O1.Multivariate GWAS on achondroplasia-like craniofacial shape variation in healthy human individuals

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Human facial shape is a complex phenotype that is largely genetically determined and with a development that involves a complex series of highly coordinated embryonic events. Naturally occurring mutations that disturb this development cause craniofacial dysmorphism and provide us with a unique window into the genetics underlying facial variation. In this work, we introduce a novel syndrome-informed facial phenotyping method to identify genomic loci associated with facial variation using achondroplasia as an example.

We compared 3D facial scans from 8,246 healthy European-ancestry individuals and 48 achondroplasia patients to calculate an achondroplasia endophenotypic score. In our healthy control sample, we performed a multivariate GWAS of these scores using canonical correlation analysis and observed 35 independent genetic loci that reached genome-wide significance ($p<5x10-8$).

Gene ontology analysis showed significant enrichment of genes involved in skeletal development. Compared to a GWAS of normal facial variation in the same cohort, we observed a higher enrichment using the achondroplasia-derived phenotype. There was an especially strong enrichment of genes involved in chondrocyte differentiation and development, chondrocyte hypertrophy and cartilage condensation, the same processes that are disturbed in achondroplasia. Furthermore, by applying these genes to a multivariate genotype-phenotype model in mice, we recovered an achondroplasia-like phenotype even without the Fgfr3 mutation that is associated with this disease in humans.

In summary, we identified 35 genomic loci that are associated with facial variation along the achondroplasia trait axis. These loci were strongly enriched for genes involved in chondrocyte differentiation and hypertrophy, the same developmental processes that are disturbed in

achondroplasia. These results highlight the great potential of our novel syndrome-informed phenotyping method to improve the understanding of the genetic interface between complex traits and Mendelian disorders.

O2.The establishment of the first reported zebrafish model for thoracic aortic dissection and rupture

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3-4 cases per 100,000 persons per year are estimated to suffer from thoracic aortic dissection (TAD). Weakening of the vessel wall of the thoracic aorta increases the risk for TAD and rupture, which associates with a high mortality rate. Current treatment options in thoracic aortic dissections (TAAD) are limited to a pharmacological reduction of hemodynamic stress and surgical repair at a critical diameter. Despite the availability of different mouse models for TAD, the underlying molecular mechanisms remain elusive.

We therefore developed a zebrafish model for aortic dissection/rupture. For this purpose, we targeted 2 genes involved in angiogenesis, SMAD3 and SMAD6. In humans, loss of function (LOF) of SMAD3 results in thoracic aortic aneurysm and dissection (TAAD), arterial tortuosity and early onset osteoarthritis. SMAD6 LOF mutations increase the risk for a bicuspid aortic valve and TAAD. In zebrafish, both SMAD3 and SMAD6 have 2 paralogues. Using CRISPR/Cas9 gene editing technology, we developed a quadruple knockout (KO): smad3a-/-;smad3b-/-;smad6a-/-;smad6b-/-. At 5 days post fertilization, quadruple KO embryos showed asymmetrical branching of the aortic arches. Survival of adult quadruple KO zebrafish was severely decreased and all but one quadruple mutants died before the age of one year. A stress-inducing protocol caused sudden death in 60% of the mutant zebrafish. Histochemical investigation of consecutive sections of the ventral aorta in quadruple mutants stained for elastin showed medial elastolysis, intramural hematomas, aortic dissections and ruptures, which was further supported by 3D reconstructions. RNA sequencing revealed upregulation of melanogenesis as well as upregulation of transcription factor mitfa, which might be involved in the pathogenesis. These observations indicate that we successfully developed the first ever reported zebrafish model for aortic dissection/rupture. This model will be highly valuable to better understand the pathogenic processes underlying TAAD and to evaluate potential therapeutic compounds.

O3.Preimplantation genetic testing (PGT) in Belgium: national recommendations for genetic centers

BeSHG PGT working group BeSHG PGT working group[1], BeSHG PGT working group BeSHG PGT working group[2], BeSHG PGT working group BeSHG PGT working group[3], BeSHG PGT working group BeSHG PGT working group[4], BeSHG PGT working group BeSHG PGT working group[5] & BeSHG PGT working group BeSHG PGT working group[6]

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PGT treatment encompasses many stages: counselling of the couple, preclinical testing, IVF treatment, embryo biopsy, genetic testing, embryo transfer and/or vitrification and clinical follow-up. An optimal coordination of all these stages demands a close collaboration between experts in assisted reproduction, embryology and genetics. Standardization and quality assurance for all Belgian assisted reproduction centers is under the supervision of the Belgian college of Reproductive Medicine (BRM). A dedicated PGT working group was initiated under the auspices of the Belgian Society for Human Genetics (BeSHG), consisting out of laboratory geneticists, clinical geneticists, gynecologists, IVF specialists and embryologists, to guarantee a high standard of care, exchange knowledge and implement a harmonized workflow in all Belgian PGT centers.

The BeSHG PGT working group developed national recommendations regarding genetic testing of in vitro fertilized embryos for single gene or chromosomal disorders. These are based on expertise and published data available at the time of composition, supplementary to the latest ESHRE PGT recommendations, and will be regularly re-evaluated and updated. The technical recommendations may be applicable for clinical and laboratory practitioners while recommendations on patient inclusion, counseling, reporting, follow-up and cost of treatment may be relevant for Belgian families, as well as for cross-border patients.

These national good practice PGT recommendations should ensure a harmonized provision of PGT service and the best care for patients seeking treatment in Belgium. This process of formulating a consensus expert opinion on best practice can be applied in other countries where PGT service is not overseen by a designated national agency.

O4.BabyDetect project: molecular newborn screening in Belgium

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Background/Objectives: Genomic technologies already play an important role in newborn screening (NBS) as a second-tier test for confirmation of first-tier biochemical assays in most of currently screened disorders. From September 2022 we have started consenting and enrolling newborns in frames of BabyDetect project to conduct a pilot genetic NBS program in Southern Belgium to explore the feasibility and acceptability of new generation sequencing NBS.

Methods: We are using targeted NGS approach to screen in three years the following number of newborns: September 2022 – 2,000, September 2023 – 17,000, September 2024 - up to 55,000 newborns in the southern Belgium. Digital informed consent is being obtained for all newborns before any research intervention. In parallel to BabyDetect, the public biochemical NBS and SMA screening data is available to compare with obtained results from the pilot.

Results: The board of dedicated professionals, both scientists and medical doctors, succeeded in preparing a potential list of genes for 126 early-onset, severe, treatable genetic disorders. tNGS panel consisting of 363 genes responsible for those disorders is designed and synthesized using Twist Bioscience technology. Ethics committee approved the study allowing electronic consenting of the first newborn in September 2022. In the period of 4 months we have already consented, sequencing and analyzing 900 newborns from Citadelle Hospital (CHR) in Liege.

Conclusion: The rapid development of innovative therapies for severe genetic conditions, which cannot be diagnosed by current metabolic NBS, underlines the need to incorporate genetic testing into the NBS. There are many gaps related to molecular NGS, which include poor coverage, diversity of captured regions, challenges in variant calling and filtering, the absence of consensus on reporting variants of uncertain significance. New pilot programs and mandatory screening programs with large samples applying genetic testing as the first-tier approach will help to clarify uncertainty related to these topics and will facilitate development of guidelines for clinical practice.

O5.The pseudoxanthoma elasticum zebrafish model contributes to novel pathophysiological insights and therapeutic strategies in ectopic mineralization.

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Pseudoxanthoma elasticum (PXE) is an autosomal recessive hallmark disorder of ectopic calcification (EC), where EC and fragmentation of elastic fibers result in skin, ocular and cardiovascular symptoms. PXE is most commonly caused by bi-alllelic pathogenic variants in the ABCC6 gene, encoding an ATP-dependent transmembrane transporter of which the substrate is unknown. With an incompletely understood pathophysiology and being an intractable disease, PXE exemplifies how disease-modeling in zebrafish can help to better understand an EC disorder and provide novel strategies for treatment.
The ABCC6 gene has two orthologs in zebrafish, abcc6a and abcc6b. We developed a complete abcc6a knockout zebrafish model using CRISPR/Cas9, showing that it has an essential role in controlling mineralization. The model developed hypermineralization of notochord and ribs starting embryonically and progressing in adulthood with development of scoliosis. This indicated a direct relation between loss of abcc6a expression and dysregulated osteogenesis.
We went on to show that an excessive DNA Damage Response was present in the abcc6a-/- fish using expression analysis of DDR/PARP1 targets with QRT-PCR. PARP1 and the ATM–p21–p53 axis were found to be significantly increased. In addition, PARP1 downstream targets IL-6, signal transducer and activator of transcription 1/3, TET1, and RUNX2 were upregulated.
Finally, we validated our PXE zebrafish as a model for compound screening in EC by showing a reduction of the hypermineralization with known effective drugs such as vitamin K1, etidronate and magnesium citrate. Based on this validation study we demonstrated for the first time that sodium thiosulphate and PARP-inhibition using minocycline are able to attenuate the PXE-related mineralization in vivo.
Overall, we demonstrate how to use the PXE zebrafish model in translational research from mechanistic insights to in vivo compound screening.

O6.Retained chromosomal integrity following CRISPR/Cas9-based mutational correction in human embryos

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Aims: CRISPR/Cas9-mediated gene correction can be used to reduce mutation transmission. However, recent genome editing experiments in human embryos have reported mosaicism and loss-of-heterozygosity (LOH, loss of one parent's DNA contribution). These LOH events were either attributed to partial/complete loss of the targeted chromosome or to correction by employing the maternal wild-type allele (gene conversion). Here, we targeted a male-infertility related mutation in human embryos, to further investigate the DNA repair mechanisms after CRISPR/Cas9-mediated double-strand break induction.

Methods: Sperm harbouring a heterozygous base-pair substitution in PLCZ1 (c.136-1G>C) was utilized. The CRISPR complexes were injected simultaneously with the sperm into donated human spare oocytes followed by assisted oocyte activation to induce fertilization. DNA was extracted from a whole embryo or individual blastomeres and amplified by whole-genome amplification. These samples were analysed through GENType, a next-generation sequencing-based method for genome-wide haplotyping and copy number profiling, and a targeted SNP assay (8,500 bps around the mutation site).

Results: In half (9/18) of the embryos originating from mutant sperm, only the wild-type allele was detected (designated “absence-of-mutation”), without signs of repair template use. In thirteen embryos analysed with GENType, of which three were absence-of-mutation embryos, the targeted chromosome was intact and no long-range LOH events were observed, while short-range LOH events could be identified. Based on the normal copy number and the presence of heterozygous SNPs flanking the CRISPR/Cas9 target site, we hypothesize that these short-range LOH events were caused by gene conversion. To investigate mosaicism, individual blastomeres were analysed from two embryos originating from mutant sperm. Both embryos were mosaic with 60% (9/15) of the blastomeres displaying solely the wild-type allele. All blastomeres contained an intact targeted chromosome without long-range LOH events.

Conclusion: Mutational correction can be obtained in human embryos by CRISPR/Cas9, resulting in short-range LOH events with a retained chromosomal integrity.

O7.Striking phenotypical differences between Ipo8 knock-out mouse models on different genetic backgrounds explored by RNA sequencing

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IPO8 encodes importin-8, a ubiquitously expressed nuclear transport receptor of the importin-β family. Importin-8 translocates cargoes such as proteins, RNAs and ribonucleoproteins from the cytosol to the nucleus. We recently identified bi-allelic loss-of-function IPO8 variants causing a syndromic form of thoracic aortic aneurysm (TAA). An Ipo8 knock-out (Ipo8-/-) mouse on a C57Bl/6N genetic background displayed root and ascending aortic aneurysms from 8 weeks of age onwards. Surprisingly, the identical Ipo8 knock-out on an Sv129 genetic background did not show any aneurysm development.
The aim of our RNA sequencing study is to identify differentially expressed genes in the aortic walls of C57Bl/6N Ipo8-/- mice compared to Sv129 Ipo8-/-, to pinpoint genes acting as TAA-drivers in the former, or protectors in the latter.
C57Bl/6N Ipo8-/- and WT, and Sv129 Ipo8-/- and WT (N=8 per group) were sacrificed at 16 weeks. The aortic root and ascending aorta were isolated and RNA was extracted. RNA-sequencing was outsourced to Novogene, while data analysis was carried out in house.
RNA-seq data analysis of the four groups was performed, surprisingly pointing out that the largest variation between the groups was due to the genetic background, and not due to the mutation itself. My preliminary data analysis points towards an important role for calcium signalling, chemokine signalling, cell adhesion and cytokine secretion to explain the difference between both genetic backgrounds. These dysregulated pathways were previously reported in TAA development. However, our model will additionally allow us to discriminate what is disease-driving from what is a secondary effect or a passenger event.
Based on the striking divergent cardiovascular phenotype of the C57Bl/6N and Sv129 Ipo8-/- strains, RNA-sequencing of aortic root and ascending aorta showed interesting differentially expressed pathways and genes, which will be investigated further. Moreover, our model emphasizes the importance of mouse genetic backgrounds in disease modelling.

O8.WES advances a genetic diagnosis in patients with differences of sex development and corroborates the role of RXFP2 in autosomal recessive bilateral cryptorchidism and infertility

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Background/Aims:
Differences of sex development (DSD) are heterogeneous conditions affecting the development of chromosomal, gonadal or anatomical sex. The diagnostic yield of whole exome sequencing (WES) studies typically does not exceed 35%. Here, we investigated the benefits of WES for the genetic diagnosis in patients with DSD.

Methods:

Between 2016 and 2023, 198 unrelated index patients with a clinical suspicion of DSD underwent WES-based panel testing interrogating the coding regions of 130 genes implicated in DSD, primary ovarian insufficiency, and hypogonadotropic hypogonadism. Variants were extracted and classified according to the ACMG guidelines. Copy number variant (CNV) analysis was performed using the ExomeDepth algorithm. Structural modeling and a cyclic AMP (cAMP) reporter gene assay were used to assess the pathogenicity of an RXFP2 (NM_130806.5) missense variant.

Results:
In 15% of patients, we identified a likely pathogenic or pathogenic rare variant in 15 distinct DSD genes, including AR, NR5A1, CYP21A2, SRY, SRD5A2, DHX37, WT1, TXNRD2, HSD17B3, HSD3B2, MCM8, TACR3, FGFR1, ATRX, and RXFP2. The majority are sequence variants, four are CNVs identified using ExomeDepth. Interestingly, in two brothers displaying bilateral cryptorchidism and infertility, an intragenic RXFP2 deletion was found in trans with a heterozygous missense variant c.229G>A, p.(Glu77Lys). The RXFP2 receptor binds INSL3 and is involved in testicular descent. The pathogenicity of the missense variant was substantiated by in silico modeling and in vitro functional analysis. The missense variant showed normal expression and ability to bind the ligand INSL3, but the absence of a cAMP signal in response to INSL3 supported loss-of-function.

Conclusion:
We demonstrate the benefit of WES-based genetic testing of DSD in a clinical context and illustrate the important additive value of CNV assessment on WES data. This finding corroborates the role of RXFP2 in autosomal recessive bilateral cryptorchidism and supports that infertility is part of the phenotype.

O9.Malfunctional CTDP1 impairs the cell cycle implying its crucial role in brain development

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Malformations of cortical development (MCDs) constitute a diverse range of disorders that are common causes of neurodevelopmental delay and epilepsy. The underlying molecular mechanism of several MCDs have been elucidated, although many still remain unknown.

Trio exome analysis in a girl with congenital arthrogryposis, unilateral auditory neuropathy, absent optic chiasma and grey matter heterotopia revealed the presence of two novel variants in the C-terminal domain phosphatase 1 (CTDP1) gene. CTDP1 functions as a phosphatase which dephosphorylates the C-terminus of RNA polymerase II (RNAPII) and may potentially be involved in MCD, re-enforced by its essential role for normal embryo development.

CTDP1 expression was analysed both at mRNA level using qPCR and at protein (FCP1) level by western blot (WB) analysis. The protein's phosphatase activity on the C- terminal domain was studied using WB. Cell cycle analysis was performed by DNA staining using propidium iodide.

qPCR data showed a significant downregulation of CTDP1 expression in patient versus controls. WB revealed a decreased protein level and more phosphorylated RNAPII in patient cells. Lastly, a disturbed cell cycle was observed in the patient compared to the controls.

Our preliminary data show that the expression of CTDP1, as well as FCP1 and the phosphatase activity are altered in the patient. As seen by qPCR, alterations caused by abnormal FCP1 function in the cell cycle are associated with disturbed cell cycle proteins (p27, cyclin B). Together, these results provide preliminary evidence that FCP1 plays a role in early brain development and cell growth by regulating the cell cycle. Functional analysis on brain organoids to further characterize the role of FCP1 in brain development is currently ongoing in our lab.

O10.Multiple recombination mechanisms drive the high incidence of 22q11.2 Deletion Syndrome

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The 22q11.2 Deletion syndrome is the most common microdeletion disorder in humans and thought to be caused exclusively by non-allelic homologous recombination (NAHR) between the low copy repeats (LCRs). However, due to the repetitive nature of the LCR22s the reference genome contains gaps, the complete sequence of the LCRs remains unclear and, as a consequence, the rearrangement breakpoints have never been mapped at a nucleotide resolution.
To explore the nature of the rearrangements, we used Fiber-FISH and long-read sequencing technologies and developed a novel haplotype-aware de novo assembly algorithm, to map the LCRs and unravel the mechanisms causing rearrangements. We mapped the rearrangement locus at the nucleotide level in six families. We identified different recombination loci driving NAHR of LCR22-A/B, -B/D, -A/C and -A/D deletions. Moreover, rearrangements involving LCR22-B cluster within a palindromic AT-rich repeat (PATRR) suggesting that PATRR-mediated mechanisms are also drivers for the instability of the 22q11.2 locus. Furthermore, haplotypes in two families suggested inversions preceding deleterious rearrangements with LCR22-B. In one case, an LCR22-A/D inversion was already present in the parent of origin leading to an LCR22-B/D deletion in the patient while, in another, a mosaic LCR22-A/B inversion was found where some of the patient's cells included the inversion while others harbored the deletion. Taken together, the results indicate that a multitude of mechanisms combined contribute to the high incidence of 22q11.2 deletion syndrome.

O11.CGG Short Tandem Repeat Expansions are Overrepresented in Neurodevelopmental Disorders

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Short tandem repeat (STR) DNA tracts make up a significant portion of the human genome, accounting for approximately 3%. The trinucleotide CGG STR is a subset of the repeat found throughout the human genome. Unlike most other STRs of alternative nucleotide composition, CGG STRs are highly enriched within and surrounding gene coding regions. CGG STRs stand out due to their association with neurodevelopmental disorders (NDDs). Large expansion mutations of these repeats trigger hypermethylation of the repeat locus which, in turn, results in transcriptional silencing of the associated gene. By applying whole genome sequencing, we interrogated 5963 CGG STR loci within a large NDD cohort of 15,996 individuals. Within these individuals, we identified a total of 419 large CGG STR expansions at 142 unique CGG STR loci within 118 different genes. Through enrichment analysis, we observed a significant involvement of these genes in DNA binding, regulation, and transcription (padj = 8.31 x 10-8) and expression within brain tissues (cerebral cortex: padj = 3.6 x 10-2, cerebellum: padj = 9.9 x 10-3). Specifically, within the individuals displaying autism spectrum disorder (ASD), we were able to identify a 2.9 increased odds ratio of large CGG STR expansion incidence in comparison to unaffected parents. We classified CGG repeat expansions within over 30 genes which have been previously linked to disorders with either an autosomal or X-linked dominant inheritance pattern. With the overwhelming majority of these being neurodevelopmental in nature. Furthermore, by crosslinking our repeat expansion results with both publicly available haploinsufficiency data and exonic variant analysis of the expansion-bearing patients we identified several strong candidate genes, such as RGPD2 and SAMD1, that may be unreported in their involvement within recessive NDDs. Here, we not only highlight strong novel gene candidates for CGG expansion disorders but through gene enrichment, haploinsufficiency, and variant analyses we solidify the link between CGG STRs and NDDs.

O12.Kyphoscoliotic Ehlers-Danlos syndrome due to pathogenic variants in PLOD1 and FKBP14: further insights into the pathophysiology and comparison of clinical characteristics.

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The autosomal recessive kyphoscoliotic Ehlers-Danlos syndrome (kEDS) is caused by the deficiency of either lysyl hydroxylase 1 (LH1) or the peptidyl-prolyl cis-trans isomerase FKBP22, two proteins participating in collagen biosynthesis in the rough endoplasmic reticulum. Deficiency of LH1 (encoded by PLOD1), which is crucial for collagen crosslinking, was the first biochemically elucidated congenital error of the human collagen metabolism. Defects in FKBP22 (encoded by FKBP14), which acts as a molecular chaperone of types III, IV, VI and X collagen, were found 40 years later in a subset of individuals presenting a kEDS phenotype with a normal LH1 function.

Clinical characteristics of kEDS include congenital muscle hypotonia, early onset kyphoscoliosis, generalized joint hypermobility and vascular fragility, and defects in both PLOD1 and FKBP14 also come with some gene-specific clinical characteristics. Despite the important phenotypic overlap between kEDS-PLOD1 and kEDS-FKBP14, the common pathophysiological pathway remains poorly understood and functional studies on patient-derived material are scarce.

We report the clinical and molecular characteristics of 14 individuals with kEDS-PLOD1 and 3 individuals with kEDS-FKBP14, and compared our findings with previously reported individuals with kEDS-FKBP14 and kEDS-PLOD1. Using patient-derived skin fibroblast cultures, we found evidence of ascorbic acid-dependent upregulation of FKBP22 in kEDS-PLOD1 skin fibroblasts and thus provide the first pathophysiological link between kEDS-FKPB14 and kEDS-PLOD1. In addition, we provided the first evidence for intracellular retention of types III and VI collagen in kEDS-FKBP14, which could explain part of the phenotype. The intracellular accumulation of these collagens was not accompanied by an upregulated unfolded protein response or autophagy using western blot or RT-qPCR analysis.

In conclusion, this study compares the phenotypic features of kEDS-FKBP14 and kEDS-PLOD1 and highlights new insights on the underlying pathophysiological mechanisms and on how these defects in collagen biosynthesis influence the matrix organization and lead to the observed phenotypes.

O13.Single-cell dissection of cervix and placenta reveal both novel and overlapping cell types

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Placental trophoblasts have been detected in cervical smears early in gestation, creating opportunities for non-invasive prenatal diagnosis. However, trophoblast isolation is limited by the lack of a cell catalogue and molecular profile of cervical smears. To establish an atlas and to explore the potential of single-cell RNA-sequencing to detect cervical trophoblasts, 10,539 single-cell transcriptomes from 12 non-invasive exocervical smears from pregnant women were profiled. In addition, we characterized 34,565 cells from six placental biopsies. We uncovered a novel extravillous trophoblast cell subtype characterized by epithelial marker genes and reduced HLA-G expression. Integration of both cell atlases demonstrated surprisingly similar expression profiles between maternal epithelial cells and placental extravillous trophoblasts, indicating that these trophoblasts retained epithelial properties without an invasive mesenchymal phenotype. Additionally, differential expression analysis identified novel markers discriminating cervical and placental cell types. Those analyses may contribute to trophoblast discovery and isolation from cervical smears.

O14.To test or not to test: the importance of genetics in the diagnostic workup of cerebral palsy

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Objectives: Cerebral palsy (CP) is the most frequent cause of motor impairment in children. Perinatal asphyxia was long thought to be the leading cause of CP. However, studies have illustrated its causation in only about 10% of patients. Hence, the role of genetic factors has gained interest in the aetiology of CP. In this research project, we systematically performed genetic investigations in a paediatric CP cohort. By using this strategy, we aim to expand the knowledge regarding the contribution of genetic variants in the development of this disorder.

Methods: Medical files of 716 patients with CP were analysed for exclusion criteria: (1) extreme prematurity (<30 weeks postmentrual age); (2) history of perinatal asphyxia; (3) other aetiology (e.g. perinatal infection, trauma, etc.); (4) parental refusal of genetic test; (5) absence of parental blood samples for trio analysis. This led to the exclusion of 310 patients. In 337 out of the remaining 406 patients, both single nucleotide polymorphism array and exome sequencing were performed. In patients with a recognizable phenotype, targeted analyses were conducted. In the remaining 69 patients, analysis is still ongoing.

Results: A genetic disorder was diagnosed in 129/337 patients, resulting in an overall genetic diagnostic yield of 38.3%. A large proportion of these patients had ≥ 1 of the following comorbidities: intellectual disability/developmental delay, epilepsy, autism spectrum disorder. In this subgroup the diagnostic yield was even higher, namely 49.6%.
Overall, the most frequently affected genes were KIF1A (8/129, 6.2%) and COL4A1 (4/129, 3.1%). Other genes with variants in >1 patient were FRRS1L, MECP2, BRAF, TSEN54, DYRK1A, RNASEH2B and RNU7-1.

Conclusion: Genetic investigations in our CP cohort led to a diagnostic yield of 38.3%. This highlights the importance of genetic testing in CP. Diagnosing these disorders is crucial for the patient's prognosis and clinical follow-up, as well as genetic counselling.

O15.Whole genome sequencing sheds light on the dark matter of the genome in patients with inherited retinal diseases

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Purpose: With whole genome sequencing (WGS) we aimed to uncover non-coding variants, structural variants (SVs) and novel candidate genes in unsolved cases with inherited retinal diseases (IRD).
Methods: WGS was performed in 82 patients (77 probands) with IRD. Coding and non-coding variants were analyzed using Seqplorer, Franklin (Genoox), ExomeDepth, Manta and Delly. Intronic variants were prioritized using SpliceAI and Alamut Visual. Non-coding variants were assessed in silico using multi-omics or in vitro functional assays. Expression of candidate genes was investigated using retinal single-cell datasets.
Results: Novel (likely) pathogenic regulatory variants were found in 4% (3/77), one of which is a promotor variant of RPGRIP1 c.-152A>C in a coding monoallelic case, predicted to disrupt an OTX2 binding site. Two 5'UTR variants were identified in a monoallelic case (BBS12 c.-11+3dup) and in a patient with macular disease (ELOVL4 c.-187T>G). Coding variants in novel candidate genes represent 6.5% (5/77), such as ACACB, encoding a player in lipid metabolism. Variants in genes that were recently implicated in mostly syndromic IRD, such as ALPK1, GRN and ITM2B, facilitated a genotype-driven diagnosis in 9% (7/77). Analysis of non-coding regions in monoallelic cases allowed to pinpoint putative deep-intronic splicing variants in 16% (12/77), illustrated by the first deep-intronic ALMS1 variant that was shown to lead to pseudo-exon inclusion by minigene assays. These variants represent novel targets for antisense oligonucleotide-mediated therapy. Finally, variants in well-established IRD genes were found in 12% (9/77), including 2 SVs (3%, 2/77).
Conclusions: We demonstrate that non-coding variants in the dark matter explain 20% of the genetic architecture of our previously unsolved IRD cohort. Overall, WGS solved up to 46% of patients and uncovered novel targets for therapy.

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O16.Differential alternative splicing analysis links variation in ZRSR2 to a novel oral-facial-digital syndrome

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Orofaciodigital (OFD) syndromes represent a group of rare genetically heterogeneous developmental disorders. OFDs are due to pathogenic variants in genes that are involved in primary cilia formation and/or function. We identified the same germline variant c.1207_1208del (p.Arg403Glyfs*24) in the last exon of ZRSR2, either occurring de novo or inherited in an X-linked recessive pattern, in 6 affected males from 4 unrelated families with OFD syndrome in association with structural brain abnormalities, ranging from alobar holoprosencephaly to pituitary abnormalities. ZRSR2, located on the X chromosome, is part of the minor spliceosome complex which recognizes minor (U12-type) introns, representing 0.35% of human introns. Although somatic pathogenic variants in ZRSR2 were associated with the development of myelodysplastic syndrome (MDS), this is the first association of germline variation in ZRSR2 with a human developmental disorder. Alternative splicing analysis of minor spliceosome gene targets in lymphoblastoid and fibroblast cell lines from an affected patient harboring the ZRSR2 variant and from unrelated controls, showed significant enrichment of minor intron retention. Among the differentially spliced targets are ciliopathy-related genes, such as TMEM107 and CIBAR1. Primary fibroblasts containing this ZRSR2 variant had abnormally elongated cilia, confirming an association between defective U12-type intron splicing and abnormal primary cilia morphogenesis.

O17.An in vitro enzymatic assay to elucidate the VUS problem in RPE65, a target for retinal gene therapy

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In 2017 and 2018, Luxturna received FDA and EMA approval as the first gene therapy product to treat patients with biallelic RPE65-mutations, causing severe inherited blindness. Eligibility for gene therapy requires a complete molecular diagnosis, which is often hampered by the identification of variants of uncertain significance (VUS), which have insufficient evidence concerning their pathogenicity. Given the role of RPE65 in the visual cycle pathway, our aim is to develop an in vitro biochemical assay to assess the pathogenicity of RPE65 VUS.
This assay is based on simulating the visual cycle in HEK293-F cells via overexpression of visual cycle enzymes including RPE65. As RPE65 is responsible for the isomerization of retinol derivatives, HPLC-based analysis of retinols is used as a read-out, in addition to RPE65 immunoblotting.
First, missense variants without prior functional assessment were compiled from our in-house database, LOVD and our collaborative network and subsequently classified according to ACMG/AMP guidelines. Overall, 53 RPE65 VUS were obtained. Six constructs have been generated by cloning the open reading frames of RPE65, CRALBP, LRAT and RDH5 in a pVitro2 backbone. Additionally, expression of these bicistronic constructs was compared to monocistronic constructs. The RPE65-CRALBP construct underwent mutagenesis for all selected VUS, followed by overexpression and immunoblotting for 28 of the VUS, allowing an assessment of their effect on RPE65 protein levels. Assessment of residual enzymatic activity of these VUS is ongoing.
Functional assessment of RPE65 VUS via the enzymatic assay as proposed here, will improve patient diagnoses and subsequently eligibility for Luxturna gene therapy.

O18.Sample Catalog: A Federated Platform for Identification and sharing of biological samples in rare disorders

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Neurodevelopmental disorders are etiologically extremely heterogeneous and individually ultra-rare genetic disorders. Hence, research on those disorders is hampered by the lack of biological material. Current platforms that share information about biological samples are based on centralized databases, lack constraints on inserted information resulting into heterogeneous data that is difficult to query. Moreover, only a limited number of laboratories pool data into centralized databases.
We present Sample Catalog, a federated platform developed to enhance collaboration among investigators engaged in research on rare disorders.
Sample Catalog enables users to share metadata about biological samples, including information such as individual's age at sampling, gender, material type, HPOs, OMIMs, and availability of genomic data. The data is retained within the institutions of origin and is accessible through the platform's web-based interface. The primary investigator (PI) is proprietor. Associates of the PI are granted access and allowed to input data to the PI's catalog.
Each sample is linked to a specific individual, and can be categorized either as private or public. Public samples can be viewed by all users, while private samples are reserved to users that are directly linked to the PI. Researchers can query the metadata of public samples in order to identify appropriate samples for their studies. Additionally, Sample Catalog facilitates the organization of data into datasets, that can be shared among researchers engaged in a joint project.
Our federated data model overcomes a major bottleneck in research on rare diseases. By using standardized HPOs and OMIM terminology the identification and localization of matched samples is made easy. Currently, the platform is being installed across Europe as part of a COST Action, MINDDS, an EU consortium strengthening neurodevelopmental rare disorder research.