingen, Deutschland

The genetic cause of congenital anomalies of the kidney and urinary tract (CAKUT) remains unexplained in most patients. In whole-exome sequencing data of two siblings with genetically unresolved multicystic dysplastic kidneys (MCDK), prioritizing variants in murine CAKUT-associated genes yielded a missense variant in the teashirt zinc finger homeobox 3 (*TSHZ3)* gene. Therefore, the role of *TSHZ3* in human CAKUT was assessed. Taken together, five different rare heterozygous *TSHZ3* variants predicted to be deleterious were identified in 9/301 (3%) CAKUT families. A 5' hotspot region of *TSHZ3* was affected significantly more frequently in CAKUT patients than in gnomAD controls ($p<0.001$). N-terminal TSHZ3 variants showed significantly altered SOX9 or myocardin binding by co-immunoprecipitation. Hydronephrosis, previously observed in *Tshz3*-null mutant mice, and MCDK were (significantly) more frequent in patients with versus without *TSHZ3* variants ($p=0.039$ and $p=0.055$, respectively). Likewise, similar to *Tshz3*-null mutant mice, the smooth muscle layer was disorganized in the renal pelvis and thinner in the proximal ureter of a nephrectomy specimen of a patient carrying a *TSHZ3* variant compared to an age-matched control by immunohistochemistry. CAKUT patients with versus without *TSHZ3* variants were significantly more likely to have developmental delay ($p=0.037$) and genital anomalies ($p=0.029$). *TSHZ3* was highly expressed in human fetal brain, skeletal muscle, and kidney, and from embryonic day 11.5 to 14.5 in murine neural tissues and the mesenchymal compartments of the developing lung, bladder and ureter as well as in the medullary stroma of the developing kidney. Our results provide evidence that heterozygous *TSHZ3* variants are associated with human CAKUT, particularly hydronephrosis and MCDK, and specific extrarenal features, i.e. developmental delay and genital anomalies. (DFG grant no. KO5614/2-1 and MA9606/1-1)

Abstract ID: 114

P6-35

Disease-specific biomarkers of pathogenic *HRAS* variants in skin

T. Nauth[1], H. Voß[2], S. Deden[1], V. Rickassel[1], H. Schlüter[2], G. Rosenberger[1]

[1]Institut für Humangenetik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Deutschland, [2]Core Facility Massenspektrometrische Proteomanalytik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Deutschland

The small GTPase HRAS is a crucial modulator in epidermal homeostasis as HRAS effector proteins control keratinocyte proliferation and differentiation. The stratified epidermis consists of various layers with basal proliferative keratinocytes followed by more and more differentiated cells. Pathogenic germline variants in *HRAS* result in the non-mosaic RASopathy Costello syndrome (CS), a rare developmental disorder with >80% of patients showing the HRAS p.G12S variant. Individuals with CS present specific dermatological features including loose, redundant and soft skin, hyperpigmentation, prematurely aged skin and others. Postzygotic *HRAS* variants have been identified in disorders of the skin, so-called mosaic RASopathies (*e.g.*, Nevus sebaceous/Schimmelpenning syndrome) and >90% of patients show the HRAS p.G13R variant. Finally, oncogenic *HRAS* variants are common in dermatological and other malignancies, and the p.G12V variant pre-dominates. Although all a.m. amino acid substitutions result in a constitutively active HRAS, unique biological consequences of different *HRAS* variants (*e.g.*, G12V vs. G13R) may underlie the phenotypical pleiotropy of *HRAS*-associated disorders. To systematically study *HRAS* variant-specific skin phenotypes, we stably expressed HRAS mutants in HaCaT keratinocytes and determined proteome-wide differences by applying immunoblotting and mass spectrometry. We identified significant differences between HRAS mutants (**i**) in the expression of differentiation and proliferation markers KRT10 and KRT14, respectively, (**ii**) in the activation of canonical AKT and MAPK signaling, and (**iii**) in the activation/expression levels of several other proteins, e.g., CAV1. Our preliminary results suggest that a specific *HRAS* variant can be determined based on its functional consequences. This underscores the existence of *HRAS* variant-specific qualities of dysregulation. Therefore, we aim to characterize proteome-wide variant-specific consequences to delineate the pathophysiological architecture of *HRAS*-associated phenotypes.

Abstract ID: 115

Premature cognitive decline in a mouse model of tuberous sclerosis

J. Krummeich[1], V. Engelhardt[2], C. Caliendo[2], A. Arlt[3], D. Aschauer[4], L. Nardi[5], K. Rolski[6], K. Vincze[6], R. Schneider[6], S. Rumpel[4], M. Schmeisser[5], S. Gerber[2], S. Schweiger[2]

[1]Institute of Pathology, University Medical Center of the Johannes Gutenberg-University, Mainz, Deutschland, [2]Institute of Human Genetics, University Medical Center of the Johannes Gutenberg-University, Mainz, Deutschland, [3]Institute of Human Genetics, University Hospital Muenster, Münster, Deutschland, [4]Institute of Physiology, University Medical Center of the Johannes Gutenberg-University, Mainz, Deutschland, [5]Institute for Microscopic Anatomy and Neurobiology, University Medical Center of the Johannes Gutenberg-University, Mainz, Deutschland, [6]Department of Biochemistry, University of Innsbruck, Innsbruck, Deutschland

Tuberous sclerosis complex subunit 2 (Tsc2) is a negative regulator of the mechanistic target of rapamycin (mTOR) signaling pathway. Loss of its function results in mTOR hyperactivity. Patients with heterozygous mutations in the *Tsc2* gene develop tuberous sclerosis (TS) which includes a broad spectrum of neuropsychiatric symptoms and intellectual disability. Clinical variability is high and symptoms develop along a longitudinal trajectory.
In a mouse model carrying a heterozygous deletion of the *Tsc2* gene, autistic features were found in adolescent animals. Neither in infantile nor in adolescent and adult animals, however, we detected any signs of cognitive impairment. Only in aging animals, impairment of long-term object recognition and episodic memory was found. Restriction of hippocampal memory function was linked to a significant reduction of immediate early gene expression after memory challenge in the hippocampus. While hippocampal projections were unchanged and also morphologically no difference to wildtype animals was picked up, in hippocampal synaptosomes, molecular markers of neurodegeneration were increased and, surprisingly, a down-regulation of the mTOR pathway cascade was detected. Hippocampal injection of insulin-like growth factor 2 (IGF2), a ligand of the insulin-growth factor receptor, that induces mTOR signaling and had been shown to improve memory function, could fully rescue cognitive impairment and immediate early gene expression in the heterozygous *Tsc2* animals.
Our data provide evidence that tuberous sclerosis is a consumptive disease that causes erosion of mTOR signaling and premature degeneration of cognitive function. It suggests that impaired neurodevelopment can lead to premature aging and, by enhancing insulin signaling, provides a novel avenue for the treatment of age-related degeneration in mTORopathies.

Abstract ID: 116

P2-3

Exome sequencing reveals clonal evolution in tyrosine kinase inhibitor-resistance cell line models with evolving mutations in *PTPN11*, *KRAS* and *NRAS*

M. Kaehler[1], P. Osteresch[1], A. Kuenstner[2,3], S. Vieth[1], D. Esser[4], I. Vater[5], H. Busch[2,3], M. Spielmann[6], I. Cascorbi[1], I. Nagel[5,1]

[1]Institute of Experimental and Clinical Pharmacology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Deutschland, [2]Medical Systems Biology Group, University of Lübeck, Lübeck, Deutschland, [3]Institute of Cardiogenetics, University of Lübeck, Lübeck, Deutschland, [4]Institute of Clinical Chemistry, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Deutschland, [5]Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Deutschland, [6]Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Lübeck, Lübeck, Deutschland

Introduction: Tyrosine kinase inhibitors (TKIs) revolutionized the treatment of chronic myeloid leukemia (CML) leading to 10-year survival rates of 83 %. TKIs, e.g. imatinib and nilotinib, inhibit the BCR/ABL1-fusion protein, the major tumor-driving aberration in CML. Nevertheless, relapses after initial treatment response occur and are associated with poor outcomes. The underlying molecular mechanisms are only partially understood. The present study aims to reveal new TKI resistance mechanisms using an in vitro-CML-TKI-resistance cell line model.

Methods: K-562, a TKI-sensitive CML cell line, was exposed to increasing concentrations of imatinib and nilotinib in biological replicates until the sublines were resistant against 2 µM imatinib or 0.1 µM nilotinib. Whole exome sequencing of 0.5 µM and 2.0 µM imatinib as well as 0.05 and 0.1 µM nilotinib resistant cell lines was performed in four and two biological replicates, respectively. Potential driver mutations were analyzed by transfection experiments with subsequent TKI treatment and apoptosis, proliferation, and viability assays.

Results: The landscape of single nucleotide variants (SNVs) of the analyzed cell lines was shown to change substantially during the development of TKI resistance. The burden of SNVs in the exomes (sum of SNVs with population allele frequency below 0.1%) increased from sensitive to TKI-resistant sublines. The number of mutations detected in the TKI-resistant K-562 cell lines (variant allele frequency (VAF) > 15%) but not in the sensitive cells (VAF < 5%) differed between 103 and 195. No recurrent variants were gained during TKI resistance development in the biological replicates. Four of the six cell lines gained known pathogenic cancer driver mutations in *PTPN11* [c.836A>G p.(Tyr279Cys)], *NRAS* [c.181C>A p.(Gln61Lys)] and *KRAS* [c.175G>T p.(Ala59Thr) and c.35G>C p.(Gly12Asp)]. All three genes' products act downstream of BCR/ABL1 in the MAPK/ERK pathway.

Conclusion: The approach used herein is apparently suitable to study clonal evolution under selection pressure by TKI treatment. One mechanism of TKI resistance in K-562 cells seems to be the restoration of constitutive activation of the MAPK/ERK pathway downstream of BCR/ABL1. Some TKI-resistant sublines gained well-known driver mutations in oncogenes, particularly *PTPN11*, *NRAS* and *KRAS,* but also candidate variants with a potential pathogenic role in TKI resistance prompting further elucidation.

Abstract ID: 118

P3-49

Case report of a hemizygous intragenic deletion of *WDR13* - further evidence for the role of *WDR13* in X-linked intellectual disability

K. Cremer[1], T. Bender[1,2], H. Engels[1], S. Heilmann-Heimbach[1], H. Hundertmark[1], P. Incardona[3], C. Perne[1], A. Schmidt[1,1], J. Trautmann[1], S. Peters[1]

[1]Institute of Human Genetics, School of Medicine, University of Bonn - University Hospital Bonn, Bonn, Deutschland, [2]Center for Rare Diseases Bonn, University of Bonn - University Hospital Bonn, Bonn, Deutschland, [3]Core Unit for Bioinformatics Data Analysis, Medical Faculty, University of Bonn, Bonn, Deutschland

Introduction: Intellectual disability (ID) has an estimated prevalence of 1.5-2%. 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 expressive speech and language development, intellectual disability (IQ 50), mild postnatal macrocephaly, obesity, behavioural abnormalities, bilateral leg spasticity with pointed foot position, unilateral maldescensus testis and chronic constipation. Furthermore, he showed minor facial dysmorphism (thin upper lip vermilion) and brachyphalangy. The patient's parents are healthy. The mother had four miscarriages in early pregnancy.

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 mother is a heterozygous carrier of the variant.

Conclusion: *WDR13* (OMIM *300512) is a poorly characterized gene previously proposed as a candidate gene for X-linked intellectual disability. However, only little is known about the role of the WD repeat-containing protein 13 in the aetiology of ID. Here we present an intragenic deletion of the X-linked *WDR13* gene in a boy with intellectual disability and his healthy mother. To our knowledge, there are only four descriptions of *WDR13* variants (intragenic deletion, microduplication encompassing WDR13, missense and nonsense variant) 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.

Abstract ID: 119

P6-2

Somatic mosaicism in *STAG2*-associated cohesinopathies: expansion of the genotypic and phenotypic spectrum

J. Schmidt[1], S. Dreha-Kulaczewski[2], M.-P. Zafeiriou[3,4,5], M.-K. Schreiber[3,5], B. Wilken[6], R. Funke[6], C.M. Neuhofer[1,7,8], J. Altmüller[9,10,11], H. Thiele[9], P. Nürnberg[12], S. Biskup[13], Y. Li[1], W.H. Zimmermann[3,4,5], S. Kaulfuß[1], G. Yigit[1,4], B. Wollnik[1,4,5]

[1]Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Deutschland, [2]University Medical Center Göttingen, Department of Pediatics and Adolescent Medicine, Göttingen, Deutschland, [3]Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Göttingen, Deutschland, [4]DZHK (German Center for Cardiovascular Research), partner site Göttingen, Göttingen, Deutschland, [5]Cluster of Excellence 'Multiscale Bioimaging, from Molecular Machines to Networks of Excitable Cells' (MBExC), University of Göttingen, Göttingen, Deutschland, [6]Klinikum Kassel, Department of Pediatric Neurology, Kassel, Deutschland, [7]Institute of Neurogenomics, Helmholtz Zentrum Munich, Munich, Deutschland, [8]Friedrich-Baur-Institute, LMU Hospital, Ludwig Maximilians University, Department of Neurology, Munich, Deutschland, [9]Cologne Center for Genomics (CCG), University of Cologne, Faculty of Medicine, University Hospital Cologne,, Cologne, Deutschland, [10]Berlin Institute of Health at Charité, Core Facility Genomics, Berlin, Deutschland, [11]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Deutschland, [12]Cologne Center for Genomics (CCG), University of Cologne, Faculty of Medicine, University Hospital Cologne, Cologne, Deutschland, [13]CeGaT GmbH, Center for Genomics and Transcriptomics, Tübingen, Deutschland

STAG2 is a component of the large, evolutionarily highly conserved cohesin complex, which has been linked to various cellular processes like genome organization, DNA replication, gene expression, heterochromatin formation, sister chromatid cohesion, and DNA repair. A wide spectrum of germline variants in genes encoding subunits or regulators of the cohesin complex have previously been identified to cause distinct but phenotypically overlapping multisystem developmental disorders belonging to the group of cohesinopathies. Pathogenic variants in *STAG2* have rarely been implicated in an X-linked cohesinopathy associated with undergrowth, developmental delay, and dysmorphic features. Here, we describe for the first time a mosaic *STAG2* variant in an individual with developmental delay, microcephaly, and hemihypotrophy of the right side. We characterized the grade of mosaicism by deep sequencing analysis on DNA extracted from EDTA blood, urine and buccal swabs. Furthermore, we report an additional female with a novel *de novo* splice variant in *STAG2*. Interestingly, both individuals show supernumerary nipples, a feature that has not been reported associated to *STAG2* before. Remarkably, additional analysis of *STAG2* transcripts in both individuals showed only wildtype transcripts, even after blockage of nonsense-mediated decay using puromycin in blood lymphocytes. As the phenotype of *STAG2*-associated cohesinopathies is dominated by global developmental delay, severe microcephaly, and brain abnormalities, we investigated the expression of *STAG2* and other related components of the cohesin complex during Bioengineered Neuronal Organoids (BENOs) generation by RNA sequencing. Interestingly, we observed a prominent expression of *STAG2*, especially between culture days 0 and 15, indicating an essential function of *STAG2* in early brain development. In summary, we expand the genotypic and phenotypic spectrum of *STAG2*-associated cohesinopathies and show that BENOs represent a promising model to gain further insights into the critical role of STAG2 in the complex process of nervous system development.

Abstract ID: 120

P2-2

Identification of regulatory variants in genes predisposing for hereditary breast and ovarian cancer

B.A. Allister[1], J.L. Lühmann[1], W. Hofmann[1], J. Klein[1], J. Kater[1], L. Wendeburg[1], B. Schlegelberger[1], M.M. Golas[2,3], D. Steinemann[1]

[1]Humangenetik-Medizinische Hochschule Hannover, Funktionelle Genomik, Hannover, Deutschland, [2]Faculty of Medicine-University of Augsburg, Human genetics, Augsburg, Deutschland, [3]University Hospital Augsburg, Comprehensive Cancer Center Augsburg, Augsburg, Deutschland

Hereditary breast and/or ovarian cancer (HBOC) syndrome is a condition with an autosomal dominant inheritance pattern. Approximately 5-7% and 10-15% of all breast cancer and ovarian cancer cases respectively can be accounted for HBOC. Currently, there are 13 core genes (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CDH1, CHEK2, PALB2, PTEN, RAD51C, RAD51D, STK11* and *TP53*) tested for pathogenic loss-of-function variants (nonsense variants, frame-shifting indels, canonical splice site variants) within routine genetic diagnostics by targeted panel or whole exome sequencing (WES). The diagnostic yield, however, is around 20% only depending on patient inclusion criteria. Thus, we hypothesized that non-coding variants and structural variants might account for a fraction of the cases. Therefore, identifying and interpreting these variants in the clinical setting is of paramount importance.

In this study we focus on identifying germline variants in HBOC associated genes, including deep intronic sequences, regulatory elements modifying the pre-mRNA splicing, mobile element insertion (MEI) as well as structural genomic variants including inversions or insertions. These alterations often escape detection in the current routine diagnostic setting via whole exome sequencing (WES) and if detected, they are frequently classified as variants of unknown significance (VUS). In order to tackle this limitation, we utilize whole genome sequencing (WGS) and optical genome mapping (OGM), which give us information that WES might missed and the positional information of structural variants at single molecule resolution respectively. Thus far, using OGM, we detected a 32 kb deletion in the *FANCM* gene (chr14:45145384-45183139), which encodes for an ATP-dependent RNA helicase and has been proposed as a breast cancer predisposition gene. No other meaningful structural variant was detected in our cohort of 115 diagnostic-negative HBOC families, so far. Furthermore, we use Mobster and Scramble to detect MEI from our WGS data, which have also been implicated of predisposing genetic diseases such as cancer and have been proven challenging to detect via WES. In our cohort, we detected several MEI candidates in HBOC core genes, which we are currently validating.

These approaches may enable us to identify those genetic aberrations in our growing cohort of diagnostic-negative HBOC cases. Altogether, this work will provide us with broader spectrum of HBOC diagnostic and therapeutic strategy.

Abstract ID: 122

P5-8

De novo reciprocal translocation of the long arms of chromosome 5 and chromosome 9 in a patient with features of Bosch-Boonstra-Schaaf syndrome

A.C. Schnause[1], J. Neesen[2], P. Dremsek[2], A. Schachner[2], A. Tzschach[1], J. Fischer[1], B. Gläser[1], K. Komlosi[1]

[1]Institut für Humangenetik, Universitätsklinikum Freiburg, Zytogenetik, Freiburg, Deutschland, [2]Institut für Medizinische Genetik, Universität Wien, Pathobiochemie und Genetik, Wien, Österreich

Introduction: Developmental delay with an apparently balanced chromosomal rearrangement (ABCR) as an only finding is challenging for genetic counselling. Optical genome mapping (OGM) is able to detect structural variants at a higher resolution than conventional cytogenetics and enables further elucidation of ABCRs.
Clinical report: We report on a 10-year-old female patient born to non-consanguineous healthy parents with initial hypotonia, global developmental delay with attention deficit disorder, delayed language development, fine motor deficits, OFC on the 5th percentile, narrow philtrum, thin upper lips and prominent nasal bridge. Ophthalmological examinations showed microstrabismus, suspicion of cerebral visual impairment, optic disc hypoplasia, up-beat nystagmus and reduced visual acuity.
Methods and result: The karyotype of the patient showed an apparently balanced de novo translocation with breakpoints in the long arms of chromosome 5 and 9 (46,XX,t(5;9)(q14;q12)). Microarray analysis showed especially in the breakpoint regions no losses or gains of genomic material. Trio-WES yielded no causative variants. FisH with the probe RP11-867H3 (5q15) spanning the NR2F1 gene showed evidence of a breakpoint near the NR2F1 gene. OGM (Bionano Genomics) was carried out for a more precise mapping of the breakpoints. Since the breakpoint on chromosome 9 was mapped to the heterochromatin, it could not be exactly defined. The localization of the breakpoint on chromosome 5 in band 5q14 was confirmed in the vicinity of the gene NR2F1.
Conclusion: Haploinsufficiency of the NR2F1 gene has been described as causative of the Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS, OMIM #615722), a rare autosomal dominant neurodevelopmental disorder characterized by mild to moderate intellectual disability, developmental delay, seizures, hypotonia, hearing impairment and optic atrophy. To date, approximately 85 patients have been reported. The variants considered pathogenic - apart from whole gene deletions - affect the coding sequence of the gene and are particularly overrepresented in the DNA-binding domain of the protein. Our patient shows features resembling BBSOAS and the haploinsufficiency of the NR2F1 gene as a consequence of the ABCR t(5;9) might explain the clinical phenotype. Further expression analyses from patient cells will be needed to show reduced expression of NR2F1 and elucidate the exact disease pathomechanism.

Abstract ID: 124

P2-23

Capture RNA-seq as supplement to DNA germline testing to increase diagnostic yield

M. Wendlandt[1], F. Scharf[1], T. Volodina[1,2], S. Angerbauer[1], V. Steinke-Lange[1,2], E. Samadashvili[1,2], A. Laner[1], T. Wohlfrom[1], E. Holinski-Feder[1,2], J. Romic-Pickl[1,2]

[1]Medizinisch Genetisches Zentrum (MGZ), München, Deutschland, [2]Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München, München, Deutschland

Introduction: Hereditary tumor syndromes are responsible for 5-10% of cancers. Accurate identification of causal genetic variants in affected patients is highly important in patient management. The diagnostic rate of whole-exome sequencing (WES), the current state-of-the-art molecular diagnostics approach, is between 30-50%. Whole-genome sequencing (WGS) is estimated to increase the diagnostic rate by 5%. To further uplift diagnostic rates, high-throughput functional studies like RNA-seq are highly desired that reclassify variants of uncertain significance (VUS).
Methods: We developed a cost-efficient high-throughput capture RNA-seq approach for the analysis of RNA phenotypes in 49 cancer-associated genes from PAXgene RNA samples.
Results: We achieved ultra-high coverage sequencing data with of ~20,000 mean target coverage and on average 88% of exons covered with >50 read depth. As proof-of principle, we could verify aberrant phenotypes in 84% (22/26) of tumor syndrome patients with known pathogenic variants, as we could successfully identify reduced aberrant splicing, allelic loss or reduced gene expression. Further, we were able to reclassify 16% (5/31) VUS as pathogenic.
Conclusion: Our workflow provides high quality RNA-Seq data, which allow the assessment of splicing events and allelic imbalance, and can be automated for high sample throughput. This work represents the basis for future implementation of targeted RNA-Seq in cancer diagnostics.

Abstract ID: 125

Combined somatic copy number alteration and fragmentation analysis for treatment monitoring in colorectal cancer patients using liquid biopsy

A. Hallermayr[1], T. Wohlfrom[1], V. Steinke-Lange[1,2], A. Benet-Pagès[1,3], F. Scharf[1], E. Heitzer[4,5,6], U. Mansmann[7], C. Haberl[8], M. de Wit[9,10], H. Vogelsang[11], M. Rentsch[12,13], E. Holinski-Feder[1,2], J.M. Pickl[1,2]

[1]MGZ - Medizinisch Genetisches Zentrum, München, Deutschland, [2]Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München, München, Deutschland, [3]Institute of Neurogenomics, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Deutschland, [4]Institute of Human Genetics, Diagnostic and Research Center for Molecular Biomedicine (Austria), Medical University of Graz, Graz, Österreich, [5]BioTechMed-Graz, Graz, Österreich, [6]Christian Doppler Laboratory for Liquid Biopsies for Early Detection of Cancer, Graz, Österreich, [7]Institute for Medical Information Processing, Biometry, and Epidemiology –IBE, LMU Munich, München, Deutschland, [8]Department of Oncology and Hematology, Barmherzige Brüder, Klinikum St. Elisabeth, Straubing, Deutschland, [9]Department of Hematology, Oncology and Palliative Medicine, Vivantes Klinikum Neukoelln, Berlin, Deutschland, [10]Department of Oncology, Vivantes Auguste-Viktoria-Klinikum, Berlin, Deutschland, [11]Department of General, Visceral, Thoracic and Endocrine Surgery, Klinikum Garmisch-Partenkirchen, Teaching Hospital, Ludwig Maximilian University Munich, Garmisch-Partenkirchen, Deutschland, [12]Department of General, Visceral and Thorax Surgery, Klinikum Ingolstadt, Ingolstadt, Deutschland, [13]Department of General, Visceral, Vascular and Transplant Surgery, University Hospital Munich, Ludwig-Maximilians University of Munich, Campus Großhadern, München, Deutschland

Introduction: Liquid biopsy for non-invasive disease monitoring of cancer patients is progressing towards routine clinical practice. So far, the main focus is on circulating tumor DNA (ctDNA) analysis, targeting actionable somatic hotspot variants to support therapy decisions. Whole-genome sequencing (WGS) of ctDNA provides a promising tool for real-time monitoring of treatment response, as well as early diagnosis for all cancer patients.
Method: We established LIquid biopsy Fragmentation, Epigenetic signature and Copy Number Alteration analysis (LIFE-CNA) using WGS with ~6x coverage in 259 plasma samples collected from healthy individuals and colorectal cancer (CRC) patients.
Results: Based on 55 healthy controls, we established distinct cutoffs for the detection of ctDNA based on global and regional fragmentation patterns, transcriptionally active chromatin and somatic copy number alterations for the analytical validation of LIFE-CNA. This enabled the accurate prediction of ctDNA in 81% of patients with localized and in 94% of patients with metastatic disease at diagnosis. Further, by adding a machine learning classifier, combining global and regional cfDNA fragmentation, to our workflow we increased the accurate prediction of ctDNA (93% of patients with localized and 94% of patients with metastatic disease at diagnosis). By following individual patients treated with surgery and / or chemotherapy throughout their course of disease, we observed that changes in ctDNA signals reliably predicted response or resistance to treatment up to 5 months prior to clinical manifestation in 77% or 100% of patients, respectively.
Conclusion: In conclusion, we developed and validated a sensitive and cost-effective method for untargeted ctDNA detection at diagnosis or recurrence, as well as treatment monitoring expanding the advantages of liquid biopsy to all cancer patients. The high sensitivity and cost-effectiveness of our approach form the basis of the implementation of LIFE-CNA into clinical practice.

Abstract ID: 126

P6-20

Functional characterization of variants of unknown significance in the Alagille Syndrome-associated genes *JAG1* and *NOTCH2*

N. Buhl[1,2], E.-D. Pfister[1], U. Baumann[1], B. Schlegelberger[2], T. Illig[3], B. Skawran[2], A. Stalke[2,1]

[1]Hannover Medical School, Pediatric Gastroenterology and Hepatology, Hannover, Deutschland, [2]Hannover Medical School, Department of Human Genetics, Hannover, Deutschland, [3]Hannover Medical School, Hannover Unified Bank (HUB), Hannover, Deutschland

Introduction: Alagille Syndrome (ALGS) is an autosomal dominant multisystemic disease predominantly affecting liver, heart, kidney, vertebrae and eyes. Two NOTCH-Signaling pathway members -JAG1 and NOTCH2- are known to be associated with ALGS. Diagnosis of this disease is challenged by incomplete penetrance and variable expressivity. Frequently, variants detected in the two genes are classified as variants of unknown significance (VUS). Functional analysis can help classify them as clearly pathogenic or benign, thus contributing to a clear diagnosis. Using *in vitro* models, we aim to functionally characterize and assess the pathogenicity of 5 *JAG1* missense VUS and 4 *NOTCH2* VUS with 3 missense variants and one in-frame deletion detected in 10 patients with clinically clear or suspected ALGS.
Methods: Wild-type (WT) pcDNA3 expression vectors for JAG1/NOTCH2 were used for mutant overexpression. Using site-directed mutagenesis, the corresponding VUS as well as control variants already known to be benign or pathogenic, were inserted. After transient transfection of HEK293T or Huh7 cells, JAG1/NOTCH2 expression was quantified by qPT-PCR and Western blot. PNGase F digestion was performed to investigate glycosylation.
Results: All VUS were shown to be overexpressed at the mRNA level in both cell lines. Western blots showed JAG1 protein expression comparable to WT for 3 VUS. A significantly lower protein level was detected for 2 VUS, one of which additionally exhibited a smaller protein size. Subsequent PNGase F digestion demonstrated a lack of glycosylation of the latter mutant. For NOTCH2, western blots showed a slight overexpression in Huh7, a significant overexpression in HEK293T but no differences between VUS and WT in both cell lines.
Conclusion: Our functional analyses suggest that 2 of the JAG1 VUS may have pathogenic effects and could be the molecular cause of ALGS. All other JAG1/NOTCH2 VUS show no pathogenic effects so far. However, protein function or localization may be impaired. To address this, we are currently investigating the ability of the mutants to activate NOTCH signaling by a luciferase-based assay. In addition, we study a potential mislocalization of JAG1/NOTCH2 within the cell by immunofluorescence staining. This functional VUS characterization allows a clearer understanding of the pathomechanism of each variant and a better assessment of its clinical relevance which may contribute to personalized therapy in the future.

Abstract ID: 127

P3-11

Clinical genetic assessment and medical care of children with tumor predisposition syndromes. An interdisciplinary approach

A.L. Nitschke[1,2], N. Dikow[1], S. Hirsch[1,2,3], J. Kunz[2,4,5], K. Grund[1], P. Lüttich[4], C. Sutter[1], K. Hinderhofer[1], C.M. van Tilburg[2,4,6,7,8], O. Witt[2,4,6,7,8], A.E. Kulozik[2,4,7,8,9], D.T.W. Jones[2,3,5,7,8], M. Hempel[1], T. Milde[2,4,6,7,8], C.P. Schaaf[1], S.M. Pfister[2,3,4,7,8], K.W. Pajtler[2,3,4,7,8]

[1]Institute of Human Genetics, University Hospital Heidelberg, Heidelberg, Deutschland, [2]Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Deutschland, [3]Division of Pediatric Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Deutschland, [4]Heidelberg University Hospital, Department of Pediatric Oncology, Hematology, Immunology & Pulmonology, Heidelberg, Deutschland, [5]Pediatric Glioma Research Group, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Deutschland, [6]Clinical Cooperation Unit Pediatric Oncology, German Cancer Research Center (DKFZ), Heidelberg, Deutschland, [7]German Cancer Consortium (DKTK), Heidelberg, Deutschland, [8]National Center for Tumor Diseases (NCT) Heidelberg, Heidelberg, Deutschland, [9]Clinical Cooperation Unit Pediatric Leukemia, German Cancer Research Center (DKFZ), Heidelberg, Deutschland

Background: Up to 10 % of pediatric malignancies are associated with cancer predisposition syndromes (CPS). At Heidelberg University Hospital and in collaboration with the German Cancer Research Center (DKFZ) an interdisciplinary expert panel consisting of pediatric oncologists, human geneticists, pediatric psychologists, and other specialists (CPS board) discusses and evaluates children suspected to have a CPS. Here we report on the required organizational structures, diagnostic rates and clinical relevance of the program.

Patients and Methods: All CPS board patients since 2018 were included for retrospective data analysis. Data on medical and family history, physical examination, molecular and histological tumor characteristics, diagnosis and surveillance recommendations were retrieved from medical records. Correlations between these parameters and positive genetic testing for CPS were assessed.

Results: 70 individuals at risk for CPS (average age 6.5 years) were discussed at CPS board meetings. 35 % of them showed no signs of CPS and sought predictive testing. 65 % were affected by one (45 %) or more (13 %) cancers or showed other non-malignant features of a CPS (42 %). Sarcomas and brain tumors were the most frequent malignancies in the affected individuals. A CPS was genetically identified in 59 % of cases. In 50 % of affected patients without a known LP/P (likely pathogenic/pathogenic) variant in the family (n=20) a CPS was confirmed genetically. In two patients, a genetic mosaicism was detected by comparative analysis of tumor and normal tissue, which would not have been identified by analysis of normal tissue alone (*IDH1* and *PIK3CA* alterations, respectively). The most frequently identified CPS were neurofibromatosis type I (27 %), familial adenomatous polyposis (17 %) and Li-Fraumeni syndrome (12 %).

The detection of LP/P variants led to surveillance and treatment recommendations, and predictive testing of family members.

Conclusion: Detection of CPS is eminent for care of children at risk and their families. Diagnostics of CPS requires detailed phenotypical data, family history, paired genotypes of tumor and control DNA, and discussion of results in an interdisciplinary setting. Our approach is intended to lay the foundation for a more systematic CPS assessment and establishment of standardized surveillance concepts for individuals with common and rare CPS.

Abstract ID: 128

APC-specific variant classification rules reduce the burden of variants of uncertain significance in ClinVar and locus-specific databases considerably

S. Aretz[1,2,3], X. Yin[1,4,5], M. Richardson[6], M. Pineda[7,8,9], D. Ritter[10,11], T. Pesaran[12], G. Capella[7,8,9], S. Tavtigian[13,14], J.-P. Plazzer[4,5], A. Laner[15], J. Boyle[14], P. Mur[8,9], T. Hansen[16], X. Shi[17], K. Mahmood[18], A. Latchford[19], I. Frayling[20], S. Plon[10,11], M. Greenblatt[21], F. Macrae[4,5], I. Spier[1,2,3]

[1]Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Deutschland, [2]National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Deutschland, [3]European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS), Bonn, Deutschland, [4]Department of Colorectal Medicine and Genetics, Royal Melbourne Hospital, Parkville, Australien, [5]Department of Medicine, University of Melbourne, Parkville, Australien, [6]6 Ambry Genetics, Aliso Viejo, Vereinigte Staaten, [7]European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS), Barcelona, Spanien, [8]Hereditary Cancer Program, Catalan Institute of Oncology – IDIBELL, Barcelona, Spanien, [9]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Instituto Salud Carlos III, Madrid, Spanien, [10]Baylor College of Medicine, Houston, Vereinigte Staaten, [11]Texas Children's Cancer Center, Baylor College of Medicine, Houston, Vereinigte Staaten, [12]Ambry Genetics, Aliso Viejo, Vereinigte Staaten, [13]Department of Oncological Sciences, School of Medicine, University of Utah, Salt Lake City, Vereinigte Staaten, [14]Huntsman Cancer Institute, University of Utah, Salt Lake City, Vereinigte Staaten, [15]Department of Genomics, Medical Genetics Center Munich, München, Deutschland, [16]Department of Clinical Genetics, Rigshospitalet, Copenhagen University Hospital, Kopenhagen, Dänemark, [17]Greenwood Genetic Center, Greenwood, Vereinigte Staaten, [18]Colorectal Oncogenomics Group, Department of Clinical Pathology, University of Melbourne, Parkville, Australien, [19]Polyposis Registry, St Mark's Hospital, London, Vereinigtes Königreich, [20]Institute of Medical Genetics, University Hospital of Wales, Cardiff, Vereinigtes Königreich, [21]Vermont Cancer Center, University of Vermont College of Medicine, Burlington, Vereinigte Staaten

Background and Aim: Pathogenic germline variants of the *APC* gene underlie familial adenomatous polyposis (FAP) – the most common gastrointestinal polyposis characterised by a high risk of colorectal cancer. Advances in high-throughput sequencing generates an extensive catalogue of *APC* variants classified as variants of uncertain clinical significance (VUS) in locus-specific databases (LSDB) and public data aggregation resources such as ClinVar. Recently, *APC*-specific ACMG/AMP variant classification criteria have been developed and validated by members of the *APC* subcommittee of the ClinGen - InSiGHT Hereditary Colon Cancer/ Polyposis Variant Curation Expert Panel (VCEP). These specifications were used for a large-scale study to reassess all *APC* variants in ClinVar and the reference *APC* LSDB.
Methods: A streamlined algorithm using the *APC*-specific classification codes was developed consisting of population, variant type, and splice prediction criteria to comprehensively re-classify all *APC* variants in ClinVar and the reference *APC* LSDB to arrive at a pathogenicity class. The results were compared with the original pathogenicity assertion in ClinVar and the LSDB. From the remaining VUS, representative subgroups were selected, which were then subject of pilot approaches to further improve classification by gathering additional data from various sources.
Results: There were 9126 *APC* variants listed in ClinVar. Using the gene-specific rules, VUS decreased from 6077 (67%) to 3832 (42%), (Likely) Benign variants increased from 1791 (20%) to 4122 (45%), and (Likely) Pathogenic variants decreased from 1258 (14%) to 1172 (13%). A prioritised list of *APC* variants that remained VUS despite obvious evidence for pathogenicity underwent to a data mining-driven work-up to collect clinical and experimental evidence.
Conclusion: The application of *APC*-specific classification criteria substantially reduced the number of VUS on ClinVar and highlights the clinical relevance of gene-specific rules. In additon, the study demonstrates the feasibility of a systematic classification approach in large variant datasets. The expert approved re-classified variants will be publicly available through ClinVar with detailed evidence housed in the ClinGen Evidence Repository. However, the lack and limited access to relevant clinical and population data still is a major obstacle for sufficient variant classification and reinforces the need for improved data sharing approaches.

Abstract ID: 130

P3-50

Rare diagnosis of acromicric dysplasia in an adult woman

N. Dragicevic Babic[1], H. Hebestreit[1], W. Kreß[1], E. Koparir[2], E. Klopocki[2]

[1]Center for Rare Diseases, University Hospital Würzburg, Würzburg, Deutschland, [2]Institute of Human Genetics, University of Würzburg, Würzburg, Deutschland

We report the case of a 22-year-old woman with severe growth retardation and obesity (length 133 cm, weight 72 kg, BMI 40.7 kg/cm²). She is the first child born to non-consanguineous parents (father from Pakistan, mother from Germany). Her two maternal half-brothers are both older and healthy. No anthropometric data are available for the pre- and immediate postnatal period. At the age of four a non-syndromic short stature and mild fusion of the labia were documented. Hearing impairment and poor eyesight were excluded. Cytogenetic analysis revealed a normal female karyotype (46, XX) at age of six. After attending a regular school during the first year, she attended a special school thereafter. Her previous diagnoses encompass depressive episodes, spinal problems and pain in both arms. An X-ray following an injury to her left foot revealed short metatarsals on the right foot.

Clinical and physical examination revealed some dysmorphic features: round face, full lips, acne vulgaris, hirsutism, a large cafe-au-lait spot on the gluteus, a larger mole on the face, very short hands, feet and fingers; a shortened toe IV on the left foot. All laboratory values were within the normal range.

Due to the suspicion of a rare genetic disorder, whole exome sequencing of patient’s genomic DNA was performed using a Nextera DNA Flex (Illumina) and xGen-Exome-Research-Panel (IDT). The analysis revealed a heterozygous missense variant in exon 41 of the *FBN1* gene , which was classified as pathogenic (Class 5). *FBN1* variants in exons 41-42 have been described in the literature as the cause of acromimic dysplasia.

Acromicric dysplasia (MIM #102370) is a rare hereditary disorder showing autosomal dominant inheritance (prevalence 1:1,000,000). It belongs to the group of acromelic dysplasias characterized by severe short stature, short limbs, joint limitations and mild facial dysmorphism. Cases with cardiac or pulmonary involvement have been described. A multidisciplinary care has been recommended .

In summary, our case shows that a whole exome sequencing is a promising approach to detect the genetic causes in rare types of short stature.

Abstract ID: 131

Survival of glioblastoma patients vaccinated with personalized peptides: a retrospective analysis

S. Biskup[1]

[1]*Saskia Biskup, Tübingen, Deutschland*

The treatment of patients with *IDH*-wildtype glioma, CNS WHO grade 4 has not seen significant breakthroughs these past years. Following standard of care, the recurrence rate is still high, and the prognosis is poor. Immunotherapeutic concepts, mostly centered around immune checkpoint inhibition, transformed treatment of solid cancers like malignant melanoma and lung cancer, however clinical trials on checkpoint inhibition in high grade gliomas have yielded negative data so far.
Methods: Tumors from 51 patients with *IDH*-wildtype glioma, CNS WHO grade 4 were analysed for somatic mutations. In agreement with their treating physician, patients added a personalized peptide vaccine to their treatment as an individual healing attempt. Patients were monitored from October 2015 until May 2022. We retrospectively evaluated their clinical courses and the results of their immune monitoring data.
Findings: Median overall survival was 32 months (95% CI: 24·9-48·8) from first diagnosis, which is significantly longer compared to a control cohort treated with Stupp's regimen. An immune response to at least one of the vaccinated peptides was detected in blood samples of 36 of 41 monitored patients. Significantly longer survival was observed for patients that exhibited a peptide specific immune response compared to those without such a response (p=·0068).
Interpretation: The personalized neoantigen-based peptide vaccines elicited durable T-cell responses in most patients. Induction of these immune responses positively correlated with survival time. This real-world observation will be translated into a clinical trial entitled NEO GBM to evaluate the specific contribution of the neoantigen vaccine.

Abstract ID: 132

Enhancing applicability of PEDIA by integrating it into the variant prioritization pipeline with open source GestaltMatcher, CADA and VarFish

M.A. Bhasin[1], A. Knaus[1], T.-C. Hsieh[1], P. Krawitz[1]

[1]*Institute for Genomic Statistics and Bioinformatics, University Hospital of Bonn, Bonn, Deutschland*

Introduction: Numerous variant effect prediction tools are available for exome-wide variant analysis for rare disorders. PEDIA supports finding the correct diagnosis in patients with rare disorders by including phenotype information into the variant interpretation. However, the current facial analysis approach, DeepGestalt, is not an open-source software and requires data transfer to Face2Gene. This increases the difficulty in integrating PEDIA into the clinics because some patients do not agree to data transfer to another site. Therefore, we propose new version of PEDIA that works with open-source tools CADA, GestaltMatcher and VarFish, facilitating on-premise usage.
Methods: 358 patients with 64 monogenic disorders were recruited for benchmarking. The sequencing data in the form of VCF files were imported into VarFish. Various filtering steps for population frequency, molecular impact and quality are then applied to each case before obtaining any scores. For facial image analysis, GestaltMatcher was used instead of DeepGestalt. The GestaltMatcher database (GMDB) stores the Frontal photos and the clinical descriptions in the form of HPO terms. GMDB sends the facial (Gestalt) scores and HPO terms to VarFish upon REST API requests. For feature analysis, CADA scores (instead of Phenomizer, BOQA, and FeatureMatch) were obtained by requesting the CADA web service from VarFish. For molecular analysis, the CADD scores were retrieved from VarFish. These scores are then sent to the PEDIA platform to generate the final combined scores. To measure the performance, the top-10 accuracy was reported. Analysis with TNAMSE (163 cases) cohort was also performed.
Results: For the test cohort, the new PEDIA system of CADA and CADD scores (obtained from VarFIsh) combined with GestaltMatcher scoring attained top-10 accuracy of 80% compared to the previous version. For the TNAMSE cohort, accuracy of 82% was achieved.
CADA integration with VarFish is now complete and will be available to the community soon. Our next step is to work on integrating PEDIA scores directly into the prioritization pipeline in the clinic so that the final PEDIA scores can be visualized by the analyst within VarFish without transferring data to other sites.
Conclusion: By integrating open-source components VarFish, CADA and GestaltMatcher, the new PEDIA approach becomes easier to be implemented into the variants prioritization pipeline in the clinic without losing performance.

Abstract ID: 133

P6-6

Gene therapy for Best vitelliform macular dystrophy based on haplotype-specific CRISPR/Cas editing

A. Milenkovic[1], L. Happel[1], I. Römer[1], D. Eisner[1], M. Weskamp[1]

[1]Universität Regensburg, Humangenetik, Regensburg, Deutschland

Purpose: Best vitelliform macular dystrophy (BVMD) is a hereditary maculopathy caused by dominant-negative mutations in the Bestrophin-1 (BEST1) gene. So far, there is no treatment available. Here, we report a suitable gene therapy approach by haplotype specific CRISPR/Cas editing.

Methods: Genetic variants across the BEST1 locus were established based on the 1000 Genomes Project (data release Feb. 2009). Single-nucleotide-based haplotypes were established, and frequencies were estimated by the Hapview software. Single guide RNAs (sgRNA) targeting single nucleotide polymorphisms (SNPs) of the mutation-associated but not the mutation-free haplotypes were designed. Efficacy and allele specificity were determined in vitro (1) by a fluorescence-based assay in HEK293T cells using the pCAG-EGxxFP / pX330-sgRNA/Cas9 plasmid system to targeted reconstitution of EGFP fluorescence and (2) by analyzing CRISPR-induced double strand breaks in RPE cells differentiated from patient-derived human induced pluripotent stem cells (hiPSCs-RPEs).

Results: Analysis of SNP heterozygosity across the BEST1 locus revealed 69 common SNPs with minor allele frequencies > 0.05. Eleven major haplotypes with a frequency > 0.01 in the general population of European descent were established. We designed 26 sgRNAs for targeting the six most common haplotypes. Quantification of reconstituted EGFP fluorescence revealed six sgRNAs with high efficacy and sufficient allele specificity. For a further 14 sgRNAs allele specificity could be increased by shortening the sgRNA sequence length from 20 to 17 and/or by incorporating an additional base mismatch into the sgRNA sequence. High editing efficacy, ranging from 29 to 68 %, was confirmed at the on-target allele in hiPSC-RPEs while the off-target allele remained unaffected for all sgRNAs tested.

Conclusion: The current study identified a set of twenty allele-specific sgRNAs to specifically target haplotypes carrying a mutated BEST gene. This approach covers a significant fraction of BD patients independent of an individual mutation. The exclusion of genome-wide off-targets in edited hiPSC-RPEs is in progress.

Abstract ID: 134

P5-9

Comparison of Bionano optical genome mapping with conventional cytogenetic tests - region-specific analyses

M. Blankenburg[1], J. Flunkert[1], O. Vedrines[1], M. Stumm[1]

[1]*Medicover Genetics GmbH, Berlin, Deutschland*

Introduction: Optical Genomic mapping (OGM) has emerged as a promising method for detecting structural variants in human genomes in recent years. It is able to detect structural variants that are difficult or impossible to identify using traditional cytogenetic methods such as conventional chromosome analysis, fluorescence *in situ* hybridization or microarrays. Bionano OGM enables the detection of structural variants of the entire genome from 500 bp to whole chromosomes and offers an approach to overcome the limitations of previous routine diagnostic methods.

Materials and Methods: The performance of OGM compared to traditional cytogenetic methods was tested in an extended validation study. For this, ultramolecular DNA was extracted from 30 blood samples with known structural variants including deletions, duplications, inversions and translocations, labeled, then the molecules were linearized in nanochannel arrays on specific chips and imaged with the Saphyr Genome Imaging Instrument. A *de novo* genome assembly was performed and the results compared to the known aberrations from traditional cytogenetic assays.

Results: Using OGM, previously determined aberrations could be confirmed with a high concordance rate. Furthermore, due to its higher resolution compared to chromosome and microarray analysis, OGM enables the respective breakpoints to be mapped more precisely in addition to the detection of structural variants.

Conclusion: In summary, our results show that OGM is a reliable method that can combine multiple diagnostic tests in one workflow and thus has the potential to possibly replace them in the future.

Abstract ID: 137

P7-10

Detection of small structural variants in patients with high clinical suspicion

A. Moscu-Gregor[1], L. Frey[1], S. Chahrokh-Zadeh[1], M. Morak[1], S. Lippert[1], O. Wachter[1], S. Lott[1], J. Philippou-Massier[1], R. Schulz[2]

[1]MVZ Martinsried GmbH, Medicover Genetics, Martinsried, Deutschland, [2]Praxis für Kinder- und Jugendmedizin, Medizinische Genetik, Achim, Deutschland

Introduction: Despite numerous technical advances in recent years and the discovery of a growing number of disease-relevant genes, many hereditary diseases remain genetically elusive. In particular, the detection of deletions and insertions (indels) ranging in size from approximately 20 bp to at least one exon remains challenging, as these cannot be detected with high sensitivity and precision using standard SNV or CNV callers. In the following, we present two cases in which we were able to uncover such intraexonic indels.
Methods: SNV and CNV analyses were performed using Twist Bioscience enrichment, Illumina® DRAGEN Bio-IT Platform, Manta caller and VarSeq® (GoldenHelix). Sanger sequencing analysis was performed using SEQUENCE Pilot (JSI) and MLPA data were analyzed using Gene Marker. Long-read sequencing was performed using PacBio.
Results: Two patients with high clinical suspicion of arrhythmogenic right ventricular cardiomyopathy (ARVC) and Rett syndrome, respectively, were referred to our laboratory for genetic testing. In both cases, NGS analysis revealed two conspicuous mapping breakpoints within exons of the disease-relevant genes DSP and MECP2, indicating the presence of intraexonic indels. However, variant detection by multiple callers was inconsistent. Thus, independent methods were used to verify the indels, confirming the respective clinical diagnoses ARVC and Rett syndrome for the patients.
Conclusion: Structural variants are increasingly considered an important class of variants, but the accurate NGS-based calling of these variants faces several computational and scientific challenges. The structural variants presented here demonstrate, that the use of NGS and implementation of multiple variant callers in conjunction with high clinical suspicion in patients can improve the detection rate of such variants and ensure an accurate and reliable diagnostic result.

Abstract ID: 138

P6-21

Mutational analyses in Egyptian and Pakistani patients with skeletal phenotypes

N. Wasif[1,2], A. Parveen[3], N. Muhammad[4], S.I. Hussain[4], Z. Ur Rehman[4], S.A. Khan[4], S. Jan[4], N. Khan[4], M. Muzammal[5], S.W. Abbasi[6], N. Kakar[7,8], A. Arif[3], N. Hassib[9], M. Mostafa[10], R. Elhossini[10], N.N. Roshdy[11], N. Muhammad[4], S. Khan[4], R. Siebert[2], O. Ammerpohl[2]

[1]Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Deutschland, [2]Institute of Human Genetics, Ulm University and Ulm University Medical Center, Ulm, Deutschland, [3]Faculty of Life Sciences, University of Central Punjab (UCP), Lahore, Pakistan, [4]Kohat University of Science and Technology (KUST), Department of Biotechnology and Genetic Engineering, Kohat, Pakistan, [5]Gomal Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, Pakistan, [6]National University of Medical Sciences, NUMS Department of Biological Sciences, Rawalpindi, Pakistan, [7]Faculty of Life Sciences and Informatics, BUITEMS, Department of Biotechnology, Quetta, Pakistan, [8]Institute of Human Genetics, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Lübeck, Deutschland, [9]Human Genetics and Genome Research Institute, National Research Centre, Orodental Genetics Department, Cairo, Ägypten, [10]Human Genetics and Genome Research Institute, National Research Centre, Clinical Genetics Department, Cairo, Ägypten, [11]Endodontics, Faculty of Dentistry, Cairo University, Cairo, Ägypten

Introduction: Populations with a high rate of consanguineous marriages are enriched for several syndromic and non-syndromic genetic traits, including skeletal and ectodermal dysplasia. Here we aimed to identify the pathogenic variants underlying such skeletal phenotypes in patients from Egypt and Pakistan.

Methods: We recruited three Pakistani and one Egyptian family with skeletal phenotypes. In one family, a non-syndromic split hand/foot malformation (SHFM) (OMIM 619959) segregated and the three others showed a syndromic brachyolmia type 5/dental anomaly, short stature (DASS) syndrome (OMIM 601216), and a distinctive clinical feature of hearing impairment. We obtained informed written consent from all the participants and the respective ethical committees approved the study. We utilized whole exome sequencing to identify the disease-causing variants and subsequent Sanger sequencing for segregation analysis.

Results: Exome sequencing revealed a heterozygous variant (c.1118del) in *UBA2*, leading to a frameshift and premature stop codon (p.Arg373Asnfs*21). The variant co-segregated with SHFM in the family in an autosomal dominant manner but was remarkably associated with reduced penetrance and variable expressivity regarding SHFM. In the three families with DASS phenotypes, we have identified a homozygous splice acceptor site variant (*LTBP3*:c.3629-1G>T; p. ?) in the Egyptian patients, while two previously reported homozygous frameshift variants (*LTBP3*:c.132delG; p.Pro45Argfs*25), and (*LTBP3*:c.2216delG; p.Gly739Alafs*7) segregated in the Pakistani patients.

Conclusion: Several reports of pathogenic variants in *UBA2* causing SHFM with and without other clinical features have recently been published (Schnur et al., 2021; Elsner et al., 2021). Moreover, mouse *Uba2* is strongly expressed in the developing limb buds (Costa et al., 2011).The studies conducted so far on the involvement of *LTBP3* in humans in various skeletal, ectodermal and cardiac phenotypes have shown no association between *LTBP3* and hearing loss; however, when *Ltpb3* expression in the developing mouse embryos was analyzed by performing in situ hybridization, a discrete expression was observed in the developing cochlea (Huckert et al., 2015). In our Egyptian patients, we are reporting hearing impairment associated with DASS for the first time.

The present study further extends the mutational spectrum and allelic heterogeneity of *UBA2*, and *LTBP3*, associated with various skeletal malformations.

Abstract ID: 139

P2-4

Highly sensitive Liquid Biopsy Duplex Sequencing complements tissue biopsy to enhance detection of clinically relevant genetic variants

A. Hallermayr[1], T.M. Neuhann[1], V. Steinke-Lange[1,2], F. Scharf[1], A. Laner[1], R. Ewald[3], B. Liesfeld[3], E. Holinski-Feder[1,2], J. Romic-Pickl[1,2]

[1]Medizinisch Genetisches Zentrum (MGZ), München, Deutschland, [2]Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München, München, Deutschland, [3]Limbus Medical Technologies GmbH, Rostock, Deutschland

Background: Liquid biopsy (LB) is a promising complement to tissue biopsy for the detection of clinically relevant genetic variants in tumor and mosaic diseases. A combined workflow enabling parallel tissue and LB analysis is required to maximize the diagnostic yield for patients.

Methods: We developed and validated a cost-efficient combined next-generation sequencing (NGS) workflow for both tissue and LB samples, and applied Duplex Sequencing technology for highly accurate detection of very low frequency variants in plasma. Clinically relevant cutoffs for variant reporting and quantification were established.

Results: We investigated assay performance characteristics for very low frequencies of clinically relevant variants. For LB analysis we achieved 100% sensitivity and 92.3% positive predictive value (PPV) for single nucleotide variants (SNVs) and 91.7% sensitivity and 100% PPV for small insertions and deletions (InDels) in clinically relevant hotspots with 0.5-5% allele frequency (VAF). We further established a cutoff for reporting variants (i.e. Limit of Blank, LOB) at 0.25% VAF and a cutoff for quantification (i.e. Limit of Quantification, LOQ) at 5% VAF in plasma to enable the accurate clinical interpretation of analysis results. With our LB approach as complement to tissue biopsy, we were able to identify the molecular cause of a clinically confirmed asymmetric overgrowth syndrome in a 10-year old child that would have remained undetected with tissue analysis as well as other molecular diagnostic approaches.

Conclusion: Our flexible and cost-efficient workflow allows analysis of both tissue and LB samples and provides clinically relevant cutoffs for variant reporting and precise quantification. Complementing tissue analysis by LB is likely to increase the diagnostic yield for patients with molecular diseases.

Abstract ID: 141

Genetic associations underlying susceptibility to SARS-CoV-2 and severity of COVID-19: results of the global COVID-19 Host Genetics Initiative

K.U. Ludwig[1], A. Schmidt[1], E.C. Schulte[2,3], COVID-19 Host Genetics Initiative

[1]Institute of Human Genetics, University Hospital, University of Bonn, Bonn, Deutschland, [2]University Hospital, Ludwig-Maximilians-Universität Munich, Department of Psychiatry and Psychotherapy, Munich, Deutschland, [3]Institute of Psychiatric Phenomics and Genomics (IPPG), University Hospital, Ludwig-Maximilians-Universität Munich, Munich, Deutschland

The SARS-CoV-2 pandemic continues to be public health emergency, and the highly variable outcome after viral contact remains a major research question. The COVID-19 Host Genetic Initiative (COVID-19 HGI) was formed in early 2020 to advance our understanding of the biology underlying SARS-CoV-2 infection and COVID-19 severity, through global collaboration and data sharing. Since its first data release, the HGI's results have been made available at covid19hg.org with no restriction of use.

In its latest release, the COVID-19 HGI comprises 219,692 cases and over 3 million controls from 82 individual studies, including some minority ethnicities. Meta-analyses were performed for three phenotypes: critical illness (respiratory support/death; 21,194 cases), hospitalization (49,033 cases), and lab-confirmed or self-reported PCR-confirmed SARS-Cov-2 infection (219,692 cases). A total of 51 distinct genome-wide significant loci were observed across all three phenotypes, some of which were overlapping between phenotypes but could be attributed to specific phenotypes using two-class Bayesian modelling.

Gene prioritization analyses highlighted three major biological pathways to be involved in COVID-19 etiology. First, candidate causal genes located within loci associated with susceptibility to SARS-CoV-2 infection are involved in viral entry pathways and include genes like ACE2, MUC5B, SFTPD, MUC16, and TMPRSS2. The second pathway is the type I/III interferon pathway, comprising candidate genes such as TYK2, OAS1/3, IFNAR1/IFNAR2, JAK1 and STAT1/STAT2 which are located within loci associated with disease severity. Importantly, results from rare variant and functional studies support a role of insufficient or delayed interferon response in severe COVID-19. Finally, several loci contain genes involved in the upkeep of healthy lung tissue such as DDP9, SFTPD, FOXP4, and MUC5B, which have been previously associated with respiratory disease, lung cancer, or interstitial lung disease. Based on the overall summary statistics, genetic correlation and Mendelian randomization (MR) analyses identified numerous shared biological pathways across traits, including established demographic factors such as high BMI or diabetes. Interestingly, evidence was found for a causal role of kidney function in COVID-19 disease severity. This novel data release substantially expands the number of loci associated with COVID-19 phenotypes and highlights key pathways involved in COVID-19 etiology.

Abstract ID: 144

Elucidating the clinical and molecular spectrum of *SMARCC2* associated NDD in a cohort of 65 affected individuals

G. Vasileiou[1], E. Bosch[1], B. Popp[2,3], E. Güse[1], C. Skinner[4], P.J van der Sluijs[5], I. Maystadt[6], A. Pinto[7], A. Renieri[7], L. Bruno[7], S. Granata[7], C. Marcelis[8], O. Baysal[8], D. Hartwich[9], L. Holthöfer[9], B. Isidor[10], B. Cogné[10,11], D. Wieczorek[12], V. Capra[13], M. Scala[14], R. Abou Jamra[3], K. Platzer[15], L. Carter[16], O. Kuismin[17], A. van Haeringen[5], R. Maroofian[18], I. Valenzuela[19], I. Cuscò[20], J.A. Martinez-Agosto[21], A.M. Rabani[21], H.C. Mefford[22], E. Pereira[23], C. Close[23], K. Anyane-Yeboa[24], M. Wagner[25], M. Hannibal[25], P. Zacher[26], I. Thiffault[27], G. Beunders[28], M. Umair[29], P.T. Bhola[30], E. McGinnis[31], J. van de Kamp[32], R.E Stevenson[4], G.WE Santen[5], P.M Campeau[33], M. Severino[34], A. Reis[1,35], A. Accogli[36]

[1]Friedrich-Alexander-Universität Erlangen-Nürnberg, Universitätsklinikum Erlangen, Institute of Human Genetics, Erlangen, Deutschland, [2]Berlin Institute of Health at Charitè, Universitätsklinikum Berlin, Centre of Functional Genomics, Berlin, Deutschland, [3]Institute of Human Genetics, UKL University of Leipzig Medical Center, Leipzig, Deutschland, [4]Greenwood Genetic Center, Greenwood, Vereinigte Staaten, [5]Leiden University Medical Center, Department of Clinical Genetics, Leiden, Niederlande, [6]Center for Human Genetics, Institute of Pathology and Genetics, Gosselies, Belgien, [7]Genetica Medica, Azienda Ospedaliera Universitaria Senese, Siena, Italien, [8]Radboud University Medical Center, Department of Human Genetics, Nijmegen, Niederlande, [9]University Medical Center of the Johannes Gutenberg University, Institute of Human Genetics, Mainz, Deutschland, [10]Service de Génétique Médicale, Centre Hospitalier Universitaire de Nantes, Pays de la Loire, Nantes, Frankreich, [11]INSERM, CNRS, UNIV Nantes, l'Institut du Thorax, Nantes, Frankreich, [12]Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Institute of Human Genetics, Düsseldorf, Deutschland, [13]Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italien, [14]University of Genoa, Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, Genoa, Italien, [15]UKL University of Leipzig Medical Center, Institute of Human Genetics, Leipzig, Deutschland, [16]Levine Children's Hospital, Atrium Health, Department of Pediatrics, Division of Medical Genetics, Charlotte, Vereinigte Staaten, [17]PEDEGO Research Unit, Medical Research Center Oulu, and University of Oulu and Department of Clinical Genetics, Oulu University Hospital, Oulu, Finnland, [18]UCL Queen Square Institute of Neurology, University College London, Department of Neuromuscular Diseases, London, Vereinigtes Königreich, [19]University Hospital Vall d'HebronMedicine Genetics Group, Valle Hebron Research Institute, Department of Clinical and Molecular Genetics, Barcelona, Spanien, [20]University Hospital Vall d'Hebron, Medicine Genetics Group, Valle Hebron Research Institute, Department of Clinical and Molecular Genetics, Barcelona, Spanien, [21]UCLA David Geffen School of Medicine, Departments of Human Genetics, Pediatrics, and Psychiatry, Los Angeles, Vereinigte Staaten, [22]Center for Pediatric Neurological Disease Research, St. Jude Children's Research Hospital, Memphis, Vereinigte Staaten, [23]Division of Clinical Genetics, Columbia University Irving Medical Center, Department of Pediatrics, New York, Vereinigte Staaten, [24]Division of Clinical Genetics, Department of Pediatrics, Columbia University Irving Medical Center, New York, Vereinigte Staaten, [25]Division of Pediatric Genetics, Metabolism and Genomic Medicine, University of Michigan Medical School, Detroit, Vereinigte Staaten, [26]Epilepsy Center Kleinwachau, Radeberg, Deutschland, [27]Genomic Medicine Center, Children's Mercy Hospital, Kansas City, Vereinigte Staaten, [28]University Medical Center Groningen, Department of Genetics, Groningen, Niederlande, [29]King Abdullah International Medical Research Center (KAIMRC), King Saud Bin Abdulaziz University for Health Sciences, King Abdulaziz Medical City, MNGHA, Medical Genomics Research Department, Riyadh, Saudi-Arabien, [30]Children's Hospital of Eastern Ontario (CHEO), Department of Genetics, Ottawa, Kanada, [31]Division of Neurology, Department of Pediatrics, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Vereinigte Staaten, [32]VU Medical Center, Department of Clinical Genetics, Amsterdam, Niederlande, [33]CHU Sainte-Justine and University of Montreal, Department of Pediatrics, Montreal, Kanada, [34]Neuroradiology Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italien, [35]Centre for Rare Diseases Erlangen (ZSEER), Erlangen, Deutschland, [36]Division of Medical Genetics, McGill University Health Centre, Department of Human Genetics, Faculty of Medicine, McGill University, Department of Specialized Medicine, Montreal, Kanada

BAFopathies are neurodevelopmental disorders (NDDs) caused by germline variants in BAF complex subunits, and include Coffin-Siris (CSS) and Nicolaides-Baraitser (NCBRS) syndromes. Recently, pathogenic variants in the BAF-subunit SMARCC2 were reported but without detailed clinical description. Here we systematically report clinical and molecular data on the largest *SMARCCA2* cohort to date, including 41 novel und 24 previously published cases. Main clinical manifestations include neurodevelopmental delay with intellectual disability (85%), behavioural disorders (57%), muscular hypotonia (69%), brain malformations (59%), feeding difficulties (47%) and facial dysmorphic features (45%). The clinical presentation lacks many of CSS and NCBRS clinical hallmarks e.g. hypoplastic corpus callosum, nail hypoplasia, and prominent interphalangeal joints, although it is listed in OMIM as CSS 8.
When analysing the molecular spectrum, we found that non-truncating and likely gene-disrupting (LGD) variants cause different clinical presentations, with the first being predominantly linked to a significantly more severe neurodevelopmental delay and the latter found primarily in cases with mild/borderline ID or even normal cognitive development (OR: 17.77; $p<0.001$). Segregation analysis of 45 *SMARCC2* variants showed that LGD alterations were mainly inherited from healthy parents, while non-truncating variants mostly occurred *de novo*. The latter clustered in two SMARCC2 functional protein domains, the SANT and core assembly domain, which represent new mutational hotspots. The two distinct neurodevelopmental manifestations and the clustering of non-truncating changes suggest distinct pathomechanisms, with

probably a gain-of-function or dominant-negative effect for the non-truncating variants.
In vitro, N-terminal missense variants showed reduced protein expression, similar to LGD loss-of-function variants. Through 3D protein modelling, missense variants in different SMARCC2 functional domains are predicted to change the dynamics of interactions with other BAF subunits. Proximity-ligation and co-immunoprecipitation assays did not reveal any large-scale impairment of interaction, indicating a more complex underlying molecular pathology that requires further investigation. In conclusion, this study significantly expanded the clinical spectrum of BAFopathies, revealed a genotype-phenotype correlation and allowed for a better classification of SMARCC2-associated variants.

Abstract ID: 145

P3-20

RYR2 and atrial fibrillation – it is not always a disease of old age

B. Boehm[1], J. Gaa[2], P. Hoppmann[3], E. Martens[3], D.S. Westphal[3,1]

[1]Klinikum rechts der Isar München, Institut für Humangenetik, München, Deutschland, [2]Klinikum rechts der Isar München, Institut für diagnostische und interventionelle Radiologie, München, Deutschland, [3]Klinikum rechts der Isar München, Klinik und Poliklinik für Innere Medizin I, München, Deutschland

Introduction: Atrial fibrillation (AF) is a common arrhythmia in elderly individuals and associated with increased risk of mortality. The pathogenesis of AF is complex and based on multiple genetic and environmental factors. Genome wide association studies identified several loci in individuals with AF of predominantly European descent, indicating the complex genetic architecture of this disease. In rare cases familial forms of AF have been described. Today, disease-causing variants in at least eleven different genes are associated with monogenic AF.

Methods: DNA for exome sequencing (ES) was extracted from peripheral blood using the Chemagic DNA Blood Kit on a chemagic 360 instrument (PerkinElmer, Baesweiler, Germany). ES was performed using a Sure Select Human All Exon 60 Mb V6 Kit (Agilent, Santa Clara, California) for enrichment and sequencing was performed on a NovaSeq 6000 engine (Illumina, San Diego, California).Reads were aligned to the University of California Santa Cruz human reference assembly (hg19) with Burrows-Wheeler Aligner (BWA) v.0.5.8. Single nucleotide variants and small insertions and deletions were detected with SAMtools v.0.1.7.

Results: A 37-year-old male individual presented to our emergency department with AF. He was diagnosed with paroxysmal AF and combined hyperlipidemia at the age of 35 years. Additionally, his family history was positive for AF. His 34-year-old brother had also been diagnosed with AF as well as nonobstructive hypertrophic cardiomyopathy. Moreover, the patient’s father was diagnosed with AF, although with onset at an advanced age. Coronary artery disease was excluded via cardiac catheter examination. Transthoracic echocardiography and cardiac MRI revealed a reduced systolic left ventricular ejection without any signs of hypertrophic cardiomyopathy. ES of the index and his brother identified a likely pathogenic missense variant in *RYR2*.

Conclusion: This case of familial AF helps to strengthen the role of *RYR2* as a disease gene in the context of AF. Genetic testing should be considered in individuals with positive family history for AF and AF in the young. As RYR2 has already been identified as a possible target for prevention and therapy of AF, the knowledge of variants in *RYR2* might become even more crucial for individual molecular therapies in the future.

Abstract ID: 146

P1-6

TERT promoter DNA methylation differs between subtypes of malignant lymphomas

A.G Kouroukli[1], A. Fischer[1], H. Kretzmer[2], E. Chteinberg[1], N. Rajaram[3], S. Glaser[1], J. Kolarova[1], P. Bashtrykov[3], S. Mathas[4], O. Ammerpohl[1], A. Jeltsch[3], R. Siebert[1], S. Bens[1]

[1]Institute of Human Genetics, Ulm, Deutschland, [2]Max Planck Institute for Molecular Genetics, Computational Genomics, Department of Genome Regulation, Berlin, Deutschland, [3]Institute of Biochemistry and Technical Biochemistry, Department of Biochemistry, Stuttgart, Deutschland, [4]Max-Delbrück-Center (MDC) for Molecular Medicine, Group Biology of Malignant Lymphomas, Berlin, Deutschland

Telomerase Reverse Transcriptase (*TERT*) promoter mutations are the most frequent non-coding drivers in solid cancers. Alternative mechanisms for *TERT* regulation include promoter DNA methylation. Published data on *TERT* promoter methylation focuses on solid cancers. Thus, we here set out to investigate *TERT* promoter DNA methylation patterns und sequence variants in different lymphoma types.

We analyzed available array DNA methylation data of the *TERT* locus from 155 hematopoietic cell lines, 110 benign B cell populations and 277 primary B cell lymphomas. We additionally investigated 30 CpGs at the *TERT* locus in 40 lymphoma cell lines and 3 lymphoblastic cell lines (BS cohort) by targeted bisulfite NGS sequencing. We analyzed publicly available RNA-sequencing data on 4 classical Hodgkin lymphoma (cHL) cell lines and supplemented these data with additional 4 cHL cell lines. Furthermore, we performed *TERT* Western blot analyses in 8 cHL cell lines. Finally, we screened the *TERT* promoter region in 40 lymphoma cell lines for Single Nucleotide Variants (SNVs).

Burkitt lymphomas show an extremely low DNA methylation at the *TERT* promoter (avg. beta value bisulfite sequencing: 0.1, avg. beta value array: 0.24) and high *TERT* RNA expression (RPKM: mean 15.70). Contrastingly, cHL cell lines are characterized by a significantly higher *TERT* DNA methylation than BL (avg. beta value bisulfite sequencing: 0.4, t-test, $p < 0.01$, avg. beta value array: 0.5, t-test, $p < 0.01$) inversely correlating to RNA and protein expression. We detected a common SNP (rs2853669) that disrupts an ETS-family general recognition site at the *TERT* promoter in 20/43 cell lines (BS cohort) screened. This SNP is present in 6/10 cHL cell lines with allelic dominance of the alternative allele in 3/6. Allele-specific DNA methylation analysis in cHL did not show significant DNA methylation differences between the alternative allele and the reference allele.

We conclude that different types of lymphomas have different *TERT* promoter DNA methylation patterns. Our data provide a baseline for further more detailed allele-specific analyses on *TERT* promoter methylation, *TERT* expression and its relation to sequence variances in lymphomas.

Abstract ID: 147

P3-51

Prenatal presentation of *ARCN1*-associated skeletal dysplasia

A. Busche[1], J. Steinhard[2], S. Ledig[1], M. Möllers[3], A. Bohring[1]

[1]Institut für Humangenetik, Universitätsklinikum Münster, Münster, Deutschland, [2]Zentrum für Pränatale Medizin und Humangenetik, Münster, Deutschland, [3]Klinik für Gynäkologie und Geburtshilfe, Universitätsklinikum Münster, Münster, Deutschland

The Short Stature-Micrognathia Syndrome (SSMG, OMIM #617164) is a rare skeletal dysplasia characterized by intrauterine growth restriction, postnatal short stature with rhizomelic shortening, and micrognathia. Other common features are mild developmental delay, preterm birth, microcephaly, and genitourinary malformations in males. SSMG is caused by heterozygous pathogenic variants in *ARCN1* (OMIM *600820) and is inherited in an autosomal dominant pattern. Up to now, only about 20 patients have been reported.

Here we present a couple referred to us in the 22nd week of gestation. Prenatal ultrasound showed intrauterine growth restriction, a small thorax, relatively shortened long bones, bowed femura, and oligohydramnios. Trio-Exom-Analysis on amniotic fluid and parental DNA revealed the heterozygous de novo variant c.862C>T p.(Arg288*) in *ARCN1* (NM_001655.5). According to ACMG-guidelines the variant was classified as pathogenic (Class 5). The couple decided to terminate the pregnancy.

We present prenatal and postmortem clinical as well as molecular data of a fetus with *ARCN1*-associated skeletal dysplasia. Bowed limbs and thoracic hypoplasia, seen in this case, have been reported rarely before. With this report we further delineate the phenotypic spectrum of *ARCN1*-associated skeletal dysplasia and want to rise attention to a rare and clinically variable condition.

Abstract ID: 148

P5-4

Prenatally detected whole gene deletion of *SF3B4* associated with Nager syndrome

M. Kuschmann[1], C. Barkhaus[1], A. Bohring[1], A. Busche[1], C. De Santis[2], J. Horvath[1], C. Müller-Hofstede[1], Y. Stratis[1], A. Röpke[1]

[1]Universitätsklinikum Münster, Institut für Humangenetik, Münster, Deutschland, [2]Universitätsklinikum Münster, Abteilung für Gynäkologie und Geburtshilfe, Münster, Deutschland

Nager syndrome is a type of acrofacial dysostosis and is associated with heterozygous mutations and deletions in the *SF3B4* gene located in the chromosomal region 1q21.2. The literature mostly describes loss-of-function mutations, but whole gene deletions have been described as well. Nager syndrome is characterized by craniofacial malformations including microretrognathia, cleft palate and ear malformations, as well as limb malformations including radial limb hypoplasia like absence or hypoplasia of the thumbs. Short stature, costovertebral, renal or cardiac malformations have been described as well, while most individuals exhibit normal intelligence. Nager syndrome has been detected prenatally due to structural abnormalities visible on ultrasound and confirmed by invasive prenatal diagnosis.
We describe a 37-year-old woman in her 13th week of pregnancy. Invasive prenatal diagnosis was performed because of an abnormal ultrasound. The fetus presented with a complex malformation syndrome with malformation of both arms, short long bones of the lower extremities, misaligned feet, and Hygroma colli. After termination of the pregnancy, additionally retrognathia and absent thumbs were observed. Both direct and long term culture of chorionic villi showed a normal female karyotype. Symptom-based panel analysis on direct culture of chorionic villi showed no pathogenic variants. Array-SNP analysis on direct culture of chorionic villi showed a de novo heterozygous loss of approx. 58 kb in the chromosomal region 1q21.2 encompassing the *SF3B4* gene. No other disease-associated genes were located in the deleted region. The phenotype of the fetus is consistent with the clinical phenotype of Nager syndrome. To our knowledge, this is the smallest prenatally detected whole gene deletion of *SF3B4* described in literature, associated with the Nager syndrome phenotype.

Abstract ID: 149

P3-5

Resolving pathogenicity of non-truncating *ARID1B* variants in Coffin-Siris syndrome

E. Bosch[1], E. Güse[1], P. Kirchner[1], M. Hebebrand[1], A.B Ekici[1], A. Reis[1], G. Vasileiou[1]

[1]*Institut für Humangenetik, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Deutschland*

Variants affecting the function of different subunits of the ATP-dependent chromatin remodelling BAF complex lead to distinct but largely overlapping neurodevelopmental syndromes called BAFopathies. The most common BAF-related disorder is Coffin-Siris syndrome (CSS), characterised by neurodevelopmental delay with intellectual disability, muscular hypotonia, short stature, agenesis of the corpus callosum, nail hypoplasia and coarse facial features. *ARID1B* is most frequently affected in CSS. To date, only heterozygous truncating *ARID1B* variants resulting in nonsense-mediated mRNA decay (NMD) are considered causative.

Exome sequencing identified a *de novo* in-frame deletion in the last exon of *ARID1B* (p.(Glu2129_Met2132del)) in a 5-year-old female individual with clinical suspicion of CSS. The variant occurred within the EHD2 domain, known to interact with the catalytic BAF subunit SMARCA4. Given its non-truncating nature it was initially classified as variant of uncertain significance. To investigate a potential clinical significance we performed RNAseq analysis from blood samples of the individual together with six other CSS cases with causative truncating *ARID1B* variants and nine controls. The transcription pattern of the in-frame deletion clustered together with that of the truncating variants and separately from controls, indicating pathogenicity. This led to its reclassification as likely pathogenic. Literature search revealed additional variants within the EHD2 domain in individuals with CSS clinical manifestations: two missense alterations, p.(Ile2031Thr) and p.(Cys1945Arg), as well as one NMD-escaping truncating variant, p.(Ser2155Leufs*33). Overexpression of the four mutants in HEK cells revealed formation of both nuclear and cytoplasmic aggregates, with the latter significantly enriched as compared to the wild type. Co-staining with vimentin and γ-tubulin revealed these cytoplasmic aggregates to be aggresomes, juxtanuclear inclusion bodies containing poly-ubiquitinylated proteins, surrounded by a vimentin cage and attached to the microtubule organisation centre. Such structures occur when the proteasome machinery is overwhelmed due to overload of misfolded proteins. Our findings suggest that non-truncating variants in the EHD2 domain of ARID1B can be clinically relevant by impairing protein folding. Interestingly, the phenotypic presentation is comparable to that of individuals with haploinsufficiency variants, indicating a common pathomechanism.

Abstract ID: 150

P3-52

Colon cancer in a 41-year-old patient with *MORC2*-related neurodevelopmental disorder

B. Herr[1], A. Quante[1], M. Quante[2], A. Decker[2], S. Timme-Bronsert[3], U. Grasshoff[4], S. Gauß[4], L. Stühn[4], B. Gläser[1], A. Tzschach[1]

[1]Institute of Human Genetics, Medical Center, University of Freiburg, Freiburg, Deutschland, [2]Department of Internal Medicine II, Medical Center, University of Freiburg, Freiburg, Deutschland, [3]Department of Pathology, Medical Center, University of Freiburg, Freiburg, Deutschland, [4]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Deutschland

Heterozygous pathogenic variants in the *MORC2* gene have been associated with axonal Charcot-Marie-Tooth disease type 2Z (MIM#616688) and developmental delay, impaired growth, dysmorphic facies, and axonal neuropathy (MIM#619090). In 2020, 20 patients with *de novo* variants in the ATPase module of *MORC2* affected by neurodevelopmental disorders with growth retardation and variable craniofacial dysmorphisms were reported, five of whom carried the variant c.79G>A (p.Glu27Lys) (PMID: 32693025). No malignancies were reported in these patients.

We report on a 42-year-old patient with short stature, microcephaly, dysmorphic facial features, severe intellectual disability and neuromuscular disorder, brain anomalies (internal and external hydrocephalus, supra- and infratentorial atrophy, basal ganglia lesions, hyperintense white matter lesions), progeroid features, hearing loss and impaired vision. At the age of 41 years stenosing colon cancer (adenocarcinoma of ascending colon) was diagnosed. The tumor showed mismatch repair deficiency and high microsatellite instability (MSI-H).

Molecular genetic testing for Lynch syndrome and further hereditary tumor syndromes showed no pathogenic or likely pathogenic variants. Whole-genome sequencing revealed the heterozygous pathogenic variant c.79G>A (p.Glu27Lys) in the *MORC2* gene.

Cancer in a *MORC2* patient may be a coincidence, but a causal relationship cannot be excluded. Reports of additional patients (especially of adults) will be needed to answer the question whether or not germline mutations in *MORC2* predispose to malignancy.

Abstract ID: 153

P2-12

Spectrum of somatic variants and clinical phenotypes in RUNX1-associated familial platelet disorder with predisposition to hematologic malignancies

A. Förster[1], M. Decker[1], Y. Behrens[1], B. Schlegelberger[1], G. Göhring[1], T. Ripperger[1]

[1]*Institut für Humangenetik, Medizinische Hochschule Hannover, Hannover, Deutschland*

Pathogenic germline variants in *RUNX1* cause familial platelet disorder with predisposition to hematologic malignancies (RUNX1-FPD), characterized by a broad phenotypic spectrum and incomplete penetrance. Causal germline variants are presumed to promote accumulation of somatic alterations in an inflammatory microenvironment that can finally lead to malignant transformation and frank leukemia. The spectrum of somatic variants is heterogeneous between individuals, even among affected family members. Published data are limited due to few patient numbers as well as small and inconsistent somatic gene panels. Thus, the interplay of predisposing germline variants and acquired variants still remain to be better elucidated to understand leukemic transformation in RUNX1-FPD. Here, we collected DNA samples of 27 individuals with likely pathogenic or pathogenic *RUNX1* germline variants from 11 families. Germline variants include 11 (41%) partial or complete gene deletion, nine (33%) frameshift, four (15%) nonsense, two (7%) splice site, and one (4%) missense variant. Among the probands, 14 (52%) showed non-malignant symptoms (e.g., (thrombo)cytopenia). Regarding malignant neoplasms, six (22%) were diagnosed with MDS, one (4%) with AML, one (4%) with MDS/AML, and two (7%) with other hematologic malignancies (e.g., CMML). Three (11%) individuals were asymptomatic at the time of sample collection with no known cytopenia and/or platelet dysfunction. As recently systematically reviewed by us, RUNX1-FPD predominantly harbor somatic variants in genes such as *RUNX1*, *TET2*, *ASXL1*, *BCOR*, *PHF6*, *SRSF2*, *NRAS*, and *DNMT3A* (PMID 35884491). To better define the spectrum of acquired variants in RUNX1-FPD, we sequenced 36 samples (i.e., bone marrow or peripheral blood) from the aforementioned 27 patients using a customized NGS panel encompassing 148 genes (i.e., entire coding regions or hot spot exons) associated with hematologic malignancies. So far, we recurrently identified somatic variants in *ANKRD26* and *BCOR*. Further data analysis including variant classification and inclusion of additional patients is presently ongoing. The present study will illustrate intrafamilial and interindividual heterogeneity of clonal hematopoiesis. It will support elucidation of malignant transformation being the prerequisite for future risk-adapted surveillance, potential chemoprevention, and indication for hematopoietic stem cell transplantation prior to overt leukemia. Funded by BMBF MyPred (01GM1911B).

Abstract ID: 154

P2-13

A functional characterization of *hsa-miR-129-5p* in triple-negative breast cancer cells

R. Steinmetz[1], T. Reinkens[1], F. Hennig[1], N. Buhl[1], V. Schäffer[1], M. Eilers[1], A. Stalke[1], B. Skawran[1]

[1]*Department of Human Genetics, Hannover Medical School, Hannover, Deutschland*

Introduction: Studies have found *hsa-miR-129-5p* (*miR-129-5p*) to be a key player in several cancerous and non-cancerous diseases. The expression of *miR-129-5p* has been reported to be reduced in several cancer types, such as triple-negative breast cancer. This has led us to want to further investigate the function of *miR-129-5p* in triple-negative breast cancer cells.
Methods: Three different triple-negative cancer cell lines (HCC38, HCC1937 and HCC1395) were transiently transfected with mimic-*miR-129-5p*. To explore proliferative behavior, we used a WST1-Assay, performed in each cell line 24 hours, 48 hours, 72 hours and 96 hours after transfection. A Caspase Glo3/7-Assay was performed to explore apoptosis simultaneously. We used a Transwell-Migration-Assay to analyze migration behavior after transfection.
Results: After overexpressing *miR-129-5p*, proliferation declined significantly in the HCC38 cells, whereas apoptosis increased significantly. In the HCC1937 cells, proliferation declined only marginally, while apoptosis increased significantly. Lastly, HCC1395 did not show any decline in proliferation after *miR-129-5p*-overexpression, nor a rise in apoptosis. Furthermore, migration declined in all three cell lines after overexpression of *miR-129-5p*. In HCC38 and HCC1937 it declined significantly. In HCC1395 this effect was less pronounced.
Conclusion: In the performed assays, *miR-129-5p* seems to be promoting anti-tumoral behavior, regarding proliferation, apoptosis and migration. The downstream-targets of *miRNA-129-5p*, mediating this effect are largely still to be investigated. As the effect of miRNA-overexpression in our studies was most significant and most consistent across cell lines in the Migration-Assay, we plan to investigate targetgenes, which encode for migration-related proteins. We plan to do this, by measuring the expression of those predicted genetical targets on mRNA-level after an overexpression of *miR-129-5p*. After finding targets, whose expression is controllable by *miRNA-129-5p*-overexpression, we want to transiently knock them down by transfection of siRNA and repeat the functional assays concerning migration, proliferation and apoptosis to determine the effect of this knockdown.

Abstract ID: 155

Saturation mutagenesis data facilitate interpretation of noncoding variants in the *IRF6* enhancer associated with orofacial clefting

R. Hollstein[1], F. Thieme[1], F. Lauber[1], A. Biedermann[1], J. Fazaal[1], M. Schubach[2], M. Kircher[3,2], K.U. Ludwig[1]

[1]Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Deutschland, [2]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Deutschland, [3]Institute of Human Genetics, University Hospital Schleswig Holstein, Campus Lübeck, Lübeck, Deutschland

Orofacial clefting (OFC) is one of the most common birth defects, occurring in about 1 in 600 live births. A major subtype is nonsyndromic cleft lip with or without cleft palate (nsCL/P), which has a multifactorial etiology. Several genes have been implicated in OFC, e.g. because of their location near GWAS loci or by rare variants found in monogenic forms. However, these only partially explain its high heritability. One component of this heritability gap could be rare variants in noncoding regions. These variants are not covered by array-based genotyping and, when identified by sequencing, are difficult to assign a pathogenic effect due their sheer numbers.

One of the best studied genes involved in OFC is *IRF6*. Common and rare variants within the gene itself and an upstream enhancer are associated with different forms of OFC, including nsCL/P. In this study, we combined two sequencing-based high-throughput methods to investigate the contribution of rare variants within the *IRF6* enhancer to OFC. We used molecular inversion probes (MIPs) to sequence the enhancer region in 699 nsCL/P cases and 511 population controls. In total, we found 11 variants in cases: three common single nucleotide variants (SNV) with an allele-frequency between 0.014 and 0.2, two 4- and 9-bp indels, and six rare SNVs.

In parallel, we performed a massively parallel reporter assay (MPRA), using a previously established saturation mutagenesis library of the *IRF6* enhancer, to analyse the effects of all possible single nucleotide exchanges within the enhancer. For this we transfected a human oral epithelial cell line (GMSM-K), HEK293T and HaCaT cells with the plasmid libraries to generate cell-type specific effect maps. These results were then used to interpret the variants observed in nsCL/P cases.

One common SNV (rs76145088) and one rare variant showed a significant decrease of enhancer activity in HaCaT ($p < 1e-5$), but not in GMSM-K or HEK293T cells. Interestingly, the other five rare variants affected the enhancer activity in either HaCat or GMSM-K at a nominal significance threshold ($0.05 > p > 1e-5$), while the two common variants had no effect ($p > 0.05$). An increase in sequencing depth might increase sensitivity and resolve these results.

In conclusion, the combination of MPRA data together with large-scale identification of risk variants is a promising tool for the interpretation of non-coding variants in orofacial clefting, and might be applicable to other human disorders.

Abstract ID: 156

P6-14

Multigene panel sequencing in highly consanguineous families and Isolated patients with congenital forms of skeletal dysplasias

N. Kakar[1,2], F.u. Rehman[3], D. Bhattacharya[4], S.B. Gandham[5], M. Goyal[6], R. Kaur[4], K. Kaur[4], K.S. Sodhi[7], C. Kubisch[8], G. Borck[9], I. Panigrahi[4], K.M. Girisha[5], U. Kornak[10], M. Spielmann[11]

[1]Institut für Humangenetik, Universitätsklinikum Schleswig-Holstein, University of Lübeck and University of Kiel, 23562, Lübeck, Deutschland, [2]Department of Biotechnology, BUITEMS, Quetta, Pakistan, [3]Department of Pathology, Bolan Medical College, Quetta, Pakistan, [4]Department of Pediatrics, APC, PGIMER, Chandigarh, Indien, [5]Department of Medical Genetics, Kasturba Medical College, Manipal University, Manipal, Indien, [6]Pediatrics Genetic & Research Laboratory, Department of Pediatrics, Lok Nayak Hospital, New Delhi, Indien, [7]Department of Radiodiagnosis, APC, PGIMER, Chandigarh, Indien, [8]Institute of Human Genetics University Medical Center Eppendorf, Hamburg, Deutschland, [9]Institute of Human Genetics, Ulm University, Institute of Human Genetics, Ulm University, Ulm, Germany, Deutschland, [10]Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Deutschland, [11]Institut für Humangenetik, Universitätsklinikum Schleswig-Holstein, University of Lübeck and University of Kiel, Lübeck, Deutschland

Skeletal dysplasias (SKDs) are a large heterogeneous group of more than 450 genetic disorders characterized by abnormal development, growth, and maintenance of bones or cartilage in the human skeleton. SKDs are often caused by mutations in early patterning genes and in many cases part of multiple malformation syndromes in combination with non-skeletal phenotypes. The aim of this study was to evaluate the diagnostic yield of multigene panel sequencing analysis of genes involved in congenital SKDs in patients from highly consanguineous families compared to isolated cases. Therefore, we performed panel sequencing of 386 bone-related genes in 7 highly consanguineous families from Pakistan and 27 isolated cases from India affected with SKDs. In the consanguineous families, we were able to identify the underlying genetic cause in five out of seven families resulting in a diagnostic yield of 71.4 %. Whereas, in the isolated cases we identified 12 causative variants corresponding to a diagnostic yield of 41 %. The genetic heterogeneity in our cohorts was very high and we identified mutations in several genes known to cause different types of SKDs.
In conclusion, panel sequencing proved to be a highly effective way to decipher the genetic basis of SKDs in highly consanguineous families with a diagnostic yield of up to 71.4%. Furthermore, our findings expand the allelic spectrum of skeletal dysplasias.

Abstract ID: 157

P2-14

Establishment of a functional assay for the classification of VUS in *FANCJ*

A. Droste[1], P. Frost[1], J. Knaup[1], M. Hess[1], L. Herrmann[1], P. Neurgaonkar[1], R. Kalb[1]

[1]*Universität Würzburg, Institut für Humangenetik, Würzburg, Deutschland*

The development of next generation sequencing (NGS) methods has improved quantity and quality of genetic analyses. The interpretation of variants of unknown significance (VUS) is still challenging, albeit required for a reliable prediction of pathogenicity and genetic counselling.

The Fanconi anemia (FA) pathway is required for the repair of DNA interstrand crosslinks. Biallelic inactivation of *FANCJ* causes Fanconi anemia, while monoallelic mutations are associated with an increased risk for hereditary breast and ovarian cancer. Here, we present a cell culture-based model to determine functional effects of VUS in *FANCJ*. Adaption of this system to other genes like *FANCA*, *FANCG* and *ATM* is in progress. These data will allow reclassifications of VUS and will provide functional data for advanced *in-silico* predictions.

Abstract ID: 158

Disruption of the topologically associated domain at Xp21.2 is related to 46,XY gonadal dysgenesis

V. Yumiceba[1], J.A Meinel[2], A. Künstner[3,4], K. Schultz[1], N. Kruse[1], F.J Kaiser[5,6], P.-M. Holterhus[7], A. Claviez[8], O. Hiort[2], H. Busch[3,4], M. Spielmann[1,9], R. Werner[2,10]

[1]Institute of Human Genetics, Universitätsklinikum Schleswig-Holstein, University of Lübeck and University of Kiel, Lübeck, Deutschland, [2]Department of Pediatrics and Adolescent Medicine, Division of Pediatric Endocrinology and Diabetes, Universität zu Lübeck, Lübeck, Deutschland, [3]Group of Medical Systems Biology, Lübeck Institute of Experimental Dermatology, Universität zu Lübeck, Lübeck, Deutschland, [4]Institute for Cardiogenetics, Universität zu Lübeck, Lübeck, Deutschland, [5]Institute of Human Genetics, Universität Duisburg-Essen, Duisburg, Deutschland, [6]Essen Center for Rare Diseases (EZSE), University Hospital Essen, Essen, Deutschland, [7]University Medical Center for Pediatric Endocrinology and Diabetes, Department of Pediatrics and Adolescent Medicine I, Universitätsklinikum Schleswig-Holstein, Kiel, Deutschland, [8]Department of Pediatrics and Adolescent Medicine I, Division of Pediatric Oncology and Hematology, Universitätsklinikum Schleswig-Holstein, Kiel, Deutschland, [9]Partner Site Hamburg/Kiel/Lübeck, German Center for Cardiovascular Disease, Berlin, Deutschland, [10]Institute of Molecular Medicine, Universität zu Lübeck, Lübeck, Deutschland

Gonadal dysgenesis (GD) refers to a subset of differences of sexual development characterised by defective gonadal formation. So far several structural or numerical anomalies of the sex chromosomes or mutations in key genes involved in the development of the gonads have been associated with GD, but the majority of GD patients GD do not receive a molecular diagnosis even after Exome sequencing. Duplications of Xp21.2 of NR0B1 were reported in non syndromic 46,XY GD cases, underlining gene dosage as the most plausible explanation. Recently a deletion upstream of NR0B1 was described in a 46,XY GD patient challenging the gene dosage hypothesis. This motivated us to characterise the 3D architecture and its functional topologically associating domains (TADs) at the Xp21.2 locus to explain the pathogenicity of structural variations (SV) causing GD.

Here we identified two novel unrelated patients with 46,XY GD harbouring SVs at the Xp21.2 locus: a complex rearrangement (inversion, duplication, deletion) upstream of NR0B1 and a 1.2 Mb tandem triplication, including NR0B1. These structural variants were confirmed by whole genome sequencing and breakpoints were resolved by Sanger sequencing. High-throughput chromosome conformation capture (Hi-C) analysis revealed rewiring of TADs. Even though both cases displayed different structural variants, they shared a minimal overlap region (195 kb) that was mapped to a TAD boundary, which insulate genes and its cognate regulatory elements from neighbour domains. The TAD boundary duplication close to NR0B1 has been associated with neo-TAD and shuffled TAD formation. The new disposition of the 3D genome could place ectopic enhancers in proximity to NR0B1, leading to enhancer hijacking and atypical NR0B1 spatio-temporal expression. Modelling of previous Xp21.2 structural variations associated with isolated GD support our hypothesis of 3D genome alterations, first due to similar minimal overlap region and secondly the predicted formation of neo-TADs as well as TAD fusion.

We present a novel mechanism on how deletions, duplications, or inversions at the NR0B1 locus can cause partial or complete GD by disrupting the cognate TAD in the vicinity of NR0B1, via a non-coding element positioning. This model not only contributes to a better GD diagnosis with CNVs at Xp21.2, but also gives deeper insight on how spatiotemporal activation of developmental genes can be disrupted by reorganised TADs causing impairment of gonadal development.

Abstract ID: 159

SEC24C deficiency causes trafficking and glycosylation abnormalities and leads to a congenital syndrome including anemia, microcephaly, and cataracts

N. Bögershausen[1], B. Cavdarli[2], M.P. Milev[3], J. Schmidt[1], Y. Li[1], D. Kaçar[4], G. Haliloğlu[5], C. Thiel[6], M. Sacher[3], G. Yigit[1], B. Wollnik[1]

[1]Institute of Human Genetics, University Medical Center Goettingen, Göttingen, Deutschland, [2]Department of Medical Genetics, Ankara Cıty Hospıtal, Ankara, Türkei, [3]Department of Biology, Concordia University, Montreal, Kanada, [4]Department of Pediatric Hematology, Ankara City Hospital, Ankara, Türkei, [5]Department of Pediatric Neurology, Hacettepe University, Ankara, Türkei, [6]Center for Child and Adolescent Medicine, Department Pediatrics I, Heidelberg University, Heidelberg, Deutschland

SEC24 proteins are important components of the coat protein complex II (COPII), which is responsible for the formation of secretory vesicles on the endoplasmic reticulum (ER) membrane for intracellular trafficking. Within COPII, SEC24 proteins are essential to cargo selection and packaging. So far, a human phenotype has only been associated with one of the four human SEC24 homologues: *SEC24D*. Here we report a novel syndrome characterized by anemia, epilepsy, microcephaly, liver dysfunction, and congenital cataracts, in a consanguineous family with four affected individuals, three of whom are deceased. Exome sequencing revealed a biallelic, truncating variant in the *SEC24C* gene in the index patient, leading to absence of the SEC24C protein in fibroblasts from the same individual. A cargo sorting assay (RUSH) showed delayed ER-Golgi transport in SEC24C deficient patient cells, indicating impaired anterograde traffic as a consequence of the loss of SEC24C. Moreover, immunoblotting showed a reduction in the abundance of SEC24C interaction partners such as SEC23B and SEC13, indicating an interesting mechanistic link to congenital dyserythropoietic anemia type II, which is caused by biallelic variants in the *SEC23B* gene. As we also observed a clinical overlap with the disease group “congenital disorders of glycosylation”, we used liquid chromatography mass spectrometry to investigate a possible influence of SEC24C deficiency on protein glycosylation and we found a slight shift in the N-glycosylation pattern, with a reduction of galactose and sialic acid residues. In summary, we describe a family with a novel rare syndrome, caused, in the index patient, by a likely pathogenic biallelic variant in *SEC24C* and show that loss of SEC24C leads to a defect in anterograde vesicle trafficking and to a possible secondary alteration of protein glycosylation.

Abstract ID: 160

P7-11

Information integration to predict chromatin folding using neural networks

S. Sys[1,2], S. Weißbach[3], C. Heidrich[4], S. Gerber[3], K. Everschor-Sitte[5]

[1]Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Institut für Humangenetik, Mainz, Deutschland, [2]JGU Mainz, Institute of Multiscale Modeling, Mainz, Deutschland, [3]Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Institute of Human Genetics, Mainz, Deutschland, [4]Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Instutute of Human Genetics, Mainz, Deutschland, [5]Universität Duisburg-Essen, Faculty of Physics, Duisburg-Essen, Deutschland

Long range sequence interactions in genomes revealed an additional spatial layer of genome organization. This layer is important for the functional compartmentalization of cells and therefore coupled to healthy cell development.
While it is possible to cover such interactions experimentally using chromatin capture methods, the computational prediction of such interactions proves difficult on the level of genetic variation. The most promising approaches currently to predict genome-folding are deep convolutional neural networks. While neural networks have been used to either predict the chromatin structure solely based on the genetic sequence or have been pre-trained in the means of transfer learning on epigenetic markers as acetylation sites, no real efforts were made to intergrate (epi-)genomic annotation and genetic sequence in one network.
Here we present a novel integrative approach using convolutional and transformer networks to predict the three-dimensional genome architecture based on genetic sequence and genomic annotations.

Abstract ID: 161

P7-6

Decipher patient needs in the process of genetic counseling to individualize the personal assistant GEN-SUPPORT

N. Ammon[1], D. Wolff[2], T. Kupka[2], S. Oeltze-Jaffra[2], C. Reichert[2], B. Schlegelberger[1], A.K. Bergmann[1], B. Vajen[1]

[1]Hannover Medical School, Department of Human Genetics, Hannover, Deutschland, [2]Hannover Medical School, Peter L. Reichertz Institute for Medical Informatics, Hannover, Deutschland

Introduction: Genetic counseling is the central element in the care of patients and families with hereditary breast and ovarian cancer (HBOC). Index patients and their families receive a wealth of information, about the nature of the disease, the consequences for the index patient and the family members. Moreover, important information about preventive measures, therapy options and support services such as psycho-oncological care is transferred during genetic counseling. We develop a mobile assistant, called GEN-SUPPORT, to assist patients during the genetic counseling process in a personalized way. Thus, the index patient will be enabled to access above-mentioned information in a personalized structured way. For the personalization, we first determined patient needs with qualitative interviews.

Material and methods: To determine patients' needs, nine qualitative interviews based on the critical incident technique were performed in 2022 with HBOC patients, who presented for genetic counseling and testing at the Department of Human Genetics (2017-2022) of Hanover Medical School. The qualitative evaluation was carried out according to the content analysis of Mayring by using the data analysis software MAXQDA.

Results: Patients demanded higher need for personalized support during the process of genetic counseling. The simplicity of language and the personalized way of transferring knowledge were relevant for most interviewed patients. Furthermore, patients often felt disoriented and lacked a clear roadmap for the next steps. Patients also increasingly asked for supportive information regarding medical genetics for both, the time before genetic counseling or testing as well as afterwards. Personal exchange with other patients, for example in the form of a forum, was particularly important to the patients.

Conclusion: There is a tremendous need for patients with HBOC for better and personalized support, which makes the use of AI-based mobile support for HBOC patients worthwhile. Providing support as well as information will improve the patients' care situation. Their health literacy would increase while their burden would decrease.

Abstract ID: 163

P1-5

Transcriptome analysis on Emery-Dreifuss muscular dystrophy primary tissue cultures reveals alterations of metabolic, fibrotic, and splicing pathways

S. Hintze[1], J.I. de las Heras[2], V. Todorow[1], B. Schoser[1], E.C. Schirmer[2], P. Meinke[1]

[1]Friedrich-Baur-Institute at the Department of Neurology, University Hospital, LMU Munich, Germany, Munich, Deutschland,
[2]Institute of Cell Biology, University of Edinburgh, Edinburgh, UK, Edinburgh, Vereinigtes Königreich

Emery-Dreifuss muscular dystrophy (EDMD) is a genetically and clinically variable disorder. Previous attempts to use gene expression changes find its pathomechanism were unavailing, so we here engaged a functional pathway analysis. RNA-Seq was performed on cells from 10 patients diagnosed with an EDMD spectrum disease with different mutations in 7 genes. Upon comparing to controls, the pathway analysis revealed that multiple genes involved in fibrosis, metabolism, myogenic signaling, and splicing were affected in all patients. Splice variant analysis revealed alterations of muscle-specific variants for several important muscle genes. Deeper analysis of metabolic pathways revealed a reduction in glycolytic and oxidative metabolism and reduced numbers of mitochondria across a larger set of 14 EDMD patients and 7 controls. Intriguingly, the gene expression signatures segregated the patients into three subgroups whose distinctions could potentially relate to differences in clinical presentation. Finally, differential expression analysis of miRNAs changing in the patients similarly highlighted fibrosis, metabolism, and myogenic signaling pathways. This pathway approach revealed a transcriptome profile that can both be used as the basis for establishing a biomarker panel for EDMD and direct further investigation into its pathomechanism. Furthermore, the segregation of specific gene changes into three distinct groups that appear to correlate with clinical presentation may be developed into prognostic biomarkers, though this will first require their testing in a wider set of patients with more clinical information.

Abstract ID: 164

P1-4

Nuclear envelope proteins in myotonic dystrophy: extending evidence for a relevant contribution to the phenotype

P. Meinke[1], V. Todorow[1], S. Hintze[1], B. Schoser[1]

[1]Friedrich-Baur-Institute at the Department of Neurology, University Hospital, LMU Munich, Munich, Deutschland

Myotonic dystrophy type 1 (DM1) is a multisystemic disorder with predominant muscle and neurological involvement. Despite a well described pathomechanism, which is primarily a global missplicing due to sequestration of RNA-binding proteins, there are still many unsolved questions regarding additional factors contributing to the disease development. One such question is the disease etiology in the different affected tissues. We observed alterations at the nuclear envelope (NE) in primary muscle cell cultures of DM1 patients before. This led us to reanalyze a published RNA-sequencing dataset of DM1 and control muscle biopsies regarding the misregulation of NE proteins. We could identify several muscle NE protein encoding genes to be misregulated depending on the severity of the muscle phenotype. Among these misregulated genes were NE transmembrane proteins (NETs) involved in nuclear-cytoskeletal coupling as well as genome organization. For selected genes, we could confirm that observed gene-misregulation led to protein expression changes. Furthermore, we investigated if genes known to be under expression-regulation by genome organization NETs were also misregulated in DM1 biopsies, which revealed that misregulation of two NETs alone is likely responsible for differential expression of about 10 % of all genes being differentially expressed in DM1. Notably, the majority of NETs identified here to be misregulated in DM1 muscle are mutated in Emery-Dreifuss muscular dystrophy (EDMD) or clinical similar muscular dystrophies, suggesting a broader similarity on the molecular level for muscular dystrophies than anticipated. This shows not only the importance of muscle NETs in muscle health and disease, but also highlights the importance of the NE in DM1 disease progression.

Abstract ID: 165

P3-53

Three sisters with Schwartz-Jampel syndrome and an additional cardiac phenotype

A. Schmetz[1], F. Distelmaier[2], F. Pillekamp[3], B. Westhoff[4], J. Schaper[5], P. Gierthmühlen[6], D. Wieczorek[1], H. Surowy[1]

[1]Institute of Human Genetics, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Deutschland, [2]Department of General Paediatrics, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Deutschland, [3]Department of Paediatric Cardiology and Pulmonology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Deutschland, [4]Department of Orthopaedics, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Deutschland, [5]Center for Rare Diseases, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Deutschland, [6]Department of Prosthodontics, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Deutschland

Biallelic pathogenic loss-of-function variants in *HSPG2* (1p36.12) cause the rare autosomal-recessive Schwartz-Jampel syndrome (SJS, OMIM # 255800), also called chondrodystrophic myotonia. *HSPG2* encodes the perlecan core protein, a key component of the extracellular matrix and basement membrane.
Three sisters aged ten, nine and six years presented with the common clinical features of SJS, short stature, enlarged knees and facial dysmorphism, but no other severe features of chondrodysplasia and myotonia. In all three sisters, whole-exome sequencing (WES) identified a homozygous likely pathogenic missense variant in the *HSPG2* gene (NM_005529.7:c.5273G>A p.(Arg1758Gln)), located in one of its immunoglobulin-like domains. Four heterozygous but no homozygous carriers of the variant have been reported in GnomAD (exomes v.2.1.1). The heterozygous carrier status of the consanguineous parents was confirmed by segregation analysis. In all three sisters, a loss-of-heterozygosity (LOH) region is present at the *HSPG2* gene locus, with a minimal extent of 11.6 Mb.
Each of the sisters was additionally diagnosed with non-compaction cardiomyopathy, a phenotype that was not previously described in connection with SJS. In all three sisters, re-analysis of the WES data for the cardiac phenotype identified an additional homozygous variant in the gene *TRIM63* (NM_032588.4:c.224G>A p.(Cys75Tyr)), encoding a muscle specific RING zinc finger E3 ubiquitin ligase (MuRF1). The affected conserved cysteine is part of its RING-finger domain and predicted to interact with cysteine residues at positions 39 and 78. Twenty-eight heterozygous but no homozygous carriers of the variant were reported in GnomAD, and heterozygous carrier status of the parents was confirmed. Biallelic loss-of-function mutations in *TRIM63* have recently been described as a cause of hypertrophic cardiomyopathy, although the reported patients were diagnosed at an older age (range 15-69 years).
The homozygous variant in *TRIM63* is the most likely explanation of the cardiac phenotype observed in the three sisters with SJS, with a possible modifying effect of the *HSPG2* variant. *TRIM63* is located 4.2 Mb downstream of *HSPG2* in the same LOH region, thus explaining the observed co-segregation of both observed phenotype entities.

Abstract ID: 166

P3-54

Novel homozygous *LAMB1* in-frame deletion in a pediatric patient with brain anomalies and cerebrovascular event

L. Toutouna[1], I. Bader[2], S. Beck-Woedl[2], K. Komlosi[1], B. Glaeser[1], U. Feige[3], N. Luetzen[3], M. Eckenweiler[4], T. Haack[2], J. Fischer[1], A. Tzschach[1]

[1]Institute of Human Genetics, University Medical Center Freiburg, University of Freiburg, Freiburg, Deutschland, [2]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Deutschland, [3]Department of Neuroradiology, University Medical Center Freiburg, University of Freiburg, Freiburg, Deutschland, [4]Department of Neuropediatrics and Muscle Disorders, University Medical Center Freiburg, University of Freiburg, Freiburg, Deutschland

Biallelic pathogenic variants in *LAMB1* are associated with autosomal recessive lissencephaly 5 (OMIM 615191), which is characterized by brain malformations (cobblestone lissencephaly, hydrocephalus), developmental delay and epilepsy. Pathogenic variants in *LAMB1* are rare, only 11 pathogenic variants and 11 patients have been reported to date. Here we report on a 6-year-old patient from a consanguineous family with profound developmental delay, microcephaly and a history of perinatal cerebrovascular event. MRI examinations showed cerebellar cystic defects, signal intensity abnormalities and hypoplastic corpus callosum. Trio-exome analyses revealed a homozygous in-frame deletion of 104 amino acids in *LAMB1* (c.(3079+1_3080-1)_(3391+1_3392-1)del, p.(Lys1027_Ala1131delinsThr)) affecting the EGF-like units 11 and 12 in domain III of the LAMB1 protein. To our knowledge, this is the first reported in-frame deletion in the *LAMB1* gene. The patient broadens the clinical and molecular spectrum of *LAMB1* associated syndromes.

Abstract ID: 167

P6-11

Functional analysis of a *MALRD1* variant associated with increased bile acid synthesis and hepatic cholestasis

V. Reichenbach[1], I. Evangelakos[1], D. Papingi[1], M. Jankofsky[2], A. Worthmann[3], C. Kubisch[1], C. Schlein[1]

[1]University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Deutschland, [2]University Medical Center Hamburg-Eppendorf, Department of Pediatrics, Hamburg, Deutschland, [3]University Medical Center Hamburg-Eppendorf, Institute of Biochemistry and Molecular Cell Biology, Hamburg, Deutschland

Introduction: *MALRD1* (MAM and LDL-receptor class A domain-containing protein 1, or *Diet1*) is an intestinal expressed gene and the encoded protein positively regulates the levels of fibroblast growth factor 15 in mice (Fgf15, the ortholog in humans is FGF19). After its intestinal production, FGF15/19 follows the enterohepatic circulation and mediates a negative feedback mechanism crucial for the inhibition of hepatic bile acid synthesis. Loss of *Diet1* in mice is associated with decreased ileal Fgf15 production and subsequent failure to inhibit hepatic bile acid synthesis that ultimately results in enhanced cholesterol conversion to bile acids. Bile acid accumulation and impaired bile flow can lead to hepatic injury and cholestasis. To date, it has not been investigated how MALRD1 or hypofunctional variants can affect bile acid-related hepatic diseases.
Methods: Whole exome sequencing was performed in a newborn infant that presented hepatomegaly and severe cholestasis. In order to analyze the common pathophysiological features accompanying the cholestatic liver disease of the patient we determined serum parameters including transaminases as well as albumin. Further, we analyzed circulating levels of FGF19 by solid sandwich ELISA and performed bile acid and lipidomic analysis of the plasma following LC-MS/MS-based approaches.
Results: We identified a putatively pathogenic homozygous missense mutation c.2740A>G [p.(Ile914Val)] in *MALRD1.* Plasma analysis revealed elevated liver transaminases and hypoalbuminemia, indicating extensive liver damage. Circulating FGF19 levels were dramatically reduced in the index patient in comparison to control individuals, pointing towards inhibition of its production and/or secretion. This was associated with a 20-fold induction of both conjugated (CBA) as well as unconjugated (UBA) bile acids in the plasma, arguing for dysfunctional FGF19-associated inhibition of hepatic bile acid synthesis.
Conclusion: In conclusion, we describe a putatively pathogenic biallelic MALRD1 variant that causes a cholestatic liver disease by dysregulating FGF19 plasma levels and leading to excess bile acid synthesis. Identification of factors that stimulate MALRD1 expression/function and sustain the FGF19-mediated inhibition of bile acid synthesis could be investigated as potential therapy for cholestatic liver diseases caused by bile acid overload.

Abstract ID: 168

P4-4

Identification of pathobiologically relevant cell types for male-pattern hair loss

S. Henne[1], N. Engelmann[1], S. Heilmann-Heimbach[1]

[1]*Institute of Human Genetics, University Hospital of Bonn & University of Bonn, Bonn, Deutschland*

Male-pattern hair loss (MPHL) is a highly heritable and prevalent form of hair loss. The phenotype is characterized by a strict androgen dependency and changes in hair cycle dynamics (i.e. shorter growth/prolonged resting). Genome-wide association studies (GWAS) have identified >350 genomic risk loci and have implicated numerous candidate genes and pathways. However, the exact cell types and hair cycle stages in which these genes and pathways exert their biological effects have not yet been elucidated. Over the past years, the availability of comprehensive single-cell (sc) RNA-Seq datasets for relevant tissues and developmental stages and algorithms that enable a systematic integration of single-cell expression and GWAS data has rendered the investigation of pathobiological mechanisms at single-cell resolution possible.

Here, we applied the single-cell disease relevance score (scDRS) approach, which identifies cells exhibiting excess expression of disease-associated genes. We used published MPHL GWAS data (Yap et al. 2018) and two independent published scRNA-Seq datasets from (i) the murine Tabula Muris Senis (TMS) FACS sc expression atlas and (ii) a scRNA-Seq dataset of the murine hair follicle (HF) during hair growth and rest (Joost et al. 2020) to identify MPHL-relevant cell types in both a broad and HF-specific context.

Our analysis revealed a significant excess expression (FDR<0.05) of MPHL-associated genes in 18 cell types from the TMS FACS atlas. The highest proportion of significant cells was observed in dorsal skin cell types both during hair growth and rest. Further associations included gonadal, marrow and subcutaneous adipose tissues, which are of interest as adipogenesis has been previously suggested to play a role in MPHL pathogenesis. The analysis of the HF-specific data identified nominally significant (P<0.05) associations with 7 cell populations, including (i) inner and outer root sheath keratinocytes and dermal sheath fibroblasts during hair growth and (ii) the dermal papilla and skin fibroblasts during hair rest. Gene set analyses of genes most highly correlated with sc disease scores in the TMS FACS and HF data sets identified 191 pathways (FDR<0.05) that seem to be relevant in specific (ferroptosis, adipogenesis) or across different hair cycle stages (androgen signaling). Together, our data provide novel insights into MPHL-relevant cell types and cellular processes and constitute a basis for systematic functional follow-up of GWAS.

Abstract ID: 169

P1-12

Features of lncRNA expressions and regulation across different brain regions of primates

M. Navandar[1], C. Vennin[2,3], B. Lutz[2,3], S. Gerber[1]

[1]Institute of Human Genetics, University Medical Center, Mainz, Deutschland, [2]Leibniz Institute for Resilience Research, Mainz, Deutschland, [3]Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University, Mainz, Deutschland

Human and non-human primates like chimpanzee have strikingly similar genomes, but differs phenotypically, behaviourally, and cognitive wise. It is fascinating to understand the molecular aspect of brain evolution, wherein functions of protein coding genes are widely studied. Looking at the complexity of the eukaryotic genome, little is known about the role of non-coding parts such as long non-coding RNAs (lncRNAs) during brain evolution. In this study, we predicted lncRNAs and analysed its transcriptional expression pattern across different brain regions of human and non-human primates (chimpanzee, gorilla and gibbon). Our analysis identified shared orthologous and non-orthologous lncRNAs having striking differences in the genomic features. Differential expression analysis of the shared orthologous lncRNAs from human and chimpanzee revealed distinct expression pattern in subcortical regions (striatum, hippocampus) and neo-cortical regions, while retaining homogeneous expression in cerebellum. Co-expression analysis of lncRNAs and protein coding genes revealed the massive proportions of co-expressed pairs in neo-cortical regions of human compared to chimpanzee. Network analysis of co-expressed pairs revealed distinctive role of the hub acting orthologous lncRNAs in region and organism specific manner. Overall, our study provides the insight of lncRNA driven gene regulatory landscape, evolutionary scenario, and a resource for candidate lncRNAs in human and non-human primate’s brain.

Abstract ID: 170

P6-13

Two cases with MBD5-associated neurodevelopmental disorder expand the known disease spectrum and highlight role of WGS in detecting non-coding CNVs

J. Fischer[1], M. Bermudez[1], D. William[1], K. Hackmann[1], L. Rossow[1], J. Porrmann[1], J. Schallner[2,3], T. Kretschmer[4], M.A. Lee-Kirsch[2,4], R. Berner[2,4], E. Schröck[1], N. Di Donato[1]

[1]Institut für Klinische Genetik, Universitätsklinikum Carl Gustav Carus an der Technischen Universität Dresden, Dresden, Deutschland, [2]Klinik und Poliklinik für Kinder- und Jugendmedizin, Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden, Dresden, Deutschland, [3]Sozialpädiatrisches Zentrum (SPZ), Universitätsklinikum Carl Gustav Carus Dresden, Dresden, Deutschland, [4]UniversitätsCentrum für Seltene Erkrankungen, Universitätsklinikum Carl Gustav Carus Dresden, Dresden, Deutschland

Introduction: Haploinsufficiency of the MBD5 gene has been identified as a cause for MBD5-associated neurodevelopmental disorder (MAND), which aside from delayed development (especially language impairment) frequently presents with epilepsy, hypotonia, microcephaly, gait ataxia and skeletal abnormalities. Pathogenic copy number variants (CNVs) involving the first five non-coding exons of MBD5 have been described, but remain difficult to be identified in patients. Furthermore, relatively frequent parental mosaicisms have been reported (14% in one publication; PMID: 33912662) making segregation analyses and predictions regarding recurrence risks challenging.
Methods: Whole genome sequencing (WGS) was carried out using DNA PCR-Free Prep (Illumina) and sequencing on Illumina NovaSeq 6000 with a median target coverage of 40-fold.
Results: Using WGS we identified two patients with an MBD5-associated neurodevelopmental disorder. Patient 1 was a 6-year-old boy with impaired cognitive development, behavioural abnormalities, corpus callosum dysplasia and dysmorphic facial features. No seizures had been reported. He harboured an 81 Kb tandem duplication involving the non-coding exon 5 ([hg38] chr2:148,287,663-148,369,006). Patient 2 was a 5-year-old girl exhibiting developmental delay, microcephaly, facial dysmorphism and gait ataxia. There was no evidence of epilepsy. A 147 Kb deletion including non-coding exons 1 and 2 of the MBD5 gene was detected ([hg38] chr2:148,012,300-148,160,144). Segregation using WGS of the parents showed low grade variant mosaicism in the father which was further analysed using break-point specific PCR/amplicon sequencing.
Conclusion: This report helps further defining the MBD5 disease spectrum, highlighting that epilepsy may not be a cardinal feature of this neurodevelopmental disorder especially in younger children. Moreover, it showcases the importance of whole genome sequencing in the detection of non-coding CNVs and its ability to specifically locate CNV breakpoints as well as low grade mosaic variants allowing for improved variant segregation.

Abstract ID: 171

P7-12

NanopoReaTA: user-friendly tool for nanopore-seq real-time transcriptional analysis

A. Wierczeiko[1,2], S. Pastore[3], S. Mündnich[3], M. Helm[3], T. Butto[3], S. Gerber[1]

[1]University Medical Center of Johannes-Gutenberg-University, Institute of Human Genetics, Mainz, Deutschland, [2]Leibniz Institute of Resilience Research (LIR) gGmbH, Mainz, Deutschland, [3]Johannes-Gutenberg-Universität, Institute of Pharmaceutical and Biomedical Sciences, Mainz, Deutschland

Introduction: In standard sequencing experiments, the experimental steps and data analysis are generally performed independently, and the data analysis is initiated by bioinformatics experts only after sequencing is complete. Nowadays, novel technologies such as Oxford Nanopore technologies, provides a unique opportunity to analyze data “live” or in real-time while the sample is being sequenced. However, the use of real-time analysis has yet been established in association with transcriptional assays.

Results: Here, we introduce NanopoReaTA, a real-time, on-demand, transcriptomic analysis toolbox that allows rapid access to transcriptomic sequenced data. The user will have access both to transcriptome-wide as well as gene-specific alterations, gaining real-time knowledge regarding the experimental setup such as the differences between distinct conditions or expression levels of individual genes. In addition, the implemented quality control functions will allow the user to monitor the data variability throughout the ongoing sequencing progress.

Material and methods: For real time analyses output, we incorporated commonly used R packages such as DESeq2 to perform analyses such principle component analysis (PCA) and differential gene expression (DGE). On the transcript level, we included differential transcript usage using DRIMSeq and transcript expression using DEXSeq. In addition, we incorporated novel functions such as targeted expression analysis which allows the user to visualize the expression of specific target genes while sequencing. All the visual outputs can be individually downloaded.

Conclusion: Ultimately, the user will be automatically guided through the application (e.g. via R Shiny App) where a simple “start pre-processing” button will initiate the real-time processing of MinKNOW output fastq files as they are being generated. Such toolbox will provide biological snapshots of the sequencing run, thereby accelerate decision-making regarding clinical cases or sequencing interventions.

Abstract ID: 172

P3-81

Approach to cohort-wide re-analysis of exome data in 1000 individuals with neurodevelopmental disorders

T. Bartolomaeus[1], I. Halfmeyer[1], B. Popp[2], M. Radtke[1], T. Helms[3], J. Hentschel[1], D. Popp[1], R. Abou Jamra[1]

[1]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Deutschland, [2]Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Center of Functional Genomics, Berlin, Deutschland, [3]Limbus Medical Technologies GmbH, Rostock, Deutschland

Introduction: Re-Analysis of nondiagnostic Exome Sequencing (ES) is recommended by the ACMG and has the potential to increase diagnostic yield in individuals with rare diseases. However, it is not systematically applied in daily routine due to restricted capacities of laboratories.

Methods: Here, we describe a systematic approach to re-analyse ES data of a cohort consisting of 1040 diagnostic and nondiagnostic individual with with severe, early-onset diseases, mainly with NDD. All cases were reprocessed by an updated bioinformatics pipeline. We applied a strict filter cascade to reveal the most promising SNVs of the whole cohort.

Results: For the 1040 individuals, the filter cascade revealed a list of 802 variants (0.77 variants per case) that had to be manually evaluated. This variant set resulted in seven novel diagnoses (0.8% of all nondiagnostic cases) and two secondary findings. Thirteen additional variants were identified by a scientific approach prior to this re-analysis and were also present in this variant set. This results in a total increase in the diagnostic yield of 2.3%. The filter cascade was optimised during the course of the study and finally resulted in a sensitivity of 85%. We retrospectively applied the criteria of our filter cascade to re-analysis cohorts in which a case-by-case approach was applied. Up to 80% of the variants found there could also be identified with our filter cascade.

Conclusion: This work is intended to provide a practical recommendation for other laboratories wanting to introduce a resource-efficient re-analysis strategy into their clinical routine.

Abstract ID: 173

P3-25

Blended phenotype in an infant with developmental delay, bilateral cleft lip and palate and coloboma due to *de novo* deletions at 2q24.2q24.3 and 6p24

M. Walther[1], M. Krumbiegel[1], A. Reis[1], U. Hüffmeier[1]

[1]*Institute of Human Genetics, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Deutschland*

Interstitial microdeletions at 2q24.2q24.3 are rare and cause a clinically heterogeneous phenotype due to different deletion sizes and affected genes. While frequent symptoms include developmental delay, hypotonia and joint laxity, further potential manifestations are microcephaly and hearing impairment.

We report on a 22-month-old girl with intrauterine growth retardation, dystrophy, delayed motor and speech development, hypotonia, microcephaly, coloboma and several dysmorphic features. In addition, bilateral cleft lip and palate was diagnosed and treated early. Bilateral hearing impairment was also suspected.

Using CMA, we detected a *de novo* deletion of 3.15 Mb at 2q24.2q24.3, harboring 11 protein-coding genes. Chromosomal and FISH analyses revealed a pericentric inversion of chromosome 2, suggesting a localization of the deletion at 2q24.2q24.3 within the inversion breakpoint on the derivative chromosome 2q.

Among genes affected by the deletion, *TBR1* represented a candidate for the developmental delay, as there is a known disease association in carriers of *de novo loss-of-function* variants (OMIM #606053). Moreover, it has a central role during murine cortical neurogenesis and is expressed spatiotemporally in human brain. For the 10 further genes, a disease association has not been described so far (n = 9) or was not relevant for the infant's phenotype (*IFIH1*).

All symptoms were previously described in individuals with comparable deletions at 2q24.2q24.3 apart from orofacial clefting and coloboma. In addition, a *de novo* 548 kb deletion at 6p24 affecting *TFAP2A* was detected. Pathogenic variants or, less common, interstitial deletions including *TFAP2A* as in this case are associated with a variable disease known as branchiooculofacial syndrome (BOFS). Among several other manifestations, anomalies like coloboma, cleft lip with or without cleft palate and hearing loss are typical symptoms in BOFS.

In summary, most of the symptoms present in the index could be assigned to either of the two microdeletions. Only hearing impairment has been associated with both disease entities.

Abstract ID: 174

P3-12

Whole-genome sequencing of 65 families with inherited retinal dystrophies

C. Kiel[1,2], V. Wenzel[1,2], A.Z. Häusler[1,2], H. Stöhr[1,2], G. Spital[3], I. Schönbuchner[4], H. Jägle[5], U. Kellner[6], B.H. Weber[1,2,7]

[1]Universität Regensburg, Institut für Humangenetik, Regensburg, Deutschland, [2]Bavarian Genomes Network for Rare Disorders, München, Deutschland, [3]Augenzentrum am St. Franziskus-Hospital, Münster, Deutschland, [4]Humangenetik Ostbayern, Regensburg, Deutschland, [5]Universitätsklinikum Regensburg, Klinik und Poliklinik für Augenheilkunde, Regensburg, Deutschland, [6]AugenZentrum Siegburg, MVZ Augenärztliches Diagnostik- und Therapiecentrum Siegburg GmbH, Zentrum für Seltene Netzhauterkrankungen, Siegburg, Deutschland, [7]Universitätsklinikum Regensburg, Institut für Klinische Humangenetik, Regensburg, Deutschland

Introduction: Inherited retinal dystrophies (IRDs) are phenotypically and genetically heterogenous, rare and mostly monogenic disorders. The DNA diagnostics yield of IRDs is estimated to be 60-70 %, leaving approximately 30-40 % of patients without genetically confirmed causation. Whole-genome sequencing (WGS) is preferentially used to identify deep-intronic and structural variants in known IRD-associated genes, or variants in novel genes so far not associated with IRDs. However, the true diagnostic value of WGS in IRDs is still unknown.

Methods: WGS was performed in 143 individuals from 65 families with different types of IRD as part of the Bavarian Genomes Network for Rare Disorders. Analysis of WGS data was performed using various bioinformatic tools, phenotype-specific criteria and presumed mode of inheritance. WGS data were also screened for structural variants.

Results: Causative mutations were identified in 12/65 IRD families. Ten of these families were found to carry mutations in genes known to be associated with syndromic IRDs. In addition, the analysis highlighted at least one promising disease-causing gene for another 13/65 families. In these families, the candidate variants are mostly second hit deep-intronic variants with a predicted splice effect in known IRD genes, others are structural variants in known IRD genes and some occur in novel putative IRD candidate genes, which are currently under further investigation.

Conclusion: Our data suggest that WGS is an effective tool to identify mutations in known IRD-associated genes that would have been missed by whole exome sequencing or targeted gene-panel analysis, e. g. complex structural mutations and deep-intronic variants. The assessment of pathogenicity of novel non-coding variants, however, is challenging and requires further time-consuming and personnel-intensive analyses.

Abstract ID: 175

P3-13

Mutations affecting Glycine-X-Y repeats in the *COL4A6* gene cause hearing loss

D. Owrang[1,2], A. Rad[3], S.-J. Lin[4], S.M. Kolb[5], G. Oprea[3], V. Skrahina[3], K. Huang[4], N. Waheed[6], M. Hussain[6], S. Riaz[6], P. Bahena[5], T. Haaf[5], G.K. Varshney[4], D. Schindler[5], B. Vona[1,2,5]

[1]University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Deutschland, [2]University Medical Center Göttingen, Institute for Auditory Neuroscience and InnerEarLab, Göttingen, Deutschland, [3]Arcensus GmbH, Rostock, Deutschland, [4]Oklahoma Medical Research Foundation, Genes & Human Disease Research Program, Oklahoma City, Vereinigte Staaten, [5]Julius Maximilians University Würzburg, Institute of Human Genetics, Biozentrum, Würzburg, Deutschland, [6]Pakistan Institute of Medical Sciences, Islamabad, Pakistan

Hereditary hearing loss is one of the most common conditions in humans that exhibits a tremendous degree of genetic and clinical heterogeneity. X-linked non-syndromic hearing loss is one of the rarest forms of deafness, thought to affect between 1 and 5% of patients with congenital hearing loss. Collagen type IV alpha-6, encoded by *COL4A6* (OMIM: 303631), remains a rare form of non-syndromic hearing loss with limited reports linking *COL4A6* to congenital X-linked non-syndromic deafness (DFNX6, OMIM: 300914). This study contributes two additional families with rare variants in *COL4A6* that impact glycine-X-Y repeats.

The first proband presented with congenital right-sided unilateral hearing loss that was diagnosed at newborn hearing screening. The only finding on temporal bone imaging was narrowing of the internal acoustic meatus. Molecular genetic testing identified a hemizygous missense variant (NM_001287758.1:c.4369C>T, p.(Pro1457Ser)) in *COL4A6* that was inherited from the asymptomatic mother. The second proband derives from a consanguineous union and has a neurological syndrome with hearing loss. Genome sequencing uncovered a dual molecular diagnosis that would explain the blended phenotype involving a novel hemizygous *COL4A6* synonymous variant (c.1767G>A, p.(Pro589Pro)) and a previously reported homozygous *DYM* (OMIM: 607461) splice variant (NM_001353214.3:c.1125+1G>T, p.?). Interestingly, he has an affected sibling who only has the *DYM* variant and a neurological phenotype, while the proband additionally reported hearing loss. The *COL4A6* variant was subjected to RNA analysis using an in vitro splice assay and confirmed leaky splicing that involved a mixture of normal splicing and exon skipping causing an in-frame deletion of sequence encoding Gly-X-Y repeats.

We present two *COL4A6* cases exhibiting an atypical clinical presentation or complex genetic background. A multiple molecular diagnosis is thought to be involved in 4.9% of molecular diagnoses, while a greater appreciation for genetic contributors of unilateral hearing loss is rapidly emerging. Furthermore, our work underscores the importance of testing synonymous variants for aberrant splicing effects and considering multiple genetic variants to unravel a blended phenotype. While *COL4A6* remains an extremely rare cause of hearing loss, our work adds to the limited existing body of work on this X-linked form of hearing loss.

Abstract ID: 176

P3-55

Vascular Ehlers-Danlos syndrome with co-occuring metachondromatosis in a German family

L. Wankner[1], M. Höller[1], L. Houet[2], A. Hotz[1], S. Alter[1], A. Tzschach[1], B. Gläser[1], K. Komlosi[1,2], J. Fischer[1]

[1]Institute of Human Genetics, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Deutschland, [2]Freiburg Center for Rare Diseases, Medical Center, University of Freiburg, Freiburg, Deutschland

Vascular Ehlers-Danlos syndrome (vEDS) is an autosomal dominant connective tissue disease characterized by arterial, intestinal, and/or uterine fragility. However, in minors vEDS is often identified due to a positive family history, with clinical manifestations such as distal joint hypermobility, easy bruising, thin skin, and clubfeet being the most common. Metachondromatosis (MC) is a rare autosomal dominant disorder characterized by the presence of multiple exostoses, that predominantly occur in digits and toes, and enchondromatous lesions. Both of these lesions are small, point towards the adjacent growth plate and usually show a spontaneous decrease in size or a regress. Loss-of-function mutations of the *PTPN11* gene have been linked to MC in several families. We describe a family with two different autosomal dominant diseases where every member is affected in a different pattern and manner.

Patients and Methods: Two sons of a family presented with joint problems as a result of different parental dominant diseases. The 16-year old son was born with clubfeet, showing easy bruisability and bleeding tendency of the skin, arthritis of the ankles and exostosis of the fingers. His 8-year-old brother showed delayed motor development with high cognitive functions, rapidly growing exostoses and a calcaneal bone cyst. Chromosome microarray analysis and NGS panel diagnostics were performed in the older son, while in his brother diagnosis was confirmed via segregation analysis.

Results: Using whole-exome sequencing we detected two different autosomal dominant diseases in the 16-year-old son segregating in the family: maternally inherited metachondromatosis due to the likely pathogenic variant c.921_933+11del, p.(?) in the *PTPN11*-gene and paternally inherited vascular Ehlers-Danlos syndrome due to the pathogenic *COL3A1* c.1618G>A, p.(Gly540Arg) variant. The *PTPN11* variant was confirmed in the younger son, while he did not carry the *COL3A1* variant.

Conclusions: When autosomal dominant diseases segregate on both sides of a family, the co-occurrence can cause an atypical phenotype in the offspring. To our knowledge, this is the first case in the literature describing the co-occurrence of vascular EDS and metachondromatosis. In this complex case, a detailed clinical family history remained the key stone in successful genetic counselling by explaining atypical phenotypic presentations in the offspring through co-occurring autosomal dominant diseases.

Abstract ID: 179

P6-22

NUDT2 loss-of-function is associated with a recessive neurodevelopmental disease with mental retardation, movement disorder, and peripheral neuropathy

R.A. Husain[1], X. Jiao[2], J.C. Hennings[3], J. Giesecke[4], M. Rautenberg[5], K. Bjørgo[6], A. Mir[7], M. Ilyas[7], S.M. Abbasi[7], S. Efthymiou[8], N. Dominik[8], R. Maroofian[8], H. Houlden[8], J. Rankin[9], A. Pagnamenta[10], M. Nashabat[11], W. Al-Tuwaijri[12], M. Fadhel[13], M. Umair[13], R. Taylor[14], E. Khouj[15], F. Alkuraya[15], W. Reardon[16], A. El-Hattab[11], M. Mekki[11], C. Beetz[17], P. Bauer[17], A. Putoux[18], G. Lesca[18], D. Sanlaville[18], D. Osmanovic[3], H.-J. Mentzel[19], C.A. Hübner[3], T.B. Haack[5], P. Huppke[1], M. Kiledjian[2], I. Rubio[4]

[1]Klinik für Neuropädiatrie, Universitätsklinikum Jena, Jena, Deutschland, [2]Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, Vereinigte Staaten, [3]Institut für Humangenetik, Universitätsklinikum Jena, Funktionelle Genetik, Jena, Deutschland, [4]Klinik für Anästhesiologie und Intensivmedizin, Jena, Deutschland, [5]Medizinische Genetik und Angewandte Genomik, Universität Tübingen, Tübingen, Deutschland, [6]Department of Medical Genetics, Oslo University Hospital, Oslo, Norwegen, [7]Department of Biological Sciences, International Islamic University, Islamabad, Pakistan, [8]Department of Neuromuscular Disorders, University Clinic London, London, Vereinigtes Königreich, [9]Royal Devon and Exeter NHS Foundation, Department of Clinical Genetics, Exeter, Vereinigtes Königreich, [10]Oxford NIHR Biomedical Research Centre, Wellcome Centre for Human Genetics, Oxford, Vereinigtes Königreich, [11]University Hospital Sharjah, Sharjah, Vereinigte Arabische Emirate, [12]Department of Pediatrics, Neurology Division, King Abdullah Specialist Children´s Hospital, Riyad, Vereinigte Arabische Emirate, [13]King Abdullah International Medical Research Centre, Riyadh, Vereinigte Arabische Emirate, [14]Translational and Clinical Research Institute, Newcastle University, Newcastle, Vereinigtes Königreich, [15]Department of Translational Genomics, King Faisal Specialist Hospital and Research Centre, Riyadh, Vereinigte Arabische Emirate, [16]Children´s Health Ireland, Clinical Genetics, Crumlin, Irland, [17]Centogene GmbH, Rostock, Deutschland, [18]Groupement Hospitalier Est, Hospices Civils de Lyon, Service de Génétique, Bron, Frankreich, [19]Institut für Diagnostische und Interventionelle Radiologie, Universitätsklinikum Jena, Jena, Deutschland

We report pediatric cases of a neurodevelopmental disorder characterized by mental and motor developmental delay, gait disorder, and peripheral neuropathy associated with variants in *NUDT2*. NUDT2 encodes a Nudix family RNA decapping enzyme. The full spectrum of variants in highly conserved regions includes highly disruptive truncating variants and two novel variants, missense c.174G>T and an in-frame deletion c.410_412del. We show that the two novel gene variants lead to a marked reduction in enzymatic hydrolase activity, strongly supporting that loss of function, either through reduced protein stability or loss of enzymatic activity is causative for the disease. Accordingly, to its RNA-uncapping activity, NUDT2-deficient patient fibroblasts exhibited a markedly altered transcriptome partly due to changes in overall mRNA stability. Our results confirm that pathogenic *NUDT2* variants with decreased mRNA decapping activity are associated with a novel recessive neurological disease in pediatric patients. This novel disease entity is reminiscent of previously reported neuropathies associated with the loss of related mRNA decapping enzymes DCPS and EDC3. Our findings further underscore the importance of physiological mRNA processing for neuronal development and homeostasis.

Abstract ID: 180

P3-56

Novel pathogenic variant in PUF60 detected in a male patient with Verheij syndrome

D. Kaschta[1], F. Gaaß[2], I. Nagel[1], M. Spielmann[1], H. Muhle[3], A. Caliebe[1], I. Nagel

[1]Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Deutschland, [2]Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Lübeck, Lübeck, Deutschland, [3]Department of Neuropediatrics, University Medical Center Schleswig-Holstein, Campus Kiel, Kiel, Deutschland

Introduction: *PUF60* encodes a nucleic-acid-binding protein, involved in pre-mRNA splicing and regulation of transcription through interaction with other proteins.
Heterozygous mutations in the PUF60 gene on chromosome 8q24.3 are associated with Verheij syndrome (VRJS). VRJS is characterized by growth retardation, delayed psychomotor development, dysmorphic facial features, and skeletal, mainly vertebral, abnormalities. Additional variable features may include coloboma, renal defects, and cardiac defects (summary by Verheij et al., 2009 and Dauber et al., 2013). This syndrome belongs to the craniofacial spliceosomopathies. So far 46 patients have been reported (Fennell et al., Am J Med. Genet 2022).
Methods: DNA extraction was done from the patient's blood. The DNA library was prepared using Illumina's *DNA PCR-Free Prep*-Kit. Whole genome sequencing was performed using a NovaSeq 6000 with a mean coverage of 82x. Variant calling was achieved using *DRAGEN* and the *MANTA* variant caller. Resulting variant analysis and interpretation was done using Illumina's *TruSight Software Suite.* The mutation was subsequentially validated using Sanger sequencing.
Results: We report on a 11-year-old boy with Verheij syndrome. His primary diagnosis was neurodevelopmental delay accompanied by a comparably short stature and unspecific facial dysmorphism (thin upper lip vermillion, a narrow palpebral fissure, mild hypertelorism, protuberant ears). The patient had generalized hypotonia, was diagnosed with autistic spectrum disorder, mild intellectual disability and suffered from sleep disturbance.
In trio whole genome sequencing we identified the heterozygous pathogenic variant c.636_640del p.(Gln249Hisfs*7) in the *PUF60* gene. Additional Sanger sequencing as well as a whole genome analysis of the parents showed that the variant was a de novo event. The deletion of four base pairs in the 9th of 13 exons of the *PUF60* gene likely results in nonsense mediated decay and thereby in loss of function of the corresponding *PUF60* allele. PUF60 has a pLI of 0,997 and loss of function variants in PUF60 are known to cause the Verheij-syndrome.
Conclusion: The variant c.636_640del p.(Gln249Hisfs*7) in the *PUF60* gene is most likely the cause of the Verheij-syndrome explaining the phenotype of the patient.

Abstract ID: 181

Morphological alterations of microglia in the *Magel2* KO mouse model of Prader-Willi and Schaaf-Yang syndromes

F. Althammer[1], M. Wimmer[1], T. Schubert[1], C. Schaaf[1]

[1]*Universitätsklinikum Heidelberg, Institut für Humangenetik, Heidelberg, Deutschland*

Prader-Willi (PWS) and Schaaf-Yang syndromes (SYS) are genetic disorders characterized by infantile hypotonia, neurodevelopmental delay and intellectual disabilities. In both diseases, deletions of or point mutations in the *MAGEL2* gene have been identified as an underlying cause and thus, the *Magel2* KO mouse model represents a suitable tool to study the pathophysiology of both diseases. While the contribution of neuronal dysfunction to the pathophysiology of the diseases is extensively studied, the role of glial cells such as microglia remains unclear. Microglia are the resident immune cells of the brain and display different morphological configurations depending on their functional state. Previously published studies reported increased brain age and elevated low-grade systemic inflammation in PWS patients, which could be indicative of microglial activation and systemic inflammation. However, potential changes in microglial morphology in mouse models of PWS have not yet been addressed. To fill this gap, we employed our previously established microglia morphometric profiler based on the Imaris software and analyzed microglial surface and filaments in different brain regions of wildtype and Magel2 KO mice. Given that the interaction between microglia and astrocytes promotes sustained neuroinflammation, we also assessed GFAP intensity as a measure of reactive astrocytes in the respective brain regions. We found significantly reduced surface area and cell volume for microglia in the hypothalamus, but not the central amygdala or the nucleus accumbens of *Magel2* KO mice. Intriguingly, we found no increase in the astrocytic marker GFAP, which potentially indicates low-grade systemic inflammation without hypertrophic, reactive astrocytes. We further investigated blood brain-barrier permeability, oligodendrocyte precursor cells and microglia-vessel interactions in the brains of *Magel2* KO and wildtype mice. While the precise underlying mechanisms and consequences of altered microglial morphology in *Magel2* KO mice remain unclear, we hypothesize that they may contribute to some of the previously described functional disruptions, and could potentially be a driving factor of PWS/SYS pathophysiology. This study provides unprecedented evidence for brain region-specific microglial activation in a mouse model of PWS and opens up possibilities for further studies of microglial functions in the developing and adult brain of *Magel2* KO mice.

Abstract ID: 182

P3-10

Specialised Dup15Q clinic at Heidelberg University Hospital

C. Gabriel[1], S. Syrbe[2], J.H. Döring[2], D. Balke[3], C.P. Schaaf[1], U. Kotzaeridou[3], M. Hempel[1]

[1]Universitätsklinikum Heidelberg, Institute of Human Genetics, Heidelberg, Deutschland, [2]Universitätsklinikum Heidelberg, Division of Pediatric Epileptology, Centre for Paediatrics and Adolescent Medicine, Heidelberg, Deutschland, [3]Universitätsklinikum Heidelberg, Division of Child Neurology and Inherited Metabolic Diseases, Centre for Paediatrics and Adolescent Medicine, Heidelberg, Deutschland

Chromosome 15q11.2-13.1 duplication syndrome (dup15q syndrome) is, with a frequency of 1 in 5000 (but ~1:584 in intellectual disability and 1:253-1:522 in autism spectrum disorder (ASD) cohorts), one of the more frequent chromosome anomalies among the rare diseases. Clinically, the syndrome is characterised by a global delay with variable intellectual disability, hypotonia, ASD and epilepsy.

Patients with dup15q syndrome need a multidisciplinary care team consisting of geneticists, neuropediatricians, epileptologists, and allied health professionals such as psychologists and physiotherapists. Seeking appointments for all their children’s needs can become exhausting for families, the care is disjointed across different hospitals and healthcare providers see very few children with this syndrome, never being able to build the necessary expertise to manage these complex patients.

To overcome these issues an interdisciplinary team at the University Hospital Heidelberg in collaboration with the parent association Dup15q e.V. established a one-stop specialised dup15q clinic. Interested families contact a genetic counsellor (GC) who ascertains the needs of the patient via questionnaires and phone calls and organizes a team of medical and allied health staff of the genetics and social paediatric centre (Sozialpädiatrisches Zentrum, SPZ) departments tailored specifically to the child’s personal needs. The patients come for a day-long outpatient visit, accompanied by their GC, who acts as a point-of-contact for patients and health professionals. The families receive personalized care by a team that discusses the patient interdisciplinarily, including support for parents and siblings. Recommendations to their physicians and other health professionals are made and care continues at their local treating sites.

This offers families a team of specialised professionals, who see a large number of patients with dup15q syndrome, and offers us as professionals the patient contact necessary to build a deep understanding of the needs of these patients and families. We also use the dup15q clinic to build up a German registry of dup15q patients, which parents and health professionals fill in on an annual basis, expanding the understanding of the syndrome.

To our knowledge, this specialized multidisciplinary dup15q clinic managed by the genetics department is a unique model in Germany and may serve as template of providing care for complex patients with rare genetic diseases.

Abstract ID: 183

Chromosomal imbalances define a site-specific pattern in distant metastases of colorectal cancer

M.M. Golas[1,2], B. Gunawan[3], A. Gutenberg[4], B.C. Danner[5], J.S. Gerdes[3], C. Stadelmann[6], L. Füzesi[3,7], T. Liersch[8], B. Sander[9]

[1]Human Genetics, Medical Faculty, University of Augsburg, Augsburg, Deutschland, [2]Comprehensive Cancer Center Augsburg, University Hospital Augsburg, Augsburg, Deutschland, [3]Institute of Pathology, University Medical Center Göttingen, Göttingen, Deutschland, [4]Department of Neurosurgery, Asklepios Hospital Harburg, Hamburg, Deutschland, [5]Department of Cardiac, Thoracic and Vascular Surgery, University Medical Center Göttingen, Göttingen, Deutschland, [6]Institute for Neuropathology, University Medical Center Göttingen, Göttingen, Deutschland, [7]General Pathology and Molecular Diagnostics, Medical Faculty, University of Augsburg, Augsburg, Deutschland, [8]Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Göttingen, Deutschland, [9]Institute of Pathology, Hannover Medical School, Hannover, Deutschland

Introduction: As cancer progresses, metastasis to secondary organs occurs, with the metastatic organs showing a cancer-specific pattern. This phenomenon, known as metastatic organotropism, is seen in colorectal cancer (CRC) as preferential spread of metastases to the liver and lungs, while spread to other organs such as the brain is seen in only a minority of patients with metastatic CRC. At the same time, CRC is an example of a cancer genetically characterized by a complex pattern of chromosomal imbalances. Despite the detailed clinical knowledge of metastatic organotropism in CRC, little is known about the chromosomal aberrations and the resulting genetic changes that promote preferential seeding of cancer cells to specific metastatic organs.
Methods: Here we examined chromosomal imbalances in primary tumors and liver, lung, and brain metastases of CRC, including a number of primary CRC-metastasis pairs. By comparing own cytogenetic data to a CRC cohort analyzed by next generation sequencing, genetic alterations were analyzed in a total of 1,106 lesions in two independent cohorts. The organotropism of cytogenetic aberrations was modelled by statistical methods including co-occurrence and oncogenetic tree modeling, similarity analysis and phylogenetic tree reconstruction. The affected molecular pathways were determined by pathway enrichment analysis.
Results: There were significant differences in the total number and kind of chromosomal imbalances observed in the respective metastatic organs, with brain metastases having the highest number of imbalances. Organotropic mapping showed differences in the frequency of specific chromosomal imbalances depending on metastatic site, which together defined site-specific patterns of organotropic imbalances in the CRC metastases. The majority of the organotropic imbalances were enriched in brain and liver metastases. Organotropic imbalances were shown to be linked to DNA repair, cell cycle as well as signaling and were associated with epithelial-mesenchymal transition. Evolutionary modeling indicated an early acquisition of the majority of the organotropic imbalances during tumor evolution.
Conclusion: Overall, our data link the phenomenon of metastatic organotropism to patterns of cytogenetic aberrations. The identified site-specific differences of chromosomal imbalances could support the identification of CRC patients with an increased risk of metastasis.

Abstract ID: 184

P3-57

Clinical and etiological differences between alopecia areata patients with and without immune-mediated comorbidities

Y. Gossmann[1], A. Friedrich[1], M. Herrera-Rivero[2], M.-T. Schmitz[3], N. Cesarato[1], S. Awasthi[4], N. Fricker[1], S. Ripke[4], M.M. Nöthen[1], S. Redler[5], R.C. Betz[1], F.B. Basmanav[1]

[1]Institute of Human Genetics, Medical Faculty & University Hospital Bonn, Bonn, Deutschland, [2]Department of Genetic Epidemiology, Institute of Human Genetics and Department of Psychiatry, Münster, Deutschland, [3]Informatics and Epidemiology, Institute of Medical Biometry, Bonn, Deutschland, [4]Department of Psychiatry and Psychotherapy, Charité Universitätsmedizin Berlin, Berlin, Deutschland, [5]Institute of Human Genetics, Medical Faculty & University Hospital Düsseldorf, Düsseldorf, Deutschland

Alopecia areata (AA) is a common form of hair loss with a lifetime risk of ~2%. Genome-wide association studies (GWAS) delivered 14 susceptibility loci, majority of which implicate genes involved in the immune response. This is in alignment with the clinical observations that AA patients are at increased risk for developing additional immune-mediated disorders, such as atopic dermatitis, asthma and autoimmune thyroid disease. In our own AA cohort, which is one of the largest world-wide with ~2700 individuals, half of our patients suffer only from AA (i.e. patients without comorbidities), while the remaining half suffer from at least one additional atopic and/or autoimmune disorder (i.e. patients with comorbidities). The aim of the current study was to investigate the clinical and etiological (i.e. genetic) differences between these two groups of patients.
We analyzed whether the age of onset of AA, disease severity, persistence of illness, therapy response and family history of AA differs between patients with vs. without comorbidities using binary logistic and/or linear regression analyses in a total of 2657 AA patients. Next, in a group of 2069 patients from whom genome-wide genotype data was available, we performed a GWAS of comorbidity status. Hereby, genotypes from 1138 patients with comorbidities (i.e. cases) were compared to 931 AA patients without comorbidities (i.e. controls).
We discovered significant differences in terms of age of onset, severity and duration of AA and noted that having concomitant atopy and Hashimoto thyroiditis had significant influences on all of the three clinical parameters, while persistence of AA was also influenced by the comorbidity status for vitiligo and psoriasis. We did not have any loci surpassing the genome-wide significance threshold, presumably due to the lack of statistical power. However, we had 6 suggestive loci ($p < 5E-8$; top SNP $p=8,7E-07$) which showed association to the manifestation of immune-related comorbidities in AA; implicating altogether 22 genes based on physical mapping and expression quantitative trait loci (eQTL) annotations.
Our results suggest that genetic factors play a role in the risk of AA patients to develop immune-related comorbidities and that the clinical course of AA is different between patients with vs. without comorbidities. The underlying biology for our findings and their translational relevance should be elucidated by future studies.

Abstract ID: 185

Pathogenic variants in the gene *SPTLC1* cause hyperkeratosis lenticularis perstans

S. Alter[1], S. Jägle[1], H.-H. Hsu-Rehder[1], H. Juratli[2], A.D. Zimmer[1], B. Wiedenhofer[3], D. Metze[4], S. Emmert[5], J. Fischer[1]

[1]Institut für Humangenetik, Universitätsklinikum Freiburg, Medizinische Fakultät, Albert-Ludwigs-Universität Freiburg, Freiburg, Deutschland, [2]Klinik für Dermatologie, Universitätsspital Basel, Basel, Schweiz, [3]Praxis für Dermatologie, Treia, Deutschland, [4]Klinik für Hautkrankheiten, Universitätsklinikum Münster, Münster, Deutschland, [5]Klinik und Poliklinik für Dermatologie und Venerologie, Universitätsklinikum Rostock, Rostock, Deutschland

In 1958, a new, rare skin disease was described for the first time by the German dermatologist Heinz Flegel. This skin disease is characterized by asymptomatic small, hyperkeratotic reddish-brown papules. Mostly, the lesions initially appear at the dorsal feet and the lower extremities. Typically, disease onset is in the fourth to fifth decade of life. The disease was named hyperkeratosis lenticularis perstans (HLP) or Flegel’s disease.
The etiology of HLP has not been fully understood. A genetic cause has been suspected for a long time. However, gene variants have not been associated with the development of HLP. In order to clarify the genetic cause of HLP, we retrospectively analyzed EDTA blood, skin biopsies, and formalin-fixed paraffin-embedded (FFPE) tissue from five patients by next-generation sequencing (NGS). In all patients we identified different rare, heterozygous germline variants in the *SPTLC1* gene (*). Interestingly, these loss-of-function variants were either small deletions or splice variants. Immunofluorescence staining of skin sections showed reduced SPTLC1 protein in HLP patients. Additionally, there were indications of a loss of heterozygosity (LOH) in affected lesions representing a second hit.
Based on our results, we conclude that pathogenic germline variants in the *SPTLC1* gene in combination with an LOH in lesional skin are responsible for the development of HLP. This is likely to result in a decrease of SPTLC1 protein, a key enzyme in sphingolipid biosynthesis, disrupting the sphingolipid signaling pathway. By elucidating the genetic cause of HLP, the way for new therapeutic approaches for this rare skin disease is paved.

Abstract ID: 186

P3-58

16q12.2 microdeletion as a rare cause of developmental delay

C. Barkhaus[1], Y. Stratis[1], A. Röpke[1], C. Müller-Hofstede[1], A. Bohring[1]

[1]Institut für Humangenetik, Universitätsklinikum Münster, Münster, Deutschland

We report on a 17-year-old boy with developmental delay, aggressive behavior patterns and distinctive facial features, including severe hypertelorism, broad nasal bridge, short philtrum and a small mouth with thin lips. The boy is the first child to non-consanguineous healthy parents. Pregnancy was unremarkable. Growth parameters at birth were slightly lower due to preterm delivery in the 37th week of gestation (BW = 2700 g, BL = 48 cm). Speech development was delayed, whereas motor development was age-appropriate. His sister, currently 14 years old, is not affected.

Being in the 13th week of another pregnancy, the mother asked for prenatal diagnosis regarding her son’s developmental disorder. Genetic testing of the boy involving karyotyping, molecular analysis of the FMR1 gene and multi-gene panel testing gave normal results. SNP array detected a deletion of about 2.7 Mb in the chromosomal region 16q12.2 comprising 27 genes. Segregation analysis in both parents confirmed de novo origin.

Case reports of patients with deletions of the proximal chromosome 16q are rare. Patients have been described to be affected by developmental delay and mental retardation. Moreover, autism spectrum disorder and aggressive behavior can occur. Craniofacial dysmorphism such as microcephaly, hypertelorism, epicanthus, broad nasal bridge and low-set ears are typical for affected patients.

The minimal region of overlap in patients reported with a 16q deletion and a similar phenotype spans 1.7 Mb on chromosome 16, encompassing IRX5, GNAO1 and NUDT21 genes. This suggests the pathogenic role of the above-mentioned genes, previously known to be of importance in diseases concerning the central nervous system (Apuzzo et al. 2020).

Since IRX5, GNAO1 and NUDT21 genes were also affected by the 16q12.2 deletion in our patient, we consider the 16q12.2 deletion the pathogenic variant that is causative for the boy’s symptoms. Based on that result we offered his mother prenatal diagnosis. SNP array on long term culture of chorionic villi was performed and the 16q12.2 deletion could not be found in the prenatal sample.

In summary, 16q12.2 deletions are a rare finding in children with developmental delay and can cause a broad spectrum of phenotypic appearance.

Abstract ID: 187

P3-30

KMT2B associated intellectual disability without dystonic features: an unusual phenotype for a dystonia gene

C.-C. Becker[1], K. Alt[2], M. Seidl[3], G. Miltenberger-Miltenyi[1]

[1]Genetikum München, München, Deutschland, [2]Genetikum, Neu-Ulm, Deutschland, [3]Klinikum Memmingen, Sozialpädiatrisches Zentrum, Memmingen, Deutschland

Introduction: Pathogenic variants in histone methylation modifier (HMM) genes cause different neurodevelopmental syndromes with overlapping features. KMT2B (MIM *606834) encodes histone methyltransferase, a HMM that plays an important role in gene expression and in cell cycle. Additionally, KMT2B seems to have a critical function in memory and learning since KMT2B knockout mice display impaired memory function. In 2016, disease-causing variants in KMT2B were first reported to cause early-onset dystonia. In 2020, Cif et al. published a cohort of 53 patients delineating a novel phenotype with global developmental delay without dystonic features (9/53). They hypothesized that - despite the previously known phenotypic variability, the non-dystonic group might be under-recognized and could account for a larger proportion of KMT2B-related disorders.

Patient and methods: We report on a 10-year old boy of consanguineous (1st grade cousins) parents who presented first signs of developmental delay at the age of 4 years. Main symptoms included intellectual disability and pathological EEG findings. Our patient has a younger healthy sister. His family history was unremarkable. We carried out chromosome analysis, arrayCGH, FMR1 analysis and TRIO-WES.

Results: During his visit in our genetics clinic the patient showed global developmental delay. Facial features included curly blonde hair and a broad nasal tip. He also had small hands with clinodactyly on both sides. Body weight was 34 kg (P25-50), length 143 cm (P50), head circumference 51.6 cm (P3-P10). Chromosome analysis revealed an unremarkable male chromosome set (46,XY). array-CGH showed no evidence of chromosomal microdeletions and/or microduplications. FMR1 gene did not reveal any alteration. On the contrary, whole exome sequencing (TRIO-WES) with symptom-based evaluation detected a novel, de novo variant in heterozygous form in the KMT2B gene: [c.1021C>T (p.Gln341Ter)]. We evaluated the variant as pathogenic (ACMG class 5).

Conclusion: Here we present a patient with KMT2B associated intellectual developmental disorder type 68 (MRD68; MIM #619934) but with no signs of dystonia. Pathogenic variants in this gene are usually associated with dystonia. To our knowledge, until now, only nine patients have been reported having exclusively a developmental disorder without accompanying dystonia. Thus, our case adds further evidence that the KMT2B gene associated non-dystonic phenotype might yet be under-recognized.

Abstract ID: 188

P6-23

Comprehensive genetic and clinical characterization of 200 patients with idiopathic Small Fiber Neuropathy

A. Lischka[1], K. Eggermann[1], N. van den Braak[2], C. Dumke[2], R. Broschwitz[2], G. Peschke[2], A. Maier[2], A. Quade[3], M. Häusler[3], B. Namer[4], A. Lampert[4], R. Rolke[5], J.B. Schulz[2], M.F. Dohrn[2,6], I. Kurth[1]

[1]Institute for Human Genetics and Genomic Medicine, Medical Faculty, University Hospital RWTH Aachen, Aachen, Deutschland, [2]Department of Neurology, Medical Faculty, University Hospital RWTH Aachen, Aachen, Deutschland, [3]Division of Pediatric Neurology and Social Pediatrics, Department of Pediatrics, University Hospital RWTH Aachen, Aachen, Deutschland, [4]Institute of Physiology, Medical Faculty RWTH Aachen University, Aachen, Deutschland, [5]Department of Palliative Medicine, Medical Faculty RWTH Aachen University, Aachen, Deutschland, [6]John P. Hussman Institute for Human Genomics and Department of Human Genetics, Miller School of Medicine, University of Miami, Miami, Vereinigte Staaten

Introduction: Small fiber neuropathies (SFN) lead to dysfunction and/or degeneration of thin nerve fibers and are clinically characterized by neuropathic pain of variable severity. SFN has a large impact on the quality of life of affected patients. The potential causes include immunological conditions, diabetes mellitus, vitamin B12 deficiency. Genetic forms are due to gain-of-function variants in genes encoding voltage-gated sodium channels. However, in the majority of patients the underlying cause remains unclear. In this registry study, we characterize the clinical phenotype of idiopathic SFN in depth and search for (novel) genetic causes.

Methods: Adult patients with idiopathic/familial SFN were broadly phenotyped by clinical examinations, skin biopsies, electroneurography, quantitative sensory testing, olfactory testing, Sudoscan, laboratory diagnostics and autonomic testing in a subgroup of patients with pathological standing test. All patients were genotyped using whole exome sequencing. Additionally, children with symptoms of SFN were included in exome sequencing, in a subset of cases as duo analyses with their affected parents.

Results: To date, exome sequencing of 169 adults and 27 children has been performed in addition to the clinical characterization. The cohort includes six families of whom at least two affected family members were analyzed, suggesting a genetic cause or predisposition. In six cases, genetic testing revealed heterozygosity for rare variants in the SFN-associated genes *SCN9A*, *SCN10A*, and *SCN11A* which were considered to be possibly pathogenic and which are currently subject of further evaluation.

Conclusion: This study presents a clinically and genetically comprehensive characterization of a large cohort of nearly 200 SFN patients. In the well-defined cohort with confirmed clinical diagnosis of SFN, after the exclusion of common non-genetic causes, exome sequencing revealed possibly pathogenic sequence variants in known SFN genes in 3% of the patients. The results suggest that the proportion of monogenetic causes of SFN is substantially lower than previously reported, reinforcing that research on hitherto unknown genetic, non-genetic and multifactorial causes for idiopathic SFN is urgently needed.

Abstract ID: 189

P1-1

Evidence of a quantum physics layer of epigenomics from modeling dissipative charge-transfer dynamics along DNA

M. Rossini[1,2], O. Ammerpohl[3], R. Siebert[3,2], J. Ankerhold[1,2]

[1]Institute for Complex Quantum Systems, Ulm University, Ulm, Deutschland, [2]IQST, Ulm University, Ulm, Deutschland, [3]Institute of Human Genetics, Ulm University Medical Center, Ulm, Deutschland

Both physiologic and disease development is strongly associated with epigenetic modifications, which link the sequence of the DNA with responses to external stimuli. Common and to most extent defining to all epigenetic mechanisms is that they are informational processes associated with DNA, which e.g. can regulate gene activity but do not alter the DNA sequence itself. Yet widely acknowledged epigenetic mechanisms rely on chemical processes, like methylation of DNA, various modifications of histones or chromatin remodeling. Nevertheless, besides chemical processes also physical principles apply to DNA. Indeed, since long it has been speculated if, and if yes to which extent, quantum mechanics is not only responsible for the building mechanisms for macromolecular structures like DNA but also for their functionality and operation.

We here investigated the hypothesis that quantum information in the form of charges can travel along and across both DNA strands under ambient (physiologic) conditions. The investigations were fostered by previous experiments showing relatively long charge mobility (up to ms) over relatively long distances (up to 2 nm) in DNA. We considered a simplified model of the DNA structure in the form of a two-strand ladder embedded in thermal environments representing solvent and/or residual molecular degrees of freedom (backbone). Focus was laid on quantum dynamical properties of single charges as well as excitons (charge-hole excitations).

For DNA-parameterized two-dimensional tight-binding lattices in silico models, we predict that even at moderate temperatures long-range tunneling may play an important role depending on the energetic profile determined by the nucleobase sequences. Especially interesting to note is that intra-strand quantum features seem to survive on relatively long time scales, leading to measurable effects on the system. Particle-hole separation and trapping are competing processes that can be triggered externally.

Our study provides theoretical evidence that quantum information transfer in DNA under ambient conditions could exist. Moreover, the model provides testable assumptions for further studies relations between charge distributions and environmental properties that may be relevant for several means of gene expression regulation like binding of transcription factors or polymerase. Thus, in essence, our study provides theoretical support for a completely novel layer of epigenetics linked to quantum physics.

Abstract ID: 190

P2-24

Integrating functional analysis into *RUNX1* germline variant classification

M. Decker[1], A. Prüne[1], A. Wittstock[1], T. Illig[2], B. Schlegelberger[1], T. Ripperger[1]

[1]Medizinische Hochschule Hannover, Institut für Humangenetik, Hannover, Deutschland, [2]Hannover Medical School, Hannover Unified Bionbank, Hannover, Deutschland

RUNX1-associated familial platelet disorder with predisposition to hematologic malignancies (RUNX1-FPD) is caused by heterozygous pathogenic germline variants *of transcription factor gene RUNX1*. Following current classification guidelines, *RUNX1* missense variants must frequently be classified as variants of uncertain significance (VUS) hindering definitive clinical conclusions. Since many *RUNX1* VUS are identified as private variants in individual families, they have not been associated with RUNX1-FPD before and no functional data is available. To support variant classification, we have established, validated and clinically applied a set of functional assays addressing heterodimerization, phosphorylation, and transactivation of RUNX1. In close collaboration with clinical colleagues seeking final variant classification for their patients, we have functionally characterized 52 variants including 10 known pathogenic and one known benign controls. Thereby, we identified transcriptional activation assays of RUNX1 target genes as suitable first-line-screening of identified *RUNX1* VUS. By integrating our functional scoring in the present ClinGen Myeloid Malignancy Variant Curation Expert Panel (MM-VCEP)-based classification scheme, 5 (12%) were classified as likely benign and 12 (29%) variants as likely pathogenic. Conclusively, functional analysis contributed to the reclassification of more than one third of the investigated RUNX1 variants. Clinical applicability was shown in the context of thrombocytopenia and myeloid malignancies as well as for incidental findings. Integrating functional analysis of *RUNX1* variants into variant classification supports clinical decision making and thereby improves patient care. Present functional assays are not applicable for C-terminal *RUNX1* variants as known pathogenic variants in this region resembled wild type activity. Additional *RUNX1* VUS are currently characterized (n=10). Future attempts focus on multiplexed assays for variant effects (MAVE), characterization of obvious functional differences of N-terminal and C-terminal variants, and analysis of synergistic effects of germline *RUNX1* variants and secondary transforming somatic variants in RUNX1-FPD.

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Abstract ID: 191

P7-13

Third generation sequencing of a skin lesion swab from a monkeypox infection

C. Hewel[1], M. Linke[1], B. Plachter[2], H. Schmidt[2], S. Schweiger-Seemann[1], S. Gerber[1]

[1]Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Institut für Humangenetik, Mainz, Deutschland,
[2]Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Institut für Virologie, Mainz, Deutschland

Genomic sequencing and surveillance continues to be invaluable for emerging and ongoing outbreak scenarios. The recent surge of monkeypox infections is no exception. Thus, we performed genomic sequencing of a local monkeypox sample on a PromethION P24. The goal was to assess strain determination, preliminary assessment of mutational pattern, viral coverage, beta-testing of adaptive sampling in host depletion mode and tracking of sample composition while sequencing, thereby evaluating ease of use, and suitability of nanopore sequencing as a method to characterise a virus in real time without lab enrichment.
Adaptive sampling to reject human host reads, and thus to passively enrich the target of interest was carried out only on 1/2 of the flow cell. The rationale was to test adaptive sampling on a PromethION type of flow cell and have a control side left.
Throughout the sequencing the metagenomic composition was queried using Centrifuge to estimate species composition. In fact, after the first 4000 reads monkeypox could be reliably detected in the sample.
A preliminary draft consensus was made. In comparison to the reference (MT903344.1) characteristic base pair substitutions can be found, which are connected to the activity of the hosts APOBEC3 enzymes and have been observed for the sequences of the current outbreak.
Nanopore sequencing has been deployed in previous outbreaks such as Zika virus or Ebola as a lightweight and fast method to rapidly characterise a sequence. Taken together with the increased throughput of a PromethION, high coverage of lowly abundant samples is feasible.

Abstract ID: 192

P8-1

Prospective evaluation of NGS based sequencing in epilepsy patients: results of 7 clinical genetic laboratories

M. Witzel[1], J. Jüngling[2], B. Schulte[2], S. Wenzel[3], S. Kleier[3], B. Eichhorn[4], C. Hennig[4], P. Lorenz[4], L. von der Heyden[5], M. Kuhn[5], M. Luedeke[5], A. Holtorf[6], S. Heinrich[6], K. Hörtnagel[6], S. Knippenberg[7], A. Teubert[7], R. Glaubitz[7], S. Biskup[2], A. Abicht[1], T. Neuhann[1]

[1]Medizinisch Genetisches Zentrum, München, Deutschland, [2]Zentrum für Humangenetik Tübingen, Tübingen, Deutschland, [3]Gemeinschaftspraxis für Humangenetik & Genetische Labore, Hamburg, Deutschland, [4]MVZ Mitteldeutscher Praxisverbund Humangenetik, Praxis am Kinderzentrum Dresden-Friedrichstadt, Dresden, Deutschland, [5]MVZ Genetikum® GmbH, Neu-Ulm, Deutschland, [6]MVZ Martinsried GmbH, Martinsried, Deutschland, [7]Amedes Genetics, Hannover, Deutschland

Background: Epilepsy is one of the most common and disabling neurological disorders. It is highly prevalent in children with neurodevelopmental delay and syndromic diseases. However, epilepsy can also be the only disease-determining symptom. The exact molecular diagnosis is essential to determine prognosis, comorbidity, and probability of recurrence and to inform therapeutic decisions.

Methods and materials: Here, we describe a prospective cohort of patients with epilepsy, evaluated in 7 diagnostic outpatient centers in Germany. Over a period of 2 months, 07/2022 through 08/2022, 317 patients with seizure related *Human Phenotype Ontology* (*HPO*) were analyzed. Evaluated data included phenotype (syndromic, non-syndromic), sequencing method and molecular results.

Results: Whole Exome Sequencing (WES) was applied in half of all patients, followed by panel testing (35%), and Trio WES (T-WES) (15%). Overall, a pathogenic variant (PV) (ACMG cl. 4 /5) was identified in 21% of patients; 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%) and patients >12y: 21% (33%). Finally, diagnostic findings in syndromic vs. non-syndromic patients revealed a significant overlap of recurring causes of monogenic epilepsies including SCN1A, CACNA1A, SETD1B, confirming the heterogeneity of the associated conditions. Median turnaround time (TAT) for a patient was three weeks (median: 22d) and did not differ significantly between panel, WES, T-WES.

Conclusions: In patients with seizures – regardless of the detailed phenotype – a monogenic cause can frequently be identified; this justifies early and broad application of genetic testing. Our data suggest that the diagnostic yield is highest in exome or trio-exome based testing, resulting in a molecular diagnosis within 3 – 4 weeks, with profound implications for therapeutic strategies and for counselling families and patients regarding prognosis and recurrence risk.

Abstract ID: 193

P4-2

Using the ends of the polygenic risk score spectrum for schizophrenia to better capture anxiety-related brain structures – a UK Biobank study

L. Sindermann[1], S. Paracchini[2], M. Nöthen[1], A. Forstner[1,3]

[1]Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Deutschland, [2]School of Medicines, University of St Andrews, St Andrews, Vereinigtes Königreich, [3]Institute of Neuroscience and Medicine (INM-1), Research Center Jülich, Jülich, Deutschland

Introduction: Mental disorders are common in the population and show overlapping genetic factors and symptomatology such as anxiety/worrying feelings. The polygenic risk score spectrum for schizophrenia (PRS_SCZ) is suggested to be linked to increased anxiety. However, its link to anxiety/worrying-related fronto-limbic brain structures is poorly understood in the general population. The aim of the present study was therefore to investigate the effect of the PRS_SCZ score on fronto-limbic brain structural alterations by particularly focusing on the ends of the PRS_SCZ score spectrum.

Methods: We focused on a subsample from the UK Biobank, including n=10699 individuals divided into the first decile (n=2885), the second decile (n=2778), the second to last decile (n=2612), and the last decile (n=2424) of the PRS_SCZ. We investigated a) regional grey matter volume (rGMV) group differences in anxiety-related, fronto-limbic regions (regions of interest: amygdala, insula, hippocampus, medial frontal cortex) correcting for age, sex, principal components 1-10, scanner site dummies 1-6, head size, head position xyz, b) its link with binary self-rated anxiety/worrying, and additionally c) the association between anxiety/worrying and the PRS_SCZ.

Results: Comparing the different PRS_SCZ groups, we detected structural differences in all fronto-limbic rGMV (F(4/10665)=225.883, p<.001, *Wilks-lambda*=.922, *partial eta²*=.078) with a decreased rGMV in the case of increased PRS_SCZ. These fronto-limbic rGMV in turn were negatively associated with anxiety/worrying (r=-.062 to r=-.035, all p<.001). Additionally, the anxiety/worrying was linked to PRS_SCZ (X^2(3)=13.515, p=.004).

Conclusion: The preliminary findings enhance our understanding of fronto-limbic alterations depending on the genetic liability for schizophrenia. Alterations in regional grey matter volumes of fronto-limbic regions may underline the link between the genetic liability for schizophrenia and anxiety. Using the ends of the genetic liability may help in future to improve personalized treatment options through better stratification of individuals. However, to clarify the strength of stratifying individuals according to their genetic liability, further replications and machine learning approaches are needed.

Abstract ID: 194

P3-15

Long-read genome sequencing and RNA sequencing resolve an intronic LINE-1 insertion in the APC gene in a so far unsolved adenomatous polyposis family

A.A. Baumann[1,2], L. Knol[3], M. Arlt[1,4,5], T. Hutschenreiter[1], A. Richter[1,2], M. Franke[1], D. William[1], K. Hackmann[1], D. Richter[3,4,5], I. Spier[6,7], S. Aretz[6,7], E. Schröck[1,2,4,5,8], H. Glimm[3,4,5], A. Jahn[1,2,4,5]

[1]Institute for Clinical Genetics, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Deutschland, [2]National Center for Tumor Diseases (NCT) Dresden, German Cancer Research Center (DKFZ), Dresden, Deutschland, [3]Department of Translational Medical Oncology, NCT Dresden and DKFZ, Dresden, Deutschland, [4]German Cancer Consortium (DKTK), Dresden, Deutschland, [5]Center for Personalized Oncology, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Deutschland, [6]Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Deutschland, [7]National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Deutschland, [8]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Deutschland

Introduction: Familial adenomatous polyposis is caused by pathogenic variants in the tumor suppressor gene Adenomatous Polyposis Coli (*APC*). The genetic cause for a family with familial adenomatous polyposis across 4 generations could not be identified by cancer gene panel sequencing (94 genes), array-CGH, exome sequencing, and investigation of specific intronic variants.
Methods: Long-read genome sequencing (PacBio), short-read genome sequencing, short-read RNA-sequencing, and further validations were performed in different tissues of multiple family members.
Results: Long-read genome sequencing (PacBio) of one family member resolved a 6 kb insertion of a LINE-1 element in intron 8 of the *APC* gene that could be detected but not deciphered with short-read genome sequencing (Illumina). The structural variant was validated on DNA level in several family members and investigation of RNA revealed aberrant splicing.
Conclusion: This case study supports the utility of long-read DNA sequencing techniques and complementary RNA approaches to tackle unsolved cases.

Abstract ID: 195

Frameshifts of intrinsically disordered regions alter nucleolar phase separation and cause genetic disease

M.A. Mensah[1,2,3], H. Niskanen[4], A.P. Magalhaes[4], S. Basu[4], M. Kircher[5,6], H.L. Sczakiel[1,2,3], A.M.V. Reiter[1], J. Elsner[1], P. Meinecke[7], S. Biskup[8], B.H.Y. Chung[9], G. Dombrowsky[10,11], C. Eckmann-Scholz[12], M.P. Hitz[10,11], A. Hoischen[13], P.-M. Holterhus[14], W. Hülsemann[15], K. Kahrizi[16], V.M. Kalscheuer[3], A. Kan[17], M. Krumbiegel[18], I. Kurth[19], J. Leubner[20], A.C. Longardt[13], J.D. Moritz[21], H. Najmabadi[16], K. Skipalova[1], L. Snijders Blok[22], A. Tzschach[23], E. Wiedersberg[24], M. Zenker[25], C. Garcia-Cabau[26], R. Buschow[27], X. Salvatella[26,28], M.L. Kraushar[4], S. Mundlos[1,2,3], A. Caliebe[6], M. Spielmann[3,6,29], D. Horn[1], D. Hnisz[4]

[1]Charité - Universitätsmedizin Berlin, Institut für Medizinische Genetik und Humangenetik, Berlin, Deutschland, [2]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, BIH Biomedical Innovation Academy, Berlin, Deutschland, [3]Max Planck Institute for Molecular Genetics, RG Development & Disease, Berlin, Deutschland, [4]Max Planck Institute for Molecular Genetics, Department of Genome Regulation, Berlin, Deutschland, [5]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Exploratory Diagnostic Sciences, Berlin, Deutschland, [6]University Hospitals Schleswig-Holstein, Institute of Human Genetics, Kiel / Lübeck, Deutschland, [7]University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Deutschland, [8]Center for Genomics and Transcriptomics (CeGaT) GmbH, Tübingen, Deutschland, [9]The University of Hong Kong, Department of Pediatrics and Adolescent Medicine, School of Clinical Medicine, LKS Faculty of Medicine, Hong Kong, Hongkong, China, [10]University Hospital Schleswig-Holstein, Department of Congenital Heart Disease and Pediatric Cardiology, Kiel, Deutschland, [11]Carl von Ossietzky University, Department of Medical Genetics, Oldenburg, Deutschland, [12]University Hospital Schleswig-Holstein, Department of Obstetrics & Gynecology, Kiel, Deutschland, [13]Radboud University Medical Center, Molecular Life Sciences, Radboud Expertise Center for Immunodeficiency and Autoinflammation and Radboud Center for Infectious Disease (RCI), Nijmegen, Niederlande, [14]University Hospital Schleswig-Holstein, Department of Pediatrics, Pediatric Endocrinology and Diabetes, Kiel, Deutschland, [15]Katholisches Kinderkrankenhaus Wilhelmstift, Handchirurgie, Hamburg, Deutschland, [16]University of Social Welfare and Rehabilitation Sciences, Genetics Research Center, Tehran, Iran, [17]Queen Mary Hospital, Department of Obstetrics and Gynaecology, Hong Kong, Hongkong, China, [18]Universitätsklinikum Erlangen, Institute of Human Genetics, Erlangen, Deutschland, [19]RWTH Aachen 52 University Hospital, Institute for Human Genetics and Genomic Medicine, Aachen, Deutschland, [20]Charité – Universitätsmedizin Berlin, Department of Pediatric Neurology, Berlin, Deutschland, [21]University Hospital Schleswig-Holstein, Department of Radiology and Neuroradiology, Pediatric Radiology, Kiel, Deutschland, [22]Radboud University Medical Center, Department of Human Genetics, Nijmegen, Niederlande, [23]University of Freiburg Medical Center, Institute of Human Genetics, Freiburg, Deutschland, [24]Helios Kliniken Schwerin, Zentrum für Kinder- und Jugendmedizin, Schwerin, Deutschland, [25]University Hospital Magdeburg, Institute of Human Genetics, Magdeburg, Deutschland, [26]The Barcelona Institute of Science 66 and Technology, Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spanien, [27]Max Planck Institute for Molecular Genetics, Microscopy Core Facility, Berlin, Deutschland, [28]ICREA, Barcelona, Spanien, [29]DZHK (German Centre for Cardiovascular Research), Lübeck, Deutschland

Background: Intrinsically disordered regions (IDRs) are drivers of protein-based phase separation. Their role in genetic disease is not fully understood. A fetus with Brachyphalangy-Polydactyly-Tibia aplasia syndrome (BPTAS) featured a *de novo* frameshift (fs) in the C-terminal IDR of HMGB1.
Objective: To elucidate the role of fs mutations in IDRs and the genetic cause of BPTAS.
Materials and methods: We identified 5 unrelated BPTAS individuals and performed genome, exome, and targeted Sanger sequencing. Droplet formation of recombinant wild-type and mutant EGFP-tagged HMGB1 was studied *in vitro*. The localization of wild-type and various mutant HMGB1 proteins expressed in U2OS cells was examined. ClinVar, Cosmic and OMIM were searched for pathogenic variants altering IDRs. Localization patterns of selected mutant variants (*CALR, DVL1, FOXC1, FOXF1, FOXL2, HMGB3, MEN1, MYOD1, PHOX2B, RAX, RUNX1, SOX2, SQSTM1*) were examined in U2OS cells.
Results: BPTAS is a variable but always severe multi-organ malformation syndrome. Each BPTAS individual carried a (*de novo*) *HMGB1* fs (c.556_559GAAG;p.(Glu186Argfs*42) or c.551_554delAGAA;p.(Lys184Argfs*44)). These fss have not been observed in the recently characterized *HMGB1*-associated neurodevelopmental syndrome clinically distinct from BPTAS. They replace the protein's conserved acidic IDR tail by a basic domain with a hydrophobic terminus. They specifically altered HMGB1's capability to form droplets and slowed its diffusion rate. Wild type HMGB1 showed a diffuse nuclear localization in living cells which contrasted with mutant HMGB1 forming discrete nuclear inclusions. These inclusions replaced the granular component of the nucleolus. Beyond HMGB1, we identified 3888 frameshifts in databases that transform a protein's C-terminal IDR tail confirming their altered nucleolar phase separation capacity in a selection of variants including Robinow syndrome-associated *DVL1* fss.
Conclusion: BPTAS is caused by *de novo* fss at the beginning of the HMGB1 IDR tail. These frameshifts alter HMGB1's phase separation capacity and mislocalize HMGB1 to the nucleolus disturbing nucleolar dynamics. We showed that such frameshifts affecting C-terminal IDRs are frequent potentially explaining the pathomechanisms of various other human genetic disorders.

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P3-79

Report of 23 new individuals with *PHIP*-associated Chung-Jansen syndrome

A. Kampmeier[1], A. Küchler[1,2], I. Parenti[1], E. Leitão[1], C. Depienne[1], J. Beygo[1], F. Kaiser[1,2], T.-C. Hsieh[3], A. Afenjar[4], S. Beck-Wödl[5], E.K Bijlsma[6], N.C Bramswig[1], M. Elbracht[7], U. Grasshoff[5], T.B Haack[5], M.K Haanpää[8,9], U. Heinrich[10], R. Abou Jamra[11], M. Koch-Hochgrebe[12], H. Koillinen[8,13], E. Lausberg[7], H. Lesmann[14], V. López-González[15], C. Macedo[16], F. Marbach[17], P. Caro[17], D. Popp[11], I. Rost[10], K. Rostasy[12], C. Ruivenkamp[6], C.P Schaaf[17], M.J. Sanchez-Soler[15], A. Schmetz[18], C. Steinborn[19], S. Weidensee[20], P. Krawitz[3], D. Wieczorek[18,21]

[1]Institut für Humangenetik, Universitätsmedizin Essen, Essen, Deutschland, [2]Essener Zentrum für Seltene Erkrankungen (EZSE), Universitätsmedizin Essen, Essen, Deutschland, [3]Institut für Genomische Statistik und Bioinformatik, Universitätsklinikum Bonn, Bonn, Deutschland, [4]Sorbonne Université, Centre de Référence Malformations et Maladies Congénitales du Cervelet et Déficiences Intellectuelles de Causes Rares, Département de Génétique et Embryologie Médicale, Paris, Frankreich, [5]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Deutschland, [6]Department of Clinical Genetics, Leiden University Medical Center, Leiden, Niederlande, [7]Institut für Humangenetik und Genommedizin, Uniklinik RWTH Aachen, Aachen, Deutschland, [8]Clinical Genetics Unit, Turku University Hospital, Turku, Finnland, [9]Department of Genomics, Turku University Hospital, Turku, Finnland, [10]Zentrum für Humangenetik und Laboratoriumsdiagnostik Dr. Klein Dr. Rost und Kollegen, Martinsried, Deutschland, [11]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Deutschland, [12]Vestische Kinder- und Jugendklinik Datteln, Abteilung für Neuropädiatrie, Datteln, Deutschland, [13]Institute of Biomedicine, University of Turku, Turku, Finnland, [14]Institut für Humangenetik, Universitätsklinikum Bonn, Bonn, Deutschland, [15]Sección Genética Médica, Servicio de Pediatría, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spanien, [16]Serviço de Genética, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar e Universitário Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal, [17]Institut für Humangenetik, Universitätsklinikum Heidelberg, Heidelberg, Deutschland, [18]Institute of Human Genetics, Medical Faculty, University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Deutschland, [19]MVZ Mitteldeutscher Praxisverbund Humangenetik, Dresden, Deutschland, [20]MVZ Mitteldeutscher Praxisverbund Humangenetik, Erfurt, Deutschland, [21]Center for Rare Diseases, Medical Faculty, University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Deutschland

In 2016 and 2018, Chung, Jansen and colleagues described a new syndrome caused by haploinsufficiency of *PHIP* (pleckstrin homology domain interacting protein, OMIM *612870) and mainly characterized by developmental delay (DD), learning difficulties/intellectual disability (ID), behavioral abnormalities, facial dysmorphism and obesity (CHUJANS, OMIM #617991). So far, *PHIP* alterations appear to be a rare cause of DD/ID. “Omics” technologies such as exome sequencing or array analyses have led to the identification of distinct types of alterations of *PHIP*, including, truncating variants, missense substitutions, splice variants and large deletions encompassing portions of the gene or the entire gene as well as adjacent genomic regions.

We collected clinical and genetic data of in total 23 individuals with *PHIP*-associated Chung-Jansen syndrome (CHUJANS) from all over Europe. Follow-up investigations (e.g. Sanger sequencing, qPCR or Fluorescence-in-situ-Hybridization) and segregation analysis showed either *de novo* occurrence or inheritance from an also (mildly) affected parent. In accordance with previously described patients, almost all individuals reported here show developmental delay (22/23), learning disability or ID (22/23), behavioral abnormalities (20/23), weight problems (13/23) and characteristic craniofacial features [i.e. large ears/earlobes, prominent eyebrows, anteverted nares and long philtrum (23/23)].

To further investigate the facial gestalt of individuals with CHUJANS, we performed facial analysis using the GestaltMatcher approach. By this, we could establish that *PHIP* patients are indistinguishable based on the type of *PHIP*alteration (e.g. missense, loss of function, splice site) but show a significant difference to the average face of healthy individuals as well as to individuals with Prader-Willi syndrome (PWS, OMIM #176270) or with a *CUL4B*-alteration (Intellectual developmental disorder, X-linked, syndromic, Cabezas type, OMIM#300354).

Our findings expand the mutational and clinical spectrum of CHUJANS. We discuss the molecular and clinical features in comparison to the published individuals and the clinical variability of the phenotype, also illustrated by variants inherited from a mildly affected parent.

This study has been accepted for publication in Frontiers in Cell and Developmental Biology and is currently in press (Kampmeier et al.).

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P2-6

Genetic and epigenetic characterization of B-cell neoplasms with *IGH*::*BCL3*-translocation

C. Drewes[1], C. Lopez[2,1], N. Okeke[1], S. Hillebrecht[1], P. Schütz[1], A. Pfaus[1], A. Mottok[1], S. Bens[1], C. Schneider[3], S. Stilgenbauer[3], E. Tausch[3], R. Siebert[1]

[1]Institute for Human Genetics, Ulm University and Ulm University Medical Center, Ulm, Deutschland, [2]Institut d'Investigaciones Biomèdiques August Pi I Sunyer (IDIBAPS), Barcelona, Spanien, [3]Division of CLL, Department of Internal Medicine III, Ulm University Medical Center, Ulm, Deutschland

The *BCL3* gene located on chromosome 19q13 encodes a transcriptional coactivator of the nuclear factor kappaB (NF-κB) family. It is recurrently deregulated in B-cell neoplasms by a juxtaposition to regulatory elements of one of the immunoglobulin loci, mostly the immunoglobulin heavy chain (*IGH*) locus on chromosome 14q32. In B-cell chronic lymphocytic leukemia (CLL), the *IGH*::*BCL3* translocation has been associated with younger age at diagnosis, more aggressive clinical course, and atypical phenotype of the tumor cells. Two different subgroups of *IGH*::*BCL3* translocation positive B-cell neoplasms have been described which differ in the number of chromosomal aberrations, *IGHV* mutation status and histopathological features [Martín-Subero et al. 2007]. Here we aimed at further molecularly characterizing *IGH*::*BCL3*-translocation positive CLL in order to investigate whether they form a distinct subtype of B-cell neoplasms.
We collected a total of 89 cases of CLL and related B-cell neoplasms with *IGH*::*BCL3*-translocation based on results of fluorescence *in situ* hybridization (FISH). For 48 samples DNA methylation and copy number aberration (CNA) analyses were performed using the 850K EPIC BeadChip array. Breakpoints at the *BCL3* and *IGH* loci were sequenced using a targeted capture strategy in nine samples. Diagnostic molecular data obtained by FISH and *IGHV* mutation analyses were integrated. Analysis of CNA in the *IGH*::*BCL3*-translocated cases showed a prominent skewing towards presence of trisomy 12 and depletion of 13q14 deletion as compared to the general CLL population, with 53% of the *IGH*::*BCL3*-translocated cases showing trisomy 12, 21.3% deletion 13q, 15% deletion 11q and 6% deletion 17p. A total of 56% of the *IGH*::*BCL3*-translocated cases showed an unmutated *IGHV* status and 21% of cases a mutated *IGHV* status, with 23 % remaining to be determined. Thus, there was an overrepresentation (total 73% of yet analysed cases) of cases with unmutated *IGHV* as compared to the general CLL population. In the DNA methylation analyses, the *IGH*::*BCL3*-translocated CLLs segregate separately from other CLLs with and without *IGH*-translocations. Targeted capture-based sequencing data showed the *BCL3*-translocations to derive from aberrant class switch recombination often involving *IGHA* segments.
Our ongoing large multi-OMICs study shows that *IGH*::*BCL3*-translocated CLL differ genetically and epigenetically from other CLL.

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P3-6

Biallelic variants in the Guanine Exchange domain expanding the spectrum of *EEF1D*-related neurodevelopmental disorders

L. Averdunk[1,2], K. Al-Thihli[3,4], H. Surowy[1], H.-J. Lüdecke[1], M. Drechsler[1], G. Yigit[5,6], L. Smorag[5], B. Al Hallak[7], Y. Li[5], J. Altmüller[8,9], T. Guthoff[10], M. Wallot[11], P. Nürnberg[12,13], B. Wollnik[5,6,14], R. Abou Jamra[15], A. Al-Maawali[3,4], D. Wieczorek[1]

[1]Institute of Human Genetics, Heinrich-Heine-University Düsseldorf, Medical Faculty, Düsseldorf, Deutschland, [2]Medical Faculty, University Hospital, Heinrich-Heine-University, Department of General Pediatrics, Neonatology and Pediatric Cardiology, Düsseldorf, Deutschland, [3]College of Medicine and Health Sciences, Sultan Qaboos University, Department of Genetics, Muscat, Oman, [4]Genetic and Developmental Medicine Clinic, Sultan Qaboos University Hospital, Muscat, Oman, [5]Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Deutschland, [6]DZHK (German Centre for Cardiovascular Research), Göttingen, Deutschland, [7]Practice for Pediatrics, Kefrenbel, Syrien, [8]Berlin Institute of Health at Charité, Universitätsmedizin Berlin, Berlin, Deutschland, [9]Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Deutschland, [10]Heinrich-Heine-University, Department of Ophthalmology Medical Faculty, Düsseldorf, Deutschland, [11]Bethanien Hospital, Department of Pediatrics, Moers, Deutschland, [12]Cologne Center for Genomics (CCG), University of Cologne, Köln, Deutschland, [13]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Köln, Deutschland, [14]University of Göttingen, Cluster of Excellence Multiscale Bioimaging, from Molecular Machines to Networks of Excitable Cells (MBExC), Göttingen, Deutschland, [15]Institute of Human Genetics, University of Leipzig, Leipzig, Deutschland

Protein translation is an essential cellular process and dysfunctional protein translation causes various neurodevelopmental disorders. The eukaryotic translation elongation factor 1A (eEF1A) delivers aminoacyl-tRNA to the ribosome, while the eEF1B complex acts as a guanine exchange factor (GEF) of GTP for GDP indirectly catalyzing the release of eEF1A from the ribosome. The gene *EEF1D* encodes the eEF1Bd subunit of the eEF1B complex.
EEF1D is alternatively spliced giving rise to one long and three short isoforms. Two different homozygous, truncating variants in *EEF1D* had been associated with severe intellectual disability and microcephaly in two families. The published variants only affect the long isoform of *EEF1D* that acts as a transcription factor of heat shock element proteins.
By exome sequencing, we identified two different homozygous variants in *EEF1D* in two families with severe developmental delay, severe microcephaly, spasticity, and failure to thrive with optic atrophy, poor feeding, and recurrent aspiration pneumonias. The *EEF1D* variants in this study are localized in the *C*-terminal GEF domain suggesting that a disturbed protein translation machinery might contribute to the neurodevelopmental phenotype. Pathogenic variants localized in both the alternatively spliced domain or in the GEF domain of *EEF1D* cause a severe neurodevelopmental disorder with microcephaly and spasticity.

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P8-2

All-in-one AAV vectors for CRISPR/SpCas9 genome editing

F. Biasella[1], A. Milenkovic[1], N. Buchhauser[1], B.H. Weber[1,2]

[1]Institute of Human Genetics, University of Regensburg, Regensburg, Deutschland, [2]Institute of Clinical Human Genetics, University Hospital, Regensburg, Deutschland

Introduction: Adeno-associated virus (AAV) vectors are important delivery vehicles for therapeutic genome editing tools but are severely constrained by their limited packaging capacity of ~ 4.8 kb. The size of the gene encoding *streptococcus pyogenes* (SpCas9) (~4.1 kb) combined with the sgRNA scaffold precludes packaging into a single AAV vector. One option to overcome this limitation is to use a dual AAV system, although simultaneous delivery of multiple vectors can limit dose and efficacy and may increase safety risks. Here, we describe an “all-in-one” AAV single vector that co-expresses SpCas9 and a single guide RNA (sgRNA) targeting exon 3 of the Bestrophin-1 (*BEST1*) gene.

Methods: To drive sgRNA expression vector constructs were generated by restriction and Gibson assembly cloning carrying a minimal CMV (100 and 159bp, respectively) or full-length RSV promoter (226 bp) for SpCas9 expression, a minimal RNA polymerase III H1 promoter (95 bp), a shortened (99 bp) and full-length U6 promoter (241 bp) in combination with a minimal poly A signal sequence (49 bp). Cleavage efficiency of sgRNA_BEST1$_{exon3}$ in all constructs was determined *in vitro* (1) by a fluorescence-based assay in HEK293T cells using an pCAG-EGxxFP vector to target reconstitution of EGFP fluorescence and (2) by analyzing CRISPR-induced double strand breaks (DSB) after virus particle production and viral transduction of HEK293T cells.

Results: Quantification of reconstituted EGFP fluorescence revealed that truncated CMV_159 bp showed similar or even higher SpCas9 activity when compared to a dual vector application, while CMV_100 bp was rather inefficient. For sgRNA activity, “all-in-one” AAV constructs using a truncated U6 promoter showed stronger EGFP expression when compared to H1 promoter constructs. Fluorescence intensity was increased by 3-fold when using the full-length U6 promoter. For the optimized “all-in-one” construct AAV-U6$_{full\text{-}lenght}$_CMV_159bp_SpCas9 high editing efficiency (~83% DSB) was confirmed after viral transduction of HEK293T cells.

Conclusion: Our study identified an “all-in-one” AAV construct with excellent genome editing properties and viral particle production. Delivery of the CRISPR/Cas9 tools in a single AAV vector is superior to splitting the required tools into two vectors and may extend the use of AAV viruses for CRISPR/Cas9 *in vivo* genome editing.

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P6-24

Role of homozygous variants in generalised pustular psoriasis and two other rare pustular diseases

M.D. Hayatu[1], A.B. Ekici[1], R. Mössner[2], V. Oji[3], S. Philipp[4], J. Prinz[5], K. Schäkel[6], W. Sondermann[7], M. Sticherling[8,9], D. Wilsmann-Theis[10], S. Uebe[1], U. Hüffmeir[1]

[1]Institute of Human Genetics, Universitätsklinikum, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Deutschland, [2]Department of Dermatology, Georg-August-University Göttingen, Göttingen, Deutschland, [3]Department of Dermatology, University of Münster, Münster, Deutschland, [4]Department of Dermatology, University of Berlin, Berlin, Deutschland, [5]Department of Dermatology and Allergology, Ludwig-Maximilian University Munich, Munich, Deutschland, [6]Department of Dermatology, University of Heidelberg, Heidelberg, Deutschland, [7]Department of Dermatology, University of Essen, Essen, Deutschland, [8]Department of Dermatology, Universitätsklinikum, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Deutschland, [9]Deutsches Zentrum Immuntherapie, Friedrich-Alexander-Universität Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Deutschland, [10]Department of Dermatology, Fachklinik Bad Bentheim, Bad Bentheim, Deutschland

Generalized pustular psoriasis (GPP) is rare, highly inflammatory, and in some cases fatal subtype of pustular psoriasis. In its most severe form, GPP is multisystemic, with flare-ups of wide-spread redness on the skin covered with sterile pustules, as well as systemic and extracutaneous symptoms. The main genetic contribution of this oligogenic disease is by bi-allelic variants in two genes, *IL36RN* and *MPO*. However, the majority of individuals with GPP do not carry a variant in these genes. Acrodermatitis continua of Hallopeau (ACH) and acute generalised exanthematous pustulosis (AGEP) are two rarer pustular diseases that are clinically and genetically similar to GPP.

As major disease-relevant genes were identified using autosomal-recessive inheritance model, we analysed single exomes of 88 individuals with GPP, ACH and AGEP for homozygous variants in new genes. Using criteria of a MAF of less than 2% and a CADD score of >15 for missense variants, we identified variants in eight autosomal and two X-linked genes in at least two individuals. Some individuals of a group of healthy controls also carried homozygous/ hemizygous variants in eight candidate genes, which we therefore excluded. For the remaining two genes, we assessed gene expression and function using different databases. While gene 1, localizing to the major histocompatibility complex I, was expressed in many different immune cells, the expression of gene 2 was mainly outside immune cells and skin tissues. Further research is ongoing to evaluate these genes as potential new candidate genes in GPP.

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P6-25

Possible association of bi-allelic variants in DHX16 with epilepsy and recurrent subdural hematoma

A. Caliebe[1], M. Spielmann[1], H. Muhle[2], F. Herzog[3], N. Larsen[4], I. Nagel[1]

[1]University Medical Center Schleswig-Holstein (UKSH), Institute of Human Genetics, Kiel, Deutschland, [2]University Medical Center Schleswig-Holstein, Department of Neuropediatrics, Kiel, Deutschland, [3]University Medical Center Schleswig-Holstein (UKSH), Department of Congenital Heart Disease and Pediatric Cardiology, Kiel, Deutschland, [4]University Medical Center Schleswig-Holstein (UKSH), Department of Radiology and Neuroradiology, Kiel, Deutschland

Introduction: Pathogenic heterozygous variants in *DHX16* are associated with neuromuscular disease and ocular or auditory anomalies with or without seizure (NMOAS, MIM 318733). To the best of our knowledge four patients with this condition have been described (Paine et al., 2019). Patients present with developmental delay, brain abnormalities (agenesis of the corpus callosum, ependymal heterotopia), seizures respectively infantile spasm, muscular hypotonia, neuropathy with joint contractures, cystic kidneys, sensorineural hearing loss, ocular abnormalities (chorioretinal lacunae, retinopathy, nystagmus, blindness), and facial dysmorphism. The gene is part of the DExD/H-box RNA helicase gene family. The pathogenic variants described so far are located in the DEXDc- and HELICc-domains.

Patient and genetic findings: We report on an 18 months old boy who was admitted to hospital at the age of eight weeks with status epilepticus, subdural haematoma, small haemorrhage of the hippocampi, and intraretinal haemorrhage. Until this episode development had been normal. At one year trepanation of the skull was performed due to recurrence of the subdural haematoma . He is affected by right-sided hemiplegia, especially imvolving the arm. Since the first event he presents with global developmental delay. When last seen at 18 months he was able to roll over and crawl. Seizures are controlled by antiepileptic medication. In repeat MRI scans supratentoriell substance loss and dilated ventricular spaces were noted. Exome sequencing was performed, revealing compound heterozygosity for two variants in *DHX16*, which both have been observed in the population (gnomAD) with a low allele count of 1 respectively 10/152,166. For both variants homozygosity has not been observed. One variant [NM_003587.5:c.718C>T p.(Arg240*)] is a stop-gain in the fifth of 20 exons inherited from the father and the other a missense variant [NM_003587.5:c.1918C>T p.(Arg640Trp)] inherited from the mother targeting the HELICc domain of DHX16 (CADD-score: 28,2).

Conclusion: We speculate that compound heterozygosity in *DHX16* for two variants rarely observed in the population could be responsible for the symptoms in the patient. As differential diagnoses shaken child syndrome or another yet undiagnosed genetic condition have to be considered.

Reference: Paine, I. et al. Paralog studies augment gene discovery: DDX and DHX genes. Am. J. Hum. Genet. 105: 302-316, 2019.

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P6-34

A homozygous variant in the *VAPA* gene leads to disturbed lipid metabolism and organellar contact sites

L. Hipp[1], F. Marbach[2], S. Ferdinandusse[3], S. Hämmerling[4], Ö. Altug-Teber[5], O. Brandau[5], F.M. Vaz[3], M. Hempel[2], D. Haas[6], M. Simons[1]

[1]Sektion Nephrogenetik, Institut für Humangenetik, Universitätsklinikum Heidelberg, Heidelberg, Deutschland, [2]Genetische Poliklinik, Institut für Humangenetik, Universitätsklinikum Heidelberg, Heidelberg, Deutschland, [3]Laboratory Genetic Metabolic Disease, Departments of Clinical Chemistry and Pediatrics, University of Amsterdam, Academic Medical Center (AMC), Amsterdam, Niederlande, [4]Zentrum für Kinder- und Jugendmedizin, Klinik für Pädiatrische Onkologie, Hämatologie und Immunologie, Pneumonologie, Universitätsklinikum Heidelberg, Heidelberg, Deutschland, [5]Synlab MVZ Humangenetik Mannheim, Mannheim, Deutschland, [6]Zentrum für Kinder- und Jugendmedizin, Klinik Allgemeine Pädiatrie, Sektion für Neuropädiatrie und Stoffwechselmedizin, Universitätsklinikum Heidelberg, Heidelberg, Deutschland

Introduction: The vesicle-associated membrane protein A (VAPA) is involved in the formation of a variety of membrane contact sites (MCS) between the endoplasmic reticulum (ER) and different subcellular organelles, including mitochondria, peroxisomes and the trans-Golgi network (TGN). While the knockout of *VAPA* is not viable in mice, we report the first patient with a homozygous variant in the *VAPA* gene, causing an early stop codon and thus repressing the expression of the VAPA protein. The patient presents with congenital retinal blindness, deafness, progressive failure to thrive and microcephaly, profound global developmental delay and oxygen-dependent intestinal lung disease due to pulmonary alveolar proteinosis.
Methods: Whole exome sequencing was performed on the patient's DNA. To investigate the pathomechanism of the *VAPA* variant, fibroblasts were obtained from a skin biopsy and analyzed by immunocytochemistry and lipidomics.
Results: Immunofluorescence staining of subcellular organelles revealed circular mitochondrial morphology compared to fibroblasts of healthy controls. This phenotype is consistent with a recently reported CRISPR/Cas9-mediated knockout of *VAPA* in RPE1 cells (M. Subr et al, BioRxiv preprint), supporting the pathogenicity of the *VAPA* variant in the patient. We further found that VAPB, another member of the *VAMP* family, accumulates at ER-mitochondria contact sites in the *VAPA* deficient fibroblasts, potentially aggravating the cellular pathology. Additionally, the structure of the TGN appeared more vesicular and dysorganized than in the control cells. Finally, lipidomic analysis of the patient’s fibroblasts revealed an accumulation of long-chained triglycerides and ether lipids, suggesting that fatty acids may not be properly transferred into peroxisomes. However, peroxisomal function itself was not impaired.
Conclusion: The role of VAPA in the formation of MCS and the lipid transfer at different subcellular MCS supports the hypothesis that an accumulative defect in the subcellular organization is driving the pathology in the patient. Investigating patient fibroblasts with a loss-of-function variant in the *VAPA* gene thus presents a unique opportunity to improve our understanding of the role of MCS in subcellular organization and lipid metabolism.

Abstract ID: 205

CUX1-related neurodevelopmental disorder: deep insights into phenotype-genotype spectrum and underlying pathology

R. Abou Jamra[1], E. Marcos-Grañeda[2], L. A Weiss[2], M. Nieto[2], Z. Tümer[3,4], K. Platzer[1], H. Oppermann[1], CUX1 Study Group

[1]Institute of Human Genetics, Leipzig, Deutschland, [2]Centro Nacional de Biotecnología, Department of Cellular and Molecular Biology, Madrid, Spanien, [3]Kennedy Center, Department of Clinical Genetics, Copenhagen, Dänemark, [4]Faculty of Health and Medical Sciences, Department of Clinical Medicine, Copenhagen, Dänemark

Introduction: Heterozygous, disease-causing *CUX1* variants are associated with global development delay or intellectual disability. This study delineates the clinical presentation in an extended cohort and infvestigates the molecular mechanism underlying the disorder in a *Cux1*$^{+/-}$ mouse model.
Methods: Through international collaboration, we assembled the phenotypic and molecular information for 33 individuals (22 unpublished cases). A *Cux1*$^{+/-}$ mouse model was used to analyze CUX1 expression in the brain and evaluate susceptibility to epilepsy.
Results: We describe 33 patients with 25 different null and four missense variants. The leading symptoms were mild to moderate delayed speech and motor development, and intellectual disability. In *Cux1*$^{+/-}$ mice, we found delayed growth, histologically normal brains, and increased susceptibility to seizures. In *Cux1*$^{+/-}$ brains, the expression of *Cux1* transcripts was half of WT animals. Expression of CUX1 proteins was also reduced, although in early postnatal animals significantly more than in adults.
Conclusion: Disease-causing *CUX1* variants result in a non-syndromic phenotype of developmental delay and intellectual disability. In some patients, this phenotype ameliorates with age, resulting in a clinical catch-up and normal IQ in adulthood. In adult *Cux1*$^{+/-}$ mice, post-transcriptional processing of CUX1 protein compensates better for the reductions of *Cux1* transcripts than in developing animals.
CUX1 Study Group includes Isis Atallah, Stefanie Beck-Woedl, Charlotte Brasch-Andersen, Christina R Fagerberg, Paolo Fontana, Zoë Frazier, Alice Gardham, Christina A Gurnett, Mona Hashim, Maria Iascone, Bertrand Isidor, Anne Marie Jelsig, Lars Kjaersgaard Hansen, Jasmine Kretzer, Ilona Krey, Alma Kuechler, Johannes R Lemke, Jonathan Levy, Fortunato Lonardo, Kari Magnussen, Robert Maiwald, Sandra Mercier, Donatella Milani, Nathan Noble, Alistair T Pagnamenta, Despoina C Pavlidou, Matthias Pergande, Simone Race, Julia Rankin, Geetanjali Rathore, Linda M Reis, Miriam S Reuter, Stephanie Riley, Martina Rinelli, Stephen W Scherer, Elena V Semina, Siddharth Srivastava, Jessica Tenney, Susanne H Vineke, Denisa Weis, Pia Zacher

Abstract ID: 206

P2-15

Malignant rhabdoid tumors in patients conceived following assisted reproduction technologies (ART) – genetic, epigenetic and clinical features

J. Kolarova*[1], K. Nemes*[2], M. Benesch*[3], P. Johann[2], M. Hasselblatt[4], C. Thomas[4], S. Bens[1], S. Glaser[1], O. Ammerpohl[1], O. Liaugaudiene[5], A. Sadeghipour[6], N. von der Weid[7], I. Schmid[8], C. Gidding[9], A. Erdreich-Epstein[10,11], C. Khurana[12], G. Ebetsberger-Dachs[13], A. Lemmer[14], Z. Khatib[15], C. Hernández Marqués[16], J. Pears[17], F. Quehenberger[18], U. Kordes[19], J.A. Biegel[11,20], R. Siebert[1], M.C. Frühwald*[2]

[1]Ulm University & Ulm University Medical Center, Institute of Human Genetics, Ulm, Deutschland, [2]University Medical Center Augsburg, Swabian Children's Cancer Center, Paediatric and Adolescent Medicine, Augsburg, Deutschland, [3]Medical University of Graz, Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Graz, Österreich, [4]University Hospital Münster, Institute of Neuropathology, Münster, Deutschland, [5]Hospital of Lithuanian University of Health Sciences, Kauno Klinikos, Kaunas, Department of Genetics and Molecular Medicine, Kaunas, Litauen, [6]Iran University of Medical Sciences, Department of Pathology, Rasoul Akram Medical Complex, Tehran, Iran, [7]University Children's Hospital Basel, Department of Pediatric Hematology and Oncology, Basel, Schweiz, [8]Dr. von Haunersches Kinderspital, Department of Pediatric Hematology and Oncology, München, Deutschland, [9]Princess Máxima Center for Pediatric Oncology, Princess Máxima Center for Pediatric Oncology, Utrecht, Niederlande, [10]Children's Hospital Los Angeles, Children's Center for Cancer and Blood Diseases and Department of Pathology, Los Angeles, Vereinigte Staaten, [11]Keck School of Medicine, University of Southern California, Norris Comprehensive Cancer Center, Los Angeles, Vereinigte Staaten, [12]Evangelisches Krankenhaus Bielefeld, Children's Center, Bielefeld, Deutschland, [13]Kepler University Hospital Linz, Department of Pediatrics, Linz, Österreich, [14]HELIOS Klinikum Erfurt, Children's Hospital, Erfurt, Deutschland, [15]Miami Children's Hospital, Department of Pediatric Hematology and Oncology, Miami, Vereinigte Staaten, [16]Hospital Infantil Universitario Niño Jesús, Department of Pediatric Oncology, Madrid, Spanien, [17]Children's Health Ireland at Crumlin, Children's Health Ireland at Crumlin, Dublin, Irland, [18]Medical University of Graz, Institute for Medical Statistics, Granz, Österreich, [19]University Medical Center Hamburg-Eppendorf, Department of Pediatric Hematology and Oncology, Hamburg, Deutschland, [20]Children's Hospital Los Angeles, Department of Pathology and Laboratory Medicine, Los Angeles, Vereinigte Staaten

Malignant rhabdoid tumors (MRTs) are embryonal tumors affecting mainly children younger than 3 years. Clinically, MRT can be categorized according to the tumor location into MRT of the central nervous system (ATRT), the kidneys (RTK) and other soft tissues (eMRT). The driving genetic event is bi-allelic inactivation of *SMARCB1* or rarely *SMARCA4*. Heterozygous pathogenic germline variations (PGV) in both genes are associated with the rhabdoid tumor predisposition syndromes 1 or 2 (RTPS1/2), respectively. Some reports have suggested that assisted reproductive technologies (ARTs) might increase the risk of childhood cancer. To examine a potential link between risk for MRT and ART we performed a multi-institutional analysis of the EU-RHAB database, complemented by MRT cases reported to EU-RHAB by collaborating partners around the world.
We compiled clinical, treatment and outcome data of 14 children with MRT following ART. To detect *SMARCB1* alterations FISH, MLPA and sequencing was performed on tumor and where possible germline samples. We determined the DNA methylation status of 10 tumor and 6 blood samples of affected patients by methylation array analysis comparing the results to corresponding controls.
A total of 8 patients were diagnosed with ATRT, 3 with extracranial, extrarenal, 1 with renal rhabdoid tumor and 2 with synchronous tumors. Metastases at diagnosis were present in 6 patients. Median age at diagnosis of patients conceived by ART was 9 months. We found a female predominance with significant (p=0.009) overrepresentation of girls (n=13) as compared to boys (n=1) when compared to the EU-RHAB cohort with MRT conceived without ART (n=213, M:F; 117:96). A PGV was detected in 5 patients. In 8 patients a gross total resection was achieved, 12 patients received in addition to surgery conventional chemotherapy. Radiotherapy was applied to 6 patients. A total of 10 patients achieved complete remission, 5 remain in continued complete remission. DNA methylation analyses of 10 tumors and 6 blood samples from patients conceived by ART showed neither evidence for altered DNA methylation subgrouping of the tumor nor for underlying germline imprinting defects, respectively.
We found no significant differences in genetic, epigenetic or clinical characteristics, apart from sex skewing, comparing MRT patients conceived after ART respectively conceived without ART. Long-term survival is achievable in cases with PGV, metastatic disease at diagnosis, or relapse.

Abstract ID: 207

P3-31

Severe psychomotor and developmental delay due to a homozygous deletion of *CTNND2*

M. Rieger[1], M. Krumbiegel[1], S. Trumpp[2], T. Rupprecht[2], C. Kraus[1], S. Braig[2], A. Reis[1], G. Vasileiou[1]

[1]Institute of Human Genetics, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Deutschland, [2]Children's Clinic, Klinikum Bayreuth GmbH, Bayreuth, Deutschland

CTNND2 encodes δ-catenin, a component of an adherens junction complex, and plays an important role in maintenance of neuronal structure and function in the cortex. Its association with neurocognitive functions is well established by *in vivo* and *in vitro* studies. To date, various heterozygous *CTNND2* aberrations, predominantly deletions, were reported in individuals with mild developmental delay, borderline to mild cognitive impairment, and behavioral disorders. No individuals with biallelic pathogenic variants were described so far.

Here we report three siblings from consanguineous parents of Syrian origin, who presented with severe neurodevelopmental disorder with profound speech and motor deficits, behavioral anomalies, microcephaly, short stature, central muscular hypotonia with hypertonia of the lower extremities, and dysmorphic facial features. Both parents were illiterate, but able to fulfill their everyday tasks. A fourth child in the family was diagnosed with mild developmental delay and borderline intellectual disability.

Copy number analysis of exome sequencing data in the three severely affected siblings revealed a homozygous microdeletion at chromosome 5p15.2 encompassing the last 19 exons of *CTNND2* and the first exon of *DAP.* The parents and the mildly affected child were heterozygous for the 5p15.2 microdeletion. *DAP* is not associated with any genetic disorder, to date. Due to the previously reported association of heterozygous *CTNND2* variants with mild developmental delay, we consider the biallelic deletion of *CTNND2* to be causative for the severe clinical manifestations of the affected siblings. The clinical phenotype of the heterozygous family members resembled that of the mildly affected heterozygous carriers in the literature. The pathogenicity of the homozygous *CTNND2* aberration is further supported by knockout mouse models, which also exhibited a severe phenotype with impairment of cognitive function as well as alterations of synaptic composition and plasticity.

Thus, we provide evidence that biallelic loss-of-function alterations in *CTNND2* result in a severe neurodevelopmental disorder compared to the mildly affected heterozygous carriers. Homozygosity is also characterized by microcephaly and short stature, not previously described in heterozygous cases. In conclusion, *CTNND2* can be considered a quantitative factor of cognitive function with functional loss of one copy resulting in mild and that of both in severe impairment.

Abstract ID: 208

P3-59

Panel versus whole exome genetic testing in a real-world cohort of patients with amyotrophic lateral sclerosis (ALS)

K.P. Lubieniecki[1], J.M. Lubieniecka[1], S. Bernsen[2], K.R. Döring[1], N. Jovancevic[1], R. Fabian[2], H.H.P. Nguyen[1], P. Weydt[2]

[1]Ruhr-Universität, Humangenetik, Bochum, Deutschland, [2]Universitätsklinikum Bonn, Klinik für Neurodegenerative Erkrankungen, Bonn, Deutschland

In total, 110 patients (41% females) with suspected diagnosis of amyotrophic lateral sclerosis (ALS) were referred for genetic testing to the Department of Human Genetics Ruhr University in Bochum between 2018 and 2021. Twenty eight percent of the patients had family history of ALS (11%) or possible other neurodegenerative disease (17%), and for 13% family history was not available. Fifty nine percent of patients had negative family history (sporadic ALS, sALS). Genetic test results have important consequences for counselling and management of ALS patients.
First, all the patients were tested for a hexanucleotide repeat GGGGCC expansion in the *C9orf72* gene, the most common known genetic cause of ALS, accounting for about 40% of fALS (familial ALS) and about 7% of sALS in the European population. Positive cases (17) were reported and excluded from further analysis. Ninety three C9orf72 negative samples underwent whole exome sequencing (WES) using Ion GeneStudio S5 Next Generation Sequencing System (Thermofisher). The resulting fastq files were aligned to the GRCh37 human reference genome. Variant calling was done using cloud-based Ion Reporter Software ver. 5.18 and AmpliSeq Exome single sample (Germline) workflow (ver 5.18). Annotation of the variants was performed using Ensemble Variant Effect Predictor (VEP) release 106. The results of WES and 76 genes virtual panel routinely used for ALS diagnostics were compared. In total 210 pathogenic/likely pathogenic (P/LP) and 973 variants with conflicting interpretation (CI) of pathogenicity were identified in WES analysis. In the analysis of gene panel, 5 P/LP and 22 CI variants were detected. WES identified additional 47 P/LP variants in genes associated with ALS symptoms that are not included in the routinely used ALS gene panel.

Abstract ID: 209

BTRR complex deficiency is a driver for genomic instability in Bloom syndrome

I.I. Gönenc[1], A. Wolff[1], A. Busley[2,3], A. Wieland[4], F. Ludewig[1,5], D.C.J. Spierings[6], A. Zibat[1], G. Salinas[1,5], M. Räschle[4], F. Foijer[6] L. Cyganek[2,3], G. Yigit[1,3], B. Wollnik[1,3,7]

[1]Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Deutschland, [2]Stem Cell Unit, Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Deutschland, [3]German Center for Cardiovascular Research (DZHK), Partner Site Göttingen, Göttingen, Deutschland, [4]Department of Molecular Genetics, Technical University of Kaiserslautern, Kaiserslautern, Deutschland, [5]NGS- Core Unit for Integrative Genomics, University Medical Center Göttingen, Göttingen, Deutschland, [6]European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, Groningen, Niederlande, [7]University of Göttingen, Cluster of Excellence Multiscale Bioimaging, from Molecular Machines to Networks of Excitable Cells (MBExC), Göttingen, Deutschland

Bloom syndrome (BS) is an autosomal recessive genomic instability-related disorder presenting with primary microcephaly, growth retardation, sunlight sensitivity, immunodeficiency, and cancer predisposition. BS is caused by biallelic loss-of-function variants in one of the four members of the BTRR complex, namely *BLM, TOP3A, RMI1*, and *RMI2*. The BTRR complex plays a role in dissolving DNA intermediates that arise during homologous recombination repair and DNA replication. Using a CRISPR/Cas9-based approach, we introduced biallelic loss-of-function mutations in *BLM*, *TOP3A,* and *RMI1* in isogenic iPS cell lines to characterize the cellular defects associated with the deficiency of each of the BTRR complex members. The pluripotency of each KO iPSC line was confirmed and genomic instability profiles were assessed on a cellular level from several aspects. Karyotype analyses and array CGH revealed normal results; however, sister chromatid exchange (SCE) rates, a prominent cytogenetic characteristic of BS, were significantly elevated in all KO iPSCs. Single-cell whole genome sequencing showed an increase in copy number alterations in BTRR-deficient cells. Next, we analyzed mitotic errors including chromatin bridges, lagging DNA, and ultrafine anaphase bridges (UFB). Interestingly, we observed increased rates of UFBs in all three KO cell lines implying that the dissolution of UFBs is dependent on a properly functioning BTRR complex. Single-cell transcriptomics revealed significant changes in specific expression profiles related to mechanisms associated with Bloom syndrome. Taken together, we successfully generated BTRR complex-deficient isogenic iPSC lines and characterized them in terms of genomic instability and transcriptome profiles on a single-cell level, providing mechanistic insights into genome instability as cause of Bloom syndrome.

Abstract ID: 211

Enhancer hijacking at the *ARHGAP36* locus is associated with connective tissue to bone transformation

U. Melo[1], J. Jatzlau[2], S. Hartmann[1], P. Knaus[2], E. Giorgio[3], M. Spielmann[1]

[1]Max Planck Institute for Molecular Genetics, Berlin, Deutschland, [2]Freie Universität Berlin, Berlin, Deutschland, [3]University of Pavia, Pavia, Italien

Heterotopic ossification (HO) is a disorder caused by abnormal mineralization of soft tissues in which signaling pathways such as BMP, TGFβ and WNT are known key players in driving ectopic bone formation. Identifying novel genes and pathways related to the mineralization process are important steps for future gene therapy in bone disorders. In this study, we detected an inter-chromosomal insertional duplication disrupting a topologically associating domain (TAD) and causing an ultra-rare progressive form of HO. This structural variant led to enhancer hijacking and misexpression of *ARHGAP36* in fibroblasts, validated here by orthogonal *in vitro* studies. In addition, *ARHGAP36* overexpression inhibited TGFβ, and activated hedgehog signaling and genes/proteins related to extra cellular matrix production. Our work on the genetic cause of this HO disease has revealed that *ARHGAP36* plays a role in bone formation and metabolism, outlining first details of this gene contributing to bone-formation and -disease.

Abstract ID: 212

P1-3

Comparative DNA methylation profiling of human and murine ALK+ LBCL with aberrant ALK expression reveals similarities to plasma cell neoplasms

S. Glaser[1], C. Schnaudt[1], R. Wagener[1,2], S. Harkins[3], A. Louissaint[3], W. Klapper[4], C. Laurent[5], L. Xerri[6], M. Ren[7], S. Sander[7], W. Wößmann[8], C. Voena[9], R. Chiarle[9,10], R. Siebert[1,2]

[1]Institute of Human Genetics, Ulm, Deutschland, [2]Institute of Human Genetics, Kiel, Deutschland, [3]Department of Pathology, Boston, Vereinigte Staaten, [4]Department of Pathology, Hematopathology Section and Lymph Node Registry, Kiel, Deutschland, [5]Institut Universitaire du Cancer, Département de Pathologie, Toulouse, Frankreich, [6]Institut Paoli-Calmettes, Pathology and Tumor Immunology, Marseille, Frankreich, [7]Adaptive Immunity and Lymphoma Group, Heidelberg, Deutschland, [8]Department of Pediatric Hematology and Oncology, Hamburg, Deutschland, [9]Department of Molecular Biotechnology and Health Sciences, Torino, Italien, [10]Boston Children's Hospital, Department of Pathology, Boston, Vereinigte Staaten

Aberrant expression of anaplastic lymphoma kinase (ALK) due to structural chromosomal alterations affecting the *ALK* gene is associated with various malignancies. *ALK* rearrangement in the human B-cell lineage is associated with ALK-positive large B-cell lymphoma (ALK+ LBCL) whereas aberrant ALK expression in the murine B-cell lineage leads to plasma cell neoplasms. In order to better understand the consequences of aberrant ALK expression in the B-cell lineage, we here performed DNA methylation profiling of human and murine ALK+ B-cell neoplasms.

DNA methylation profiles of 6 ALK+ LBCLs and 3 plasmacytomas from the NPM-ALK transgenic mouse model (Chiarle et al., 2003) were generated using the Infinium® MethylationEPIC BeadChip or Infinium® Mouse Methylation BeadChip (Illumina Inc., San Diego, CA, USA). For comparison, we mined array-based DNA methylation data from multiple myeloma (MM), diffuse large B-cell lymphomas (DLBCL), ALK+ and ALK- anaplastic large cell lymphomas (ALCL) as well as benign B- and T-cell subpopulations. Differentially methylated loci for human and mouse samples were identified using the OMICS Explorer 3.6 (Qlucore; Lund, Sweden). For further downstream analysis, gene ontology enrichment analysis and dimensional reduction using an UMAP were applied.

UMAP and phylogenetic analyses revealed high similarities of the DNA methylation profiles of ALK+ LBCL with MM. Furthermore, K-means clustering showed a separation of MM and ALK+ LBCL from DLBCL and ALK+ and ALK- ALCL based on two CpGs modules, which are characterized by a hypermethylation of poised promoter regions in module 1 for DLBCL and a hypomethylation of a heterochromatin in module 2 for MM and ALK+ LBCL. These two modules refer to DNA methylation patterns representing an earlier (module 1) and late (module 2) stage in B-cell development.

Comparing DNA methylation profiles of ALK+ LBCL in human and plasmacytomas from the NPM-ALK mouse model, a lower median DNA methylation of both tumors compared to their benign control groups could be identified. Finally, tumors from both species share a hypermethylated signature including genes involved in B-cell activation and immune response-regulating cell surface receptor signaling pathway.

In summary, DNA methylation profiling of human ALK+ LBCL grouped the tumors with plasma cell neoplasms. Moreover, comparative DNA methylation analyses identified common gene modules in murine and human ALK+ B-cell neoplasms affected by epigenetic changes.

Abstract ID: 214

P2-1

Integrated mRNA and miRNA expression profiling on archived tissue of high-grade B-cell lymphomas with 11q aberrations

G. Di Stefano[1], E. Chteinberg[2], H. Loeffler-Wirth[3], S. Hillebrech[2], R. Wagener[2], S. Bens[2], K. Oehl-Huber[2], S. Dahlum[2], D. Abramov[4], B. Burkhardt[5], A. Louissaint Jr.[6], H. Horn[7], A. Mottok[2,8], R. Santi[1], I. Oschlies[9], W. Klapper[9], K. Schafernak[10], Y. Zhang[11], A. Rosenwald[8], H. Binder[3], G. Ott[7], L. Leoncini[12], C. Del-Val[13,2], R. Siebert[2]

[1]Pathology Section, Careggi University Hospital and Department of Health Sciences, University of Florence, Florence, Italien, [2]Institute of Human Genetics, Ulm University and Ulm University Medical Center, Ulm, Deutschland, [3]IMISE, University of Leipzig, Leipzig, Deutschland, [4]Department of Pathology, Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russische Föderation, [5]Pediatric Hematology and Oncology, University Hospital Muenster, Muenster, Deutschland, [6]Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts, Vereinigte Staaten, [7]Department of Clinical Pathology, Robert-Bosch-Krankenhaus and Dr. Margarete-Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Deutschland, [8]Department of Pathology, University of Würzburg, Würzburg, Deutschland, [9]Section Haematopathology and Lymph Node Registry, Department of Pathology, Christian-Albrechts-University Kiel, Kiel, Deutschland, [10]Department of Pathology and Laboratory Medicine, Phoenix Children's Hospital, Phoenix, AZ, Vereinigte Staaten, [11]Memorial Sloan Kettering Cancer Center, New York, NY, Vereinigte Staaten, [12]Section of Pathology, Department of Medical Biotechnologies, University of Siena, Siena, Italien, [13]Department of Computer Science, University of Granada, Granada, Spanien

Introduction: In the 5th edition of the WHO classification of tumors of the haematolymphoid system, high-grade B-cell lymphoma with 11q aberrations (HGBL-11q) has been upgraded to a distinct entity of malignant B-cell lymphomas. A typical pattern of gains and losses on the long arm of chromosome 11 genetically characterizes this lymphoma. The differential diagnosis from other morphologically somewhat related high-grade B-cell lymphomas (HGBL), like diffuse large B (DLBCL) or Burkitt lymphoma (BL), thus is of increasing importance. Gene expression profiling data of HGBL-11q are still limited; therefore, the following study aimed to characterize the mRNA and miRNA expression of HGBL-11q on archived materials and compare it to other HGBL.

Methods: Transcriptional profiling was performed on formalin-fixed paraffin-embedded (FFPE) archived tissues of 27 HGBL-11q, 7 BL, 132 DLBCL, and 10 tonsils by the HTG EdgeSeq using 298 genes selected for their importance in B-cell lymphomas and 2083 miRNAs. Besides UMAP analyses, an unsupervised machine learning approach, PGMRA, was applied to analyze and integrate mRNA and miRNA expression. The identified miRNAs were used to build a miRNA-mRNA interaction network using available databases.

Results: The unsupervised UMAP based on the 298 selected genes led the HGBL-11q category to cluster separately from BLs and DLBCLs. Furthermore, by applying PGMRA analysis on the 298 genes and the 2083 miRNAs, five gene groups containing 140 mRNA and 143 miRNAs were identified and mainly associated with HGBL-11q; also, HGBL-11q was distinguished in two main subsets. Considering databases of the 143 miRNA interactions with miRNAs and proteins, a network of 16 interaction modules could be constructed. Interestingly, the whole network was connected to six miRNAs and the miRNAs expression distinguished the two main HGBL-11q subgroups: one more like BL and the other more akin to DLBCL.

Conclusion: Our investigation of FFPE-tissues classifies HGBL-11q as a distinct entity using UMAP analysis. Moreover, PGMRA shows HGBL-11q to be a less homogeneous group than BL with two main subsets: precisely, with one HGBL-11q group more like BL and the other more similar to DLBCL. Transcription profiling analysis highlights different biological characteristics in HGBL and may offer new insights to overcome these overlapping features between HGBL despite the very different genetic aberrations.

Abstract ID: 215

P7-14

Clinvar-This: ClinVar API Submission Made Easy

M. Holtgrewe[1]

[1]Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Core Unit Bioinformatics (CUBI), Berlin, Deutschland

Introduction

ClinVar is an essential resource for rare disease genetics research and clinical diagnostics. A central feature of ClinVar is allowing diagnostics and research centers to upload variants and their clinical/pathogenicity assessment. In particular, as German health insurances reimburse centers for submission to such public databases. Historically, ClinVar submission involved filling complex spreadsheets or preparing XML files for upload.

NCBI introduced a RESTful (representational state transfer) application programmable interface (API) for ClinVar submission in May 2021 and removed XML-based submission in November 2022. The two main advantages of the REST API are (1) allowing the fully automated submission to ClinVar and (2) variants submission is not delayed by NCBI ClinVar curator inspection. It is thus possible to submit variants and obtain an SCV identifier within a very few hours.

However, using the API is not trivial as its documentation leaves open some process details and by itself requires preparation of JSON (JavaScript Object Notation) documents.

Results

We present our software “clinvar-this” that provides both a Python-based command line interface (CLI) tool and a Python client API for submission to ClinVar. The CLI tool allows the easy submission of variants from a minimal TSV (tabulator separated values) format standard VCF (variant call format) files, GA4GH PhenoPackets, and ClinVar spreadsheet templates.

Methods

The Python software clinvar-this is available as Open Source software under a permissive license. See https://github.com/bihealth/clinvar-this.

Conclusion

We hope that it facilitates the submission of variants to ClinVar either by use of the command line client or the integration of the Python library code into centers’ variant analysis platforms.

Abstract ID: 216

P6-33

Modelling 16p11.2 microduplication syndrome: transcriptome analysis of human iPSC-derived neural cells highlights defects in early neural progenitor

M. Bertin[1], D. Pieh[1], M. Navandar[1], S. Kaseberg[1], M. Linke[1], S. Davydenko[2], J. Winter[1], K.-E. Mengel[3], M. Dewenter[1], S. Strand[4], K. Komlosi[5], O. Tüscher[2], S. Gerber[1], S. Schweiger[1]

[1]Institute of Human Genetics, Mainz, Deutschland, [2]Department of Psychiatry and Psychotherapy, Mainz, Deutschland, [3]Department of Pediatrics, Mainz, Deutschland, [4]Department of Internal Medicine I, Mainz, Deutschland, [5]Institute of Human Genetics, Freiburg, Deutschland

Microdeletions and microduplications of a 600kb genomic region on chromosome 16p11.2 have been associated with a spectrum of neurodevelopmental impairments including Autism Spectrum Disorder (ASD) and schizophrenia (SCZ). 16p11.2 CNV carriers manifest opposite neuroanatomical phenotypes and physiological conditions, with microdeletion carriers reporting macrocephaly and high body mass index, while microduplication being associated with microcephaly and low body mass index. Although several studies have looked at different cells from 16p11.2 microdeletion and microduplication patients, it remains unclear what molecular signatures drive the phenotype.

Here we report a German family with three members affected by a heterozygous microduplication of 554 kb at 16p11.2 presenting with a variable spectrum of neuropsychiatric, neurodevelopmental, and connective tissue symptoms. To further investigate gene dosage effects of the microduplication in the nervous system, skin biopsies were obtained from the affected daughter and unaffected mother of the family, and Human Dermal Fibroblasts (HDFs) were reprogrammed into induced Pluripotent Stem Cells (iPSCs). iPSCs were differentiated into neural progenitor cells (NPCs) and neurons and used for a bulk RNA sequencing approach.

Expression analysis of genes affected by the microduplication revealed dose-dependent gene de-regulation increasing with neural differentiation status, pointing towards premature differentiation during early development and a reduction of stem cell capacity in the patient. This fits to the microcephaly picture in the patient and other carriers of a similar microduplication. Furthermore, gene ontology analysis together with protein-protein interaction network characterization of de-regulated genes shows a substantial disruption of the ERK1/2 signalling cascade in all cells analysed from the patient (iPSCs, NPCs, and neurons), again with a crescendo towards differentiated neurons.

This data suggests impairment of the ERK1/2 pathway as the driver of molecular aberrations seen in the 16p11.2 microduplication patients and inhibitors of this pathway may be promising candidates for molecular intervention.

Abstract ID: 217

De novo variants in *KDM2A* cause a syndromic neurodevelopmental disorder

K. Platzer[1], E. Anderson[2], S. Kour[2], D. Rajan[2], K. Stals[3], D. Donnelly[4], S. O'Sullivan[4], J. Mantovani[5], P. Zacher[6], S. Drunat[7], J. Cohen[8], K. McWalter[9], P. Sanchez[10], K. Grand[10], H.-G. Kim[11], R. Belles[12], K. Lichtenbelt[13], A. Rauch[14], I. Thiffault[15], F. Tran-Mau-Them[16], A. Garde[17], R. Rabin[18], J. Pappas[18], H. Mefford[19], U. Bhan Pandey[2]

[1]Institut für Humangenetik, University of Leipzig Medical Center, Leipzig, Deutschland, [2]Childrens Hospital of Pittsburgh, Department of Pediatrics, Pittsburgh, Vereinigte Staaten, [3]Royal Devon and Exeter NHS Foundation Trust, Exeter Genomics Laboratory, Exeter, Vereinigtes Königreich, [4]Northern Ireland Regional Genetics Centre, Belfast Health and Social Care Trust, Belfast, Vereinigtes Königreich, [5]Division of Child Neurology, Washington University School of Medicine, St. Louis, Vereinigte Staaten, [6]Epilepsy Center Kleinwachau, Radeberg, Deutschland, [7]Hôpital Universitaire Robert Debré, Département de Génétique, Paris, Frankreich, [8]Kennedy Krieger Institute, Department of Neurology and Developmental Medicine, Baltimore, Vereinigte Staaten, [9]GeneDx, Gaithersburg, Vereinigte Staaten, [10]Division of Medical Genetics, Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, Vereinigte Staaten, [11]Neurological Disorders Research Center, Qatar Biomedical Research Institute, Doha, Katar, [12]Geisinger Health System, Medical Genetics, Danville, Vereinigte Staaten, [13]University Medical Center Utrecht, Department of Genetics, Utrecht, Niederlande, [14]Institute of Medical Genetics, University of Zurich, Zurich, Schweiz, [15]Genomic Medicine Center, Children's Mercy Hospital, Kansas City, Vereinigte Staaten, [16]Equipe Génétique des Anomalies du Développement, Université de Bourgogne-Franche-Comté, Dijon, Frankreich, [17]Centre de Référence Anomalies du Développement et Syndromes Malformatifs, Dijon, Frankreich, [18]NYU Grossman School of Medicine, Department of Pediatrics, New York, Vereinigte Staaten, [19]Center for Pediatric Neurological Disease Research, St. Jude Children's Research Hospital, Memphis, Vereinigte Staaten

Lysine-demethylase 2A (*KDM2A*) is responsible for demethylation of lysine residues on histone tails that constitutes a key dynamic chromatin modification impacting gene regulation. Variants in genes of the epigenetic machinery, including multiple KDMs, have been described to cause neurodevelopmental disorders. Therefore, *KDM2A* was an appealing candidate gene for further investigation after the initial identification of a *de novo* missense variant in an individual with a neurodevelopmental disorder. Through international collaboration and matchmaking, we identified heterozygous *de novo* variants in *KDM2A* in twelve unrelated individuals with an overlapping phenotype of mild to severe intellectual disability and a spectrum of epilepsy, microcephaly, autism and facial dysmorphism. *De novo* variants comprise seven missense variants and five truncating variants. We provide several lines of evidence for causation through (1) *in silico* prediction and structural modelling of variants in a gene constraint for both missense and truncating variants; (2) an aberrant subcellular distribution pattern of mutated KDM2A with patient specific variants in a mammalian cell model; (3) expression of patient specific mutant KDM2A in Drosophila melanogaster caused a more severe external eye degenerative phenotype as compared to WT KDM2A and (4) aberrant genomewide methylation profiles in blood derived patient DNA. Further testing of developmental abnormalities and motor defects in Drosophila melanogaster are ongoing. Taken together, we firmly establish heterozygous *de novo* variants in *KDM2A* as a novel cause of a syndromic neurodevelopmental disorder.

Abstract ID: 218

P3-60

Delineation of *AMMECR1* as a candidate gene for idiopathic short stature

C.T. Thiel[1], A. Schulz[1], A. Fink[1], C. Kraus[1], S. Uebe[1], A.B. Ekici[1], M. Krumbiegel[1], A. Reis[1,2]

[1]Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Deutschland, [2]Centre for Rare Diseases Erlangen (ZSEER), Erlangen, Deutschland

Short stature is a common medical concern in childhood and affects 3 % of the general population. It is thought to be controlled by a multitude of genetic variants with a broad range of effect sizes. We recently demonstrated that rare variants play significant role in the etiology of idiopathic short stature by confirming variants in known short stature associated genes in more than 16 % of these patients. In addition, we identified 63 candidate genes for short stature.
In a study group of 605 patients with idiopathic short stature we identified an affected male with an *de novo* InDel-Variant in the X-linked *AMMECR1* gene, leading to a frameshift p.(Arg152Aspfs*11). The variant was detected in the patient's transcriptome by reverse transcriptase RT-PCR. Using quantitative real-time qPCR, we observed significantly decreased expression of the *AMMECR1* gene in lymphocytes of this individual. Immunofluorescence showed that the protein was expressed at a lower level in patient-derived lymphoblastoid cells (LCLs) and resided in the nucleus. These results implicate mRNA decay and loss-of function as the underlying disease mechanism.
Disease causing variants in *AMMECR1* have been recently described in three independent reports to be associated with growth deficit, hearing impairment, elliptocytosis, and nephrocalcinosis. Even though, no additional symptoms beside short stature were observed in the case reported here, we provide supporting evidence for *AMMECR1* as a novel candidate gene for idiopathic short stature.

Abstract ID: 219

P4-10

The role of rare genetic variants enrichment in epilepsies of presumed genetic etiology

D. Le Duc[1], L. Bundalian[1], J. Lemke[1]

[1]*Institut für Humangenetik, Universitätsklinikum Leipzig, Leipzig, Deutschland*

Previous studies suggested that severe epilepsies like e.g., developmental and epileptic encephalopathies (DEE) are mainly caused by ultra-rare genetic variants. For milder phenotypes, it remained unclear to what extent rare or common variants contribute to the phenotype. To determine the importance of rare genetic variants for different epilepsy types, we analyzed a whole-exome sequencing cohort of 9,170 epilepsy-affected individuals and 8,436 controls. To reveal a potential difference across different epilepsies, the cohort was split in three groups: severe DEEs, genetic generalized epilepsy (GGE), and non-acquired focal epilepsy (NAFE). We required qualifying rare variants (QRVs) to occur in controls at a minor allele frequency ≤ 1:1,000, to be predicted as deleterious (CADD≥20), and to have odds ratio in epilepsy cases ≥2. We identified genes enriched with QRVs in DEE (n=21), NAFE (n=72), and GGE (n=32). The strongest enrichment was present in NAFE and the weakest in DEE. This suggests that rare variants may play a more important role for NAFE than DEE pathophysiology. Moreover, we found that QRV-carrying genes are involved in brain extracellular matrix for NAFE. The present study confirms an involvement of rare variants for NAFE, while in DEE and GGE, the contribution of such variants seems to be limited.

Abstract ID: 220

P6-26

Identification of a large inversion disrupting the dystrophin gene in a 24-year-old with Duchenne muscular dystrophy by optical genome mapping

L.S. Erbe[1], W.M. Gerding[1], S. Janßen[2], M. Kneifel[3], C. Köhler[2], M. Vorgerd[3], T. Lücke[2], S. Ossowski[4], H.H.P. Nguyen[1], S. Hoffjan[1]

[1]Ruhr-Universität Bochum, Abteilung für Humangenetik, Bochum, Deutschland, [2]Ruhr-Universität Bochum, Klinik für Kinder- und Jugendmedizin, Abteilung für Neuropädiatrie, Bochum, Deutschland, [3]Ruhr-Universität Bochum, Universitätsklinikum Bergmannsheil, Klinik für Neurologie, Heimer-Institut für Muskelforschung, Bochum, Deutschland, [4]Universität Tübingen, Institut für Medizinische Genetik und Angewandte Genomik, Tübingen, Deutschland

Introduction: Duchenne muscular dystrophy is one of the best characterized and most severe neuromuscular diseases. The common genetic alterations are well understood and can usually be detected by MLPA or NGS. Nevertheless, there are a few patients with a clear clinical phenotype, matching muscle biopsy and positive family history in whom no variant in the *DMD* gene can be detected. As an option for these unresolved cases, optical genome mapping (OGM) can be applied. It is suitable for finding particularly large structural variants that cannot be identified by standard diagnostic methods.
Materials/methods: We present a 24-year-old patient with a Duchenne-typical symptom complex, highly elevated CK values (up to 17,000 U/l), and a positive maternal family history. Muscle biopsy at the age of one year showed pathologically altered muscle tissue with an active dystrophic process, and dystrophin was not detectable in Western blot analysis. Routine diagnostics including MLPA and whole exome sequencing did not reveal a pathogenic variant. Therefore, OGM was performed on the patient´s DNA using the Bionano Direct label and stain kit, the Bionano Genomics Saphyr instrument and bioinformatically processed and analysed using the Bionano Solve 3.7 and Bionano Acces 1.7.1.1 software.
Results: OGM analysis revealed a hemizygous inversion of approximately 1.28 Mb on the short arm of chromosome X, partially affecting the *DMD* gene. One breakpoint is located downstream and the other one within the *DMD* gene. Therefore, a truncating variant can be assumed, which leads to a defective dystrophin synthesis and confirms the clinical diagnosis of Duchenne muscular dystrophy. Breakpoint region analysis is currently under way.
Discussion: Inversions affecting the *DMD* gene are a rare, but already described cause of Duchenne muscular dystrophy. OGM appears to be a quick and suitable method to detect such structural variants and may help to clarify more unsolved cases and lead to new pathogenetic insights.

Abstract ID: 221

P3-61

Characterization of CNVs and SNVs in Known and Candidate Genes in Patients with Idiopathic Short Stature

A. Fink[1], S. Uebe[1], A.B. Ekici[1], C. Kraus[1], M. Krumbiegel[1], A. Reis[1,2], C.T. Thiel-Hirschmann[1]

[1]Humangenetisches Institut, Erlangen, Deutschland, [2]Center for Rare Diseases Erlangen (ZSEER), Erlangen, Deutschland

Short stature is a common medical concern in childhood and affects 3% of the general population. It is thought to be controlled by a multitude of genetic variants with a broad range of effect sizes. We recently demonstrated that rare variants play a significant role in the etiology of idiopathic short stature by the identification of 63 candidate genes in 254 individuals. To replicate these results, we performed exome sequencing with SNV and CNV analysis in additional 99 affected individuals with idiopathic short stature. Exome data of 77 individuals passed quality control for CNV screening in 1,287 short stature-associated genes and the 63 candidate genes. CNVs were validated by MLPA, splice variants by RT-PCR.

Of the 12 identified CNVs in 9 individuals, 4 CNVs in 4 cases were confirmed by MLPA. While 3 CNVs included known short stature genes (*GRIA4*, *ARX – POLA1 and PHF6*), one CNV in the candidate gene *LAMA5* was identified. The overall prevalence of short stature-associated CNVs in this cohort was 5.2 % (4/77), confirming that CNVs are an important contributor to short stature.

For SNV analysis, variants were filtered by population frequency and quality, based on coverage and allele count ratios. Variants were prioritized by *CADD* score, known expression in chondrocytes, association with growth phenotypes in mouse or zebrafish, overlap with GWAS hits, functional annotations and inheritance. From initially 4,191 variants in 99 affected individuals, 23 (from 20 individuals) were considered likely disease causing based on ACMG criteria. For 2 splice variants no effect on the transcript was observed by RT-PCR in lymphocytes and were excluded for further consideration. Of the remaining 21 variants, 19 variants were identified in genes known for short stature, 2 are located in recently by our group described candidate genes (1 missense variant in *PIK3CA2* and 1 non-frameshift deletion in *SMARCA5*).

In summary, 22 affected individuals (22%) carried a *de novo* or an inherited variant (SNV or CNV), in a known short stature associated gene segregating with the phenotype in the family. None was compound heterozygous for a SNV and CNV. In addition, variants in 3 further candidate genes were identified.

Our results underline the phenotypical spectrum of known short stature genes and confirmed at least 3 candidate genes for idiopathic short stature. Overall, in 23.2% (23/99) of the patients in this study a variant associated with idiopathic short stature was identified.

Abstract ID: 222

P3-1

Single centre experience with diagnostic whole genome sequencing in 70 patients with rare diseases

D. William[1], Z. Kowalzyk[1], K. Hackmann[1], M. Bermúdez[1], J. Fischer[1], A. Jahn[1], M. Franke[1], M.C. Roa Bravo[1], L. Rossow[1], A.-S. Onuk[1], S. Hütter[1], J. Porrmann[1], J. Schallner[2,3], T. Kretschmer[4], M.A. Lee-Kirsch[2,4], R. Berner[2,4], E. Schröck[1], N. Di Donato[1]

[1]Institut für Klinische Genetik, Universitätsklinikum Carl Gustav Carus an der Technischen Universität Dresden, Dresden, Deutschland, [2]Klinik und Poliklinik für Kinder- und Jugendmedizin, Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden, Dresden, Deutschland, [3]Sozialpädiatrisches Zentrum (SPZ), Universitätsklinikum Carl Gustav Carus Dresden, Dresden, Deutschland, [4]UniversitätsCentrum für Seltene Erkrankungen, Universitätsklinikum Carl Gustav Carus Dresden, Dresden, Deutschland

Introduction: Whole genome sequencing (WGS) is on the verge of becoming the method of choice for the diagnostics of rare diseases. The streamlined library preparation increases automation in sample processing and improves cost efficiency. Additionally, WGS is free of enrichment biases, lowering the risk of missing variants due to their location at loci that are notoriously difficult to enrich in targeted NGS approaches.

Methods: A cohort of 70 patients received diagnostic WGS at the Institute for Clinical Genetics at the University Hospital Carl Gustav Carus in Dresden. Most patients (n=50) presented with a neurodevelopmental disorder, tumour risk syndrome (n=3) or other syndromic disorders with normal intelligence (n=18). 50% of the patients had previous genetic work up with exome sequencing. The Illumina DNA PCR-Free prep kit was used for library preparation and 150nt paired-end sequencing was done with a median coverage of 40 reads. Data was processed by using the Illumina Trusight Software Suite with custom annotation files.

Results: We identified pathogenic variants in 16 patients, 10 of which had previously received WES, increasing the diagnostic yield in the exome cohort by 28%.

28 patients showed variants of unknown significance (VUS) requiring segregation and/or mRNA or metabolic analysis. 10 pathogenic variants represented intronic SNVs, CNVs, and one structural variant that would not have been discovered by any other method. WGS analysis was especially expedient in patients with a focused clinical question, e.g. bilateral retinoblastoma, CdLS, Menkes syndrome, and ciliary dyskinesia with one heterozygous variant in *DNAH9*. Furthermore, WGS enabled the diagnosis of cartilage-hair-hypoplasia associated with biallelic variants in the RNA-coding *RMRP* gene that is not enriched in any of the commonly used exome kits. Importantly, WGS enabled exclusion of a recessive disorder in patients with one heterozygous pathogenic variant, in which we could confidently demonstrate the absence of any additional rare variants, including intronic regions and UTRs.

Conclusions: Our results demonstrate the clear diagnostic advantage of WGS in patients with rare diseases by increasing the diagnostic yield by nearly 1/3. We also highlight the importance of the thorough clinical evaluation prior to the genetic testing. Functional characterization of non-coding variants will further improve the diagnostic yield of WGS and consequently benefit patients and their families.

Abstract ID: 223

Effects of *TXNIP*-variants to systemic and cellular lipid and glucose handling

J.-J. Scholz[1], S. Piel[1], S. Wortmann[2,3], I. Evangelakos[1], M. Hempel[4], D. Karall[5], S. Weidemann[6], B. Reinhart-Steininger[7], K. Steinbrücker[8], A. Wredenberg[9], J. Mayr[10], T. Stulnig[7], A. Knisely[11], E.J.S. Arnér[12,13], H. Prokisch[14,15], B. Schoser[16], J. Heeren[17], C. Kubisch[1], A. Worthmann[17], C. Schlein[1]

[1]Institute for Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland, [2]University Children's Hospital, Paracelsus Medical University (PMU), Salzburg, Österreich, [3]Radboud Center for Mitochondrial Medicine, Department of Pediatrics, Amalia Children's Hospital, Nijmengen, Niederlande, [4]Institute for Human Genetics, Heidelberg University Hospital, Heidelberg, Deutschland, [5]University Children's Hospital, Medical University Innsbruck, Inssbruck, Österreich, [6]Institute of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland, [7]Clinical Division of Endocrinology & Metabolism, Medical University of Vienna, Vienna, Österreich, [8]University Children's Hospital, Paracelsus Medical University (PMU), Salzburg, Österreich, [9]Department of Medical Nutrition, Karolinska Institutet, Novum, Karolinska University Hospital, Stockholm, Schweden, [10]University Children's Hospital, Salzburger Landeskliniken and Paracelsus Medical University Salzburg, Salzburg, Österreich, [11]Department of Pathology, Medical University of Graz, Graz, Österreich, [12]Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Schweden, [13]Department of Selenoprotein Research, National Institute of Oncology, Budapest, Ungarn, [14]Institute of Neurogenomics, Helmholtz Zentrum München, Neuherberg, Deutschland, [15]Institute of Human Genetics, Technical University Munich, Munich, Deutschland, [16]Department of Neurology, Friedrich-Baur Institute, Ludwig Maximilians University, Munich, Deutschland, [17]Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland

Introduction: Disturbances in cellular energy metabolism can lead to storage diseases, for example triggered by glycogen or lipid mishandling. Most of the known storage diseases are mechanistically explained by reduced function of catabolic or anabolic enzymes, however, also uncontrolled nutrient uptake may lead to increased energy storage. Thioredoxin-binding protein (TXNIP) is a multifunctional protein involved in cellular glucose uptake via endocytosis of glucose transporters, but also in redox reactions. The aim of this study is to investigate the impact of TXNIP in human glucose and lipid metabolism and to pave the way for therapeutic interventions by investigation in preclinical models.
Methods: The clinical presentation and biosamples of neonatal and adult patients with loss-of-function and missense variants in *TXNIP* were investigated. For this purpose, gene and protein expression analysis as well as LC/MS-based lipidomic approaches were performed. Moreover, histology via H&E staining and electron microscopy of liver and muscle of an adult patient were examined. To study a potential therapy for pathogenic variants in *TXNIP*, patient fibroblasts as well as *TXNIP*-knockout mice were treated with ritonavir, a strong glucose transporter inhibitor, previously used as a HIV and COVID19 medication.
Results: Patients with variants in *TXNIP* showed a broad spectrum of clinical features. All investigated patients presented lactate acidosis, whereas hypoglycemia, combined dyslipidemia, loss of strength, cardiomyopathy and seizures were variable clinical phenomenon. Electron microscopy of muscle and liver biopsies revealed glycogen and lipid deposits, confirming increased glycogen storage and *de novo* lipogenesis as well as irregularly shaped mitochondria. Liver and muscle gene expression of an adult patient revealed a high *de novo* lipogenesis, most likely due to an uncontrolled glucose uptake and subsequent need to store the intracellular energy. Mice treated with ritonavir showed signs of rescued hypertriglyceridemia, hypoglycaemia and reduced gene expression of lipid synthesis-markers in the examined organs, whereas wild type mice did not benefit from ritonavir treatment.
Conclusions: We introduce *TXNIP* to be associated with pathogenic glycogen storage and subsequent dyslipidemia. Current investigations targeting pathogenic muscle and hepatic lipid deposition imply a possible treatment option with the glucose transporter inhibitor ritonavir.

Abstract ID: 224

P1-13

Comprehensive RNA sequencing demonstrates that endothelial differentiation of *CCM1* knockout iPSCs triggers a specific gene expression signature

R.A. Pilz[1], D. Skowronek[1], L. Mellinger[1], U. Felbor[1], M. Rath[1,2]

[1]University Medicine Greifswald, and Interfaculty Institute of Genetics and Functional Genomics, University of Greifswald, Department of Human Genetics, Greifswald, Deutschland, [2]MSH Medical School Hamburg, Department of Human Medicine and Institute for Molecular Medicine, Hamburg, Deutschland

Introduction: Cerebral cavernous malformation (CCM) is a neurovascular disease that can lead to seizures and stroke-like symptoms. The familial form is caused by heterozygous germline variants in the *CCM1*, *CCM2,* or *CCM3* gene. It is well established that a second-hit mechanism plays a pivotal role in CCM development. However, it is still poorly understood whether the second somatic inactivation is sufficient to initiate CCM development or whether additional exogenous factors are also required.

Methods: We here differentiated *CCM1* knockout induced pluripotent stem cells (*CCM1*$^{-/-}$ iPSCs) into early mesoderm progenitor cells (eMPCs) and endothelial cells (ECs). Using RNA sequencing, we studied differential gene expression between *CCM1* knockout and wild-type cells in all three developmental stages.

Results: Notably, CRISPR/Cas9-mediated loss of *CCM1* expression led to hardly any gene expression differences in iPSCs and eMPCs. However, after differentiation into ECs, we found significant deregulation of signaling pathways known to be involved in CCM pathogenesis. Deregulated genes were associated with angiogenesis, vessel formation, and cell motility. Key players of CCM pathogenesis, namely KLF2 and KLF4, were highly deregulated only in *CCM1*$^{-/-}$ ECs. Our results demonstrate that
(1) CCM1 is dispensable for EC differentiation but essential for the maintenance of endothelial quiescence and
(2) CCM1 apparently does not play a critical role in iPSCs and eMPCs.

Conclusion: Only a microenvironment of proangiogenic cytokines and growth factors triggers the establishment of a characteristic gene expression signature upon *CCM1* inactivation. Consequently, *CCM1*$^{-/-}$ precursor cells may exist that develop a cellular and molecular phenotype only when entering the endothelial lineage. In conclusion, supporting factors for the endothelial dysfunction of *CCM1*$^{-/-}$ ECs must also be addressed in CCM therapy development.

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Abstract ID: 225

P4-3

Genome-wide association study of therapy response in alopecia areata

M. Herrera-Rivero[1], Y. Gossmann[2], N. Cesarato[2], S. Awasthi[3], N. Fricker[2], S. Ripke[3], S. Redler[4], M.M. Nöthen[2], R.C. Betz[2], F.B. Basmanav Ünalan[2]

[1]Department of Genetic Epidemiology, Institute of Human Genetics and Department of Psychiatry, University of Münster, Münster, Germany, Münster, Deutschland, [2]Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Deutschland, [3]Department of Psychiatry and Psychotherapy, Charité Universitätsmedizin Berlin, Campus Mitte, Berlin, Germany, Berlin, Deutschland, [4]Institut für Humangenetik, Medizinische Fakultät und Universitätsklinikum Düsseldorf, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Deutschland

Introduction: Alopecia areata (AA) is a one of the most common causes of hair loss in the population with a lifetime risk of about 2%. AA is an autoimmune disorder whereby the target of the T-cell mediated attack is the hair follicle. Accordingly, the mainstay of treatment is immunosuppression with corticosteroids or immunomodulation. Other options include photochemotherapy or repurposed drugs such as minoxidil. The efficacies of the available therapeutic modalities range from low to moderate and there are groups of patients who do not respond to one or any of the treatments, which pose significant financial, temporal and life quality burdens to the health care system and/or patients. The aim of the current study was to identify molecular biomarkers associated with the likelihood of a positive response to therapy, which can provide a basis for patient stratification and also point towards novel targets for adjuvant therapy strategies.
Methods: We performed to date the first genome-wide association study (GWAS) of therapy response in AA by using the genome-wide genotype data of 1.500 AA patients who had received at least one type of therapy based on their self-reports. The study was performed in in an exploratory fashion whereby a series of GWASs were performed. Briefly, therapy response was defined (1) regardless of the type of therapy (general therapy response) and (2) according to the type of treatments (e.g. response to corticosteroids, to any type of immunomodulatory therapy, etc.) patients had received.
Results: None of the GWASs yielded genome-wide significant results ($p < 5E-8$), which is presumably due to the small sample size and lack of statistical power. However, many overlapping as well as unique loci across the GWASs were identified which showed suggestive association with therapy response ($p<1E-5$; top SNP: 3,53E-07). Many of these loci implicate biologically relevant genes involved in immune response (e.g. *NFKB1,* p=4,68E-06) or skin/hair intrinsic mechanisms. Gene enrichment analysis revealed that the implicated genes are significantly enriched in pathways such as the glucocorticoid receptor regulatory network, pathways in cancer and T cell receptor signaling.
Conclusion: Our results deliver novel biological insights into the therapy response in AA and hold translational value with regards to precision medicine.

Abstract ID: 226

GestaltMatcher supports delineation of novel syndromes to facilitate lumping and splitting decision-making by facial phenotype descriptors

H. Klinkhammer[1,2], H. Lesmann[1,3], S. Moosa[4], A. Hustinx[1], B. Javanmardi[1], J.-M. Li[1], M.M.C. Chui[5], C.C.Y. Mak[5], L. Averdunk[6], F Distelmaier[6], B.H.Y. Chung[5], P. Krawitz[1], T.-C. Hsieh[1]

[1]Institut für Genomische Statistik und Bioinformatik, Universitätsklinikum Bonn, Bonn, Deutschland, [2]Institut für Medizinische Biometrie, Informatik und Epidemiologie, Universitätsklinikum Bonn, Bonn, Deutschland, [3]Institut für Humangenetik, Universitätsklinikum Bonn, Bonn, Deutschland, [4]Stellenbosch University, Division of Molecular Biology and Human Genetics, Stellenbosch, Südafrika, [5]University of Hong Kong, Department of Paediatrics and Adolescent Medicine, School of Clinical Medicine, LKS Faculty of Medicine, Hong Kong, Hongkong, China, [6]Klinik für Allgemeine Pädiatrie, Neonatologie und Kinderkardiologie, Universitätsklinikum Düsseldorf, Düsseldorf, Deutschland

Introduction: Lumping and splitting is a longstanding discussion in clinical genetics. Accurately categorizing the disorders will help patients receive the appropriate treatment earlier. Because many pleiotropic genes cause more than one disorder, it is crucial that the phenotypic data can help split different phenotypes in the early stage. On the other hand, lumping different disorders into one phenotypic series can broaden the phenotypic spectrum. Since next-generation phenotyping, such as GestaltMatcher, can quantify the facial syndromic similarities among patients and supports clustering analysis of any photograph, GestaltMatcher can be utilized to lump and split phenotypes. Therefore, we present two cohorts as examples to demonstrate how GestaltMatcher lumps a disorder into a phenotypic series and splits two phenotypes caused by the same gene.
Methods: We compiled a dataset of 7,459 images from 5,995 patients diagnosed with 449 rare disorders. We first utilized GestaltMatcher to encode each photo to a facial phenotype descriptor. Each photo is positioned in the Clinical Face Phenotype Space (CFPS), where distances between images define facial syndromic similarity. We further developed a statistical method based on the pairwise distances of images by comparing the distance distribution of two given cohorts with the distribution of cohorts from the same disorder and different disorders. We presented two cohorts for the lumper and splitter analysis: Cohort-1 contained 33 patients with disease-causing mutations in *Gene-X*, and Cohort-2 consisted of five patients with disease-causing mutations in *Gene-Y*.
Results: With clustering analysis in the CFPS, we demonstrated how GestaltMatcher can split *Gene-X* into two phenotypes and lump *Gene-Y* into the phenotypic series of Rothmund-Thomson syndrome. The statistical analysis showed that the distance distribution between two groups of *Gene-X* was similar to the distribution from two different disorders. On the molecular level, the pathogenic mutations of *Gene-X* are subgrouped into two different exons differing in Nonsense-mediated decay, and the two subgroups also showed different methylation signatures. Additionally, we found statistical evidence for the similarity of Gene-Y and Rothmund-Thomson syndrome and suggested the lumping of *Gene-Y*.
Conclusion: GestaltMatcher opens the door to the exploration of unknown phenotype-genotype associations and further facilitates the lumping and splitting decision-making.

Abstract ID: 227

P5-3

Complex triplication rearrangement (DUP-TRP-DUP) in a patient with severe neurodevelopmental disorder

D. Popp[1], H. Faust[1], A. Panzer[2], R. Abou Jamra[1]

[1]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Deutschland, [2]DRK Kliniken Berlin Westend, Epilepsiezentrum und Neuropädiatrie, Berlin, Deutschland

We present a 5-year old male patient with neurodevelopmental disorder, clinically Dravet like, with febrile and afebrile generalized seizures, which culminated once in a status epilepticus, severe mental retardation and stagnation of development, microcephaly, strabismus, unsteady gait and aggressiveness. Electroencephalography showed generalized epileptic discharges. Brain imaging revealed several unspecific focal, perivascular lesions and unspecific volume reduction of white matter. The family history is unremarkable. We performed single exome sequencing which revealed a complex X-chromosomal structural variant.

Subsequent to exome sequencing as part of our routine diagnostics, the variant was investigated further by Nanopore long-read sequencing targeting the complex structural variant by adaptive sampling. Furthermore, segregation analysis of the mother was performed by qPCR of the *IQSEC2* gene that was found triplicated in the index patient.

By exome sequencing we found a 432 kb triplicated X-chromosomal region that includes the genes *GPR173*, *TSYPL2*, *KANTR*, *KDM5C*, *IQSEC2* and *SMC1A* (exon 20-25) and that was flanked by duplications of the genes *FAM156B* and *FAM156A* upstream and of exon 17-19 of the gene *SMC1A* downstream. Such structural variants – a triplication flanked by duplications - are denoted as DUP-TRP-DUP. Pathogenic variants in *KDM5C*, *IQSEC2* and *SMC1A* are associated with intellectual development disorder, seizures, microcephaly, strabismus and brain malformations. Hence, this variant explains the patient’s phenotype very well. This DUP-TRP-DUP occurred *de novo* as the mother has an unremarkable dosage of the *IQSEC2* gene.

Analysis of exact breakpoints and soft-clipped long reads revealed that this structural variant is an inverted triplication flanked by duplications (DUP-TRP/INV-DUP). Complex structural rearrangements like DUP-TRP/INV-DUP are often mediated by repetitive sequences. In line with this, the duplicated genes *FAM156B* and *FAM156A* constitute a pair of inverted repeats that might have played a role in the DUP-TRP/INV-DUP formation.

In summary, we assessed the DUP-TRP/INV-DUP described above as pathogenic for this patient. This structural variant has not been reported in the literature yet, even though other X-chromosomal DUP-TRP(INV)-DUP are known. Beyond routine exome sequencing, long-read sequencing allowed a targeted in-depth characterization of this DUP-TRP/INV-DUP by easing the breakpoint and inversion analysis.

Abstract ID: 228

P4-6

Identification of marker chromosome mosaicism in a newborn with VACTERL association

J. Ćomić[1,2], U.A. Schatz[1], G. Leszinski[1], S. Langer-Freitag[1], D. Sollacher[1], I. Sikora[1], M. Brugger[1], M.C. Braunisch[3,4], M. Shoukier[5], K.M. Riedhammer[2,1], J. Hoefele[1]

[1]Institute of Human Genetics, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, München, Deutschland, [2]Department of Nephrology, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, Munich, Germany., München, Deutschland, [3]Institute of Human Genetics, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, Munich, Germany, München, Deutschland, [4]Department of Nephrology, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, München, Deutschland, [5]Prenatal Medicine Munich, Department of Molecular Genetics, University Hospital, Munich, Germany, München, Deutschland

Introduction: The term VACTERL represents an acronym for a broad spectrum of congenital anomalies such as: vertebral anomalies (V), anorectal anomalies (A), cardiac anomalies (C), tracheoesophageal fistula or atresia (TE), renal anomalies (R), and limb anomalies (L). Only a limited number of monogenic and chromosomal causes are known. We here present the case of a male neonate with VACTERL association and a small supernumerary marker chromosome (sSMC).
Methods and results: The pregnancy occurred after intracytoplasmic sperm injection because of decreased sperm count and paternal gonosomal mosaicism. Fetal ultrasound showed multiple anomalies compatible with VACTERL association. Prenatal trio exome sequencing on amniocytes and parental blood was performed and inconspicuous. Prenatal chromosome analysis on amniocytes showed a male karyotype with a sSMC in 10% of the analyzed mitoses. Array analysis revealed unremarkable findings and no evidence of coding sequences in the identified sSMC. The parents did not carry a sSMC. Postnatal examination is planned to further examine this phenomenon.
Conclusion: Marker chromosomes are *de novo* in 70% of cases and often appear in mosaic state. Some syndromes are known to be caused by sSMCs, e.g., Pallister-Killian syndrome. So far, only single cases with VACTERL association have been described carrying a sSMC. These markers might be responsible for the development of the VACTERL spectrum. Further analysis using optical genome mapping (bionano Genomics) will provide a simple and reliable method for assessing chromosomal aberrations at a much higher resolution and could probably provide more insight into the molecular basis of the VACTERL association.
Keywords: VACTERL association, marker chromosome, exome sequencing, chromosome analysis, Bionano.

Abstract ID: 229

Heterozygous variants in LAMA5 are associated with short stature by affecting the focal adhesion pathway

A. Schulz[1], S. Uebe[1], A.B. Ekici[1], A. Reis[1,2], C. Thiel[1]

[1]Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Deutschland, [2]Centre for Rare Diseases Erlangen (ZSEER), Erlangen, Deutschland

Short stature is a common medical concern in childhood and affects 3 % of the general population. It is thought to be controlled by a multitude of genetic variants with a broad range of effect sizes. We recently demonstrated that rare variants play significant role in the etiology of idiopathic short stature by the identification of 63 candidate genes in 254 individuals. In the most promising candidate gene, *LAMA5*, we identified heterozygous *LAMA5* variants in seven patients (three missense, one nonsense, three splice variants) from a study group of 605 patients (1.2%) with idiopathic short stature.
LAMA5 encodes for the laminin alpha 5 subunit. Laminins self-assemble to form a network present in all basal laminae and are connected to the cell by receptors of the integrin family. Integrin receptors modulate signal transduction cascades that affect gene expression, for instance phosphorylation of focal adhesion kinase (FAK). This kinase contributes to a variety of cellular processes, including cell survival, proliferation, apoptosis, adhesion, migration and mechano-transduction. Therefore, LAMA5 was considered as a candidate gene for idiopathic short stature.
We observed significantly altered expression pattern of *LAMA5* in lymphycytes of four of our seven patients, whereas FAK-signalling was negatively affected in 5/7 patients as shown in ELISA. The stimulation of FAK was shown to be independent of the amount of translated LAMA5 protein, but rather dependent on the functional effect of the respective variants. We hypothesize that hypophosphorylation of FAK leads to reduced stimulation of osteoblast proliferation and increased apoptosis of osteoblasts and osteocytes. FAK signalling and/or basement membrane organization could be impaired in these individuals based on several mechanisms, including altered binding to integrin receptors, reduced cell attachment, incorrect protein folding and changes in the interaction with other proteins.
In conclusion, our results underline *LAMA5* as a novel gene for idiopathic short stature and implicate an altered focal adhesion pathway as a novel pleiotropic pathomechism in short stature.

Abstract ID: 230

P1-2

Functional analyses of *SEPHS2* variants link ferroptosis to cholesterol metabolism

J. Ridder[1], L. Abramians[1], S. Kotschi[2], I. Evangelakos[1], M. Musfeldt[1], F. Haumann[1], T. Bierhals[1], K. Rading[1], J. Tokarz[3], R. Santer[4], K. Dyar[3], C. Kubisch[1], A. Worthmann[5], A. Bartelt[2], C. Schlein[1]

[1]University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Deutschland, [2]Ludwig-Maximilians-University of Munich, Institute for Cardiovascular Prevention (IPEK), München, Deutschland, [3]Helmholtz Center Munich, Institute for Diabetes and Cancer (IDC) Research Group Metabolic Physiology, München, Deutschland, [4]University Medical-Center Hamburg-Eppendorf, Department of Pediatrics, Hamburg, Deutschland, [5]University Medical Center Hamburg-Eppendorf, Department of Biochemistry and Molecular Cell Biology, Hamburg, Deutschland

Introduction: Selenophosphate synthase 2, encoded by *SEPHS2*, plays a key role in the biosynthesis of the 21 st proteinogenic amino acid selenocysteine. Oxidation is essential for a wide range of biological processes; however, reactive oxygen species can lead to reduced cellular function. Oxidation of lipids compromises plasma membrane function and thereby the integrity of a cell and leads to a process called ferroptosis, a form of cell death. Selenoproteins, such as GPX4, are actively decreasing the membrane lipid peroxidation and thereby, protect from ferroptosis-induced cell death.
Methods: Trio exome sequencing was performed in DNA of a 4-year-old male patient of the intensive care unit of the children’s hospital was performed. Untargeted metabolomic analysis was performed in primary patient fibroblast to determine changes lipid compositions. With LC-MS/MS-based lipidomic analysis was used to quantify ferroptosis associated lipid changes and fibroblast function was analyzed by gene expression and western blot analysis.
Results: WES-sequencing revealed compound-heterozygous missense variants in *SEPHS2*. Clinical chemistry showed servere rhabdomyolosis, kidney and liver failure. Untargeted metabolomic analysis of *SEPHS2* overexpression cells unravelled a metabolomic signature of cholesterol and phospholipid involvement. Patient fibroblasts showed a drop in phosphatidylcholines and phosphatidylethanolamines upon ferroptosis activation. In addition, cell membrane components such as cholesteryl esters were also significantly reduced, whereas lipoprotein receptors were strongly induced. Cells undergoing ferroptosis were shown to take up more LDL, which we mechanistically evaluated to be protective against ferroptosis.
Conclusion: In the current project, we introduce *SEPHS2* as a candidate gene for rhabdomyolysis and combined kidney and liver failure. We showed that fibroblasts from the index patient show higher membrane lipid peroxidation associated with an increased cholesterol demand. High cholesterol levels circumvent ferroptosis leading to the presumption that cholesterol is the main lipid class targeted by ferroptotic cell death.

Abstract ID: 231

P3-62

Implementation of a high-risk Familial Breast and Ovarian Cancer prevention network in sparsely populated Western Pomerania

U. Felbor[1], N. Hosten[2], S. Wurm[3], D. Gürtler[3], L. Stark[1], R.K. Schmutzler[4], M. Rath[1,5]

[1]University Medicine Greifswald, and Interfaculty Institute of Genetics and Functional Genomics, University of Greifswald, Department of Human Genetics, Greifswald, Deutschland, [2]Institute of Diagnostic Radiology and Neuroradiology, University Medicine Greifswald, Greifswald, Deutschland, [3]Institute for Community Medicine, University Medicine Greifswald, Department of Prevention Research and Social Medicine, Greifswald, Deutschland, [4]Center for Familial Breast and Ovarian Cancer, Center for Integrated Oncology (CIO), Medical Faculty, University of Cologne and University Hospital Cologne, Cologne, Deutschland, [5]MSH Medical School Hamburg, Department of Human Medicine and Institute for Molecular Medicine, Hamburg, Deutschland

Introduction: The German Consortium Hereditary Breast and Ovarian Cancer (GC-HBOC) consists of 23 academic centers striving to provide high-quality regional care for affected individuals and healthy at-risk family members.
Methods: According to the standard operating procedures defined by the GC-HBOC, a certified Familial Breast and Ovarian Cancer Center was implemented at the University Medicine Greifswald despite the COVID-19 pandemic.
Results: Over a four-year period from 2018 to 2021, genetic analyses were performed in a total of 658 individuals, including 41 males, which paved the way to local annual risk-adapted breast cancer surveillance for 91 women in 2021. Certification according to ISO 9001:2015 and to OnkoZert was achieved in 2021 and 2022, respectively. Facilitators and barriers to implementation and growth of a high-risk breast and ovarian cancer service network integrating four interdisciplinary breast cancer centers and two gynecological cancer centers in the North Eastern part of Germany are discussed.
Conclusion: Our experience demonstrates that with interdisciplinary collaboration of dedicated local experts, support from the GC-HBOC, and fruitful clinical and scientific collaborations it is possible to establish a high-risk breast and ovarian cancer service even in a sparsely populated region.

Abstract ID: 232

De novo variants in *CNOT9* cause a neurodevelopmental disorder with or without epilepsy

R. Abou Jamra[1], L. von Wintzingerode[1], R. Bekheirna[2], B. Ben-Zeev[3], C. Cesario[4], K.M Chan[2], C. Depienne[5], O. Elpeleg[6], M. Iascone[7], W.V Kelley[8], M.-C. Nassogne[9], M. Niceta[10], L. Pezzani[11], N. Rahner[12], N. Revencu[13], T. Santiago-Sim[14], M. Tartaglia[10], M.L Thompson[8], M. Trivisano[15], J. Hentschel[1], H. Sticht[16], H. Oppermann[1]

[1]Institute of Human Genetics, Leipzig, Deutschland, [2]Baylor College of Medicine/Texas Children's Hospital, Department of Molecular and Human Genetics, Houston, Vereinigte Staaten, [3]Sheba Medical Center, Pediatric Neurology Institute, Ramat Gan, Israel, [4]Ospedale Pediatrico Bambino Gesù, Translational Cytogenomics Research Unit, Roma, Italien, [5]Institute of Human Genetics, Essen, Deutschland, [6]Hadassah, Department of Genetics, Jerusalem, Israel, [7]Azienda Ospedaliera Papa Giovanni XXIII, Laboratory of Medical Genetics, Bergamo, Italien, [8]HudsonAlpha Institute for Biotechnology, Huntsville, Vereinigte Staaten, [9]Cliniques universitaires Saint-Luc, Reference Centre for refractory Epilepsy, Brussels, Belgien, [10]Ospedale Pediatrico Bambino Gesù, Genetics and Rare Diseases Research Division, Roma, Italien, [11]Azienda Ospedaliera Papa Giovanni XXIII, Paediatric Department, Bergamo, Italien, [12]MVZ Institute for Clinical Genetics and Tumor Genetics, Bonn, Deutschland, [13]Cliniques universitaires Saint-Luc, Center for Human Genetics, Brussels, Belgien, [14]GeneDx, Gaithersburg, Vereinigte Staaten, [15]Ospedale Pediatrico Bambino Gesù, Department of Neuroscience, Roma, Italien, [16]Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute of Biochemistry, Erlangen, Deutschland

Introduction: CNOT9 is a part of the protein complex CCR4-NOT that has been connected to neurodevelopmental delay (NDD) through several of its components. The study aim at adding heterozygous *de novo* variants in one further member of this protein complex, *CNOT9*, as novel cause of NDD.
Methods: Individuals were clinically examined by pediatricians or human geneticists. Variants were identified using exome or genome sequencing. The impact of the variants was evaluated using *in silico* molecular modelling and/or based on functional analyses of the specific variants that is already available in the literature.
Results: We report seven individuals carrying *de novo* missense variants in *CNOT9*; p.(Arg46Gly), p.(Pro131Leu), p.(Arg227His) and, recurrent in four unrelated individuals, p.(Arg292Trp). All affected persons have developmental delay and/or intellectual disability, with five of them showing seizures. Other symptoms include muscular hypotonia, facial dysmorphism, and behavioral abnormalities. Molecular modeling predicted that the variants are pathogenic and would lead to reduced protein stability or impaired recognition of interaction partners. Functional analyses in previous studies already showed a pathogenic effect of p.(Pro131Leu) and p.(Arg227His).
Conclusion: We propose *CNOT9* as a novel gene for neurodevelopmental disorder and epilepsy. Confirmation of our results is needed by independent studies, including functional characterization to understand the pathomechanisms. Further genes of the complex are plausible candidates and may be confirmed in future studies.

Abstract ID: 233

P7-15

Long read sequencing identifies novel pathogenic intronic variants in Gitelman syndrome

K. Neveling[1], D. Viering[2], M. Hureaux[3,4], F. Latta[2], M. Kwint[1], A. Blanchard[3,4], M. Konrad[5], R.J. Bindels[2], K.-P. Schlingmann[5], R. Vargas-Poussou[3,4,6], J.H. de Baaij[2]

[1]Radboudumc Nijmegen, Department of Human Genetics, Nijmegen, Niederlande, [2]Radboudumc Nijmegen, Radboud Institute for Molecular Life Sciences, Department of Physiology, Nijmegen, Niederlande, [3]Reference Center for Hereditary Kidney and Childhood Diseases, Paris, Frankreich, [4]Hôpital Européen Georges-Pompidou, Department of Genetics, Assistance Publique Hôpitaux de Paris, Paris, Frankreich, [5]University Children`s Hospital, Department of General Pediatrics, Muenster, Deutschland, [6]Sorbonne Université, INSERM, Université de Paris, CNRS, Centre de Recherche des Cordeliers, Paris, Frankreich

Introduction: Gitelman syndrome is a salt-losing tubulopathy characterized by hypokalemic alkalosis and hypomagnesemia. It is a recessive disorder caused by homozygous or compound heterozygous pathogenic variants in SLC12A3, which encodes the Na^+-Cl^- cotransporter (NCC). In ±10% of patients with Gitelman syndrome, current genetic techniques fail to detect a second pathogenic variant. The aim of this study was to identify a second pathogenic variant in introns, splice sites or promoter to increase the diagnostic yield.

Methods: Long read sequencing of SLC12A3 was performed in 67 individuals with Gitelman syndrome in whom a single (likely) pathogenic variant was detected previously. Additionally, we sequenced 28 individuals with one variant of uncertain significance or no candidate variant. Pathogenicity of novel identified intronic variants was assessed by midigene splice assays.

Results: Of 67 patients in whom one (likely) pathogenic variant was known previously, a second (likely) pathogenic variant was identified in 45 (67%). Patients in whom two (likely) pathogenic variants were identified had a biochemically more severe phenotype than other patients. Sixteen intronic variants outside of canonical splice sites were detected (nine different variants, mostly deep intronic, six novel), while 29 patients had an exonic variant or canonical splice site variant. Midigene splice assays of the previously known c.1670-191C>T variant and intronic candidate variants demonstrated aberrant splicing patterns.

Conclusion: Intronic pathogenic variants explain a significant part of the missing heritability in Gitelman syndrome. Long read sequencing should be considered in diagnostic workflows for Gitelman syndrome.

Abstract ID: 234

P5-12

Mild manifestations of trisomy 13

A.T. Abad Perez[1], O. Blankenstein[2,3], L. Cardellini[4], J. Grünhagen[5], J. Helmuth[5], D. Horn[1], K. Skipalova[1], V. Raile[2], M. Stahl[6,2,3], N. Ehmke[6,1]

[1]Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt- Universität zu Berlin, Institut für Medizinische Genetik und Humangenetik, Berlin, Deutschland, [2]Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt- Universität zu Berlin, Dept of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Berlin, Deutschland, [3]German Center for Lung Research (DZL), Associated Partner Site Berlin, Berlin, Deutschland, [4]Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humbold-Universität zu Berlin, Center for Chronically Sick Children, Berlin, Deutschland, [5]Labor Berlin Charité Vivantes GmbH, Dept of Human Genetics, Berlin, Deutschland, [6]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Deutschland

Trisomy 13, also known as Patau syndrome, is a multiple congenital malformation and developmental disorder characterized by anomalies of the central nervous system, heart, and genitourinary organs. Characteristic features include microphthalmia, cleft lip and palate and polydactyly. It is caused by the presence of an extra copy of chromosome 13. Individuals with mosaic trisomy 13 can have a milder phenotype.

Here, we describe a female infant with bilateral complex eye malformation with optic coloboma, unilateral postaxial polydactyly, muscular hypertonia, hearing loss and facial dysmorphism. She was born at term after an uneventful pregnancy with normal birth measurements. There were no signs of cleft lip or palate, and cerebral sonogram was unremarkable. The postnatal adaption was complicated by congenital infection, feeding difficulties, exocrine pancreatic insufficiency, thrombocytopenia and atrial septal defect type II. After initial intensive treatment, her condition stabilized and she was discharged to ambulant care. At the age of 6 months partial head control had been achieved, her growth parameters were within the normal range and her motor development was delayed. Array-CGH and karyotyping from blood cells showed free trisomy 13 without evidence of mosaicism.

Trisomy 13 is associated with poor prognosis and many affected individuals die shortly after birth or within the first year of life. The median survival age is 7-10 days. Few individuals with full trisomy 13 surviving this period are known. It has been suggested that life expectancy could be related to the absence of severe organ malformations, including heart defects and holoprosencephaly.

The herein reported individual is remarkably mildly affected, highlighting the phenotypic variability of trisomy 13. We summarize the phenotype of Patau patients with long-term survival and propose that trisomy 13 should be considered as a differential diagnosis in individuals with coloboma and polydactyly, even when growth deficiency is absent.

Abstract ID: 235

P3-29

First patient with X-linked developmental disorder caused by a partial duplication of the *ZDHHC9* gene

V.K. Berge[1], I. Hüning[1], C. Zühlke[1], M. Spielmann[1,2], B. Hanker[1]

[1]Institute of Human Genetics, University of Lübeck, Lübeck, Deutschland, [2]Institute of Human Genetics, University of Kiel, Kiel, Deutschland

To date more than 100 genes are known to be involved in X-linked intellectual disability (XLID). Among those is the Zinc Finger DHHC-Type Containing 9 (*ZDHHC9*) which encodes palmitoyltransferase and plays a significant role in the posttranslational modification of NRAS and HRAS and therefore in the cellular concentration and distribution of these proteins. A change within these is suggested to cause *ZDHHC9*-associated X-linked developmental disorder (MRXSR) although the exact pathogenic mechanism is not yet understood. So far, no similarities of symptomatology with RASopathies have been described.

ZDHHC9-associated X-linked intellectual developmental disorder is characterized by impaired intellectual development of varying degree, speech and language difficulties, behavioral problems, facial dysmorphism, epilepsy, hypotonia and marfanoid habitus.

We report on a 6-year-old boy with a developmental disorder, speech difficulties, selective mutism, temporary tall growth and hypotonia. He presented with body measurement in high normal range. He had no distinctive dysmorphic features besides deep-set eyes. An endocrinological examination had excluded an underlying endocrinopathy. Seizures had not been detected at any time. Cerebral MRI had not been conducted.

A karyogram showed a normal male karyotype (46,XY). Array-CGH detected a submicroscopic gain of 113 kb in Xq26.1 (128834919_128947700) (GRCh37/hg19) encompassing exons 6 to 10 of the *ZDHHC9* gene in the patient. Array-CGH of the mother revealed that the duplication was of maternal origin. She was an unaffected carrier.

Systematic review of the literature showed only 11 described pathogenic variants so far with regard to *ZDHHC9*-associated X-linked intellectual developmental disorder. Neither exon duplications nor partial duplications have previously been described. Taking the assumed pathogenic mechanism into account, this variant is likely to be pathogenic and causing the boy's phenotype. Our patient's phenotype apart from a missing marfanoid habitus overlaps with four other patients described in the literature with *ZDHHC9* variants and intellectual developmental disorder. Therefore, this is the first reported patient with a developmental disorder, speech difficulties, hypotonia, selective mutism and temporary tall growth as well as a partial duplication in the *ZDHHC9* gene.

Abstract ID: 237

P2-5

Targeted RNA analysis classified potential germline splice variants in tumour risk genes

A.K. Sommer[1], X. Yin[1,2,3], S. Pasternack-Ziach[1], R. Raff[1], U. Moog[4], K. Grund[4], D. Schäfer[5], G.S. Leszinski[6], C.-E. Ott[7], F. Erger[8], M. Henneke[9], I. Spier[1,10], S. Aretz[1,10]

[1]Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Deutschland, [2]Royal Melbourne Hospital, Department of Colorectal Medicine and Genetics, Parkville, Australien, [3]University of Melbourne, Department of Medicine, Parkville, Australien, [4]Institute of Human Genetics, Heidelberg University Hospital, Heidelberg, Deutschland, [5]Institute for Human Genetics, University of Frankfurt, Frankfurt, Deutschland, [6]Institute of Human Genetics, Technische Universität München, München, Deutschland, [7]Institute of Medical Genetics and Human Genetics, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Deutschland, [8]Institute of Human Genetics, University Hospital Cologne, Köln, Deutschland, [9]University Medical Center Goettingen, Department of Pediatrics and Adolescent Medicine, Göttingen, Deutschland, [10]Center for Hereditary Tumor Syndromes, University of Bonn, Bonn, Deutschland

Background and Aim: A correct genetic diagnosis is pivotal to the health care of patients with tumour risk syndromes (TRS). The identification of causative pathogenic germline variants helps to navigate the differential diagnosis of TRS and guide further predictive testing, which enable timely treatment and preventive measures. However, in a substantial fraction of patients, variants of unknown clinical significance (VUS) are identified. To evaluate their functional impact, we conducted transcript analyses of multiple potential splice variants in selected cases of suspected TRS and reassessed their pathogenicity prediction.

Methods: Between 2012 and 2022, targeted RNA analysis of variants with potential splice effects was performed. Total RNA was extracted from leukocytes using the PAXgene Blood RNA Kit, and reverse-transcribed into cDNA. Specific primers were designed for each region of interest and PCR-amplified cDNA product was Sanger sequenced. Gel electrophoresis of the amplification product was subsequently performed to identify any abnormal bands, which were then excised, re-amplified and sequenced.

Results: We confirmed pathogenic splice effects of 14 different germline variants in seven genes (*APC, MUTYH, CDH1, LZTR1, PMS2, PTEN, SDHD*) including twelve single nucleotide variants (SNVs), one deletion of three exons and one duplication of two exons. Most SNVs (9/12, 75%) led to single exon skipping. In two other cases, the insertion of a short intronic sequence, and in one case, multiple aberrant transcripts (skipping of one or multiple exons) were observed. Segregation was examined in ten cases where DNA from (un-)affected family members was available. In seven of these (70%), further affected carriers were identified within the family and in one case the variant was confirmed to be *de novo*.

Discussion: The functional analysis of potential splice variants helps to facilitate an accurate genetic diagnosis and consequently improve patient care. Although, these analyses are often time-consuming and dependent on sample availability, they are highly relevant to variant classification, notably within the ACMG/AMP classification system. The fulfilment of function criteria (PS3, BS3) can be crucial to pass the evidence thresholds that enables a classification as (likely) pathogenic or benign.

Abstract ID: 238

P7-16

Pathogenicity prediction of missense variants using AlphaFold2-derived features

A. Schmidt[1], S. Röner[2], K. Mai[1], H. Klinkhammer[3,4], M. Kircher[2,5], K. Ludwig[1]

[1]Institute of Human Genetics, University Hospital Bonn, University of Bonn, Bonn, Deutschland, [2]Berlin Institute of Health (BIH) at Charité – Universitätsmedizin Berlin, Berlin, Deutschland, [3]Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, University of Bonn, Bonn, Deutschland, [4]Institute of Medical Biometry, Informatics and Epidemiology, University Hospital of Bonn, University of Bonn, Bonn, Deutschland, [5]Institute of Human Genetics, University Hospital Schleswig-Holstein (UKSH), University of Lübeck, Lübeck, Deutschland

Missense variants are a major cause of Mendelian diseases, but their classification as (likely) pathogenic or (likely) benign remains challenging. Recently, the structure of the human proteome was predicted with high accuracy using the artificial intelligence system AlphaFold2. We therefore studied if structures predicted by AlphaFold2 can improve the accuracy of computational pathogenicity prediction for missense variants.
To address this question, we used AlphaFold2 predicted structures of the wild-type proteome and extracted features for each amino acid and its neighborhood. We used these features and trained a random forest classifier to distinguish between common (proxy-benign) and singleton (proxy-pathogenic) missense variants derived from gnomAD.
The random forest model yielded a novel AlphaFold2-based pathogenicity prediction score, which we named AlphScore. Features of particular importance to AlphScore were solvent accessibility, amino acid network related features, features describing the physicochemical environment, and AlphaFold2's quality parameter (pLDDT). The performance of AlphScore alone was lower than that of existing scores used for missense prediction, such as CADD or REVEL. However, when AlphScore was linearly combined with those scores, the integrated scores outperformed the individual scores, as measured by the prediction of missense variants from ClinVar and deep mutational scan data.
Overall, our data indicate that pathogenicity prediction of missense variants can be improved by the integration of AlphaFold2 structures.
Parts of this work are available as preprint: https://doi.org/10.1101/2022.03.05.483091

Abstract ID: 239

P3-63

ATXN2 gene variants in neurodegenerative diseases

J.M. Lubieniecka[1], K.P. Lubieniecki[1], C. Hippert[1], K.R. Döring[1], N. Jovancevic[1], S. Bernsen[2], R. Fabian[2], P. Weydt[2], H.H.P. Nguyen[1]

[1]Ruhr-Universität, Humangenetik, Bochum, Deutschland, [2]Universitätsklinikum Bonn, Klinik für Neurodegenerative Erkrankungen, Bonn, Deutschland

ATXN2 encodes an RNA binding protein involved in mRNA translation and regulation. It has also been shown to be a modifier of TD43 toxicity in yeast, flies, and mouse models. Large polyglutamine (CAG) expansions are causative of spinocerebellar ataxia type 2 (SCA2) and intermediate expansions are considered a risk factor for amyotrophic lateral sclerosis (ALS). We have evaluated genetic variants (SNVs and indels) detected in the *ATXN2* gene during routine diagnostic exome sequencing at the Human Genetics department of Ruhr University. In total 371 exomes from patients with various diagnosis (92 ALS, 24 SCA, 21 spastic paraplegia (SPG) and 234 other, non-neurodegenerative diseases), were evaluated. In this sample set, 71 *ATXN2* variants were detected, of which 27 were of high or moderate impact, not reported as benign/likely benign in ClinVar, and were present in control populations (gnomAD) at a frequency of less than 5%. Of these 27 variants 74% were in exon 1 of *ATXN2* gene within the polyglutamine repeat region. Majority of the exon 1 variants are predicted to affect the length of the repeat region, and this prediction was confirmed by repeat expansion analysis in some of the cases. In two of the ALS patients medium size expansions were detected, which would qualify the patients for *ATXN2* antisense oligonucleotides clinical trial (NCT04494256). We have also noticed an enrichment of *ATXN2* exon 1 variants in patients with ALS (14%), SPG (19%), and SCA (25%) as compared to patients with other diagnosis (6%), suggesting a potential role for these variants in the pathology of this group of related neurodegenerative diseases (possibly underlying the phenotypic similarities). Considering that the *ATXN2* gene is a target of potential new treatment for ATXN2-related disorders, further investigation of variants detected in this gene, and especially in its repeat region, is important not only for diagnostics but also disease management.

Abstract ID: 240

P3-32

Combined partial monosomy 9p and partial trisomy 20p: a case report and review of the literature

D. Wand[1], I. Filges[1]

[1]*Institut für Medizinische Genetik und Pathologie, Medizinische Genetik, Basel, Schweiz*

Here, for the first time, we report a case with a chromosomal aberration involving a 9p terminal deletion with an 20p duplication.
The deletion of distal 9p is a rare syndrome and characterized by dysmorphic features, developmental delay, and ambiguous genitalia. The phenotype shows variable expressivity and is related to the size of the deletion. The clinical presentation has been described in over 100 different case reports. Most cases of patients with Trisomy 20p syndrome are derived from parental chromosome translocation. More than 30 cases have been published. The manifestation of this syndrome are rather variable. Clinical features are craniofacial dysmorphism, abnormality in heart, brain and developmental delay. We describe a 4 month old male with multiple malformations. The cytogenetic and microarray analysis revealed an unbalanced chromosomal aberration with a deletion in the short arm of chromosome 9 at 9p24.3p24.1 associated with a duplication in the short arm of chromosome 20 at 20p13p11.1. We compared the clinical picture of our patient with some cases of isolated 9p deletion and 20p duplication, similar in size, in order to better define the phenotype- genotype association of this rare chromosomal aberration.

Abstract ID: 241

P2-16

Unmet need for interdisciplinary cooperation: case series of patients diagnosed with hereditary leiomyomatosis and renal cell cancer (HLRCC)

M. Kachanov[1], A. Möllring[1], N. Dikow[2], H. Brennenstuhl[2], S. Aretz[3], E. Holinski-Feder[4], L. Budäus[5], G. von Amsberg[5,6], G. Sauter[7], B. Beck[8], A. Volk[1]

[1]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Deutschland, [2]Institute of Human Genetics, University Hospital Heidelberg, Heidelberg, Deutschland, [3]Institute of Human Genetics, Medical Faculty, University of Bonn, and National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Deutschland, [4]MGZ Medical Genetics Center, Munich, Deutschland, [5]Martini-Klinik, Prostate Cancer Center Hamburg-Eppendorf, Hamburg, Deutschland, [6]University Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Department of Hematology and Oncology, Hamburg, Deutschland, [7]University Medical Center Hamburg-Eppendorf, Department of Pathology, Hamburg, Deutschland, [8]Institute of Human Genetics and Center for Molecular Medicine, University of Cologne, Faculty of Medicine and University Hospital of Cologne, Cologne, Deutschland

Objective: Despite the first description more than two decades ago, hereditary leiomyomatosis and renal cell carcinoma (HLRCC) remains underdiagnosed. The recent inclusion of the HLRCC-associated renal cell carcinoma (RCC) in the latest 2016 WHO classification of the urinary system tumors, as well as the implementation of fumarate hydratase deficiency immunohistochemical testing at histopathological examinations of uterine leiomyomas, provide a deeper assessment of HLRCC cases. This may lead to an increase in patients with diagnosed fumarate hydratase deficiency in tumor tissue seeking genetic counseling.

Material and Methods: We assessed a multi-institutional cohort of 13 patients (10 families) diagnosed with HLRCC who underwent genetic testing of the *FH* gene.

Results: Within 10 index patients, following HLRCC-associated conditions led to genetic counseling and testing: 3 patients were primarily diagnosed with RCC with or without cutaneous or uterine manifestations, 5 patients were diagnosed due to cutaneous and uterine manifestations which led to hysterectomy and only 2 patient was diagnosed with primary exclusively cutaneous manifestations of leiomyomatosis. The affected relatives of the index patients (n=3) demonstrated cutaneous and uterine HLRCC manifestations. In cases of multiple HLRCC manifestations, the genetics referral was mainly considered after histopathological work-up and not based on HLRCC-suspicious clinical findings.

Within the patients included, a novel germline mutation of *FH* gene (c.893_904+7delCTGCACTTACAGGTTAGTG) was identified in 4 patients from 3 unrelated families.

Conclusions: Our series strengthens a further need for interdisciplinary cooperation between clinical genetics, urology, gynecology, dermatology and pathology to provide early identification of HLRCC patients with an emphasis on the incremental role of clinical examination of the patients affected by tumor syndromes such as HLRCC. Advances in the histopathological workup of RCC and uterine leiomyomas may increase the number of HLRCC patients seeking genetic counseling.

Abstract ID: 242

P3-16

Towards targeted therapy in Li-Fraumeni syndrome: case report of a 62-year old patient with osteosarcoma

V.I. Gaidzik[1], C. Seeling[1], K. Oehl-Huber[2], S. Dahlum[2], R. Marienfeld[3], A. Beer[4], R. Mayer-Steinacker[5], T.F. Barth[3], P. Möller[3], T. Seufferlein[6], H. Döhner[1], S. Stilgenbauer[5], R. Siebert[2]

[1]Universitätsklinik Ulm, Klinik für Innere Medizin III, Ulm, Deutschland, [2]Universitätsklinik Ulm, Universität Ulm, Institut für Humangenetik, Ulm, Deutschland, [3]Universitätsklinik Ulm, Institut für Pathologie, Ulm, Deutschland, [4]Universitätsklinik Ulm, Klinik für Nuklearmedizin, Ulm, Deutschland, [5]Universitätsklinik Ulm, Comprehensive Cancer Center, Ulm, Deutschland, [6]Universitätsklinik Ulm, Klinik für Innere Medizin I, Ulm, Deutschland

Li-Fraumeni syndrome (LFS) is a tumor disposition syndrome caused by a germline pathogenic variant of the tumor suppressor gene TP53. LFS can be suspected in a personal/family history of cancer, especially when so called core cancers of LFS have been diagnosed including osteosarcoma (OS). There is an increased risk of secondary malignancy and adverse side-effects after radiation and/or conventional genotoxic chemotherapy, so alternative treatment options are needed. In our molecular and familial tumor board (MoFa) of the Comprehensive Cancer Center Ulm, we discuss these patients aiming for alternative treatment options.

We present here a case report of a 62-year-old women with diagnosis of osteoblastic OS in the right skullcap. There was no familial cancer history, however, the patient had a history of breast cancer (left) at the age of 44 years (surgery, adjuvant radio- and chemotherapy), followed by a ductal carcinoma in situ of the breast four years later. Another five years later, patient was diagnosed with BC (right), treated by surgery and endocrine therapy. In addition, patient has multiple sclerosis treated with methotrexate.

The OS was resected and an adjuvant chemotherapy in analogy to Euro-B.O.S.S. protocol was initiated. Due to the tumor history of the patient, sequencing of possible tumor disposition related genes was performed after human genetic counseling and revealed a class 5 (pathogenic) variant in TP53 supporting suspected LFS. After receiving germline genetic results and due to severe side effects, chemotherapy was stopped after 3 cycles. As there were still FDG-PET positive lesions in the resection areal of the OS, we initiated in our MoFa next generation sequencing as well as OncoScan copy number variation arrays of the OS sample. Here, we could identify a homozygous loss of 9p21.3 including the gene CDKN2A. After discussion of the patient in the MoFa the consensus was an off label treatment with CDK4/6 inhibitor Palbociclib as a maintenance therapy within a curative treatment strategy. Palbociclib was well tolerated and terminated after 22 months with no evidence of disease. The patient is still in complete remission 30 months after diagnosis of OS.

In summary, evaluation of personal/family history is essential to prevent adverse therapy effects in tumor disposition syndromes as LFS. Furthermore, we show that loss of CDKN2A might be a therapeutic rationale in patients with OS for which adjuvant therapy is not feasible.

Abstract ID: 243

Homozygous loss-of-function variants in *FILIP1* cause autosomal recessive arthrogryposis multiplex congenita with microcephaly

F. Schnabel[1,2], E. Schuler[3], A. Al-Maawali[4,5], A. Chaurasia[6,7], S. Syrbe[3], A. Al-Kindi[4,5], G.S. Bhavani[8], A. Shukla[8], J. Altmüller[9,10,11,12], P. Nürnberg[9,12], S. Banka[7,13], K.M. Girisha[6], Y. Li[1], B. Wollnik[1,14,15], G. Yigit[1,15]

[1]Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Deutschland, [2]Institute of Human Genetics, University of Leipzig Hospitals and Clinics, Leipzig, Deutschland, [3]Division of Paediatric Epileptology, Centre for Paediatrics and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Deutschland, [4]College of Medicine and Health Sciences, Sultan Qaboos University, Department of Genetics, Muscat, Oman, [5]Genetic and Developmental Medicine Clinic, Sultan Qaboos University Hospital, Muscat, Oman, [6]Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Department of Medical Genetics, Manipal, Indien, [7]Division of Evolution, Infection and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, Vereinigtes Königreich, [8]Kasturba Medical College, Manipal Academy of Higher Education, Department of Medical Genetics, Manipal, Indien, [9]Cologne Center for Genomics, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Deutschland, [10]Core Facility Genomics, Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Berlin, Deutschland, [11]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Deutschland, [12]Center for Molecular Medicine Cologne, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Deutschland, [13]Manchester Centre for Genomic Medicine, St Mary's Hospital, Health Innovation Manchester, Manchester University NHS Foundation Trust, Manchester, Vereinigtes Königreich, [14]Cluster of Excellence Multiscale Bioimaging, from Molecular Machines to Networks of Excitable Cells (MBExC), University of Göttingen, Göttingen, Deutschland, [15]German Centre for Cardiovascular Research, Partner Site Göttingen, Göttingen, Deutschland

Arthrogryposis multiplex congenita refers to a wide range of clinically and etiologically heterogeneous disorders characterized by congenital joint contractures in at least two body areas. The underlying etiology mainly includes neurological and muscular disorders. Here, we report five affected children from three independent families sharing an overlapping phenotype with multiple joint contractures affecting shoulder, elbow, hand, hip, knee and foot as well as scoliosis, reduced palmar and plantar skin folds, microcephaly and facial dysmorphism. In an exome sequencing approach, we were able to identify homozygous truncating variants in *FILIP1* in all patients. *FILIP1* is a modulator of filamin homeostasis regulating the initiation of cortical cell migration in the developing neocortex and fundamental for differentiation processes in cross-striated muscle cells during myogenesis. In summary, we provide clinical and genetic evidence that biallelic deleterious variants in *FILIP1* are causative of a novel autosomal recessive disorder and expand the spectrum of genetic factors related to arthrogryposis.

Abstract ID: 244

P3-64

Combined features of short-rib-polydactyly syndrome and oral-facial-digital syndrome in a fetus: clinical diagnosis allows targeted genetic testing

M. Messner[1], I. Mutz-Dehbalaie[2], S. Sprung[3], C. Überbacher[1], J. Zschocke[1], C. Fauth[1]

[1]Institute of Human Genetics, Medical University Innsbruck, Innsbruck, Österreich, [2]Medical University Innsbruck, Department of Gynecology and Obstetrics, Innsbruck, Österreich, [3]Institute of Pathology, Innpath Tirol Kliniken, Innsbruck, Österreich

Background: Ciliopathies caused by pathogenic variants in *KIAA0586* show a broad spectrum of clinical phenotypes, ranging from mild manifestations of Joubert syndrome to severe phenotypes with lethal outcome like short-rib thoracic dysplasia 14 with polydactyly. *KIAA0586* plays an important role in primary ciliogenesis and hedgehog signaling which are essential for the embryonic development of organs and skeleton. The short-rib-polydactyly syndrome and the oral-facial-digital syndrome also belong to the subgroups of ciliopathies. Skeletal characteristics for the short-rib-polydactyly syndrome are the short limb dwarfism, short ribs with thoracic hypoplasia and polydactyly, whereas the oral-facial-digital syndrome shows abnormalities of the face, oral cavity and digits.

Case: We report on the 2nd pregnancy in a couple of Romani descent. Prenatal ultrasound at the 22nd week of gestation disclosed polyhydramnios, short limbs, postaxial polydactyly of hands, hypoplastic thorax, unilateral cleft lip and palate, dysmorphic cerebellar vermis, dilatation of the fourth cerebral ventricle and a ventricular septal defect of the heart. Because of the severe malformations, the parents opted for pregnancy termination. Thorough postmortem fetal examination showed additional findings, including preaxial polydactyly of the halluces and a bifid and lobulated tongue.

Based on the characteristic fetal phenotype – which combines symptoms of short-rib-polydactyly syndrome and oral facial-digital syndrome – and the ethnic background of the family, homozygosity for the previously described splice variant c.1815G>A in the *KIAA0586* gene was suspected and confirmed by targeted Sanger sequencing.

Discussion and Conclusion: The variant c.1815G>A in the *KIAA0586* gene has previously been reported in six fetuses/neonates with an early lethal ciliopathy characterized by cerebral anomalies, short ribs and limbs, polydactyly and anomalies of the oral cavity. All affected individuals were of Eastern European or Romani origin (founder mutation). The present case confirms that homozygosity for this particular variant leads to a characteristic clinical phenotype that combines features of short-rib-polydactyly syndrome and oral facial-digital syndrome.

Keywords: *KIAA0586*, short-rib-polydactyly syndrome, oral-facial-digital syndrome

Abstract ID: 245

P7-7

Automated DNA extraction for detection of tandem repeat expansions with nanopore sequencing

H. Faust[1], J. Hentschel[1], D. Popp[1]

[1]*Universitätsklinikum Leipzig, Institut für Humangenetik, Leipzig, Deutschland*

Long-read technologies such as nanopore sequencing provide new opportunities detecting tandem repeat expansions and increase the diagnostic yield of next generation sequencing. However, using long-read sequencing in a diagnostic setting requires the costly establishment of an adapted workflow from wet lab to dry lab. Especially, a DNA extraction is necessary, that provides extensive read lengths for identifying large repeat expansions. We evaluated the standard DNA extraction method in our laboratory regarding suitability for nanopore sequencing. Therefore, we extracted DNA from EDTA-blood of four persons using an automated magnetic bead-based DNA extraction method (MagCore® HF 16 Plus II), and determined DNA concentration and DNA integrity. We evaluated the read length distribution of these samples in nanopore sequencing. Thereby, we measured mean N50 values of approximately 9 kb (after DNA extraction of fresh blood as well as of EDTA-blood stored at 4 °C or -20 °C). Additionally, we performed targeted nanopore sequencing based on adaptive sampling of samples with known repeat expansions. In summary, the measured read length distribution was sufficient for the detection of large tandem repeat expansions with adaptive sampling. The routine automated magnetic bead-based DNA extraction in our lab requires only minimal hands-on sample preparation, enables a high throughput, is cost-effective and, therefore, simplifies the establishment of long-read sequencing in a diagnostic as well as in a research setting.

Abstract ID: 248

DOCK4 loss-of-function variants lead to neurodevelopmental delay

V. Bothe[1], C. Herbst[1], M. Wegler[1], H. Sticht[2], S. Bakhtiari[3], M. Nizon[4], A.C. Hurst[5], I. Thiffault[6], A. Kurolap[7], H. Oppermann[1], R. Abou Jamra[1]

[1]Institute of Human Genetics, University of Leipzig Medical Center, University of Leipzig, Leipzig, Deutschland, [2]Institute of Biochemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Deutschland, [3]Barrow Neurological Institute, Phoenix Children's Hospital, Phoenix, Departments of Child Health, Neurology, Cellular, and Molecular Medicine and Program in Genetics, University of Arizona College of Medicine, Phoenix, Vereinigte Staaten, [4]Université de Nantes, CNRS, INSERM, l'Institut du Thorax, Service de Génétique Médicale, Nantes, Frankreich, [5]University of Alabama at Birmingham, Birmingham, Vereinigte Staaten, [6]Genomic Medicine Center, Children's Mercy Hospital, Kansas City, Vereinigte Staaten, [7]The Genetics Institute and Genomics Center, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

DOCK4 is a member of the dedicator of cytokinesis (DOCK) family. It is a membrane-associated cytoplasmic GTPase protein that is involved in regulation of adherence junctions between cells.
Through Trio-Exome analysis, matchmaking platforms and international collaboration we recruited a cohort of 7 individuals with mild to severe neurodevelopmental disorder (NDD). Four individuals have a de novo missense variant and, interestingly, movement disorders. The three individuals with a null-variant (one inherited, two still unknown as parents are not available) show a rather mild phenotype of NDD. Further common symptoms comprise muscular hypotonia, autism spectrum disorder, seizures, MRI abnormalities, and microcephaly. The current data thus suggests a kind of genotype-phenotype correlation, though this has to be proven.
Molecular modelling of the missense variants suggest critical disturbance of the globular structure of *DOCK4*. By using neurite outgrowth assays with Neuo-2A cells, we functionally evaluated the variants. After transfection of corresponding expression plasmids, Neuo-2A cells exhibited significantly reduced neurite length compared to overexpression of the wild type *DOCK4*. Furthermore, CRISPR-Cas9-mediated *Dock4*-knock-out Neuro-2A cells, reflecting the null-variants , show reduced neurite length compared to wild type Neuro-2A cells. The functional data suggests at this early stage a loss-of-function mechanism of the missense variants.
In summary, our results including clinical, molecular and functional data suggest that loss-of-function-variants in *DOCK4* cause a variable spectrum of a NDD. Further work to understand the phenotypic spectrum, the pathomechanisms and to expand the cohort is needed and ongoing.

Abstract ID: 249

P3-66

Next-Generation sequencing identifies a novel de novo pathogenic variant in the DYRK1A gene causing syndromic intellectual disability and microcephaly

D. Berner[1], K. Alt[1], V. Strohhäcker[1], A. Hentrich[1], H.-M. Büttel[1], D. Gläser[1]

[1]MVZ Genetikum GmbH, Molekulargenetik, Neu-Ulm, Deutschland

Introduction: Dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) is a kinase, which catalyzes the phosphorylation of serine and threonine residues on exogenous substrates. Pathogenic single-nucleotide variants in DYRK1A, including nonsense variants, result in haploinsufficiency of the gene and are supposed to cause autosomal dominant mental retardation. Here, we report a 31-year-old female patient with intellectual disability, microcephaly and dysmorphic features including short philtrum, prominent columella and hypotelorism. The family history was unremarkable.
Materials and Methods: We performed chromosome analysis, array-CGH and clinical exome sequencing (CES) in the patient followed by Sanger sequencing to validate variants in the patient and her parents.
Results: Chromosome analysis and array-CGH gave normal results. Next-generation sequencing identified a heterozygous nonsense pathogenic variant c.299T>A (p.Leu100*) in exon 3 of DYRK1A which results in a premature termination codon at amino acid position 100. The pathogenic variant was not found in large exome databases (1000g, ExAc, gnomAD), and segregation studies by Sanger sequencing confirmed de novo occurrence.
Conclusions: Pathogenic variants in the DYRK1A gene are a rare cause of syndromic intellectual disability with microcephaly and can be detected by next-generation sequencing with phenotype-based evaluation strategies.

Abstract ID: 250

P2-17

Comparison of glioblastoma tissue *vs* glioblastoma stem-like cells: genetic, biophysical and bioinformatical investigations

V.-P. Brandt[1], H. Holland[2], E. Streubel[3], S. Höhme[4], C. Sander[5], U. Nestler[5]

[1]University of Leipzig - Saxonian Incubator for Clinical Translation (SIKT) and Department of Neurosurgery, Leipzig, Deutschland, [2]University of Leipzig - Saxonian Incubator for Clinical Translation (SIKT) and Faculty of Medicine, Leipzig, Deutschland, [3]University of Leipzig - Faculty of Physics and Earth Science, Peter Debye Institute for Soft Matter Physics, Soft Matter Physics Division, Leipzig, Deutschland, [4]University of Leipzig - Institute for Computer Science / IZBI, Leipzig, Deutschland, [5]University of Leipzig - Department of Neurosurgery, Leipzig, Deutschland

According to WHO classification 2016, Glioblastoma (GB) is defined as high grade glioma with an astrocytic differentiation. Glioblastoma stem-like cells (GSCs) are discussed in context with tumour initiation, maintenance and recurrence as well as cancer therapy resistance. So far, comparative genetic and computational model-based studies on Glioblastoma and/or GSCs are rare. Therefore, we performed genetic analyses using SNP array and subtelomere FISH as well as biophysical measurements and bioinformatical spatio-temporal modeling to provide a deeper understanding of different characteristics of GCSs and GB tissue. The tissue samples were recovered by surgery and GSCs were obtained by culturing in serum-free media. Stem cell characteristics of GSCs were analysed by applying the immunoflourescent markers Nestin and SOX2. Genetic differences of GB *vs* GSCs were identified using genome wide high resolution SNP array (Affymetrix CytoScan 750 Array) and subtelomere FISH. For investigations on growth behaviour, we analysed viscoelastic properties and performed Live-Cell Imaging for 72 hours followed by bioinformatic model calculations. Detected chromosomal aberrations in glioblastoma tissue correlated to previously described genetic aberrations, e.g. gain of 7p22.2-q34, gain of 7q36.1-q36.3, as well as loss of 10p15.3-q26.3. Subtelomere FISH analyses revealed losses of chromosome 3, 4, and 6, whereas these aberrations were not found in primary tumour tissue. In contrast to the growth behaviour of GB, we identified compact and largely collectively moving cell spheroids of GSC in the periphery of the spheroid with striving for fusion. Biophysical investigations including viscoelastic measurements (with Atomic Force Microscopy) and Live-Cell Imaging confirmed differences between GSCs and GB tissue. Our results increase the knowledge about growth processes and genetics in glioblastoma *vs* GSCs. More comparative analyses of glioblastoma *vs* GSCs are necessary to improve therapy options.

Abstract ID: 251

P1-9

Engineering of an inducible iPSC line to study imprint establishment at the PWS-SRO

J. Haake[1], M. Kaufmann[1], L. Steenpass[1]

[1]DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Human and Animal Cell Lines, Braunschweig, Deutschland

Imprinted genes are controlled by imprinting centers which are differentially methylated, resulting in allele specific gene expression. Defects occurring on the only active chromosome result in imprinting disorders, such as Angelman syndrome (AS). AS is a neurodevelopmental disorder caused by the absence of functional UBE3A in the brain, which is exclusively expressed by the maternal allele. The imprinting center on chromosome 15 contains two functional elements, the PWS-SRO, which includes the *SNRPN* promotor, and the AS-SRO, 35kb upstream to the PWS-SRO. The AS-SRO element is an oocyte-specific promoter and is needed for establishment of DNA methylation at the PWS-SRO. In an iPSC-based human in vitro system, we want to investigate if AS-SRO mediated transcription across the PWS-SRO causes DNA methylation at the imprinting center. By replacing the AS-SRO with an inducible promotor that is regulated by a doxycycline-responsive transactivator protein (rtTA), transcription initiation at the AS-SRO can be manipulated. An iPSC line derived from fibroblasts of a patient with AS due to a large deletion was used for genetic engineering. Using CRISPR/Cas9, first the rtTA expression cassette was integrated into the AAVS1 safe harbor locus and second, the AS-SRO element was replaced by the inducible promoter. Because the used iPSC line carries only the unmethylated paternal imprinting center, all targeting events locate to the same chromosome. After adding doxycycline, transcription across the PWS-SRO was observed. Transcripts spliced onto exon 2 of the *SNRPN* gene as observed for the oocyte-specific transcripts. Next, for an in-depth analysis of this transcriptionally highly active region whole transcriptome RNA-seq will be performed and acquisition of DNA methylation at the PWS-SRO will be analyzed by targeted deep bisulfite sequencing. Results of these analyzes will be presented. The described in vitro iPS cell model could elucidate the link between transcription and establishment of DNA methylation.

Abstract ID: 252

P6-4

In vitro modelling of Sorsby fundus dystrophy

K. Plößl[1], C. Bischof[1], H. Stöhr[1], B. Weber[1,2]

[1]Universität Regensburg, Institut für Humangenetik, Regensburg, Deutschland, [2]Universitätsklinikum Regensburg, Institut für klinische Humangenetik, Regensburg, Deutschland

Introduction: Sorsby fundus dystrophy (SFD) is a rare autosomal dominant macular dystrophy which may lead to bilateral loss of central vision in the fourth to fifth decade of life. While it is known that SFD is caused by mutations in the *TIMP3* gene encoding the tissue inhibitor of metalloproteinases 3 (TIMP3) protein, the pathomechanism of SFD still remains elusive. To gain a further understanding of SFD pathobiology, we generated in an *in vitro* model of patient derived retinal pigment epithelium (RPE) cells.

Methods: Peripheral blood mononuclear cells (PBMCs) were obtained from three individuals who were diagnosed clinically and genetically with SFD. PBMCs were reprogrammed to induced pluripotent stem cells (iPSC) and differentiated into RPE cells. SFD-patient derived RPE cells as well as three control cell lines not showing pathogenic variants in *TIMP3* were characterized by immunocytochemistry, Western blot analyses and quantitative reverse transcriptase (qRT) PCR.

Results: We generated SFD-patient derived RPE cell lines harboring the following pathologic variants in *TIMP3*: NM_000362.5(TIMP3):c.92C>G, p.(P31R), NM_000362.5(TIMP3):c.484 G>A, p.(E162K), and NM_000362.5(TIMP3):c.545A>G, p.(Y182C). After six weeks in culture *TIMP3* mRNA expression, intracellular as well as extracellular matrix deposited TIMP3 was analyzed although no differences were seen between SDF lines and controls. TIMP3 protein from the p.(P31R) line showed an aberrant pattern in Western blot analyses, indicating that this variant may influence posttranslational processing of the protein. Under basal culture conditions SFD patient derived RPE cells showed slightly but statistically significantly higher mRNA expression of oxidative stress response genes *SOD2* and *NOQ1*.

Conclusion: Our iPSC-RPE cell lines from three SFD patients can be used to shed further light on the molecular pathology of this disease. Given the different genetic variants represented in the cell lines including the newly described TIMP3-P31R variant, it will help to uncover both, pathological features shared between SFD patients, as well as genotype-phenotype correlations.

Abstract ID: 254

P7-3

aRgus: multilayer visualization of non-synonymous SNVs & pathogenicity score modeling for enhanced vulnerability assessment

J. Schröter[1], T. Dattner[1], J. Hüllein[2], A. Jayme[3], V. Heuveline[3], G. Hoffmann[1], S. Kölker[1], D. Lenz[1], T. Opladen[1], B. Popp[4], C. Schaaf[5], C. Stafuner[1], S. Uhrig[2], D. Hübschmann[2], H. Brennenstuhl[5]

[1]Universitätsklinikum Heidelberg, Zentrum für Kinder- und Jugendmedizin, Heidelberg, Deutschland, [2]Nationales Zentrum für Tumorerkrankungen, Computational Oncology, Molecular Precision Oncology Program, Heidelberg, Deutschland, [3]Interdisciplinary Center for Scientific Computing, Engineering Mathematics and Computing Lab (EMCL), Heidelberg, Deutschland, [4]Univerität Leipzig, Institut für Humangenetik, Leipzig, Deutschland, [5]Universität Heidelberg, Institut für Humangenetik, Heidelberg, Deutschland

Introduction: Increased application of high-throughput sequencing methods is leading identification of variants in disease-associated and candiate genes reported to public databases. Automated visualization of these data can contribute to understanding and interpretation of biological relevance to human disease. However, this often requires advanced bioinformatics skills.

Methods: Here, we present aRgus, an R/Shiny based webtool for user-friendly exploitation of multiple genetic and protein related databases. aRgus enables intuitive integration and visualization of multi-layered variant data from databases including Ensembl, Simple ClinVar, UniProt, gnomAD, and dbNSFP. Mathematical modeling of *in-silico* pathogenicity scores allows global assessment of protein regions with high susceptibility to pathogenic variants.

Results: aRgus automatically determines a MANE canonical transcript of a *gene-of-interest* and generates six independent plot types:

1) an unspliced transcript plot showing chromosomal position of variants,
2) a protein plot revealing domain/region association,
3) and 4) mutational constraint plots of pathogenic and likely pathogenic, as well as gnomAD variants,
5) mathematical modeling of *in-silico* scores of all biologically possible variants, and
6) group-wise statistical comparison of such.

In addition, aRgus provides a tabular view of all variants stored in Simple ClinVar and visualizes all biologically possible SNPs with associated pathogenicity scores with color-coded individual thresholds in tabular form for quick assessment of overall pathogenicity.

Conclusion: aRgus enables assessment of pathogenicity of individual genetic alterations leading to amino acid substitutions in correlation to their regional distribution in a corresponding protein up to single amino acid resolution. The application of aRgus helped to improve variant and genetic understanding in multiple disease studies considering inborn errors of metabolism, neurometabolic diseases, as well as genetic epilepsies. In addition, aRgus provides a new method for target-identification for *in-vitro* knock out experiments by enabling identification of protein regions with computationally predicted susceptibility to biological relevant SNPs. Therefore, aRgus enables an improved and holistic understanding of previously poorly described and studied proteins involved in the development of diseases and holds potential for widespread use due to its extensive user-friendliness.

Abstract ID: 255

Deep learning-assisted selection of regulatory variants for high-throughput experimental testing

M. Schubach[1], P.M. Dash[1], C. Deng[2,3], J. Shendure[4,5], N. Ahituv[2,3], M. Kircher[1,6]

[1]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Deutschland, [2]University of California San Francisco, Department of Bioengineering and Therapeutic Sciences, San Francisco, Vereinigte Staaten, [3]University of California San Francisco, Institute for Human Genetics, San Francisco, Vereinigte Staaten, [4]University of Washington, Department of Genome Sciences, Seattle, Vereinigte Staaten, [5]Howard Hughes Medical Institute, Seattle, Vereinigte Staaten, [6]University Medical Center Schleswig-Holstein, Institute of Human Genetics, Lübeck, Deutschland

Despite large efforts to collect and curate genetic variants, clinical assertions (i.e., benign or pathogenic) are only available for a minority of known variants, especially in the non-coding genome. The NIH/NHGRI Impact of Genomic Variation on Function (IGVF) consortium was initiated in 2021 with the aim to understand how coding and non-coding variation affects genome function and subsequently phenotype. As one of the IGVF Characterization Centers, we will measure activities of at least a million regulatory variants or regions using high-throughput assays such as Massive Parallel Reporter Assays (MPRAs), CRISPRi/a, and Perturb-seq by 2026.
While most effects will be tested in cardiomyocytes and neurons, derived from an iPSC line, in this initial experiment we are testing >170,000 promoter proximal regulatory variants using MPRA in WTC11, HepG2 and K562 cells. The dataset will provide an unbiased reference for machine learning and overcome the shortage of quantitative effect readouts, limited to specific loci or standing variation. For this purpose, we built a library testing a deep learning derived set of possible proximal promoter variants. We selected variants predicted to have high/low tissue-specific or agnostic effects, by a convolutional neural network (CNN), trained on active and inactive open chromatin from multiple cell types. Benchmarking our model, we confirmed that the predicted effects of rare variants are significantly higher than those of common variants as well as variants in random regions. Furthermore, it outperforms methods in predicting saturation mutagenesis data. Using CNN interpretation techniques, we identified critical regulatory mechanisms, like key motifs in cell-type-specific transcription.
Initial HepG2 results show a library complexity of >40 million MPRA constructs. Sequencing over a billion DNA and RNA counts, we observe an average of >50 barcodes per variant allele and high reproducibility between three replicates. First analyses confirm the CNN-defined high and low effect variant groups. We anticipate that the experiments in WTC11 and K562 cells will provide insights into cell type-specific effects. We conclude that CNNs have a high potential in predicting regulatory effects, a critical step forward in genome-wide interpretation of genetic variants. This and other IGVF datasets will provide important, large, and unbiased readouts of genomic variants, enabling the assessment and further development of respective models.

Abstract ID: 256

P2-18

An inherited common PTPN11 variant associated with neuroblastoma but without characteristic features of Noonan syndrome

J. Hoyer[1], S. Schuhmann[1], C. Kraus[1], G. Fecker[2], M. Metzler[2], A. Reis[1]

[1]Humangenetisches Institut, Erlangen, Deutschland, [2]Kinder- und Jugendklinik, Erlangen, Deutschland

Neuroblastoma, particularly affecting children up to age 5, may arise in the adrenal medulla or along the paraspinal sympathetic ganglia. The majority of neuroblastomas occur sporadically, but some cases present inherited pathogenic variants in risk genes (PHOX2B, ALK, KIF1B). Various cancer predisposition syndromes also increase the risk of developing neuroblastoma (Noonan, Neurofibromatosis, Li-Fraumeni).

We report on a girl that developed intrapelvic neuroblastoma at the age of 2 years. She was treated with high dose polychemotherapy, MIGB therapy, isoretinoin, autologous stem cell transplantation and percutanous radiotherapy until the age of 4. At the age of 8 age years she developed papillary thyroid cancer which was most probably therapy related. She was referred to our genetic clinic at the age of 9 years and 5 months due to suspicion of a cancer predisposition syndrome. She attended a special needs school. Clinical manifestations included short stature (height < 1st centile), a short neck and a broad nasal tip but no organic defects. Exome sequencing revealed a variant c.1226G>C in exon 11 of *PTPN11* predicting the amino acid exchange p.(G409A). Though short stature was also present in her mother the variant was shown to be paternally inherited.

Noonan syndrome is the most common RASopathy, with a prevalence rate in livebirths of 1:1,000 to 1:2,500, half of them carrying a *PTPN11* mutation. More than 90 different mutations were described, so far. The variant identified has a relatively high gnomAD frequency = 1:7,812 (16 / 125,000). Due to this quite common prevalence and being inherited from an unaffected father, the variation was initially classified as VUS, in accordance with ClinVar annotation. Moreover the girl presented neither a heart defect nor Noonan dysmorphic facial features. Nevertheless, this variant was previously described as causing a milder, partial Noonan phenotype with variable short stature and without congenital heart defect in two families with five and three affected individuals, respectively. In the latter family, the index patient carried an additional Noonan-associated *SHOC2 de novo* pathogenic variant and was more severely affected. At age 1 month spinal neuroblastoma was diagnosed.

In summary we report the second case of neuroblastoma associated with this common *PTPN11* variant but without characteristic Noonan syndrome features, indicating that this variant might be an underdiagnosed cause of neuroblastoma.

Abstract ID: 257

2000 trio exome sequencing on research base: output and outlook

A. Golod[1], B. Büttner[1], A. Finck[1], C. Klöckner[1], J. Luppe[1], S. Martin[1], E. Rosenhahn[1], S. Schließke[1], L. Torban[1], M. Wegler[1], L. von Wintzingerode[1], J. Lemke[1], J. Hentschel[1], K. Platzer[1], R. Abou Jamra[1]

[1]*Institute of Human Genetics, Leipzig, Deutschland*

Introduction: When routine NGS is inconclusive and consent is available, we perform exome sequencing (ES) on a research basis to identify novel morbid genes for rare disorders. As we now move to genome sequencing, we summarise our experience with research exomes.
Material and Methods: Between 2017 and 2022, we performed NGS analyses in 9476 cases. Phenotypically, 4337 have neurodevelopmental disorders (NDD), and the remaining cases are distributed over 15 groups, e.g. neurology, growth, etc. Eighteen hundred cases consented to be included in research analysis to identify novel disease genes. Of these, 1550 were analysed as trio and 264 as single ES.
Sequencing was performed using standard methods. The software used was Varvis®. We evaluated the candidate genes using an automated scoring system (AutoCaSc). Eleven students have analysed the data in the context of their medical theses.
Results: In 689 cases, we have identified one candidate gene, in 405 cases two or more candidate genes, and in 706 cases we did not identify candidate genes. We uploaded 600 of the candidates to GeneMatcher. For 360 genes, we had contact with other groups. Of these, 45 were published with our contribution as novel disease genes, and 66 genes were funnelled into ongoing studies. *De novo* variants (581 out of 1746 candidates, 33%) were the easiest to be confirmed (33 of 45 published genes, 73%, reflecting the advantage of trio analyses), followed by homozygous variants (11 of 45, 24%). Almost all published cases have NDD, reflecting our research focus but also the easiness to identify the genetic causes of severe phenotypes. Thirty five, i.e. 78%, of the published genes were identified before 2020, signalling a slowdown in gene discovery using exomes and the need for different strategies and techniques.
Discussion: Unsolved cases from routine genetic diagnostics are still an underutilized area for research and rare disease gene discovery. We encourage all to take advantage of their own data or share it with research focused centres. We observe gaps in autosomal recessive as well as in rather mild (i.e. affected persons can reproduce) disorders. To close these gaps, further increase in the willingness to share data is needed. Moving to genomes would enable identifying novel causes such as variants outside the exons, repeat expansions, and structural variants.

Abstract ID: 258

P6-8

Functional analysis of potential splice site variants in craniosynostosis causing genes

A. Borst[1], D. Horn[2], T. Schweitzer[3], E. Kunstmann[1], N. Pluta[1], E. Klopocki[1]

[1]Institute of Human Genetics, University of Würzburg, Würzburg, Deutschland, [2]Institute for Medical and Human Genetics, Charité Universitätsmedizin Berlin, Berlin, Deutschland, [3]University Hospital of Würzburg, Department of Pediatric Neurosurgery, Würzburg, Deutschland

Variants in *TCF12*, encoding a bHLH transcription factor, and in *FGFR2 (Fibroblast growth factor receptor 2)* can cause craniosynostosis phenotypes in affected patients. In this type of congenital skull malformation, one or more cranial sutures are prematurely fused. Besides *TCF12* and *FGFR2*, mutations in a number of other genes can be causative for this rare disease. In some of these genes, splice site variants have already been described as causative for craniosynostosis. Splice site mutations are well-known to have a major impact on protein function in a broad range of genetic disorders. However, often only *in-silico* splice prediction tools are used to classify novel splice site variants. Further functional analyses of these novel variants are often lacking, due to the unavailability of patient-derived RNA or suitable primary cell cultures.
In this study we aim to functionally classify four potential *TCF12* and *FGFR2* splice site variants, using minigene splice and luciferase assays. To validate the *in-silico* predicted changes in the splicing process, an *in-vitro* minigene splice assay was performed. The assay was conducted in U-2 OS cells by transfection with a pSPL3b cam vector containing the respective *TCF12* or *FGFR2* variant carrying exon and flanking intronic regions. We were able to detect aberrant splicing of the respective genes caused by all tested variants in previous analyses. Two variants led to skipping of the entire exon, while the other variants resulted in either the partial inclusion of the intronic sequence or the partial omission of an exon at the mRNA level.
TCF12 changes on mRNA level imply a frameshift and premature stop codon for two variants and an in-frame deletion for the other two variants on protein level. To analyse the transcriptional activities of the resulting aberrant TCF12 proteins we performed luciferase assays. These experiments revealed a significant reduction in the transcriptional activity of the truncated proteins compared to wildtype TCF12. Overall, we were able to validate the changes in the splicing product caused by the *TCF12* and *FGFR2* variants using an *in-vitro* minigene assay. Moreover, we could demonstrate a functional effect on the TCF12 transcription factor activity using a luciferase assay system. In conclusion, the combination of functional *in-vitro* assays allows to reliably classify three craniosynostosis associated *TCF12* variants as likely pathogenic.

Abstract ID: 259

P3-17

Using genome sequencing to diagnose unsolved cases exemplified by an atypical case of Rett syndrome

S. Schuhmann[1], G. Vasileiou[1], C. Thiel[1], A. Reis[1], Bavarian Genomes Network for Rare Diseases

[1]*Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg FAU, Erlangen, Deutschland*

Exome sequencing (ES) has become the first-tier diagnostic approach in diagnosing Mendelian disorders, especially those with high genetic heterogeneity, e.g. neurodevelopmental disorders. Despite huge improvements in recent years, still some 50% remain without a diagnosis. To identify this “missing heritability” genome sequencing (GS) is proposed as a second-tier approach in unsolved cases. The ongoing project Bavarian Genomes addresses this problem and aims at identifying causative genetic variants in unsolved individuals with rare disorders using GS in combination with various other genomic techniques and an interdisciplinary data interpretation approach.

We included in this project a six-year-old male individual from healthy non-consanguineous parents with severe developmental delay, inability to speak, unsteady gait, muscular hypotonia, microcephaly, short stature, cryptorchidism and cardiac arrhythmia. Chromosomal analysis showed a 47,XXY karyotype as an incidental finding. Trio ES and copy number variant (CNV) analysis of exome data did not reveal any pathogenic alteration. Performing GS we now identified a *de novo* 1.92 kb deletion spanning a part of exon 4 and the 3’ untranslated region of the *MECP2* gene, leading to a predicted loss of 235 coding basepairs (78 amino acids). This CNV was not identifiable in ES because of the partial nature of the deletion of this exon and the absence of untranslated sequences from the target. Intragenic deletions of *MECP2* often localized in exon 3 or 4 are encountered in 5-10% of Rett syndrome individuals. Because of the Klinefelter syndrome chromosomal constellation, the clinical manifestations of this male individual resembled that of the female Rett syndrome cases. Occurrence of atypical Rett syndrome in Klinefelter individuals is well described in the literature.

Our case highlights the benefits of GS in sequencing of regions not included in the exome target and in detection of small CNVs, missed by either chromosomal microarray analysis or ES. GS is thus superior to re-analysis of ES data, as evidenced by recent studies, which reported up to three times higher yield. A systematic evaluation of GS for diagnosing unsolved cases of rare disorders within the Bavarian Genomes Network is ongoing.

Abstract ID: 260

P2-10

Ongoing V(D)J recombination leads to structural aberrations in B-lymphoblastic leukemia/lymphoma with *IGH::IL3* fusion

D. Marx[1,2], S. Balachandran[2,1], L.J. Russell[3], K. Nebral[4], R. Panzer-Grümayer[4], N. Schub[5], M. Gramatzki[5], A. Caliebe[2], M. Spielmann[1], E.M. Murga Penas[2]

[1]Institute of Human Genetics, University Hospital Schleswig-Holstein/University of Luebeck, Lübeck, Deutschland, [2]Institute of Human Genetics, University Hospital Schleswig-Holstein/Christian-Albrechts University Kiel, Kiel, Deutschland, [3]Translational and Clinical Research Institute, Newcastle University, Newcastle-upon-Tyne, Vereinigtes Königreich, [4]St. Anna, Children´s Cancer Research Institute, Vienna, Österreich, [5]Division of Stem Cell Transplantation and Immunotherapy, 2nd Medical Department, University Hospital Schleswig-Holstein Campus Kiel/Christian-Albrechts University Kiel, Kiel, Deutschland

Introduction: Structural variants (SV) that drive leukemogenesis in ALLs are important prognostic factors. Some recurrent deletions in ALL, as *SLX4IP* and *IKZF1*, are mediated by a mechanism based on the RAG endonucleases. However, for the rare B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3)/*IL3::IGH* fusion, that is diagnosed under 1 % of all ALL, neither the molecular genetic characterization of SV nor the pathomechanisms leading to these SV have been completely elucidated. We report a molecular genetic analysis of SV in two cases of B-ALL with t(5;14)/*IL3::IGH*.

Methods: DNA from two cases with the diagnosis of a B-ALL with t(5;14)/ *IL3::IGH* was available for molecular analyses. MLPA for one case revealed deletions in *BTG1*, *CDKN2A/B*, *IKZF1* and *RB1*. Whole genome sequencing (WGS) was performed with NovaSeq6000 and data were analyzed using a pipeline developed by the Institute of Human Genetics (CAU University Kiel, Germany). Screening for cryptic recombination signal sequences (cRSS) was done manually and using the Boyer-Moore-horspool string search algorithm.

Results: WGS analyses detected 21 SV, all of them deletions, in 17 genes that are recurrently altered in ALLs, including all deletions detected by MLPA. A recurrent deletion encompassing exons 18 to 27 of *RB1* was seen in both cases. Deletions of *RB1* have not been described for the B-ALL with t(5;14)/ *IL3::IGH*, so far. Both cases also showed a deletion of *BLNK*, that was intronic in one case and spanned exons 1 and 2 in the other case. Further deletions targeted the *BTLA, BTG1, IKZF1, SLX4IP* and *ADD3* genes and occurred in breakpoint hotspots found in other ALL subtypes, indicating a shared mechanism. Since RAG recombination is a reported mediator of deletions in ALL, breakpoint regions were screened for cRSS, that could be identified at the breakpoints of 17 out of 21 deletions.

Conclusions: Deletions of the *RB1* and *BLNK* genes occurred recurrently in B-ALL with t(5;14)/ *IL3::IGH*. Additional deletions were found in 15 genes that are recurrently altered in other ALLs. Breakpoints of 17 deletions showed the hallmarks of RAG activity, indicating a mechanism driven by RAG1/2, that has been described for other ALL subtypes. Interestingly, deletions of *BLNK* have been reported to cause an ongoing V(D)J recombination by inhibition of a negative feedback and therefore might lead to a prolonged activity of RAG1/2. This could be implicated in the generation of the detected deletions in these two cases.

Abstract ID: 261

P7-5

Single-cell RNA phenotyping of a mouse model for hypothyroidism reveals a pivotal role of thyroid hormone receptor alpha for hypothalamic development

V. Sreenivasan[1], R. Dore[2], J. Resch[2], J. Maier[2], C. Dietrich[3,4], J. Henck[1,4], S. Balachandran[1,5], M. Spielmann[1,4,5]

[1]Universitätsklinikum Schleswig Holstein, University of Lübeck and University of Kiel, Institute of Human Genetics, Lübeck, Deutschland, [2]University of Lübeck and Universitätsklinikum Schleswig Holstein Campus Lübeck, Center of Brain Behavior and Metabolism (CBBM), Institute for Endocrinology and Diabetes, Lübeck, Deutschland, [3]Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Core Facility Genomics, Berlin, Deutschland, [4]Max Planck Institute for Molecular Genetics, Human Molecular Genomics Group, Berlin, Deutschland, [5]DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Lübeck/Kiel, Lübeck, Deutschland

Introduction: The thyroid hormone is a key regulator of physiological processes, including growth and brain development in early life. Congenital hypothyroidism due to genetic causes or due to shortage of iodine in the diet results in severe mental retardation. Of the two thyroid hormone receptor isoforms alpha and beta, TRα1 is the most relevant isoform for brain development and is present in almost all neurons. Mice harboring mutations in TRα1 display abnormalities in several autonomic functions, which was partially attributed to a developmental defect in hypothalamic parvalbumin neurons. However, whether other cell types in the hypothalamus are similarly affected remains unknown.
Aims: To use single-nucleus RNA sequencing to obtain an unbiased view on the importance of TRα1 for hypothalamic development, in terms of the cellular diversity and their gene expression profiles that cause mental retardation.
Methods: We studied the effect of hypothyroidism in mice heterozygous for the TRα1R384C mutation, which reduces the affinity of the receptor to the thyroid hormone by 10-fold.
Results: From a cell composition level, the single-cell sequencing data revealed that the mutation had a surprisingly little effect, with the cell numbers of all major cell types and neuronal subtypes unaffected by the receptor mutation. However, the data showed a skewed effect of the mutations on the transcriptome of the hypothalamic oligodendrocytes, with the misexpression of ~100 genes. Using selective reactivation of the mutant TRα1 during specific developmental periods, we found that early postnatal thyroid hormone action seems to be crucial for proper hypothalamic oligodendrocyte development.
Conclusions: Taken together, our findings underline the well-known importance of postnatal thyroid health for brain development and provide an unbiased roadmap for the identification of cellular targets of TRα1 action in mouse hypothalamic development.

Abstract ID: 262

P6-12

Functional analysis of novel ZRS enhancer variants associated with preaxial polysyndactyly

R. Hark[1], A. Borst[1], M.-T. Schnürer[1], G. Strobl-Wildemann[2], W. Klein[3], E. Klopocki[1]

[1]Institute of Human Genetics University of Würzburg, Würzburg, Deutschland, [2]MVZ Humangenetik Ulm GbR, Ulm, Deutschland, [3]Genetikum, Stuttgart, Deutschland

In recent years, long-range regulatory elements have increasingly come into focus, making it more evident that mutations in these regions can be disease-causing. Due to disrupted transcription factor binding sites in enhancer sequences such genetic variations can result in pathogenicity comparable to mutations in the corresponding regulated genes. One prominent element is the enhancer called ZRS (ZPA regulatory sequence), which is located in intron 5 of the *LMBR1* gene, approximately 1 Mb upstream of its target gene *SHH*. SHH is essential during vertebrate limb development and especially regulates anterior-posterior patterning in limb buds. Nucleotide substitutions or microduplications within or of the ZRS region have been reported to cause limb malformations, such as preaxial polysyndactyly (PPD). Until today, at least 18 different single nucleotide substitutions in the ZRS have been described to cause PPD.

The aim of this study was to analyze functional effects of two novel ZRS variants by applying luciferase assays. We initially identified these variants by Sanger sequencing in PPD patients and allocated the so far undescribed base substitutions in close proximity to the published Cuban ZRS variant (*LMBR1*: c.423+4917G>A; Lettice et al. 2003). Both heterozygous variants occurred *de novo* in the PPD patients. For the luciferase assays, the corresponding ZRS regions were cloned into the pGL4.27 luciferase vector. After transfecting the plasmids into HEK293T cells, luciferase activity was measured and compared to wild-type ZRS sequence to detect potential increased enhancer activity of the tested variants. This experiment indicated changes in luciferase activity for both unclassified variants compared to the wild-type ZRS. Moreover, one variant showed enhanced transcriptional activity levels comparable to the Cuban ZRS variant, which has already been classified as pathogenic. Our results, therefore, suggest that one novel ZRS variant is causing increased enhancer activity and presumably deregulation of *SHH* expression in the PPD patient. Furthermore, the luciferase assay results confirmed functional relevance and thus, allow us to reclassify one of the two variants as likely pathogenic.

Abstract ID: 263

P1-10

Functional characterization of microRNA-375-3p in triple-negative breast cancer

F. Hennig[1], P. Hengstenberg[1], A.-K. Schneider[1], T. Reinkens[1], V. Schäffer[1], M. Eilers[1], A. Stalke[1], B. Schlegelberger[1], T. Illig[1,2], B. Skawran[1]

[1]Hannover Medical School, Department of Human Genetics, Hannover, Deutschland, [2]Hannover Medical School, Hannover Unified Biobank (HUB), Hannover, Deutschland

Introduction: Breast cancer (BC) is the most common malignancy worldwide with the highest number of cancer-related deaths in women. Triple-negative breast cancer (TNBC) represents 15-20% of all BCs and is a subtype defined by a lack of expression of the estrogen receptor (ER), progesterone receptor (PR) and the human epidermal growth factor 2 (HER2). It is characterized by high aggressiveness, early onset and a poor prognosis.
Aberrant expression of microRNAs (miRs) plays a significant role in cancer. Several publications show a downregulation of hsa-miR-375-3p (miR-375) in different types of cancer. A link between this miR and tamoxifen resistance and response to neoadjuvant therapy in breast cancer has also been reported.
The aim of this study was to analyze the role of miR-375 in TNBC and its therapeutic potential.
Methods:TNBC cell lines were transiently transfected with both miR-375 mimics and siRNA. Targets of miR-375 were identified using mRNA sequencing and confirmed via Ago2-RNA immunoprecipitation (RIP) and luciferase reporter assay. Real-Time PCR and Western blotting were used to determine the expression of the gene of interest. Cell viability and apoptosis were measured with WST-1 proliferation reagent and Caspase 3/7 assay respectively. Transwell assay was used for cell migration.
Results: Public datasets show downregulation of miR-375 in TNBC. Treatment with miR-375 mimics inhibited cell proliferation and migration and promoted apoptosis in TNBC cell lines. mRNA sequencing of HCC1937 cells treated with miR-375 mimics showed significant downregulation of 1288 genes. After identification of genes with potential binding sites and a literature search on the oncogenic potential of these genes, C-terminal binding protein 2 (CTBP2) was selected for further analysis. Expression of CTBP2 was downregulated on mRNA and protein level after treatment with miR-375 mimics. Luciferase reporter assay showed a direct regulation of CTBP2 by miR-375. This direct binding was confirmed by Ago2-RIP. Knockdown of CTBP2 with siRNA showed no effect on cell proliferation and apoptosis, but suppressed migration.
Conclusion: miR-375 acts as a tumor suppressor by affecting cell proliferation, apoptosis and migration and regulates CTBP2 negatively. Knockdown of CTBP2 inhibits cell migration. CTBP2 is known to be involved in the TGF-β pathway. Whether there is a connection between miR-375, CTBP2 and the TGF-β pathway will be investigated in future experiments.

Abstract ID: 264

P5-13

double ring chromosome 2 mosaicism due to large interstitial deletion 2q32.1q36.1 in a boy with muscular hypotonia

F. Roessler[1], A.-C. Teichmann[1]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, University of Leipzig, Humangenetik, Leipzig, Deutschland*

Introduction: In our genetic diagnostics unit we received both Heparin and EDTA blood for genetic testing of a 1-year-old boy with considerable muscular hypotonia and motor developmental delay.
Methods: Testing for myotonic dystrophy, spinal muscular atrophy type 1, and Prader-Willi syndrome as well as exome sequencing for single nucleotide variants (SNV) remained inconclusive. We further performed karyotyping and genome-wide copy number variation (CNV) analysis based on exome sequencing data.
Results: CNV analysis revealed a 37 megabases large duplication of the chromosomal region 2q32.1q36.1 encompassing 218 genes. Based on ACMG guidelines we classified this CNV as pathogenic. A duplication of this size has not yet been detected in the general population. Smaller *de novo* duplications with a complete overlap have been reported as (likely) pathogenic and associated with muscular hypotonia, developmental delay, ventricular septal defects and hearing impairment (decipher database patients #363238 and #402186). Unexpectedly, chromosome analysis displayed a mosaic karyotype mos 48, XY, del (2)(q32q36), + r(2)(q32q36)x2[19]/47, XY, de l(2)(q32q36), + r(2)(q32q36)[24] representing a large deletion of chromosomal material on the long arm of chromosome 2, reappearing as a ring chromosome of the deleted segment 2q32 to q36. In approximately half of the analyzed cells, we detected two of these ring chromosomes in accordance with the observed duplication in CNV analysis. Segregation analysis in the boy’s parents is pending.
Conclusion: We assume that this large chromosomal variation is causative for the boy’s current symptoms. Retrospective phenotyping for further impairments appears indicated. Evaluating the future prognosis will be challenging for the lack of data on this particular genetic anomaly. To further characterize the ring chromosome, to check for formation of a neocentromer, and to define the exact break points, additional FisH analysis and genome sequencing are ongoing. This case illustrates the diagnostic limitations of exome based CNV analysis concerning mosaic and structural chromosomal anomalies. Despite the benefits of next generation sequencing it underlines that geneticists should still be able to detect, analyse and understand chromosomal abnormalities using cytogenetic methods.

Abstract ID: 265

P3-21

Prenatally detected complex mosaicism in a baby of uncertain sex

F. Stock[1], S. Ueberberg[1], J. Beygo[1], W. Trawicki[2], T. Liehr[3], F.J. Kaiser[1,4], A. Kuechler[1]

[1]Institut für Humangenetik, Universitätsklinikum Essen, Essen, Deutschland, [2]Institut für Labormedizin und Klinische Genetik, MVZ Rhein/Ruhr, Essen, Deutschland, [3]Institut für Humangenetik, Universitätsklinikum Jena, Jena, Deutschland, [4]Essener Zentrum für Seltene Erkrankungen (EZSE), Universitätsmedizin Essen, Essen, Deutschland

We present the case of a baby born with a complex chromosomal mosaicism consisting of three abnormal cell lines. A NIPT in early pregnancy had indicated a male foetus, yet ultrasonography showed indifferent genitalia and other abnormalities (increased nuchal translucency, mesomelic shortening of long bones, large hands and feet, a hypoplastic aortic arch, a Blake's pouch cyst, and oligohydramnios). After amniocentesis in the 30th week of gestation karyotyping revealed two cell lines: 57% of the cells contained a dicentric fusion chromosome consisting of the nearly entire Y chromosome and the entire p-arm of chromosome 12, 43% of the cells contained a single X chromosome and a marker chromosome including the centromere of chromosome 12. In the third trimester a large intraabdominal tumour (later diagnosed as juvenile granulosa cell tumour originating in the ovary) became evident, therefore the baby was born via caesarean section at 36^{+4} weeks. Birth measurements were within the normal range. The genitals seemed intersexual with clitoral enlargement and oedematous labia, womb and vagina were present. No testicles were palpable. Postnatal karyotyping from lymphocytes (I) and fibroblasts (II) each showed mosaicism with three similar cell lines, yet in a different distribution. The karyotypes 46,X,+mar (I: 86%, II: 43%) and 46,X,der (Y)t(Y;12) (I: 7%, II: 55%) appear in both cell types, the third cell line is 45,X (I, 7%) and 47,X,+mar,+mar (II, 2%) respectively. We specified these results by microarray using DNA from both cell types. Those findings revealed the combination of a male karyotype with a partial trisomy 12 (duplication of the entire p-arm) and a monosomy X (Turner-karyotype, predominantly female). On the last clinical assessment at the age of six months the baby's growth and motor development were considered appropriate for age, elevated FSH indicated beginning hypergonadotropic hypogonadism. The parents were informed about the uncertain prognosis concerning mental development and gender identity.

Abstract ID: 266

P3-67

Prenatal case report: modified whole exome sequencing revealed a homozygous mutation in a RNA-coding gene causing MOPD1

T. Richter[1], A. Pohle[1], M. Weber[1], K. Grudzus[1], D. Gläser[1], H. Meyberg[2]

[1]MVZ Genetikum GmbH, Neu-Ulm, Deutschland, [2]Praxis für pränatale Diagnostik und Therapie, Ludwigsburg, Deutschland

We report on a 26-year-old woman, which had a conspicuous fetal ultrasound with an intrauterine growth retardation, slow weight gain, a reduced amount of amniotic fluid, clubfoot on the left side and the right hand in fixed bending position in the 25th week of pregnancy. An amniocentesis took place and the subsequently performed conventional chromosome analysis and array-based comparative genomic hybridization could not explain the fetal ultrasound. Therefore, we performed a trio whole exome sequencing and identified in DNA from amniotic cells the homozygous mutation n.51G>A in the RNU4ATAC gene (transcript ENST00000580972). The parents were both heterozygous carriers of this mutation. The RNU4ATAC gene does not encode for a protein but for a RNA molecule involved in splicing processing. The already described mutation n.51G>A is located in a highly conserved domain (5`stem loop) in which homozygous mutations are known as the cause of an autosomal recessively inherited microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1, OMIM #210710). MOPD1 belongs to the rare diseases with so far approximately 50 cases reported worldwide. This disease is characterized with a severe intrauterine growth retardation, severe microcephaly, facial abnormalities, short extremities, arthrogryposis, oligohydramnios and brain anomalies. A fetal MRI was performed in the 30th week of pregnancy and showed an almost complete agenesis of the corpus callosum and a disturbance of gyration. Since the RNU4ATAC-based clinical phenotype is consistent with the abnormalities in the fetal ultrasound, we interpreted this mutation causative for the clinical symptomatic. Despite the poor clinical prognosis, the parents decided to give birth to the child. The girl was born in august 2021 in the 40th week of pregnancy by spontaneous delivery with a birth weight of 1428 g, a body length of 40.5 cm and a head circumference of 26 cm and was discharged into the care of her parents after 18 days.

In general, genetic diagnostics primarily focuses on protein coding genes. However, in the current case we were only able to identify the pathogenic mutation in the RNU4ATAC gene by using a customized whole exome sequencing kit with a spike-in of special RNA-coding regions. Prospectively, analysing not only protein coding regions, but also RNA-coding genes and non-coding regions will become more and more relevant.

Abstract ID: 267

P1-7

Characterizing isoform-specific activities of thyroid hormone receptor α

N. Härting[1], G.S. Hönes[2], F. Kilpert[1], J. Eckhold[1], J. Mittag[3], T. Gemoll[4], F.J. Kaiser[1]

[1]Institut für Humangenetik, Universitätsklinikum Essen, Universität Duisburg-Essen, Essen, Deutschland, [2]Klinik für Endokrinologie, Diabetologie und Stoffwechsel, Universitätsklinikum Essen, Universität Duisburg-Essen, Essen, Deutschland, [3]Institut für Endokrinologie und Diabetes, Universität zu Lübeck, Lübeck, Deutschland, [4]Sektion für Translationale Chirurgische Onkologie und Biomaterialbanken, Universität zu Lübeck, Lübeck, Deutschland

Thyroid hormone receptors function as hormone-regulated transcription factors that mediate the effect of thyroid hormones (TH) on target gene expression. Consequently, genetic variants within the *THRA* gene encoding the thyroid hormone receptor α (TRα) can result in resistance to thyroid hormones associated with bradycardia, skeletal dysplasia, constipation, dyspraxia and neurodevelopmental delay. While most pathogenic genetic variants within the *THRA* gene only affect the TRα1 protein isoform exerting the canonical thyroid hormone receptor function, the physiological relevance of variants affecting exclusively the TRα2 isoform, that does not bind thyroid hormones, remains elusive. As the physiological function of TRα2 is rather unknown, no functional consequences of genetic alterations in TRα2 have been described.
Functional studies to characterize specific roles for TRα2 have been hampered by the lack of reliable antibodies. Therefore, we added different fusion tags to the C-termini of both isoforms to allow specific detection and discrimination. Initially, ectopically expressed TRα1 and 2 protein complexes enriched by immunoprecipitation from HEK293 cell extracts were analyzed by mass spectrometry to obtain protein-binding profiles for both isoforms. By this, well-known binding partners of TRα1 were found, confirming the validity of the experimental approach. Moreover, novel shared as well as isoform-specific binding proteins have been identified indicating a specific TH-independent function for TRα2 in the regulation of gene expression. By subsequent validation experiments using different TRα1 and 2 protein fragments, we gained insights into homo- and heterodimerization properties and identified the C-terminal part of TRα2 as the main protein-binding domain. Currently, we are finalizing the generation of iPS cells expressing endogenously tagged TRα isoforms generated by a CRISPR/Cas9-based approach. IPSCs will be differentiated into selected cells types for further analyses to obtain isoform-specific protein-protein interaction profiles of the endogenous receptors complemented by genome-wide DNA binding profiles. Combining these data sets will give us new insights into tissue-specific activities of TRα2 and support the evaluation of genetic variants of unknown significance affecting the TRα2 isoform.

Abstract ID: 268

P6-27

Further evidence for an in-frame deletion in the *HP* gene to cause hepatopathy due to ectopic endoplasmic reticulum storage

A. Stalke[1,2], N. Buhl[1,2], B. Skawran[1], U. Baumann[2], E.-D. Pfister[2]

[1]Hannover Medical School, Department of Human Genetics, Hannover, Deutschland, [2]Hannover Medical School, Pediatric Gastroenterology and Hepatology, Hannover, Deutschland

Introduction: Haptoglobin (Hp) is a plasma protein mainly produced in the liver. Variants in the Haptoglobin-encoding *HP* gene can be associated with low Hp levels in serum (MIM#614081). Hepatic abnormalities are an atypical consequence of *HP* variants, and have been reported only for a single case[1] carrying a homozygous *HP* in-frame deletion. We report on two further children carrying the same homozygous variant as this initial case. This special variant presumably leads to ectopic Hp storage in the endoplasmic reticulum (ER) preventing the protein from being released to blood and is potentially responsible for the hepatic phenotype.

Methods: Whole exome sequencing data of patients with hepatopathies but without (likely) pathogenic variants in our diagnostic hepatopathy panels were evaluated for further potential genetic causes of their liver disease. Two cases with the same *HP* variant were pursued further and serum Hp levels were determined. A liver biopsy sample was investigated using electron microscopy (EM).

Results: In two patients (1 male), we detected the homozygous *HP* variant NM_005143.5:c.703_705delAAG p.(Lys235del) (gnomAD MAF: 0.013%), previously reported by Gunzer et al[1]. They have described a 3-year-old girl with undetectable Hp serum levels, elevated transaminases, bridging portal fibrosis, steatosis and enlarged ER cisterns in the liver. Histological findings had regressed at the age of 7 years except fibrosis. Similarly, our two patients showed undetectable Hp serum levels (<0.1g/L), elevated transaminases and steatosis in the course. Furthermore, the boy’s liver histology at the age of 2 years showed advanced liver fibrosis and strikingly wide ER cisterns in EM imaging. Fibrosis regressed at the age of 10 years. His heterozygous 18-year-old brother presented with elevated transaminases and vesicular ER structures in the EM imaging, but without further abnormalities. For the girl, liver biopsy at the age of 5 years showed no fibrosis. EM imaging has not been performed, so far.

Conclusion: Two further cases confirm the observation of Gunzer et al. [1] that the *HP* variant c.703_705delAAG is not only associated with anhaptoglobinemia, but also with a hepatic phenotype, likely due to Hp accumulation in the ER of hepatocytes. To validate this link, we intend to overexpress the mutant Hp protein in a liver cell line and to test for ER colocalization using immunofluorescence staining.

References: 1.Gunzer et al. PMID:34358398

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Abstract ID: 269

P4-7

Analysis of non-syndromic cleft lip with or without cleft palate GWAS candidate genes in single-cell expression data from human embryonic development

A. Siewert[1], S. Henne[1], J. Heggemann[1], S. Heilmann-Heimbach[1], E. Mangold[1], K.U. Ludwig[1]

[1]*Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Deutschland*

Non-syndromic cleft lip with or without cleft palate (nsCL/P) occurs due to molecular defects of craniofacial development, which takes place between the fourth and twelfth week post-conception. NsCL/P is one of the most common birth defects and has a multifactorial etiology. To date, more than 45 genetic risk loci have been identified by genome-wide association studies (GWAS) which harbor more than 400 positional candidate genes. However, the effector cell types are still mostly unknown. Recently, we established an analysis pipeline for nsCL/P candidate genes in single-cell RNA sequencing (scRNA-seq) data from embryonic mice (Siewert et al., submitted) and identified epithelial or mesenchymal-derived cell types as candidate effector cell types, with many genes showing expression in only a fraction of the respective murine cell types. Given the challenges in transferability of murine data to human development, we here pursue a more systematic approach using human single cell expression data and in-house GWAS summary statistics.

For this purpose, we will use a recently published method, single-cell disease relevance scores (scDRS; Zhang et al. 2022, Nat. Genet.). This method examines the joint gene expression of a set of genes in single cells and thus can potentially identify cell-type level heterogeneity and sub-cell types in which the candidate gene set is more strongly expressed. We will apply the scDRS method to a recently published scRNA-seq data set that was collected from human embryos at four to six weeks of development, including developing facial structures (Xu et al. 2021, bioRxiv). The results from this analysis will be presented at the conference and are expected to give more insights into potential gene networks and sub-cell types that are involved in craniofacial development and contribute to nsCL/P risk.

Abstract ID: 270

P3-68

De novo missense variant in *PBX1* in a patient with 46, XY gonadal dysgenesis

S. Ledig[1], J. Horvath[1], F. Tüttelmann[2]

[1]Universitätsklinikum Münster, Institut für Humangenetik, Münster, Deutschland, [2]Universitätsklinikum Münster, Institut für Reproduktionsgenetik, Münster, Deutschland

Disorders of gonadal development are very heterogeneous concerning the genetic causes. XY gonadal dysgenesis (XY-GD) is characterized by a failure of testis differentiation leading to streak gonads and female genitalia and can occur as an isolated form or as part of a complex syndrome. Chromosome aberrations as well as variants in several genes like *SRY*, *NR5A1*, *WT1*, *DMRT1*, *DHH*, *MAP3K1*, *SRD5A2*, *NR0B1* and *SOX9* altogether are causal for about 40-45% of XY gonadal dysgenesis.
Here we report on a 35 years old woman with XY gonadal dysgenesis, double-kidney on the left side, ectropion of the urethra and movement restriction of her arms. She carries the heterozygous missense variant c.332T>C p.(Met111Thr) in *PBX1* as identified by whole-exome analysis. Subsequent analysis of her parents revealed that this *PBX1* variant has arisen *de novo*. Heterozygous deletions and loss-of-function variants of *PBX1* are associated with phenotypically variable CAKUT (congenital anomalies of kidney and urinary tract) syndrome (OMIM# 617641), which among others is characterized by malformations of the kidneys and urethra, but can also be associated with ambiguous genitalia and a disturbed sexual development in karyotypically male patients.
Interestingly, two other cases with XY gonadal dysgenesis with further extragonadal malformations and a causative heterozygous missense variant p.(Arg235Gln) in *PBX1* have been published. Therefore, to the best of our knowledge, we report here the third case of XY gonadal dysgenesis and a causative *de novo* missense variant in *PBX1*, suggesting that missense variants in *PBX1* are a rare cause of XY gonadal dysgenesis and the gene should be considered when investigating such cases.

Abstract ID: 271

P3-2

Compound-heterozygous *GRIN2A* null variants associated with severe developmental and epileptic encephalopathy

V. Strehlow[1], C. Rieubland[2], S. Gallati[2], S. Kim[3], S.J. Myers[3], V. Peterson[3], A.J. Ramsey[4], D.D. Teuscher[3], S.F. Traynelis[3], J.R. Lemke[1]

[1]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Deutschland, [2]Division of Human Genetics, University Children's Hospital, Inselspital, Bern, Schweiz, [3]Emory University School of Medicine, Department of Pharmacology and Chemical Biology and Center for Functional Evaluation of Rare Variants, Atlanta, Georgia, Vereinigte Staaten, [4]University of Toronto, Department of Pharmacology and Toxicology, Toronto, Ontario, Kanada

Introduction: N-Methyl-D-aspartate receptors (NMDARs) are expressed throughout the brain, mediating excitatory neurotransmission important for development, learning, memory, and other higher cognitive functions. NMDARs are di- or triheterotetrameric ligand-gated ion channels composed of two glycine-binding GluN1 (encoded by *GRIN1*) and two glutamate-binding GluN2 subunits (encoded by *GRIN2A–D*). Four genes encoding NMDAR subunits (*GRIN1, GRIN2A, GRIN2B*, and *GRIN2D*) have been linked to human disease. *GRIN2A* heterozygous missense and null variants (e.g., truncating, frameshift, splice variants) have been associated with disease and are clearly depleted in the general population.
Methods: We collected phenotypic information on affected family members following a standard clinical questionnaire, which is used in the GRIN Registry (http://grin-portal.broadinstitute.org/). A venous blood sample of the index girl was analyzed by panel sequencing (TruSight One Sequencing Panel, Illumina) within routine diagnostic settings. Parental samples have been tested by conventional Sanger sequencing.
Genetic variants were functional analyzed by electrode voltage clamp recordings in Xenopus laevis oocytes and by measuring β-lactamase hydrolysis of nitrocefin in mammalian human embryonic kidney (HEK) cells.
Results: We report on an 8-year-old girl with severe developmental and epileptic encephalopathy due to the compound heterozygous null variants p.(Gln661*) and p.(Leu830Pro*fs**2) in *GRIN2A* resulting in a knockout of the human GluN2A subunit of the N-methyl-D-aspartate receptor. Both parents had less severe *GRIN2A*-related phenotypes and were heterozygous carriers of the respective null variant. Functional investigations of both variants suggested a loss-of-function effect.
Conclusion: This is the first description of an autosomal recessive, biallelic type of *GRIN2A*-related disorder. Nonetheless, there are marked parallels to two previously published families with severe epileptic encephalopathy due to homozygous null variants in *GRIN1* as well as various knockout animal models. Compared to heterozygous null variants, biallelic knockout of either GluN1 or GluN2A is associated with markedly more severe phenotypes in both humans and mice. Furthermore, recent findings enable a potential precision medicine approach targeting *GRIN*-related disorders due to null variants.

Abstract ID: 272

P2-19

Diagnostic yield and clinical relevance of expanded germline genetic testing for HBOC patients

J. Henkel[1], A. Laner[1,2], M. Locher[1,2], T. Wohlfrom[1], B. Neitzel[1], K. Becker[1,2], T. Neuhann[1,2], A. Abicht[1,2,3], V. Steinke-Lange[1,2], E. Holinski-Feder[1,4,2], N. Consortium[2]

[1]Medical Genetics Center (MGZ), Munich, Deutschland, [2]NASGE, Nationale Allianz für seltene genetische Erkrankungen, Hannover, Deutschland, [3]Friedrich-Baur-Institute, Department of Neurology, Munich, Deutschland, [4]Klinikum der Universität, Department of Medicine IV, Munich, Deutschland

As a first step, we report the results of a retrospective germline analysis of nearly 7,000 individuals suspicious of hereditary breast- and ovarian cancer (HBOC) according to the German AGO Guidelines carried out in one of the contributing NASGE (Nationale Allianze für Seltene Genetische Erkrankungen) center. Genetic testing was performed by next-generation sequencing using 123 cancer-associated genes based on the Illumina TruSight® Cancer Sequencing Panel. In about 20% of cases at least one variant was reported (ACMG/AMP classes 3-5). Of those 56.3% were ACMG class 4 or 5 and 43.7% were an ACMG class 3 (VUS). We defined a virtual 14 gene HBOC core gene panel and compared this to one national and different internationally recommended gene panels, German HBOC Consortium, ClinGen expert Panel, Genomics England PanelsApp. Diagnostic yield of pathogenic variants (class 4/5) varied from 7.8% to 11.6% depending on the panel evaluated, with the 14 gene Panel having a yield of 10.8 %. Furthermore, we evaluated a workflow for a periodic re-evaluation of variants of uncertain clinical significance (VUS), reclassifying app. 50% of all VUS into either benign or pathogenic. We also show data concerning the differential diagnosis of hereditary cancer syndromes not caused by pathogenic variants in the core panel genes as 123 tumor genes had been analysed.

As a next step, we incorporated app. 30,000 data sets analysed across different German diagnostic laboratories, which are part of the NASGE network. The NASGE aims to bundle competence in the diagnosis and care of people with rare genetic diseases and thereby improve the quality of care. In doing so, NASGE is a platform for the exchange of information, knowledge, experience and references. The resulting information is made available to both colleagues and patients in databases.

Abstract ID: 273

P6-31

Homozygous disruption of *PPM1D* induces defective DNA repair and genomic instability in a patient with syndromic microcephaly

G. Yigit[1,2], N. Rosin[1], S. Hasse-Kamping[1], A. Küchler[3], J. Altmüller[4,5,6], H. Thiele[4], P. Nürnberg[4], D. Wieczorek[7], B. Wollnik[1,2,8]

[1]Institut für Humangenetik, Universitätsmedizin Göttingen, Göttingen, Deutschland, [2]DZHK (German Center for Cardiovascular Research), partner site Göttingen, Göttingen, Deutschland, [3]Institut für Humangenetik, Universität Duisburg-Essen, Essen, Deutschland, [4]Cologne Center for Genomics (CCG), University of Cologne, Faculty of Medicine, University Hospital Cologne, Köln, Deutschland, [5]Berlin Institute of Health at Charité, Core Facility Genomics, Berlin, Deutschland, [6]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Deutschland, [7]Institut für Humangenetik, Universitätsklinikum Düsseldorf, Düsseldorf, Deutschland, [8]Cluster of Excellence Multiscale Bioimaging, from Molecular Machines to Networks of Excitable Cells (MBExC), University of Göttingen, Göttingen, Deutschland

Maintenance of DNA integrity is essential for accurate transmission of the genetic information from generation to generation. To ensure DNA integrity and deal with DNA lesions, mammalian cells have developed evolutionarily highly conserved DNA damage response (DDR) pathways to specifically identify DNA lesions, recruit transducers that initiate downstream signaling and activate effectors which regulate cell cycle progression, activate DDR repair pathway, or trigger apoptosis and cellular senescence. Mg2+/Mn2+ dependent protein phosphatase 1D (PPM1D) is a Ser/Thr protein phosphatase that plays an essential role as a regulator of DDR and cellular stress responses by, e.g., dephosphorylating and inhibiting tumor repressors including ATM, CHK1 and CHK2, and p53. Recently, *de novo* truncating variants in the last two exons of *PPM1D* have been identified as cause of an intellectual disability syndrome.

Using whole-exome sequencing, we identified a homozygous pathogenic variant, c.826+1G>A, in *PPM1D* in an affected individual with a severe syndromic phenotype characterized by facial dysmorphism, severe microcephaly, intellectual disability, short stature, seizures, and an immunological phenotype. We showed that this variant located at the donor splice site in intron 3 of *PPM1D* induces defective splicing of *PPM1D* mRNA, mainly resulting in mRNA instability and RNA decay and most likely inducing loss of PPM1D function. On a cellular level, we observed that PPM1D deficiency leads to hypersensitivity to DNA double-strand break (DSB)-inducing agents and defective DSB repair, whereas other DDR repair pathways like, e.g., nucleotide excision repair were not affected. This is in contrast to previous studies using cells derived from individuals with *de novo* heterozygous *PPM1D variants, which show* normal p53 activation and DDR after induction of DSBs. In conclusion, we show that bi-allelic loss-of-function variants in *PPM1D* cause a severe syndromic phenotype characterized by severe microcephaly, intellectual disability, short stature, seizures, and an immunological anomalies that differs markedly from the phenotype of patients with heterozygous *de novo* truncating variants the last two exons of *PPM1D*, thus providing a further example of a gene in which the type and the location of mutation and its functional effect determine the inheritance pattern and phenotypic outcome of the disease.

Abstract ID: 274

P3-69

Joubert syndrome in two siblings – novel truncating mutation

S. Weidensee[1], S. Riedel[2], C. Haase[3], M. Klaus[2], B. Eichhorn[2]

[1]MVZ Mitteldeutscher Praxisverbund Humangenetik GmbH, Erfurt, Deutschland, [2]MVZ Mitteldeutscher Praxisverbund Humangenetik GmbH, Dresden, Deutschland, [3]Helios Klinikum Erfurt, Sozialpädiatrisches Zentrum, Erfurt, Deutschland

Mutations in *CEP290* gene cause Joubert syndrome 5, a clinically and genetically heterogeneous ciliopathy, characterized by midbrain-hindbrain malformation especially molar tooth sign (MTS), psychomotor delay, hypotonia, ataxia, oculomotor apraxia, and neonatal breathing abnormalities. However, the phenotype within as well as among families is highly variable. Here, we report a girl with prenatally diagnosed cerebral malformation with encephalocele, Dandy-Walker malformation and artificial ventilation after birth. By the age of 22 months, she presented developmental delay, language delay and facial dysmorphism (dolichocephaly, frontal bossing, deep-set eyes, depressed nasal bridge, low-set ears).

Her younger sister prenatally showed an enlargement of the brain cavities, but otherwise uncomplicated development until birth and normal postpartum adjustment. After initial diagnosis of nystagmus with lateral head movement of the new born by the paediatrician, a central coordination disorder was developed. Cerebral MRI shows optic nerve hypoplasia and hypoplasia as well as dysplasia of vermis cerebelli, suggesting molar tooth sign.

At the age of 7 months, she also showed facial dysmorphism with telecanthus, bilateral epicanthus, low-set ears and strabism.

After chromosome and Array CGH analysis were found to be inconspicuous, an exome analysis for both siblings in comparison with the parents was performed.

Both children carried the well-described mutation c.1936C>T / p.(Gln646*) in the *CEP290* gene (NM_025114.4) causing, in homozygous or compound heterozygous state, a Joubert syndrome. Additionally, they harbour the previously undescribed variant c.6170_6173del / p.(Arg2057Asnfs*9). The compound heterozygosity of these both variants was confirmed by the parental genotype. The molecular evidence for a Joubert syndrome is in accordance with the clinical symptoms, despite the Dandy-Walker malformation of the older girl, which may be masking the molar tooth sign.

There is a strong evidence for the disease causing effect of the unknown mutation in the *CEP290* gene, due to the confirmation of the compound heterozygosity in the two siblings together with characteristic clinical symptoms of Joubert syndrome.

Abstract ID: 275

P6-28

Description of a homozygous deletion in *COL7A1* in three non-related families

K. Echle[1], S. Alter[1], A. Hotz[1], J. Kopp[1], C. Has[2], D. Kiritsi[2], J. Fischer[1]

[1]Universitätsklinikum Freiburg, Institut für Humangenetik, Freiburg im Breisgau, Deutschland, [2]Universitätsklinikum Freiburg, Klinik für Dermatologie und Venerologie, Freiburg im Breisgau, Deutschland

Introduction: Dystrophic Epidermolysis bullosa (DEB) is a rare skin-disease characterized by blistering and scarring of the skin and mucous membranes in response to mechanical forces, which is caused by pathogenic alterations (SNP or CNV) of the Collagen Type-VII gene (*COL7A1*, OMIM *120120). Type VII collagen is the main constituent of anchoring fibrils located in the skin below the basal lamina that provide stable dermal-epidermal adhesion. DEB can be inherited in an autosomal dominant (DDEB, OMIM *131750) or autosomal recessive (RDEB, OMIM *226600) manner, depending on the type and position of the alteration within the *COL7A1* gene which results in a highly heterogenic phenotype of the disease (and clinical features).
Methods and Results: Here we describe three non-related patients with identical, homozygous deletions of exons 13-24 in *COL7A1*. Data from Next Generation Sequencing as well as a breakpoint analysis of *COL7A1* was utilized to exactly determine the position and length of the observed deletion. In addition, MLPA was performed to further confirm the homozygosity of the deletion.
Conclusion: Breakpoint analysis revealed the exact position of the breakpoint within Intron 12 of *COL7A1*, comprising a total of 4,063 deleted basepairs (c.1637-240_3255del4063) resulting in premature stop codon 29 amino acids downstream of the composed sequence of Exons 12/25.

Abstract ID: 276

P6-7

Detailed analysis of alpl/Tnap function in zebrafish and establishment of a new *in vivo* model constituting the rare disease hypophosphatasia

R. Hark[1], A. Borst[1], B. Ohlebusch[2,1], E. Riekert[1], K. Marnet[3,1], F. Jakob[3], E. Klopocki[1], S. Graser[3], D. Liedtke[1]

[1]Institute for Human Genetics, University of Würzburg, Würzburg, Deutschland, [2]Zoological Institute, University of Cologne, Köln, Deutschland, [3]Bernhard-Heine-Centrum für Bewegungsforschung, Lehrstuhl für Regeneration Muskuloskelettaler Gewebe, University of Würzburg, Würzburg, Deutschland

Hypophosphatasia (HPP) is a rare, hereditary disease caused by mutations in the *ALPL* gene coding for the ectoenzyme tissue-nonspecific alkaline phosphatase (TNAP). The different HPP manifestations, ranging from perinatal lethal forms to Odontohypophosphatasia, prominently display severe defects during dental and bone mineralization. Apart from these, the disease can sporadically cause craniosynostosis and neurological disorders like epileptic seizures, anxiety, and depression. Currently, cell culture and mouse models for experimental investigation of HPP symptoms are deployed, but to date lack affirmation in a non-mammalian species. Our work aims to establish the zebrafish (*Danio rerio*) as auxiliary *in vivo* vertebrate model system for detailed functional investigations of Tnap and visualization of tissue-specific effects of HPP.
We identified a single gene homologue corresponding to *ALPL* in the zebrafish genome (*alpl*) and conducted detailed expression analyses at different developmental stages by whole mount *in situ* hybridization (ISH), qPCR, and combined ISH-immunostaining. Zebrafish embryos showed prominent *alpl* expression in distinct brain regions, nephros, retina, tooth buds, and fins. In the adult zebrafish brain, *alpl* mRNA and Tnap activity were detected in restricted regions, like the amygdala, an organ which is linked to anxiety disorders in humans. Additionally, *alpl* expression was observed in proliferating areas of adult neurogenesis and implying a potential function within neural stem cells. Further functional experiments utilizing zebrafish embryos showed that inhibition of Tnap function results in consistent developmental alterations and these display prominent mineralization and neural effects. Interestingly, sensory neurons and their regeneration are affected after reduction of Tnap function. Finally, we developed zebrafish transgenic lines to investigate different HPP symptoms, by either a general or a tissue-restricted knockdown or overexpression of different human *ALPL* variants. Preliminary results from these lines imply that the zebrafish is a useful option to model HPP, to analyze molecular consequences of reduced Tnap function, and to visualize altered developmental processes in different tissues. The establishment of these molecular techniques in zebrafish paves the way for future tissue-specific investigations and consequently helps providing new molecular insights into functional consequences of HPP-causing *ALPL* mutations *in vivo*.

Abstract ID: 277

P3-70

KaRhab – a registry for cardiac rhabdomyomas

V.-M. Herrmann[1], J. Herrmann[1], R. Wagner[2], B. Wannenmacher[3], M. Weidenbach[3], V. Strehlow[1]

[1]Institut für Humangenetik des Universitätsklinikums Leipzig, Leipzig, Deutschland, [2]Praxis Dr. Robert Wagner, Leipzig, Deutschland, [3]Abteilung für Kinderkardiologie des Herzzentrums Leipzig, Leipzig, Deutschland

Cardiac rhabdomyoma (RHM) is considered as one of the most common benign heart tumors in the pediatric field. Nevertheless, due to the low prevalence of cardiac tumors in childhood in general, the experience with this disease entity in clinical practice remains low. In most cases, cardiac RHM are associated with Tuberous Sclerosis Complex (TSC), a genetic disorder caused by pathogenic variants in *TSC1* or *TSC2*. Because cardiac RHM are characterized by distinctive sonographic findings that may be apparent before birth, they may even be the first indication of TSC and not infrequently lead to the initiation of prenatal genetic testing. Moreover, the growing understanding of the regulatory role of *TSC1* and *-2* gene products in the mTOR pathway has recently led to the focus on mTOR as a therapeutic target not only in TSC but also in cardiac RHM. Nevertheless, information on cardiac RHM is available only in the form of single case reports, whereas large-scale studies with high case numbers are lacking.

To address this need, we established a registry for patients with cardiac RHM to collect both clinical and genetic data. Its user-friendly design allows both physicians and patients or their relatives to answer the questionnaire online with the help of the digital tool REDCap. At this time, the questionnaire is available in German, English and French. Inquiries include the characteristics of the presenting cardiac RHM and their induced clinical symptoms, further course, necessary treatment interventions, genetic diagnostics and other aspects of a possibly associated TSC. Because the study design allows for follow-up contact, it also ensures longitudinal annual data collection.

With the help of the resulting register an overview of time course, symptomatology and outcomes of different treatment strategies or genetic diagnostics in patients with cardiac RHM will be obtained. With the aim of achieving a sufficiently large group size we will be able to gain comparative and objectified knowledge on cardiac RHM.

In the absence of large-scale studies, it is challenging to provide an overview on cardiac RHM. This registry aims not only to fill the gap of a concise characterization of cardiac RHM but also to facilitate information sharing between clinicians, geneticists, patients and caregivers. Beyond that, it will be also possible to establish a multinational cohort that can serve as a basis for future research.

Abstract ID: 278

P2-20

Monitoring cancer treatment response with two different NGS-based strategies

B. Küchler[1], A. Linge[2,3,4,5], M. Krause[2,3,4,5], E. Troost[2,3,4,5], J. Wilk[1], M. Friedemann[6], M. Menschikowski[6], J. Plaschke[1]

[1]Medical and Scientific Genetic Analyses Dresden (MEWIGEN), Dresden, Deutschland, [2]German Cancer Research Center (DKFZ), Heidelberg, Deutschland, [3]German Cancer Consortium (DKTK), partner site Dresden, Dresden, Deutschland, [4]Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Department of Radiation Therapy and Radiation Oncology, Dresden, Deutschland, [5]OncoRay – National Center for Radiation Research in Oncology, Dresden, Deutschland, [6]University Hospital Carl Gustav Carus, Technische Universität, Dresden, Institute of Clinical Chemistry and Laboratory Medicine (IKL), Dresden, Deutschland

Head and neck squamous cell carcinoma (HNSCC) are aggressive, genetically complex and difficult-to-treat. To date, surgery and radiation therapy (RT, often with chemotherapy, CRT) are the primary treatment options. However, these treatments are associated with significant morbidity and compromised quality of life. Resistance to RT represents one of the factors for poor loco-regional control (LRC) rates in a subset of patients with locally advanced HNSCC. Sequencing projects have identified mutations in many genes, but studies correlating alterations with clinical outcome are scarce and often contradictory. Similarly, for stage II or III rectal cancer, it is recommended to perform surgery after neoadjuvant CRT (nCRT, outside of clinical studies). However, approximately ¼ of patients who receive nCRT achieve a pathologic complete response and may be eligible for less radical surgery or even a non-surgical treatment. Specific mutations that allow a stratification according to the expected response to RT could not be established yet.

We pursued two different strategies to evaluate DNA mutations as indicators of RT efficiency. For HNSCC, a panel of 96 genes and 209 repetitive regions was analysed by Next Generation sequencing (NGS) in the tumour DNA of 77 patients. Identified mutations were evaluated for an association with HPV status, hypoxia-associated gene signatures and clinical features including overall survival (OS), LRC and the occurrence of distant metastases (DM). So far, we found that mutations in the genes *TP53* and *NOTCH1* as well as *NOTCH3* and two histone methyltransferase genes tend to have an impact on the OS. For *TP53,* we found that the outcome depends on the type of gene alterations present. Here, we also found a correlation with the degree of tumour hypoxia and the occurrence of DM. To assess the treatment efficiency in RC, we analysed a panel of 13 genes or hotspot mutations in circulating tumour DNA by NGS in 16 selected patients for which samples of different time points during the course of disease/treatment had been available. Although, the tumour recurrence could partially be predicted by the persistence of specific mutations mainly in the *TP53* gene, a single mutations alone is not sufficient to judge therapy response in each case. Such mutations may have an additional value if analysed together with other prognostic factors. This work was funded by the Federal Ministry of Education and Research (BMBF) as part of the PRÆMED.BIO project.

Abstract ID: 279

P3-18

Novel de novo *POLR3B* variant responsible for demyelinating Charcot-Marie-Tooth disease

S. Hütter[1], A.-S. Onuk[1], G. Pommer[1], J. Porrmann[1], E. Schröck[1,2], K. Hackmann[1]

[1]Institute for Clinical Genetics, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Deutschland, [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Deutschland

The second-largest subunit of RNA polymerase III is encoded by *POLR3B.* Heterozygous pathogenic missense variants in *POLR3B* have recently been identified to cause autosomal-dominant demyelinating Charcot-Marie-Tooth disease type 1I (CMT1I, OMIM # 619742), a disorder significantly different to the previously described hypomyelinating leukodystrophy type 8 (OMIM # 614381), which is caused by biallelic pathogenic variants in the same gene. Demyelinating CMT1I is associated with gait disturbances and a varying range of intellectual disability. Additional symptoms such as demyelinating sensorimotor peripheral neuropathy, pyramidal signs, seizures, spasticity and tremor can be present. To our knowledge, nine unrelated individuals have been described with *de novo* heterozygous missense variants in the *POLR3B* gene.
We report the case of a ten-year-old girl who presented with moderate intellectual disability, behavioral abnormality, white matter abnormalities and gait disturbance. Because of an increased CSF opening pressure, a pseudotumor cerebri has been diagnosed and she receives a therapy with acetazolamide. By trio exome sequencing, a novel *de novo* heterozygous likely pathogenic missense variant in *POLR3B* NM_018082.6:c.1304G>T, p.(Gly435Val) was identified, compatible with the phenotype of the patient. This adds a new variant to the genetic spectrum of autosomal-dominant demyelinating CMT1I. In addition, our patient has an increased CSF opening pressure, which has not been reported in formerly described cases. It is unclear whether this symptom is related to demyelinating CMT1I or occurred independently.

Abstract ID: 280

P3-71

NXN-related Robinow-syndrome – expansion of the clinical and genetic spectrum

E. Schreiner[1], J. Blatterer[1], H. Tichy[1], S. Bierbaumer[1], S. Hammer[1], A. Schwerin-Nagel[2], M. Artl[1], K. Wagner[1], M. Speicher[1], S. Verheyen[1]

[1]D&F Institut für Humangenetik Graz, Graz, Österreich, [2]Kinderklinik Graz, Neuropädiatrie, Graz, Österreich

Robinow Syndrome (RS), also referred to as *fetal face syndrome* due to its characteristic craniofacial features, was described in 1969 by Meinhard Robinow. Causative variants in six genes involved in the noncanonical WNT-pathway have been reported, following autosomal dominant (*WNT5A, FZD2, DVL1, DVL3*) and autosomal recessive (*ROR2, NXN*) inheritance. *NXN*-encoded Nucleoredoxin acts as a negative regulator in this pathway. So far, four patients in three families carrying biallelic loss-of-function variants in *NXN* have been reported. We present the fifth case of RS due to a homozygous *NXN*-variant in a male child of a consanguineous family.

The genetic evaluation was initiated after birth due to omphalocele, incomplete duplex kidney, valvular pulmonary stenosis, atrial septal defect, micropenis, and testicular atrophy. Karyotype and array analysis showed normal results. Further clinical evaluation at the age of two years showed short stature, a high forehead, microcephaly, prominent eyes, long philtrum, triangular mouth, and gingival hyperplasia. Moreover, developmental delay with an emphasis on speech development was noted. Phenotype-based exome analysis revealed a homozygous missense variant [NM_022463.5:c.787C>T, p.(Arg263Trp)] which leads to an exchange of a highly conserved arginine to tryptophan in the Thioredoxin-domain. In terms of physical and chemical properties, arginine differs strongly from tryptophan. *In silico* analyses indicate a negative impact on protein function. The variant has not been described in the literature or mutation databases. Segregation analysis revealed a heterozygous carrier status in both parents, the healthy older sister did not carry the variant. However, applying the ACMG criteria, the variant could only be classified as a variant of unknown significance (VUS).

The patient's phenotype is consistent with specific, previously reported clinical features of RS. However, unlike the other four patients with *NXN*-related RS, our patient does not have (relative) macrocephaly but microcephaly. Still, based on the high phenotypic similarity and segregation data in the family, we believe that the identified variant is the underlying cause. However, functional studies are necessary to prove this hypothesis. Our findings support the implication of *NXN* as an RS-associated gene and potentially expand the mutational and phenotypic spectrum in *NXN*-related RS.

Abstract ID: 281

P2-7

Liquid Biopsy in a clinical setting - evidence from real world data

S. Biskup[1], M. Schulze[1]

[1]Zentrum für Humangenetik und CeGaT Tübingen, Tübingen, Deutschland

In a routine clinical setting we sequenced 450 cell free DNA (cf-DNA) samples from patients with >40 different tumor entities using panel- or whole exome sequencing and tumor/normal comparison. Up to date, we detected somatic variants in 176 of all samples analysed (41,7% positive rate). This number is surprisingly high. The tumor content within the cf-DNA ranged from 5-80% based on somatic variant frequency. The 10 most frequent tumor entities in our cohort cover 76% (320 samples) of all samples ranging from breast cancer (83 samples) to glioma (11 samples). We were able to detect a significant number of somatic variants for which an approved therapeutic option (FDA/EMA approved) exists (% will be udated on time of presentation).

In conclusion we will provide evidence for the use of cell free DNA sequencing for patients with advanced cancer presentated to molecular tumor boards. Cell free DNA sequencing has huge advantages for patients as it is minimal invasive (a blood draw only) but the clinical relevance has not been shown so far.

Abstract ID: 282

P3-72

An inherited inversion detected by exome sequencing causes ATP6V0A2 related cutis laxa

L. Segebrecht[1,2], P. Bahena[1], M. Jäger[1,2], S. Mundlos[1,3], C. Thiel[4], D. Seelow[1,5], U. Kornak[1,6], B. Fischer-Zirnsak[1,3]

[1]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Medical Genetics and Human Genetics, Berlin, Deutschland, [2]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Deutschland, [3]Max Planck Institute for Molecular Genetics, FG Development & Disease, Berlin, Deutschland, [4]University Hospital Heidelberg, Department 1, Center for Child and Adolescent Medicine, Heidelberg, Deutschland, [5]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Exploratory Diagnostic Sciences, Berlin, Deutschland, [6]University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Deutschland

Autosomal recessive cutis laxa type 2A (ARCL2A) is a rare connective tissue disorder characterized by congenital lax and wrinkled skin, osteopenia, seizures, and intellectual disability of various degrees. This disease is due to pathogenic variants in *ATP6V0A2*, encoding the V-type H+ ATPase subunit a2. Biochemically, this condition is characterized by a combined N- and O-linked glycosylation defect (CDG type II). Here we report on a 3-year-old female individual from Mexico presenting with cutis laxa, joint hypermobility, facial dysmorphism, and global developmental delay. Isoelectric focusing and LC-MS analysis of serum proteins showed abnormal glycans indicating CDG-II, which led to the clinical diagnosis of ARCL2A. We performed Exome Sequencing and found no causative variant in the suspected gene using our standard pipeline. However, closer inspection of the *ATP6V0A2* locus with the IGV revealed reads with a wrong orientation in exon 19, which had their mate pair approximately 97kb upstream of the *ATP6V0A2* locus in intron 35 of *DNAH10* and vice versa. Using breakpoint-specific PCR and quantitative PCR we verified an inversion of ~97 kb, a short deletion of 71 bp and an insertion of 3bp in a homozygous state in the affected individual. In the clinically unaffected mother, we found this event in a heterozygous state. The predicted consequence for the a2 subunit of the V-type H^+ATPase was premature termination: p.(L788*). For DNAH10, in which homozygous loss-of-function pathogenic variants have recently been described as the cause of spermatogenic failure in men, we predict nonsense mediated decay due to loss of 44 of 79 exons.

In conclusion, we found, to our knowledge for the first time, an inversion affecting the *ATP6V0A2* locus and thereby we expand the clinical and molecular spectrum of ARCL2A. In addition, these data suggest that structural variants with at least one breakpoint within the enriched region can be detected in Exome Sequencing and other enrichment-based NGS data.

Abstract ID: 284

P3-3

SysNDD is an expert-curated map of neurodevelopmental disorders to study gene disease connections

B. Popp[1,2], H. Van Esch[3], T. Kleefstra[4], F. Mari[5], M. Amin[6], S. Caraffi[7], Z. Tümer[8,9], F. Altay[10], A. Schenck[11], A. Verloes[12], C. Zweier[10]

[1]Institute of Human Genetics, University of Leipzig Hospitals and Clinics, Leipzig, Deutschland, [2]Berlin Institute of Health at Charité, Universitätsmedizin Berlin, Center of Functional Genomics, Berlin, Deutschland, [3]Center of Human Genetics, University Hospital Leuven, Leuven, Belgien, [4]Radboudumc and Donders Center for Medical Neuroscience, Department Human Genetics, Nijmegen, Niederlande, [5]Medical Genetics, University of Siena, Policlinico S. Maria alle Scotte, Siena, Italien, [6]INSERM, US14-Orphanet, Paris, Frankreich, [7]S.O.C. Genetica Medica, AUSL-IRCCS di Reggio Emilia, Reggio Emilia, Italien, [8]Copenhagen University Hospital, Department of Clinical Genetics, Copenhagen, Dänemark, [9]University of Copenhagen, Department of Clinical Medicine, Copenhagen, Dänemark, [10]Inselspital, Bern University Hospital, University of Bern, Department of Human Genetics, Bern, Schweiz, [11]Radboud University Medical Center, Nijmegen, Niederlande, [12]ERN ITHACA, APHP-Robert Debré University Hospital, Université de Paris, Genetics Department, Paris, Frankreich

Introduction: High-throughput sequencing has greatly advanced gene identification for neurodevelopmental disorders (NDD). However, NDDs' extreme heterogeneity, complexity, and variability in inheritance patterns and phenotypes can still hinder diagnostics, counseling, and research.

Methods: SysNDD, developed from the previous SysID database, is a freely available database and webtool at www.sysndd.org or https://sysndd.dbmr.unibe.ch. It provides expert-curated, up-to-date gene-inheritance-disease relationships (“entities”). These are annotated with 33 HPO-based phenotypes. Our method utilizes an application programming interface (API; R plumber) to facilitate data sharing and mapping across gene, phenotype, and disease-focused databases. We are collaborating with ERN-ITHACA and Orphanet to create a curation pipeline for expert volunteers to review and update all entities.

Results: The database currently contains 1,623 confirmed (definitive and moderate evidence) NDD genes, 1,256 candidate (limited evidence) genes, and a total of 3,487 entity summaries. Gene panels can be compiled and downloaded after browsing tabular data and filtering by phenotypes. All entities, genes, and diseases have overview pages with e.g. short clinical synopses. SysNDD allows researchers to conduct analyses like comparisons with other databases, investigations of the number of published genes over time, and phenotype correlations. Functional and phenotype-based gene clustering and their correlations with inheritance and syndromicity are presented.

Conclusion: The SysNDD tools enable investigating the landscape of NDDs, and their interoperability will improve diagnostics and care for individuals with rare NDDs. Integration of published case-specific variants and phenotypes will be the next feature integrated into this resource.

Abstract ID: 285

P3-8

Detection of 5q-Spinal Muscular Atrophy by short-read next-generation sequencing - unexpected results

A. Abicht[1], S. Kleinle[1], V. Scholz[1], S. Rost[1], T. Wohlfrom[1], S. Gehling[1], F. Scharf[1], A. Benet-Pagès[1], E.-C. Prott[2], S. Grinzinger[3], A. Hotter[4], V. Haug[5], S. Niemeier[6], T. Hagenacker[7], K. Goldhahn[8], A. von Moers[8], M.C. Walter[9], P. Reilich[9], K. Eggermann[10], F. Kraft[10], I. Kurth[10], H. Erdmann[1], E. Holinski-Feder[10], T. Neuhann[1]

[1]MGZ - Medizinisch Genetisches Zentrum, München, Deutschland, [2]Praxis für Humangenetik, Wuppertal, Deutschland, [3]Christian-Doppler-Klinik Salzburg, Universitätsklinik für Neurologie, Salzburg, Deutschland, [4]Department of Neurology, Medical University Innsbruck, Innsbruck, Deutschland, [5]Klinik und Poliklinik für Kinder- und Jugendmedizin, Neuropädiatrie, Unimedizin Mainz, Mainz, Deutschland, [6]Klinikum Westbrandenburg, Neuropädiatrie, Potsdam, Deutschland, [7]University Hospital Essen, Department of Neurology and Center for Translational Neuro- and Behavioral Sciences (C-TNBS), Essen, Deutschland, [8]DRK-Kliniken Berlin, Klinik für Kinder- und Jugendmedizin, Berlin, Deutschland, [9]LMU Klinikum Munich, Friedrich-Baur-Institute, Department of Neurology, Munich, Deutschland, [10]Uniklinik RWTH Aachen, Institute of Human Genetics and Genomic Medicine für Humangenetik und Genommedizin, Aachen, Deutschland

Background: The importance of early diagnosis of 5q-Spinal muscular atrophy (5q-SMA) has heightened as early intervention can significantly improve clinical outcomes. In 96% of cases, 5q-SMA is caused by a homozygous deletion of SMN1. Around 4 % of patients carry a SMN1 deletion and a single-nucleotide variant (SNV) on the other allele. Traditionally, diagnosis is based on multiplex ligation probe amplification (MLPA) to detect homozygous or heterozygous exon 7 deletions in SMN1. Due to high homologies within the SMN1/SMN2 locus, sequence analysis to identify SNVs of the SMN1 gene is unreliable by standard Sanger or short-read next-generation sequencing (srNGS) methods.
Objective: The objective was to overcome the limitations in high-throughput srNGS with the aim of providing SMA patients with a fast and reliable diagnosis to enable their timely therapy.
Methods: A bioinformatics workflow to detect homozygous SMN1 deletions and SMN1 SNVs on srNGS analysis was applied to a diagnostic patient cohort undergoing whole exome and panel testing for suggested neuromuscular disorders. SNVs were detected by aligning sequencing reads from SMN1 and SMN2 to an SMN1 reference sequence. Homozygous SMN1 deletions were identified by filtering sequence reads for the „gene-determining variant“ (GDV).
Results: 10 patients were diagnosed with 5q-SMA based on (i) SMN1 deletion and hemizygous SNV (2 patients), (ii) homozygous SMN1 deletion (6 patients), and (iii) compound heterozygous SNVs in SMN1 (2 patients).
Conclusions: Applying our workflow in srNGS-based panel and whole exome sequencing (WES) is crucial in a clinical laboratory, as otherwise patients with an atypical clinical presentation initially not suspected to suffer from SMA remain undiagnosed.

Abstract ID: 286

Prenatal trio-exome-analysis: a powerful routine diagnostic tool

U. Ahting[1], M. Isau[2], R. Wimmer[2], J. Flunkert[2], M. Wüstemann[3], I. Staboulidou[3], R. Schwerdtfeger[3], B. Schulze[4], M. Stumm[2], K. Hoertnagel[1]

[1]MVZ-Martinsried GmbH, Martinsried, Deutschland, [2]Medicover Genetics GmbH, Berlin, Deutschland, [3]Zentrum für Pränatalmedizin Hannover, Hannover, Deutschland, [4]Medicover Humangenetik Berlin Lichtenberg, Hannover, Deutschland

Introduction: Between August 2021 and November 2022 we performed a total of 310 prenatal trio exome analyses due to sonographic fetal abnormalities with an average turnaround time of 13.2 days. In 86 cases a disease-causing variant could be found (27.7%). Most frequent leading symptoms were increased nuchal translucency (n=92), heart (n=68), skeletal (n=54) and brain (n=40) abnormalities.
Method: Prenatal trio exome analysis.
Results: To figure out the additional diagnostic yield that trio exome analysis contributes to standard prenatal diagnostic procedures, we evaluated the data from one prenatal medical center in Hannover in detail. Between August 2021 and November 2022 circa 13300 prenatal ultrasound tests were performed, circa 700 (~5%) showed worrisome ultrasound findings, which is in line with reported data (Anderson N. et al. 1995. AJR Am J Roentgenol. 1995 165(4):943. PMID: 7676997). 498 invasive prenatal probes (302 CVS, 196 amniocenteses) were taken and cytogenetically analyzed. 118 of them had an abnormal karyotype, with trisomy 21 (n=55), 18 (n=22) and 13 (n=8) being the most frequent aberrations.
For 96 pregnancies (19.3% of 498) trio exome analysis was applied, comparing the extraembryonal/fetal DNA to the parents. 30 cases (31.3%) showed likely pathogenic (class 4) or pathogenic (class 5) single nucleotide variants (n=26) or copy number variants (n=4) as an explanation for the sonographic abnormalities. In three additional cases, likely pathogenic or pathogenic findings were reported which could not explain the phenotype. 21 of the variants occurred *de novo*, 9 were inherited. 20 of the diseases we found were autosomal dominant. Additionally, we detected 7 autosomal recessive and 3 X-linked diseases. We reported 20 heterozygous, 6 homozygous, 3 hemizygous cases and 1 compound heterozygous case.
Conclusion: Prenatal trio exome analysis is a powerful method that provides a substantial contribution to prenatal diagnostics with an additional detection rate of around 30% to cases with sonographic abnormalities. The high percentage of *de novo* variants detected here leads to the assumption that many pathogenic variants would probably have escaped detection using singleton instead of trio exome analysis.

Abstract ID: 287

P3-73

Mutations in Follistatin-like 1 (*FSTL1*) may be associated with a connective tissue disorder

O. Klaas[1], J. Horvath[1], A. Bohring[1]

[1]*Universitätsklinikum Münster, Institut für Humangenetik, Münster, Deutschland*

Introduction: The highly conserved glycoprotein FSTL1 (OMIM *605547) is involved in many vertebrate signaling pathways and is suspected to control skeletal and lung organogenesis via inhibition of interactions with bone morphogenetic protein (BMP). Animal models showed diverse pathological effects in Fstl1-knockout studies, but no clear pathological variants in humans were reported thus far and no human congenital disease is associated with FSTL1 mutations, to the best of our knowledge.

Case Report: Here we report on a 4-year-old female patient originally referred to genetic analysis with suspected Marfan syndrome. Symptoms include congenital joint contractures, dysphagia, scoliosis, severe myopia, ectopia lentis, arachnodactyly, pes planovalgus, muscular hypotonia and mitral valve prolapse. Sequence analysis revealed no pathological variants in genes associated with Marfan syndrome or related disorders. Trio whole exome analysis then revealed two rare compound-heterozygous mutations in FSTL1.

The mutation c.805G>A p.(Gly269Arg) alters the last base of the ninth exon of FSTL1. In silico evaluation strongly indicates an effect on canonical splicing of FSTL1, which is currently investigated via mRNA analysis.

The missense mutation c.758G>A p.(Cys253Tyr) in the same exon shows high predicted pathogenicity in silico as well. This variant alters a highly conserved cysteine residue, which may affect the cysteine-rich FSTL1 protein structure.

Subsequently, we analyzed unresolved cases with similar symptoms in our database for additional potentially pathogenic FSTL1 mutations and identified a 6-year-old boy, also referred to us with suspected Marfan syndrome. He carries a rare mutation also affecting a cysteine residue (c.764G>A p.(Cys255Tyr)) as well as a rare deep-intronic mutation (c.582-97G>C). Parental testing of these variants is pending. He exhibits a milder phenotype with tall statue and ectopia lentis. We have also reached out using the GeneMatcher platform to identify additional potential cases and learned of two patients with heterozygous mutations and similar, albeit milder, phenotypes suggesting two phenotypic entities distinguishable by inheritance.

Conclusion: We suspect mutations in FSTL1 to be associated with a very rare hereditary connective tissue disorder showing a complex phenotype with autosomal-recessive inheritance and subtle signs of disease in mutation carriers.

Abstract ID: 288

P3-26

Another case of Cutis Marmorata Teleangiectatica Congenita caused by postzygotic *GNA11* mutation

K. Komlosi[1], M. Blandfort[2], A. Hotz[1], S. Alter[1], J. Fischer[1]

[1]*Institute of Human Genetics, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Deutschland,*
[2]*Neuropediatrics and Medical Genetics, Landau, Deutschland*

Introduction: Cutis marmorata teleangiectatica congenita (CMTC, Van Lohuizen syndrome) is a rare, sporadic, congenital cutaneous vascular disorder characterized by coarse-meshed capillary malformations arranged in asymmetrically distributed patches. Associated features are variable and may include body asymmetry with hyper- or hypoplastic limbs, syndactyly, cleft palate, and glaucoma. Because of the sporadic occurrence and mosaic arrangement of lesions, the hypothesis proposed 30 years ago by Rudolf Happle was that a lethal mutation is the underlying cause manifesting in patients due to postzygotic mosaicism. Recently, in three children with widespread asymmetric CMTC and variably associated anomalies including glaucoma, choroidal capillary malformation, and body asymmetry the postzygotic *GNA11* mutation c.547C>T (p.Arg183Cys) was found in mosaic in saliva and lesional cutaneous tissue of affected individuals.

Clinical report: We report on a 10-year-old boy born to consanguineous parents from northern Afghanistan with extreme CMTC over nearly the entire skin since birth. Additionally the child had bilateral glaucoma, discrete right hemihypertrophy and right cortical dysplasia with focal seizures since the age of 4 years.

Methods and Results: DNA from a lesional skin biopsy, buccal smear and peripheral blood from the affected child was isolated and a targeted NGS panel with high sequencing depth was performed. The mutation c.547C>T, p.(Arg183Cys) in the *GNA11* gene was detected in mosaic with 3.3% (1149x) in skin, 3.4% (938x) in buccal smear and in 1% (1123x) in peripheral blood leukocytes. The same variant was recently reported as a postzygotic pathogenic variant in CMTC and was previously reported in 3 patients with capillary malformations with a mosaic of 0,3-5% in affected tissue. We also detected the variant in mosaic previously in our cohort of patients with capillary malformations and overgrowth.

Conclusion: The recurrent postzygotic mutation in *GNA11* explains the phenotype of the patient and thus enables precise genetic counseling and recurrence risk estimates in the parents. The case underlines the importance to analyze affected tissue and blood using NGS with high sequencing depth in CMTC. Our case further supports the role of postzygotic mosaic *GNA11* mutations in the etiology of CMTC and the classification of CMTC as a further entity in the *GNA11*-Related Capillary Nevus (GNARCAN) spectrum.

Abstract ID: 289

P3-27

Whole genome sequencing offers new possibilities for the identification of disease-causing gene variants escaping detection by whole exome analysis

M.A.H. Hofrichter[1], C.W. Remmele[1,2,3], A. Koparir[1], D. Bengl[1], M. Dittrich[1], S. Flandin[4], W. Shehata-Dieler[4], T. Haaf[1]

[1]Institute of Human Genetics, Julius-Maximilians-University, Würzburg, Deutschland, [2]Center for Rare Diseases, University Hospital Würzburg, Würzburg, Deutschland, [3]Bavarian Genomes Network for Rare Diseases, Bavaria, Deutschland, [4]Comprehensive Hearing Center, Department of Otorhinolaryngology, University Hospitals, Würzburg, Deutschland

Reduced costs and technical improvements have revolutionized high-throughput sequencing in recent years. Especially, whole genome sequencing uncovers new possibilities in terms of variant detection and interpretation. For this reason, the Bavarian Genomes Network for Rare Diseases was established to generate multi-omics datasets of undiagnosed patients and their relatives. The potential to analyze different genomes within this project is especially advantageous for heterogeneous disorders like hearing loss (HL). Many genes have been described for this disease, but most of the estimated 250 HL genes remain to be elucidated. The detection of new candidate genes is one of the main focuses in the study of hereditary HL. However, this requires that all known HL genes have already been investigated in every case and no disease causing variant could be observed.

Exome analysis, one of the standard methods of investigation, presents limitations that leave cases unresolved. Herein, we introduce an example, which illustrates how whole genome sequencing analysis overcomes the restrictions of previous exome analysis and disclosed the case.

For this purpose, whole genome sequencing of two affected siblings with non-syndromic HL and their unaffected mother was performed within the Bavarian Genomes Network for Rare Disease and the data were subsequently analyzed. Both children display since birth moderate to severe bilateral sensorineural HL affecting all frequencies.

Interestingly, a new splice variant in the gene *PTPRQ* (NM_001145026.2, c.6453+2dup) (DFNB84A) could be discovered, which is carried by all investigated family members. Furthermore, structural variant analysis identified a large unknown deletion, including intronic sequences and an entire exon (NM_001145026.2, c.6602+81_6738+394del). This structural variant was only observed in the affected siblings. Since there are many loss of function variants in *PTPRQ* already known to cause HL, the here presented compound heterozygous variants could explain the HL phenotype in the affected family members. In exome analysis, neither the deletion nor the splice variant could be found due to low coverage of the deleted region and to sequence errors in the splice region.

In this study, we present an example showing the advantages of whole genome sequencing to identify undetected variants in samples previously analyzed via exome sequencing even in known HL genes.

Abstract ID: 290

P3-22

Refining the phenotypic spectrum of Poretti-Boltzhauser syndrome caused by biallelic variants in *LAMA1*

B. Greiten[1], F. Rust[2], I. Nagel[2], M. Spielmann[1,2], I. Hüning[1]

[1]Institute of Human Genetics, University of Lübeck, Lübeck, Deutschland, [2]Institute of Human Genetics, University of Kiel, Kiel, Deutschland

Poretti-Boltzhauser syndrome is a neuroophthalmological autosomal-recessive disorder caused by biallelic mutations of *LAMA1. LAMA1* encodes the laminin subunit α1. The syndrome is characterized by cerebellar ataxia, delayed language and motor development and specific ophthalmologic and neuroradiologic signs. Patients can have oculomotor apraxia, strabismus, myopia and retinal dystrophy. Characteristic neuroradiological findings are cerebellar cysts, cerebellar dysplasia, hypoplasia of vermis cerebelli and enlargement of the 4th ventricle. Typically, the symptoms are not progressive. Less than 40 patients have been described so far.

We report on a 2 year and 2 months old boy with Poretti-Boltzhauser syndrome. In late pregnancy ultrasound revealed a small right kidney as well as a decrease in weight gain and amniotic fluid production. Postnatal adaptation was normal but generalized hypotonia was noted starting in infancy. The patient could not walk or speak yet. However, his speech perception seemed to be normal so far. There were two parietooccipital encephaloceles that were confirmed by a cranial MRI which also showed a dysplasia of the vermis cerebelli and an altered posterior cranial fossa. The boy had craniosynostosis, a congenital oculomotor apraxia and mild facial dysmorphisms consisting of a prominent forehead, a slight epicanthus, low-set ears and a broad nasal ridge. He had partial syndactyly of the 2nd and 3rd toes of both feet. As a secondary finding, the patient had ureteropelvic junction stenosis and hydronephrosis II-III° on the right side. Both renal malformations and craniosynostosis are not part of the phenotypic spectrum of Poretti-Boltzhauser syndrome.

Whole exome sequencing showed the heterozygous probably pathogenic variant c.9036_9047del; p.(Asn3013_Ile3016del) and the heterozygous pathogenic variant c.6151C>T; p.(Arg2051*) in the *LAMA1* gene (NM_005559.4). The parents carry one of the variants each. The phenotype of our patient overlaps with the phenotype of the patients in the literature to some extent. However, only one patient with an encephalocele has been described so far and only few patients without cerebellar cysts and cerebellar dysplasia. Thus, the clinical findings in our patient refine the phenotype of Poretti-Boltzhauser syndrome.

Abstract ID: 291

P4-8

Use of soft-clustering algorithms to reduce the genetic complexity of chronic kidney disease

A. Eoli[1], S. Ibing[2], G. Nadkarni[3], C. Schurmann[2,4], H.O. Heyne[1], E. Böttinger[2]

[1]Hasso-Plattner Institute, Personalised and Genomic Medicine, Potsdam, Deutschland, [2]Hasso-Plattner Institute, Personalised Medicine, Potsdam, Deutschland, [3]Icahn School of Medicine at Mount Sinai, Department of Medicine, Division of Nephrology, New York City, Vereinigte Staaten, [4]Bayer AG, Division Pharmaceuticals, Open Innovation & Digital Technologies, Berlin, Deutschland

Chronic kidney disease (CKD) is a condition that causes a gradual loss of kidney function and affects approximately 9.1% of the global population. The disease is highly complex, and its genetic disease-causing mechanisms are not entirely understood. In this work, we use a multivariate analysis technique to deconstruct CKD heterogeneity. First, we applied Non-negative matrix factorization (NMF), a soft-clustering algorithm, to discover overlapping clusters of 434 independent genetic variants contributing to CKD risk and 229 associated traits, identified from published multi-ancestry genome-wide association studies (GWAS). Second, we used the algorithm's weights to calculate cluster-specific polygenic scores (cPGS). We then aimed to identify phenotypes associated with each cluster by performing a phenome-wide association study (PheWAS) on 31.701 Bio*Me* biobank participants and by checking the clusters' correlation with curated disease phenotypes. These analyses were performed both across ancestries and for the individual continental populations of African (n=5,771), American (n=5,433), and European (EUR, n=7,722) ancestry. NMF identified nine clusters that may reflect different aspects of CKD: the clusters’ top-weighted traits pointed to kidney function (urea, creatinine levels), pulse and hemoglobin levels, lipid metabolism, type 2 diabetes (T2D), inflammation, and body weight. The PheWAS confirmed the top-weighted traits in most clusters. Furthermore, despite using only CKD risk-increasing alleles, the clusters showed different correlations between an increasing cPGS and different disease outcomes. Three clusters were associated with increased risk for diseases like T2D, hypertension, and reduced kidney function, while others showed a protective effect instead. Although results reached more often significance when validating across ancestries, significant ancestry-specific results were also identified for the EUR cohort. While all CKD-associated genetic variants were risk-increasing, associations with reduced kidney function and CKD-related diseases, such as T2D and hypertension, were found only for a smaller subset of variants and they differed across genetic ancestry groups. With our results, we thus use genetics to gain insights into CKD’s underlying disease biology, reduce CKD complexity, and better understand population-specific risk factors.

Abstract ID: 292

P6-29

Mouse models to study the pathophysiology of GMPPB-associated disorders

M. Schurig[1], P. Franzka[1], C. Hübner[1]

[1]*Institute of Human Genetics, University Hospital Jena, Jena, Deutschland*

Glycosylation is the most common post-translational protein modification and can affect protein stability and conformation. It plays a prominent role in cell-to-cell communication, cell matrix interaction, adhesion, protein targeting and folding. Abnormal glycosylation of proteins can induce deleterious effects as observed in congenital disorders of glycosylation (CDGs), which often result in serious, sometimes fatal malfunctions of different organ systems such as brain and muscle. GDP-Mannose-Pyrophosphorylase B (GMPPB) associated neuromuscular disorders are typical examples. GMPPB catalyses the conversion of Mannose-1-Phosphate to guanosine-diphosphate-(GDP)-mannose (activated mannose form), the key substrate for glycosylation. Patients with mutations in *GMPPB* suffer from neurological abnormalities, visual impairments, gait disorders and most prominently muscle dystrophy.

In order to investigate the underlying pathophysiology and to generate a suitable model to assess new therapeutic options we generated three mouse models. Our first mouse model harbours a point mutation in the catalytic domain of Gmppb (Gmppb knock-in), the second model is characterized by a large deletion in the catalytic domain (Gmppb knock-out), while the third model is a compound-heterozygous model (Gmppb knock-out/knock-in). While homozygous Gmppb knock-out mice are embryonically lethal and die between embryonic day 3 and 8, homozygous knock-in animals show a mild neuromuscular phenotype. This phenotype is aggravated in Gmppb knock-out/knock-in animals. While the abundance of *Gmppb* knock-in transcripts is unchanged in tissues of Gmppb knock-in mice, the abundance of Gmppb protein is reduced, suggesting that the protein stability is diminished.

Abstract ID: 293

Mutant SSU processome component *C1orf131* causes ribosome biogenesis dysfunction leading to primary microcephaly

A.I.A. Khayyat[1,2,3], M. Asif[1,4], R. Bascetin[5], U. Abdullah[6], A. Aldeeri[7], Z. Dardas[8], C. Joret[5], B. Budde[1], S. Alawbathani[1], F. Ullah[9], P. Wagle[10], Z. Ali[11], A.A. Noegel[3], W. Höhne[1], E.U.H. Makhdoom[1,12,13], T. Georgomanolis[1], D. Calame[8], S.M. Baig[12,14,15], J.R. Lupski[8,16,17], T.W. Yu[7], D.L. Lafontaine[5], P. Nürnberg[1,4], M.S. Hussain[1,4]

[1]Cologne Center for Genomics (CCG), University of Cologne, Cologne, Deutschland, [2]King Saud University, Biochemistry Department, Riyadh, Saudi-Arabien, [3]Center for Biochemistry, Medical Faculty, University of Cologne, Cologne, Deutschland, [4]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Deutschland, [5]RNA Molecular Biology, Fonds de la Recherche Scientifique (F.R.S.-FNRS), Université libre de Bruxelles (ULB), Gosselies, Belgien, [6]University Institute of Biochemistry and Biotechnology (UIBB), PMAS-Arid Agriculture University, Rawalpindi, Pakistan, [7]Division of Genetics and Genomics, Boston Children's Hospital, Boston, Massachusetts, Vereinigte Staaten, [8]Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, Vereinigte Staaten, [9]Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Vereinigte Staaten, [10]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Deutschland, [11]Centre for Biotechnology and Microbiology, University of Swat, Swat, Pakistan, [12]Human Molecular Genetics Laboratory, Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE) College, PIEAS, Faisalabad, Pakistan, [13]Neurochemicalbiology and Genetics Laboratory (NGL), Department of Physiology, Faculty of Life Sciences, Government College University, Faisalabad, Pakistan, [14]Aga Khan University, Department of Biological and Biomedical Sciences, Karachi, Pakistan, [15]Pakistan Science Foundation (PSF), 1- Constitution Avenue, Islamabad, Pakistan, [16]Human Genome Sequencing Center, BCM, Houston, Vereinigte Staaten, [17]Texas Children's Hospital, Houston, Vereinigte Staaten

The involvement of ribosome biogenesis dysfunction in the aetiology of primary microcephaly has only started to be unveiled. Here we report a novel nucleolar protein involved in primary microcephaly and intellectual disability in several affected family members born to Pakistani consanguineous parents. A combination of linkage analysis and whole-exome sequencing revealed a homozygous missense variant in *C1orf131,* which encodes a component of the small subunit (SSU) processome. Through international collaboration, we identified five different potentially pathogenic missense variants of *C1orf131* in four unrelated patients manifesting microcephaly and intellectual disability. We show that C1orf131 is a nucleolar protein that relocates to the perichromosomal region (PR) during mitosis. Patient-derived primary fibroblasts display reduced immunoreactivity to C1orf131, whose association with the PR was lost, leading to mitotic catastrophes and nucleolar abnormalities. Similar effects were observed upon overexpression of mutant C1orf131 or following protein knockdown. Furthermore, patient primary fibroblasts showed nucleolar hypersensitivity to the transcriptional blockade, and C1orf131 variants affected ribosome biogenesis deeply, with significant effects on pre-rRNA processing. Having established the C1orf131 interactome, we further report that it is part of both nucleolar and centrosomal proteomes. Intriguingly, the 18S rRNA acetyltransferase NAT10 showed impaired interaction with C1orf131 variants. Finally, transcriptomic profiling of the mutant fibroblasts revealed genes essential for nervous system development, such as those involved in dendritic morphogenesis, synapse assembly and organisation, axon guidance, axonogenesis, glial cell proliferation and differentiation, to be differentially expressed. In conclusion, our data uncovered a novel nucleolar component essential for normal brain development and function.

Abstract ID: 294

de novo MAP3K3 variants in patients with abnormalities of the lymphatic system cause attenuation of MAP3K3/MEKK3 downstream signaling pathways

I.G. Kreimer[1], T. Holling[1], T. Herget[1], F. Kortüm[1], I. Ivanovski[2], K. Steindl[2], A. Rauch[2], K. Kutsche[1]

[1]University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Deutschland, [2]University of Zürich, Institute of Medical Genetics, Schlieren, Schweiz

MAP3K3 (MEKK3) is a protein kinase and regulates gene expression through downstream effectors. Loss of Map3k3 is associated with vascular abnormalities in mice. In humans, the recurrent somatic *MAP3K3* variant NM_203351.3:c.1416C>G/p.(Ile472Met) causes verrucous venous malformation and sporadic cerebral and spinal cord cavernous malformations. MAP3K3^{I472M} strongly activates ERK5, ERK1/2, JNK, and p38 signaling indicating a gain-of-function effect.
Trio whole exome sequencing identified the *de novo MAP3K3* heterozygous variant NM_203351.3:c.1949A>G/p.(His650Arg) in patient 1 with failure to thrive, valvular pulmonary stenosis, atrial septal defect with left-to-right shunt, and cutaneous hemangiomas. She developed severe chylothorax, pericardial effusion, and ascites and died at the age of 3.5 months due to multi-organ failure. We identified patient 2 with the *de novo* heterozygous *MAP3K3* splice variant NM_203351.3:c.872-1G>T. At age 3.6 years, she showed persistence of the congenital lymphedema and normal psychomotor and intellectual development. Lymphedema affected the periorbital region and limbs. *MAP3K3* transcript analysis using leukocyte-derived cDNA of patient 2 revealed wild-type transcripts and aberrantly spliced pre-mRNAs, with mRNAs lacking the *in-frame* exon 11 or the first 14 nucleotides of exon 11 and those harboring the last 28 nucleotides of intron 10 between exons 10 and 11.
We expressed Flag-tagged MAP3K3^{WT}, MAP3K3^{I472M}, MAP3K3^{H650R}, and the kinase-dead mutant MAP3K3^{K422A} in HEK293T cells. While MAP3K3^{WT} and MAP3K3^{I472M} were detected with a molecular weight of ~100 kDa, MAP3K3^{H650R} and MAP3K3^{K422A} had an observed molecular mass below 100 kDa. A phosphatase assay revealed phosphorylation of MAP3K3^{WT} and MAP3K3^{I472M}, whereas MAP3K3^{H650R} and MAP3K3^{K422A} were not phosphorylated. Quantitative analysis revealed strongly increased levels of MAP3K3^{I472M} by 2.4-fold and reduced levels of MAP3K3^{H650R} to 17% compared with wildtype under steady-state culture conditions. Next, transiently transfected HEK293T cells were starved followed by serum stimulation for 30 min. In cells expressing MAP3K3^{WT} and MAP3K3^{I472M}, we identified strong phosphorylation of the downstream kinases p38, ERK1/2, and JNK, while cells expressing MAP3K3^{K422A} did not show kinase activation. MAP3K3^{H650R} expression caused a slight activation of the three downstream kinases. Together, our data suggest that *de novo MAP3K3* hypomorphic variants underlie a spectrum of lymphatic abnormalities.

Abstract ID: 295

P3-74

How to take a short cut – from genetics to specific metabolic diagnostics

J. Beuschlein[1], T. Völkl[2], A.-K. Blume[2], S. von Schoenaich[3], K. Gahle[1], N. Hirschberger[1], M. Shoukier[1], J. Stoffels[2]

[1]Pränatal-Medizin München, Humangenetik, München, Deutschland, [2]KJF Klinik Josefinum, Interdisziplinäres Sozialpädiatrisches Zentrum (SPZ), Klinik für Kinder- und Jugendmedizin, Augsburg, Deutschland, [3]Hessing Förderzentrum für Kinder und Jugendliche, Augsburg, Deutschland

Introduction: Combined malonic and methylmalonic aciduria (CMAMMA, OMIM-P: 614265) is a rare metabolic disease based on homozygous or compound heterozygous variants in the *ACSF3* gene (OMIM-G: 614245). The *ACSF3* gene encodes for an acyl-coenzyme A (CoA) synthetase which plays an important role in activating fatty acids. Fatty acids are an essential component of membranes and are involved in metabolism and energy storage. Clinically, CMAMMA shows a wide heterogeneity of symptoms from asymptomatic to severely affected patients with developmental delay, especially when other triggers are present. Until now, there are only 30 causative mutations listed in the Human Gene Mutation Database (HGMD 2022.3) and CMAMMA is not included in the current expanded newborn screening in Germany.
Methods: We describe a case of a 2-year old girl of consanguineous Iraqi parents. She presented with secondary microcephaly, severe development delay, clinically mild intellectual disability and speech impairment. Furthermore, dysmorphic signs include downslanted palpebral fissures, turricephalic skull, connatal pes equinovarus on the left and pes calcaneus on the right. Moreover, she showed signs of ataxia, growth impairment (small for gestational age with a birth weight of 640 g) and reduced feeling of hunger. Since she was prematurely born in the 27+4 week of pregnancy, a cMRI was performed showing no complications of preterm birth. The audiological screening detected abnormal results.
Results: Mendeliome Clinical Exome Sequencing revealed the homozygous variant NM_001243279.3: c.1470G>C, p.(Glu490Asp) in the *ACSF3* gene which we consider to be likely pathogenic according to recent ACMG criteria. Metabolic diagnostics were performed and confirmed our diagnosis of CMAMMA. Due to an early suspected genetic diagnosis, a dietary modification was implemented after a short time (limiting dietary protein intake to 0.8 – 1.3 g/kg BW, addition of an appropriate amino acid mixture to cover the protein requirement and to ensure an adequate energy supply in order to avoid a catabolic metabolic situation).
Conclusion: This case highlights the importance of including further metabolic conditions in recent newborn screening programs.

Abstract ID: 296

P5-10

Two cases of Xq28 duplications with uncommon pathomechanisms

M. Apelt[1], T. Heinrich[2], C. Hertzberg[3], D. Korinth[1], E.-K. Suk[1]

[1]Praxis für Humangenetik, Zytogenetik, Berlin, Deutschland, [2]MZV für Humangenetik und Molekularpathologie, Rostock, Deutschland, [3]Vivantes Klinikum Neukölln - DBZ, Berlin, Deutschland

Duplications in Xq28 involving *MECP2* are causative for *MECP2* duplication syndrome. The main clinical features include developmental delay, facial dysmorphism and hypotonia mainly affecting males. Symptoms are masked in carrier females due to skewed X-inactivation and the underlying aberrations are unveiled in affected sons. Here, we present two cases of Xq28 duplications with unusual origins.

In the first case, array CGH for a boy with developmental delay, small height and a Dandy-Walker malformation showed a 13Mb Xp22.33 deletion and a 478kb Xq28 duplication. Chromosome and FISH analysis presented a rearranged X chromosome with the duplicated region attached to the short arm (46,Y,der(X)(qter-->q27.2::p22.33-->qter)). It was hypothesised it arose from a pericentric inversion in a maternal X chromosome and a recombination event. However, chromosome and FISH analysis of the mother's chromosomes revealed that she carries the same derivative X chromosome (46,X,der(X)(qter-->q27::p22.3-->qter)), resulting in a 50% recurrence risk for affected sons. As the mother shows no known symptoms and fertility is not impacted, it was recommended that female relatives be tested for the derivative X chromosome to evaluate their risk of affected sons. Furthermore, as the original carrier of the inverted X chromosome and the recombination event cannot be tracked to a specific female ancestor, all carriers' X chromosomes should be labelled as derivative, not recombinant.

The second case of a boy with developmental delay showed a 1 MB Xp22.33/Yp11.32 deletion and a 2,4 Mb Xq28 duplication, again indicating a recombinant X chromosome. FISH with subtelomeric probes showed the Xqter signal to be located on the short arm of the Y chromosome though. In fact, the deletion encompasses the PAR1 and is located on the Y chromosome. The karyotype was defined as 46,X,der(Y)t(Y;X)(p11.32;q28). This hinted at an infrequent translocation t(X;Y)(q;p) in the father. We conducted chromosome analyis and subtelomeric FISH for the respective regions on the paternal chromosomes and found a normal karyotype without the translocation; thus, it arose *de novo* in the affected son. A similar case with an unbalanced *de novo* Xq-Yp translocation was reported by Lin *et al*. in 2014. Had the father carried the translocation, no normal offspring would be possible as all sons would have the same karyotype and symptoms as the firstborn and all females would have Rett syndrome resulting from the *MECP2* deletion.

Abstract ID: 298

P3-28

Comparison of three patients with variants in histone methyltransferase complex genes SETD1B, KMT2A and KMT2B

G. Miltenberger-Miltenyi[1], K. Alt[2], M. Kuhn[2], S. Geuer[2], C.-C. Becker[1]

[1]genetikum München, München, Deutschland, [2]genetikum, Neu-Ulm, Deutschland

Introduction: The histone methyltransferase complex is a group of histone-modifying enzymes (HME), which mediate the methylation of histone H3 on lysine 4 (H3K4). Pathogenic variants in histone methylation modifier (HMM) genes cause various neurodevelopmental syndromes with partially overlapping phenotypes. Animal studies demonstrated a regulatory role of the SETD1B HME on the Kmt2a- and Kmt2b histone methyltransferase genes.

Patients and Methods: We present three patients seen in our clinic with (likely) pathogenic variants in the genes SETD1B, KMT2A and KMT2B. Patient 1 is a 3-year-old son of non-consanguineous parents. He presented with generalized epilepsy and global developmental delay. Patient 2 is a 10-year old female, 2nd daughter of consanguineous parents. She showed global developmental delay, hypoventilation and hypertrichosis, starting at the age of 2 years. Patient 3 is a 10-year old boy of consanguineous parents. He presented with intellectual disability, pathological EEG and impaired fine-motor skills. In all three cases, we carried out chromosome analysis, arrayCGH, and TRIO-WES.

Results: Chromosome analysis and arrayCGH did not show any pathologic results in any of the three cases. In Patient 1, TRIO-WES revealed a novel, de novo variant (class 4) in heterozygous form in the SETD1B gene [c.5474G>A (p.Arg1825Gln)]. Patient 2 had a novel, de novo variant (class 5) in heterozygous form in the KMT2A gene [c.165dup (p.Ser56fs)]. Patient 3 carried a novel, de novo variant (class 5) in heterozygous form in the KMT2B gene: [c.1021C>T (p.Gln341Ter)].

Conclusions: Functional studies observed associations among the different proteins of the histone methyltransferase complex, such as KMT2A, KMT2B and SETD1B. Clinically, patients with variants in these genes show general developmental delay, yet with some distinct features. Epilepsy is typical in SETD1B patients, while additional hypertrichosis is common in KMT2A, whereas patients with pathogenic KMT2B variants potentially develop dystonic features. Our cases demonstrate that TRIO-WES is a solid method of choice in the genetic diagnosis of patients with general developmental delay; furthermore, we show that the clinical symptoms might indicate towards the affected gene in diseases of the histone methyltransferase complex.

Abstract ID: 299

P3-75

The clinical utility of noninvasive prenatal testing (NIPT) for detection of fetal unbalanced translocations

M. Shoukier[1], J. Beuschlein[1], C. Bagowski[1], I. Bedei[2], R. Axt-Fliedner[2], D. Huhle[3], S. Busche[1]

[1]Pränatal-Medizin München, Humangenetik, München, Deutschland, [2]Universitätsklinikum Gießen und Marburg, Zentrum für pränatale Medizin und fetale Therapie, Zentrum für Frauenheilkunde & Geburtshilfe, Gießen, Deutschland, [3]Praxis für Humangenetik Leipzig, Leipzig, Deutschland

Introduction: Reciprocal translocations are caused by a break in two chromosomes with mutual exchange of the resulting fragments. The translocation in a healthy carrier is often not diagnosed until various miscarriages or fetal anomalies take place due to an unbalanced arrangement in germ cell formation. Invasive methods (e.g. chorionic villus sampling (CVS) or amniocentesis) are the current gold standard for prenatal diagnosis of fetal unbalanced translocations (FUT). In the current study we investigate the suitability of non-invasive prenatal testing (NIPT) for detection of FUT.

Methods: Patient A carried a reciprocal translocation (46,XX,t(5;18) (p15.2;q22.1)). She had a history of recurrent miscarriages. The fetus showed increased nuchal translucency in 10th week of pregnancy. The fetus of patient B was diagnosed with intrauterine growth retardation, corpus callosum agenesis and ventricular septum defect in 25th week of pregnancy leading to invasive genetic diagnostic testing. Molecular analysis displayed an unbalanced translocation of patient B's fetus detected by NGS and confirmed by chromosomal array-CGH analysis (46,XX,der(13)t(10;13)(p14; q33)). Genome-wide NIPT was performed on two patients using the Illumina VeriSeq NIPT Solution v2 according to manufacturer's instructions, generating 9.1 and 9.9 million reads for patient A and patient B, respectively.

Results: In the blood sample of **patient A** NIPT identified a heterozygous deletion on chromosome 5 (5p15.33p15.2 del) and a heterozygous duplication on chromosome 18 (18q21.33q23 dup). The deletion size is approximately 14.70 Mb, whereas the duplication size is approximately 18.70 Mb. The NIPT results were confirmed using FISH analysis on native CVS (ISCN 2020: 46,XX,ish der(5)t(5;18)(p15.2;q22.1)(D5S23/D5S721-,VIJyRM2050++)). **For patient B** NIPT identified a heterozygous deletion on chromosome 13 (13q33.1q34 del) of approximately 12.55 Mb and a heterozygous duplication on chromosome 10 (10p15.3p14 dup) of approximately 10.80 Mb. Compellingly, the breakpoints were precisely determined and identical to the cytogenetic results in both cases.

Conclusion: The combination of qualified ultrasound investigation and genome-wide NIPT can yield a valuable tool for high precision non-invasive identification of FUT and can influence the decision for invasive diagnostics in couples with known risk for FUT. Due to the small number of cases, further studies are warranted.

Abstract ID: 300

Potential clinical utility of polygenic risk scores in disease prognosis

J. Wanner[1,2], N. Mars[2], Z. Yang[2], S. Ripatti[2], A. Ganna[2], H.O. Heyne[1,2,3], FinnGen, INTERVENE

[1]Hasso Plattner Institut, Universität Potsdam, Potsdam, Deutschland, [2]Finnish Institute for Molecular Medicine (FIMM), University of Helsinki, Helsinki, Finnland, [3]Hasso Plattner Institute, Mount Sinai School of Medicine, New York, Vereinigte Staaten

Background: Most research on polygenic risk scores (PRSs) focuses on incidence of diseases, not their prognosis. In the clinic, prediction of disease outcomes happens more frequently in at-risk than in healthy individuals. Here, we explore the utility of PRSs for disease prognosis using electronic health records of 430,897 Finns.
Methods: First, we investigate clinical scenarios where a patient presents with an unspecified symptom or a milder disease that may be a sign of a more severe disease. For example, we explore cases of angina pectoris (AP) at risk to develop coronary artery disease (CAD) and cases of unspecified first seizure at risk to develop generalized epilepsy.
Results: In 1,141 individuals with a single unspecified seizure, we found that individuals with the top 10% of generalized epilepsy PRS showed an increased risk (HR 2.01, 95% CI 1.2-3.36, p=7.5*10-3) of developing epilepsy 10 years after the event compared to the rest of the cohort, with a larger effect in young adults (age < 40). Furthermore, we show that the cumulative lifetime incidence of generalized epilepsy reaches ca 30% in individuals within the top 10% of epilepsy PRS and a previous unspecified seizure, while individuals without a previous unspecified seizure remain at a lower risk (ca 0.5%). Additionally, we investigated the interaction of PRSs and a previous milder disease or symptom. In the example of CAD, we found a substantial attenuation of the PRS effect in people with AP (0.73, 95% CI 0.712 - 0.752, p=5.63*10-109) compared to the rest of the cohort. We investigated the predictive value and attenuation of incidence PRSs on disease prognosis in multiple other diseases, such as stroke after atrial fibrillation, type 2 diabetes after gestational diabetes, and inflammatory bowel disease after irritable bowel syndrome. We found a substantial attenuation in disease prognosis for some but not all diseases due to selection bias and genetic correlation. Analyses are replicated in a biobank network (n>1.2 Million).
Conclusion: By an increase in absolute risk after a first milder or unspecific event we thus highlight possibilities (and potential pitfalls) to potentially improve the clinical utility of disease incidence PRS in applications of disease prognosis.

Abstract ID: 302

P6-15

Whole genome sequencing uncovers a 8,8 kb deletion between *L1CAM* and *AVPR2* in a boy with Hirschsprung disease and nephrogenic diabetes insipidus

I. Bader[1], K. Buder[2], S. Beck-Wödl[1], M. Sturm[1], P. Stöbe[1], O. Rieß[1], T. Haack[1]

[1]Universitätsklinikum Tübingen, Institut für Medizinische Genetik und Angewandte Genomik, Tübingen, Deutschland,
[2]Universitätsklinikum Tübingen, KfH-Nierenzentrum für Kinder u. Jugendliche, Tübingen, Deutschland

Pathogenic loss-of function variants and missense-variants in *AVPR2* (Arginine Vasopressin Receptor 2) are a known cause of X-linked nephrogenic diabetes insipidus. Pathogenic loss-of function variants and missense-variants in *L1CAM* (L1 Cell Adhesion Molecule) are a known cause of X-linked hydrocephalus with or without Hirschsprung disease. A contiguous-gene-deletion syndrome with a clinical picture that comprises nephrogenic diabetes insipidus and hydrocephalus with or without Hirschsprung disease, has been described in two male patients that harbor deletions (approx. 62 kb and 29 kb respectively) that affect coding regions of both adjacent genes *(AVPR2* and *L1CAM*) (PMID: 18553546, 17318848).
Here we describe a 1,5 year old boy with Hirschsprung disease and nephrogenic diabetes insipidus that harbors an 8,8 kb Deletion in Xq28 (NC_000023.11:g.[153883503_153892327del];[0]) located in between the two adjacent genes *L1CAM* and *AVPR2*.
The deletion affects the 5’ UTR exon 1 of *L1CAM* but no annotated protein coding exons of *L1CAM*. The deletion terminates 11 kb before the 5’-end of the nearest annotated *AVPR2*-trancript (NR_027419.2). The deletion does not affect any annotated coding or non-coding transcripts of *AVPR2*; that is why the deletion was uncovered only by whole genome sequencing and was undetectable in whole exome sequencing data of the patient.
The unusual combination of the specific and rare symptoms of the patient raises the hypothesis that this 8,8 kb deletion affects chromosomal material that regulate the expression of both genes. Our data indicate that whole genome sequencing can uncover potentially disease causing CNVs in non protein-coding regions in patients with a specific clinical picture. Further analyses, i.e. RNA-expression of *AVPR2* and *L1CAM* in blood and fibroblasts of the patient need to be performed to demonstrate dysregulation of the genes.

Abstract ID: 303

P4-9

Gene-based association analysis of transcription factors involved in differential regulation of bipolar susceptibility genes during neurodevelopment

P. Thirunavukkarasu[1], S. Herms[1,2,3], B. Burger[1], S. Cichon[1,4,5]

[1]University Hospital Basel and University of Basel, Department of Biomedicine, Basel, Schweiz, [2]University Hospital Bonn, Institute of Human Genetics, Bonn, Deutschland, [3]University Hospital Bonn, Life&Brain Center, Bonn, Deutschland, [4]University Hospital Basel, Institute of Medical Genetics and Pathology, Basel, Schweiz, [5]Research Center Juelich, Institute of Neuroscience and Medicine (INM 1), Jülich, Deutschland

Bipolar disorder (BD) is a genetically complex neuropsychiatric disorder with a lifetime prevalence of 1-2% and an estimated heritability of ~60-85%. In the last years, genome-wide association studies (GWAS) and exome sequencing studies have identified common and rare risk variants for BD. These variants, together with environmental factors, most likely act in a combinatorial manner leading to the development of BD. There is strong evidence that several of the identified BD susceptibility genes are involved in the regulation of neurodevelopmental processes, strongly suggesting that such processes play a role in the etiology of BD.

In the present study, we aimed to extend our knowledge of bipolar susceptibility genes involved in neurodevelopmental processes. We selected BD susceptibility genes which are differentially expressed in neurodevelopment and identified transcription factors (TFs) regulating these genes. We then tested the identified TFs for association with BD. Our hypothesis was that TFs involved in differential regulation of BD susceptibility genes during neurodevelopment are enriched for BD association signals.

We extracted the BD susceptibility genes from the largest recent studies (BD PGC3, 2021) (Palmer et al. 2021) as our initial gene-set. Using RNA-seq data from BrainSpan atlas, we examined the differential regulation of expression of this initial gene-set in brain development. Then, using ChEA datasets, we examined the TFs which regulated these differentially expressed susceptibility genes. Using VEGAS, a gene-set based enrichment tool, the PGC3 BD GWAS summary statistics was used to perform gene-based association tests for these TFs.

From the recent-GWAS and exome-wide study in BD, we curated 435 BD susceptibility genes. Using the developmental transcriptome data from BrainSpan database, we observed that 33 susceptibility genes were differentially regulated in brain development. Using the ChEA datasets, we observed that these 33 susceptibility genes were in turn regulated by 54 TFs. Gene-based association tests produced significant p-values for 14 out of 54 TFs (26%) which is significantly higher than expected by chance. Our results suggest that our in-silico approach, retrieving more genes involved in the biological network of known BD susceptibility genes, may be a promising approach to identify more disease-associated genes and get a more comprehensive picture of the biological networks involved.

Abstract ID: 304

Biallelic loss-of-function variants in *CYHR1* cause syndromic microcephaly

M. Asif[1,2], A.I.A. Khayyat[1,3], H.-M. Pogoda[4], S. Alawbathani[1], A. Sanner[5], T. Georgomanolis[1], J. Haasters[6], B. Budde[1], C. Becker[1], H. Thiele[1], D. Winter[5], H.G. Martin[6], M. Hammerschmidt[2,4], M. Wagner[7,8,9], P. Nürnberg[1,2], M.S. Hussain[1,2]

[1]Cologne Center for Genomics (CCG), University of Cologne, Cologne, Deutschland, [2]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Deutschland, [3]King Saud University, Biochemistry Department, Riyadh, Saudi-Arabien, [4]Institute of Zoology, Developmental Biology Unit, University of Cologne, Cologne, Deutschland, [5]Institute for Biochemistry and Molecular Biology, Medical Faculty, University of Bonn, Bonn, Deutschland, [6]Hospital Universitario de Salamanca, Salamanca, Spanien, [7]Dr. von Hauner's Children's Hospital, University of Munich, Department of Pediatric Neurology, Developmental Medicine and Social Pediatrics, Munich, Deutschland, [8]Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Deutschland, [9]Institute for Neurogenomics, Helmholtz Zentrum München, Neuherberg, Deutschland

The human brain is a highly sophisticated and complex organ, yet to be completely understood. Investigation of neurodevelopmental disorders may unravel protein networks crucial for brain development. We recruited five patients from three unrelated families from Yemen, Germany, and Spain, manifesting global developmental delay, variable degrees of microcephaly, and hypotonia. Exome sequencing identified two homozygous frameshift variants, NM_138496.1:c.959_960delTT;p.(Phe320Cysfs*18), NM_138496.1:c.1036_1037delCT;p.(Leu346Glyfs*49) in four patients from two independent families and an intronic variant NM_138496.1:c.486-2A>C;p.(?) in a patient from the third family in *CYHR1*, encoding a protein of unknown function. The intronic variant impairs the canonical splice donor site and results in the formation of an aberrant transcript (p.(Val205Glyfs*18)). Endogenous CYHR1 protein showed predominantly nuclear and weak cytosolic expression observed in HeLa, MCF-7, U-373, and human primary fibroblasts. However, we observed a complete absence of CYHR1 in primary fibroblasts derived from two independent patients. Furthermore, transiently expressed wild-type CYHR1 was exclusively observed in the nucleus; however, the immunoreactivity of the mutant protein was reduced and found in the cytoplasm, which was recovered by treating the cells with cycloheximide. Pulldown assay suggested 110 interacting partners of CYHR1, which were enriched in mRNA processing and autophagy-related pathways. Intriguingly in patient-derived fibroblasts, autophagy markers LAMP1, LAMP2, and p62 are drastically increased, whereas LC3β and ATG16L showed reduced expression. Posthoc, whole-transcriptome and proteome profiling also suggested the involvement of CYHR1 in mRNA processing and autophagy-related pathways. Knockdown of cyhr1 in *zebrafish* revealed a completely necrotic and/or dysmorphic brain compared to the control. Surprisingly, iPSCs colonies generated by patient fibroblasts could only achieve the pre-iPSCs phase as compared to wild-type fibroblasts where multiple colonies were obtained in three independent sets.

Our findings suggest that loss-of-functions variants of *CYHR1* cause autosomal recessive neurodevelopmental delay. Hence, CYHR1 is likely to be a novel key factor in human brain development by playing crucial roles in autophagy and mRNA processing.

Abstract ID: 305

Face2HPO: automated HPO labeling of syndromic faces

A. Hustinx[1], T.-C. Hsieh[1], B. Javanmardi[1], P. Krawitz[1]

[1]*Institute for Genomic Statistics and Bioinformatics, Bonn, Deutschland*

Introduction: In the last decades of the internet we have seen an exponential growth of new data.
While most of the data can be labeled by laymen, medical data like syndromic faces require expert knowledge. On top of helping patients, clinicians are expected to work on this labor-intensive labeling process.
In this work we aim to automatically label syndromic faces with their Human Phenotype Oncology (HPO)-terms, as well as provide new explainable artificial intelligence (XAI) insights.
Methods and Results: We present a deep convolutional neural network (DCNN) capable of automatically labeling syndromic faces with their respective HPO-terms. We leverage the tree-like knowledge graph of HPO to allow multiple levels of abstraction, referred to as HPO-levels.
These HPO-levels help us deal with data scarcity of underrepresented cases in the GestaltMatcher DataBase (GMDB).
More importantly, the HPO-level can help us give new insights in the decision making process of our black-box DCNN.
Previously we had to directly explain a model’s diagnosis of disorders, which is extremely difficult and results were hard to interpret. However, using our HPO-classifier we can directly infer the regions of interest, making it far easier to explain.
Conclusion: Not only can this tool facilitate clinicians with their diagnosis or administration, it can also assist GMDB users when adding new patient photos or supplementing old ones with more information.
Lastly, the use of HPO terms in our model inherently makes the process more explainable than most other diagnostic tools.

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P8-3

POLG assoc. mitochondriopathies - Prel. results of a recruiting patient fibroblast study and human neurons show reduced mtDNA and therapeutic options

Ç. Çakmak[1], I. Mantey[1,2], F. Langerscheidt[1], H. Zempel[1]

[1]Institute of Human Genetics and Center for Molecular Medicine Cologne, Cologne, Deutschland, [2]Institute of Human Genetics, Magdeburg, Deutschland

Mitochondria are essential cellular organelles, crucial for neuronal function, and the only organelles in humans with their own set of DNA (mtDNA). Mutations in the only mammalian mitochondrial DNA polymerase, Polymerase-γ (Polγ), result in a spectrum of neurogenetic disorders (POLG-spectrum disorders, POLG-SDs), including Alpers-Huttenlocher syndrome (AHS), an autosomal recessive deadly disease with a carrier frequency reported as high as 2% in the European population. In AHS, patients suffer from severe neurological symptoms like epilepsy, stroke-like infarctions, and usually decease during infancy or early childhood due to status epilepticus or liver failure. There is no curative therapy. Here, we aim to model and characterize POLG-SD in human disease-relevant neurons and patient derived fibroblasts, and rescue the cellular phenotype via genetic and pharmacological strategies. We test i.a. genetic and pharmacological stimulators of mitochondrial biogenesis (MB) which may be translatable into clinical application.

We are introducing the most common POLG-SD mutation (A467T) using (CRISPR/Cas9-based) Cre-LoxP system into i.a. induced pluripotent stem cells (iPSCs) easily differentiable into disease-relevant cortical neurons. Additionally, we are modelling mtDNA depletion by lentivirus/RNAi-knockdown of POLG in iPSC- and SH-SY5Y-derived neurons. We approach rescuing mtDNA amount, mitochondrial and neuronal function via using i.a. off-the-shelve drugs and overexpression of i.a. TWINKLE, PGC-1α and TFAM, all known to stimulate MB. As there is no therapeutic study attempt in patients or patient-derived samples, we started our own study by collecting POLG-SD affected patients´ fibroblasts and characterising the pathological features of the POLG-SDs such as decreased mtDNA amount and ATP production. We will determine promising drug candidates identified in the human cell models (A467T mutation introduced human neurons) on patient-derived fibroblasts to rescue the cellular disease phenotype with the aim to delay or prevent disease onset in future human trials. So far, we have promising drug candidates which are rescuing MB and mtDNA content in carrier-derived and POLG-SD patient-derived fibroblasts and pharmacologically mtDNA depleted neuroblastoma cell lines. Successful therapeutic strategies will serve as templates also for more common forms of neurodegenerative diseases in part caused by mitochondrial dysfunction, such as Alzheimer and Parkinson Disease.

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GestaltMatcher Database – a medical imaging database for rare Mendelian disorders

H. Lesmann[1], S. Moosa[2], T. Pantel[3], J.-M. Li[3], S. Rosnev[3], A. Hustinx[3], B. Javanmardi[3], A. Knaus[3], T. Kamphans[4], W. Meiswinkel[4], M. ten Hagen[3], P. Caro[5], C. Velmans[6], M. Höller[3], I. Abdelrazek[7], G. Elmakkawy[7], K. Alaadin[7], K.C. Coetzer[2], F. Ebstein[8], S. KÜRY[9], E. Abdalla[7], M. Elbracht[10], C. Knopp[10], C. Graziano[11], A. Arlt[12], B. Artem[13], A. Uwineza[14], F. Marbach[5], R.A. Jamra[15], M. Nöthen[1], G. Lyon[16], P. Krawitz[3], T.-C. Hsieh[3]

[1]Institute of Human Genetics, University Hospital of Bonn, Bonn, Deutschland, [2]Division of Molecular Biology and Human Genetics, Stellenbosch University, Stellenbosch, Südafrika, [3]Institute for Genomic Statistics and Bioinformatics, University Hospital of Bonn, Bonn, Deutschland, [4]GeneTalk, Bonn, Deutschland, [5]Institute of Human Genetics, Heidelberg University, Heidelberg, Deutschland, [6]Institute of Human Genetics, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Deutschland, [7]Department of Human Genetics, Medical Research Institute Alexandria University, Alexandria, Ägypten, [8]Institute für Medizinische Biochemie und Molekularbiologie (IMBM), University Medizin Greifswald, Greifswald, Deutschland, [9]Institute de Biologie, CHU de Nantes Hôtel-Dieu; l'Inst. du Thorax, INSERM, CNRS, Université de Nantes, Nantes, Frankreich, [10]Institute for Human Genetics and Genomic Medicine, Medical Faculty RWTH University, Aachen, Deutschland, [11]Unit of Medical Genetics, Department of Medical and Surgical Sciences, Policlinico St. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italien, [12]Institute of Human Genetics, University Hospital of Muenster, Muenster, Deutschland, [13]Research Centre for Medical Genetics, Moscow, Russische Föderation, [14]University Teaching Hospital of Kigali, Kigali, Ruanda, [15]Institute of Human Genetics, University Medical Center, Leipzig, Deutschland, [16]NYS Institute for Basic Research in Developmental Disabilities, Department of Human Genetics and George A. Jervis Clinic, New York, Vereinigte Staaten

Introduction: The performance of next-generation phenotyping (NGP) with artificial intelligence (AI), such as GestaltMatcher, is improved with the size and diversity of the training set. Properly labeled training data is the bottleneck in developing NGP applications and for clinicians to learn about the disorders from the images. Therefore, we developed GestaltMatcher Database (GMDB) for machine-readable medical image data that complies with the FAIR-principles and improves the openness and accessibility of scientific findings in Medical Genetics. GMDB is not only a valuable resource for developing NGP technology but also the teaching material for next-generation clinicians.
Methods: An entry in GMDB consists of a medical image such as a portrait, X-ray, or fundoscopy, and machine-readable meta information such as a clinical feature encoded in HPO terminology or a disease-causing mutation reported in HGVS format. Data was first collected from the literature. Currently, clinicians and individuals recruited from patient support groups provide their previously unpublished data. For this patient-centered approach, we developed a digital consent form. GMDB is a modern publication medium for case reports that complements preprints, e.g., on medRxiv. To enable inter-cohort comparisons, we implemented a research feature in GMDB that computes the pairwise syndromic similarity between hand-picked cases.
Results: We compiled an image collection of 12,496 images from 7,815 cases with 750 disorders through a community-driven effort. Most of the data were collected from over 1,974 publications. Besides, clinicians from 21 worldwide hospitals and eight patient support groups have contributed 361 previously unpublished cases. In addition, digital consent in GMDB has led to increasing adoption of the approach by patients. The web interface enables browsing medical images by searching for the gene or phenotype. Moreover, users can link their patient in GMDB to the patient described in their medRxiv paper because the patient photos are not allowed to be shown in medRxiv. The research app within GMDB was used to generate syndromic similarity matrices by GestaltMatcher approach to characterize 13 novel phenotypes.
Conclusion: GMDB is the first FAIR database for NGP technology where data are findable, accessible, interoperable, and reusable. Therefore, GMDB connects clinicians with a shared interest in particular phenotypes and improves the performance of AI.

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P3-76

Mutational spectrum of suspected Charcot-Marie-Tooth disease in 399 patients

V. Klaschka[1], M. Großmann[1], A. Bier[1], S. Reif[1], M. Timmer[1], C. Jänecke[1], J. Tchertov[1], B. Küchler[2], J. Plaschke[2], S. Krüger[1]

[1]Gemeinschaftspraxis für Humangenetik, Dresden, Germany (Member of Praxisnetz Humangenetik Deutschland), Dresden, Deutschland, [2]MEWIGEN, Dresden, Deutschland

Charcot-Marie-Tooth disease (CMT or HMSN, hereditary motor and sensory neuropathy) is the most common hereditary neuromuscular disease with a prevalence of about 1 in 2500 and genetically very heterogeneous. CMT refers to a group of disorders characterized by a progressive chronic motoric and/or sensory polyneuropathy with phenotypical overlap. Some subtypes of CMT also show other symptoms like spinocerebellar ataxia, optic atrophy or hearing loss.
We analysed all together 399 patients with suspected CMT using Sanger sequencing and MLPA or multigene panel Next-generation sequencing since 2008. We detected 170 pathogenic or likely pathogenic mutations in different genes confirming the diagnosis in 42.5 % of patients.
As previously described most causative mutations were detected in the genes *PMP22* (67.1 %, n=114), *GJB1* (6.5 %, n=11), *MPZ* (5.9 %, n=10) and *MFN2* (4.7 %, n=8). In our cohort these four genes accounted for 84.2 % of genetically positive patients. Among the 114 *PMP22* mutations, 49.4 % (n=84) are duplications, 15.9 % (n=27) deletions and 1.8 % (n=3) point mutations. Further mutations were found less frequent in the genes *NEFL* (2.4 %, n=4), *EGR2*, *GDAP1* and *LRSAM* (each 1.8 %, n=3), *FIG4* and *TRPV4* (each 1.2 %, n=2) as well as in *DNM2*, *GARS1*, *HSPB1*, *IGHMBP2*, *LMNA*, *MORC2* and *PMP2* (each 0.6 %, n=1). Interestingly we have not yet detected any mutations in the genes *HINT1* or *SH3TC2* which are described as belonging to the most commonly involved genes. In contrast we identified mutations in the recently described genes *SORD* and *MME* (each 1.2 %, n=2).
Compared to other studies there were minor differences in the detection rate in the various rare genes, which can certainly be attributed to the different phenotype spectrum of the patients. Nevertheless our study shows a typical gene distribution of the genotypic spectrum of CMT. Our cohort may contain more patients with borderline or subclinical CMT phenotypes. Therefore we suggest the analysis of additional or new genes in previously unexplained, non-typical or subclinical cases to confirm the clinical diagnosis. For CMT patients with *SORD* mutation a specific therapy already exist. Possibly, other therapies for specific CMT mutations will be available in the future.
In summary we propose primarily the MLPA-analysis of *PMP22* to detect whole gene duplications or deletions and secondly an NGS-multigene panel to cover point mutations in CMT patients in the more than 100 genes involved in the pathogenesis of CMT.

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Deep learning for pediatric Bone Age assessment on patients with genetically-confirmed bone disorders

S. Rassmann[1], A. Hustinx[1], T.-C. Hsieh[1], A. Keller[2], K. Mohnike[3], P. Krawitz[1], B. Javanmardi[1]

[1]Institute for Genomic Statistics and Bioinformatics, Bonn, Deutschland, [2]Kinderzentrum am Johannisplatz, Leipzig, Deutschland, [3]Medizinische Fakultät, Otto-von-Guericke-Universität Magdeburg, Magdeburg, Deutschland

Introduction: Assessing skeletal maturity via determining the bone age (BA) is a valuable tool for diagnosing and monitoring pediatric growth disorders. Especially for timing hormone treatment and orthopedic interventions, BA estimates are crucial. However, manual BA assessment is time-consuming and suffers from high intra-and inter-rater variability. While numerous Machine Learning approaches were proposed, few were validated for BA assessment on children with skeletal malformations. Particularly skeletal dysplasia co-occurring with growth disorders are an additional challenge for BA assessments, and even commercial tools fail to generalize to some of them.
Method: We trained a custom deep Convolutional Neural Networks using the data set released with the RSNA bone age challenge. The model is trained without human prior in an end-to-end fashion and, therefore, does not rely on normal bone morphology. We evaluated our approach on (1) the test set of the RSNA competition (n=200, annotated by six raters), (2) an external test with healthy children from the Los Angeles Digital Hand Atlas (DHA, n=1382, two raters), and (3) a private test set comprising children with several genetically confirmed (Turner, Noonan, and Silver Russell syndrome, Léri–Weill dyschondrosteosis/ SHOX mutation, Hypochondroplasia, Achondroplasia, and Pseudohypoparathyroidism) and non-confirmed (Intrauterine Growth Retardation) developmental disorders (n=429 with double ratings and n=443 with single ratings) from two university hospitals in Germany.
Results: Our Deep-Learning based BA models achieve an accuracy that is on-pair with state-of-the-art approaches at a mean absolute error (MAE) of 3.87 months on the RSNA test set. We show generalization to an unseen healthy test cohort on the DHA with an MAE of 5.88 months. Further, we demonstrate that our approach outperforms human raters on patients with skeletal dysplasias with an MAE of 5.41 months w.r.t. to the average of two ratings, while the inter-rater variability was > 9 months. Further, we show that our test-retest reliability on the disorder dataset (< 2.85 with CI [2.62, 3.42] months) is at least as good as human raters (< 3.08 with CI [2.67, 3.63] months). Hence, the proposed method is suited for longitudinal analysis of children with skeletal dysplasias. A user-friendly interface is made available through a web-based tool.

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P2-21

Implementation of microarray-based genome-wide somatic CNV analyses into molecular tumor board recommendations for targeted cancer therapy

K. Oehl-Huber[1], S. Dahlum[1], R. Marienfeld[2], A. Kestler[3], A. Beer[4], B. Rack[5], F. Zengerling[6], P. Möller[2], T. Seufferlein[3], S. Stilgenbauer[7], V. Gaidzik[8], R. Siebert[1]

[1]Institut für Humangenetik, Ulm, Deutschland, [2]Institut für Pathologie, Ulm, Deutschland, [3]Innere Medizin I, Ulm, Deutschland, [4]Nuklearmedizin, Ulm, Deutschland, [5]Universitätsfrauenklinik, Ulm, Deutschland, [6]Urologie, Ulm, Deutschland, [7]CCCU, Ulm, Deutschland, [8]Innere Medizin III, Ulm, Deutschland

The aim of the molecular tumor board (MTB) is to reveal molecular targeted therapy options especially for patients with rare or advanced tumors without approved treatment alternatives. Genomic alterations potentially pointing to potential therapy targets include, besides small variants like single nucleotide variants, also structural alterations including among others copy number variants (CNV). Particularly focal alterations and breakpoints of copy number changes can be informative in this regard. Therefore, the molecular and familial tumor board (MoFa) at the Comprehensive Cancer Center Ulm (CCCU) early on implemented the results of genome-wide CNV microarray analyses of DNA extracted from FFPE tumor tissue samples to identify possible structures for targeted cancer therapy. We here performed a retrospective survey of the results of these CNV analyses including but not restricted to cases included also in the Center of Personalized Medicine (ZPM) in Ulm.
We evaluated the analyses performed in the time period from 1/1/2021 until 30/11/2022. During that period, we analyzed DNA from 103 FFPE tumor samples of various cancer types such as breast cancer, colorectal cancer and glioblastomas in the MoFa setting. The turn-around time from DNA reception to report was usually about 5 working days. Samples were processed using the OncoScan CNV Array workflow, enabling the identification of copy number gains, losses, CNN-LOHs and breakpoints on a genome-wide scale in FFPE tissue.
In 12 cases, no aberrations could be detected due to low tumor cell content or a lack of deletions/amplifications in the tumor tissue. From the remaining 91 cases, we could identify potential molecular targets for therapy in 84% of cases. The molecular alterations detected by the CNV analyses pointed most frequently to a potential therapeutic use of cell cycle inhibitors (in 53% of cases), PI3K/AKT/mTOR signaling pathway inhibitors (35%), PARP inhibitors (26%) and FGFR inhibitors (14%).
Our findings indicate that array-based CNV analysis have a low technical failure rate, a considerably low turn-around time and a high probability to detect alterations potentially targetable in the MTB setting and, thus, can reliably complement sequencing-based approaches particularly with targeted panels.