Abstracts BeSHG meeting 2025

13-14 March 2025

(Aula Magna - Louvain-la-Neuve)

O1 - PRKD2 as a candidate gene modulating T cell activation in familial Systemic sclerosis

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Systemic sclerosis (SSc) is a rare, chronic condition characterized by vascular, immunological and connective tissue abnormalities. Genetics is the strongest risk factor, but no monogenic or oligogenic causes have been demonstrated, with family-based studies hampered by the low frequency of familial clustering (≤1.6% of cases). We performed whole exome sequencing (WES) on blood-DNA from twelve affected individuals, from six families. The goal of our research is to identify and functionally validate genes that may drive SSc pathogenesis in these families.

WES data was filtered for genes with variants that are: (i) shared by both affected individuals within each family, (ii) absent-to-ultra rare in the general population, and (iii) predicted to affect protein function in silico. 31-47 genes per family satisfied these criteria; of these, only three were shared by more than one family: TTN, COL12A1 and PRKD2.

PRKD2 encodes an intracellular serine threonine kinase that participates in T cell receptor (TCR) signaling, thymic selection of T cells, and T-dependent B cell responses and autoantibody production. Loss of PRKD2 has also been reported to decrease bleomycin-induced fibrosis in mice. Preliminary data from Jurkat (CD4+ T) heterozygous knock-in cell-lines (generated by CRISPR/Cas9-HDR) suggest that both of the variants identified in SSc families result in increased NF-kB activation and expression of T cell activation markers (such as CD69 and PD1) upon TCR stimulation. One of the two variants also seems to increase NFAT signalling upon TCR stimulation. These data support a pathogenic role for PRKD2 in T cells in SSc, with functional differences between the two variants identified possibly contributing to phenotypic differences between patients.

O2 - Revealing the developmental-genetic basis for the human cranial vault

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The human cranial vault is a complex trait that protects the brain and closely interacts with the cranial base and face due to shared developmental and genetic inputs. Morphological variation in the vault is both clinically and evolutionarily relevant, because it is linked to a range of disorders (e.g., craniosynostosis) and adaptations to selective pressures (e.g., childbirth constraints, increases in brain size). Yet the genetics of the vault are often overlooked at the expense of the brain, and even when studied, these investigations tend to be underpowered as radiation exposure and other imaging artifacts can limit sample sizes. Here, we acquire 3-D, spatially dense measures of vault shape from the UK Biobank (N=48,564) via data-driven phenotyping and complete a multivariate genome-wide association study. We identified 1,579 genome-wide significant loci primarily enriched for processes related to embryonic and postnatal skeletal development, as well as embryonic and skeletal system morphogenesis. The discovered loci highlight key craniofacial transcription factors, including known loci near RUNX2, DLX5, and TBX15, and novel loci near TWIST1/2, MSX1/2, ALX4, and RUNX1. We additionally identified LRP5, a major Wnt signaling receptor linked to bone density phenotypes and craniosynostosis, and confirmed several other loci associated with sagittal and metopic craniosynostosis, including BMP7. Our second most significant association was with BNC2, one of the most well-supported candidates for adaptive Neanderthal introgression. In total, we found 96 other genes in regions of Neanderthal introgression, some of which recapitulated hallmark morphological features of Neanderthals. Finally, roughly 50% of vault shape loci overlapped with brain shape loci, and their overall average shape effects were highly concordant. Altogether, these results provide the most statistically robust insights into the developmental-genetic basis for human cranial vault variation to date and further underscore how genes associated with craniosynostosis also contribute to “normal” vault variation.

O3 - Biallelic GAD2 variants cause a early-onset developmental encephalopathy

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Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian adult brain and is highly involved in the development and plasticity of the nervous system. GABA is synthesized by glutamic acid decarboxylase (GAD), which exists in two isoforms, GAD67 and GAD65, encoded by the GAD1 and GAD2 genes, respectively. GAD1 is critical for maintaining basal GABA levels, while GAD2 rapidly adapts to increased GABAergic demand. While biallelic variants in GAD1 have been linked to syndromic developmental and epileptic encephalopathy (DEE) characterized by neonatal-onset seizures, congenital malformations, and hypertonia. Interestingly, GAD2 variants have not been reported in humans thus far.

In this study, we identified multiple families with biallelic GAD2 variants associated with developmental encephalopathy. Affected individuals, all born to consanguineous parents, exhibited no abnormalities in perinatal history or microcephaly at birth. Epilepsy manifested during late neonatal or early infancy stages, predominantly as focal seizures. Brain MRIs were unremarkable, yet all cases showed developmental delays ranging from moderate to severe.

To further substantiate our clinical observations, we generated a GAD2 knockout (KO) mouse model and subjected it to extensive behavioral and seizure susceptibility assessments. The GAD2 KO mice displayed hyperactivity, heightened anxiety, repetitive behaviors, and increased seizure susceptibility, consistent with clinical features observed in affected individuals. These results parallel clinical observations in patients providing further support for the relevance and translational potential of our mouse model.

In summary, we report the first cases of biallelic GAD2 variants linked to developmental encephalopathy and provide compelling evidence for their pathogenicity. The striking parallels between human clinical phenotypes and findings from the GAD2 KO model underscore the relevance of this gene in neurodevelopment and epilepsy.

O4 - Unravelling shared pathomechanisms in syndromic thoracic aortic aneurysm disorders using single-cell RNA sequencing

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Thoracic aortic aneurysm (TAA) is characterized by progressive enlargement of the aorta, increasing the risk of dissection. Current pharmacological treatments are limited and often require high-risk surgical procedures. This highlights the need for better understanding of the disease mechanisms to develop new therapies to stop or reverse TAA. The transforming growth factor β (TGF-β) signalling pathway is key in TAA, with mutations in this pathway linked to syndromic TAAs (sTAAs), such as Marfan syndrome (MFS), caused by FBN1 mutations, and IPO8-related sTAA, caused by IPO8 mutations.

We analysed single-cell RNA sequencing data from aortic samples (ascending aorta and root) of the TAA mouse models C57Bl6J Fbn1C1041G/+ and C57Bl6N Ipo8-/- , and their respective wild-type (WT) littermates to identify convergent mechanisms. Samples were collected before and after aneurysm development to distinguish driving from compensatory mechanisms.

In the aneurysmal tissue samples, several top overlapping differentially expressed genes (DEGs) encoding for extracellular matrix (ECM)-related proteins, such as Timp1, Fbln2, Tnc, Fn1, and Col8a1, were identified in the vascular smooth muscle cell (VSMC) cluster. Enrichment analysis revealed involvement of focal adhesions, ECM, and cytoskeleton in Fbn1C1041G/+ and Ipo8-/- VSMCs, which are linked to mechanotransduction. Differences between WT and mutant mice were less pronounced in non-aneurysmal tissue samples. A few shared DEGs were identified, including Tnc, Sncg, Wif1, Rbp4 and Smad6. Additionally, we pinpointed a shared VSMC subcluster enriched at the late timepoint, which showed a greater upregulation of ECM and focal adhesion genes (Tnc, Fn1, Itga5), along with a higher expression of TGF-β-related genes (Bmp4, Tgfb1, Tgfb2, Serpine1) and lower expression of contractile genes (Myh11, Cnn1).

In conclusion, dysregulation of mechanotransduction may be an important shared pathomechanism in Marfan syndrome and IPO8-related sTAA. Furthermore, a subset of VSMCs with a more extreme phenotypic switch might be involved in the TAA pathogenesis of both disorders.

O5 - Clinical evaluation of long-read sequencing-based episignature detection in developmental disorders

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Disease-specific genome-wide methylation changes characterize a subset of developmental disorders. These episignatures inform about underlying pathogenic mechanisms and can be used to assess the pathogenicity of genomic variants and confirm clinical diagnoses. Currently, episignature detection requires the use of indirect methylation profiling microarrays. As long-read-sequencing (LRS) enables the detection of single nucleotide variants (SNVs), structural variants (SVs), and base modifications, we aimed to assess its potential for simultaneous episignature and genomic variant detection.

Twenty patients, with thirteen distinct developmental disorders having known episignatures, were subjected to genome-wide nanopore sequencing. We first evaluated the compatibility of known episignatures with nanopore sequencing and subsequently developed a support vector machine for each developmental disorders.

Methylation levels at episignature loci co-clustered disease samples and microarray-based disease reference. Our classifier identified the correct episignature in 17/20 blinded samples. The three remaining patients were classified as control by both our classifier and commercially available microarray-based episignature assay. All pathogenic SNVs and SVs detected by standard-of-care methods were identified. In addition, we were able to evaluate disease-associated imprinted regions as well as X-inactivation.

This study demonstrates nanopore sequencing is suitable for episignature detection and underscores the value of LRS for concurrent haplotyped genomic and epigenomic analyses.

O6 - Functional validation of novel TIE1 variants as causes for primary lymphedema

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Primary lymphedema (PL) is a chronic, debilitating disease for which there is no cure, characterized by lymph accumulation leading to swellings. To establish molecular therapies for PL, it is essential to characterize variants of unknown significance (VUSs) in vitro to assess whether they have a damaging impact or not, and by which mechanism. We recently reported the molecular impact of three loss-of-function variants in the tyrosine kinase receptor TIE1 causing late-onset forms of lymphedema, both in human patients and mouse models (Brouillard, Murtomäki, Leppänen, et al., J Clin Invest, 2024). This establishes TIE1 as a new PL-causing gene. We screened our cohort of >900 PL index patients for novel possible pathogenic variants in TIE1, using our in-house developed Highlander software. We identified 22 new VUSs, including nine susceptible to alter splicing. Prior to functional validation of missense variants, RNA stability was explored for six of these variants in patient cells, and seven variants were cloned in a minigene vector. Preliminary results show that one of the VUSs leads to the loss of a donor site resulting in splicing alteration. We mutagenized 19 of the TIE1 missense variants in expression constructs and studied both global expression and membrane localization in transfected cells. Two had significantly reduced global TIE1 expression levels compared to WT, whereas two showed a significant increase. Flow cytometry demonstrated significantly reduced cell surface expression for one, despite a global expression level comparable to WT. This suggests the latter to be sequestrated inside the cells.

Among the patients carrying these 22 VUSs, the onset of PL was at an older age than observed for other PL-associated genes. This makes us hypothesize that the TIE1 variants predispose to PL and need a concomitant genetic or environmental factor to induce PL. Functional studies are needed to clarify this.

O7 - Unraveling VUS in BRAF: A path to improve diagnosis, prognosis, and therapy in cancer patients

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Introduction/aim: BRAF is a key regulator in the RAS-MAPK/ERK pathway and thus a crucial target for cancer therapies. The surge in massive parallel sequencing and extended gene panels revealed, next to (likely) pathogenic variants, numerous variants of unknown significance (VUS), complicating clinical decision-making regarding therapy. Functional characterization of variants in clinically relevant genes, such as BRAF, is essential to bridge the gap between genetic findings and clinical application. This research aims to explore the biological and clinical implications of established and newly identified BRAF VUS variants, to improve BRAF-targeted therapies.

Methods: From a cohort of 5000 genetically tested cancer patients, 12 non-V600E BRAF variants and one FNBP1::BRAF fusion were selected for functional testing. Variants were introduced into plasmids using site-directed mutagenesis or Golden Gate cloning and confirmed by sequencing. HEK-293T cells were transfected with these plasmids either individually or in combination with CRAF. After 48 hours, proteins were isolated and p-ERK levels were analyzed by Western blot. Cells transfected with the fusion were treated with BRAF/MEK inhibitors for 2 hours to assess p-ERK inhibition.

Results: Cells transfected with FNBP1::BRAF fusion, identified in a glioma patient, exhibited elevated ERK activation compared to BRAFwild type, with p-ERK levels similar to BRAFV600E-transfected cells. Treatment with MEK inhibitor (Trametinib) or a BRAF inhibitor (Dabrafenib) successfully reduced p-ERK levels compared to those observed in wild-type controls. Among the 12 missense variants, 5 induced ERK hyperactivation only in presence of CRAF. The remaining variants showed no ERK hyperactivation, irrespective of CRAF presence.

Conclusion: This study identified the FNBP1::BRAF fusion as a potential glioma driver, which had not been functionally characterized before. Trametinib and Dabrafenib effectively reversed the constitutive ERK-phosphorylation induced by FNBP1::BRAF expression. Moreover, 5 of 12 studied

missense variants are potentially likely pathogenic non-V600 BRAF variants, based on the observed ERK hyperactivation in the presence of CRAF.

O8 - A novel form of autosomal dominant spondylocostal dysostosis in three unrelated families caused by the same heterozygous pathogenic variant in MESP2

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Spondylocostal dysostosis (SCD) is a rare skeletal dysplasia characterized by congenital vertebral segmentation defects occurring during embryonic development. SCD patients typically present with disproportionate short stature, scoliosis, vertebral segmentation defects (e.g. hemivertebrae, butterfly vertebrae) and rib abnormalities. SCD typically shows an autosomal recessive inheritance and several causal genes have been identified. These genes play a crucial role in somitogenesis and the subsequent formation of the vertebral column. Families with an autosomal dominant form of SCD have been reported, but only in one family a heterozygous pathogenic variant in TBX6 has been identified.

We have collected three unrelated families with an autosomal dominant form of SCD that were negative for heterozygous variants in TBX6. Whole-genome sequencing (WGS) on DNA from a Belgian family with dominant SCD (Family I) led to the identification of a heterozygous pathogenic MESP2 variant (c.268G>A; p.Glu90Lys) segregating with the SCD phenotype. The c.268G>A variant has not been reported in gnomAD and prediction programs uniformly classify it as disease-causing. Interestingly, by using Franklin (Genoox), we learned that the same MESP2 variant had been identified in a Brazilian trio (Family II) with a dominant form of SCD. Moreover, we recently identified a Spanish family with SCD (Family III) with this same variant. The p.Glu90Lys variant affects a highly-conserved amino acid residue in the basic helix-loop-helix (bHLH) domain of the MESP2 transcription factor. Luciferase assays with a Lfng reporter were performed, investigating the regulation of LFNG expression by wildtype and p.Glu90Lys MESP2. These data strongly suggest a dominant negative effect of p.Glu90Lys MESP2.

In conclusion, we provide the first evidence that a heterozygous variant in MESP2 can cause an autosomal dominant form of SCD. Further studies are currently undertaken to validate the functional effects of this mutation on vertebral segmentation, both in vitro and in vivo.

O9 - Using the Genetically Encoded Calcium Indicator NCaMP7 in hiPSC-derived cardiomyocytes to model Brugada Syndrome

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Human induced pluripotent stem-cell derived cardiomyocytes (hiPSC-CM) combined with Genetically Encoded Calcium Indicators (GECI) provide a promising platform for cardiac disease modelling. GECI are fluorescent proteins capable of fluorescence emission upon binding calcium ions, enabling imaging of calcium transients in living cells. Here we used NCaMP7, a recently developed green-fluorescent GECI, in hiPSC-CM derived from a Brugada Syndrome patient with a specific Belgian founder mutation in SCN5A, its isogenic control and an unrelated control individual.

HiPSC-CM differentiated following an in-house optimized protocol were transduced with NCaMP7-containing AAV2 for a duration of 24 hours. hiPSC-CM were imaged using live cell spinning disk confocal microscopy for up to 30 days and were subjected to baseline recording followed by stimulation with anti/pro-arrhythmic compounds verapamil, dofetilide and flecainide.

NCaMP7 was successfully introduced into hiPSC-CM and enabled visualization of the cellular calcium transients. A high dynamic range ($\Delta F/F0 \approx 100$) with negligible bleaching was observed. We measured a decrease in the dynamic range (up to 50%) over the course of the experiment, likely caused by the transient nature of the transfection.

Our model was validated by demonstrating a dose-dependent decrease in beatrate upon treatment with verapamil ($p < 0,001$) and tachyarrhythmic behaviour upon treatment with dofetilide in concentrations above 2nM (two-fold increased beatrate; $p < 0,001$).

Furthermore, treatment with flecainide led to loss of contractions in BrS hiPSC-CM (in 33% of recordings), but not in control hiPSC-CM. Arrhythmic beating in our hiPSC-CM cultures at baseline was observed in patient cells (up to 38,9% of recordings), but also in control lines (up to 25,4% of recordings),with substantial variability between multiple differentiations of the same hiPSC-line.

NCaMP7 enabled reliable, long-term, visualization of calcium transients in hiPSC-CM. The model's response to anti/pro-arrhythmic compounds was validated and BrS hiPSC-CM showed more arrhythmic beating and a higher sensitivity to flecainide.

O10 - Cardiovascular effects of fibrillin impairment in zebrafish

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Background: Fibrillins play a crucial role in maintaining structural stability and regulating extracellular signaling pathways of connective tissues. Fibrillinopathies commonly cause ocular and skeletal abnormalities, but the most severe complications are in the cardiovascular system. That is also the case for Marfan syndrome (MFS), which patients are especially susceptible to progressive aortic dilation, dissection, and rupture.

Objectives: We generated a zebrafish MFS model to improve the understanding of the cardiovascular consequences of fibrillin impairment.

Methods: Zebrafish mutants of the fibrillin-2b gene (fbn2b-/-) were subjected to detailed cardiovascular phenotyping by fluorescent microscopy in embryonic stages, and cardiac ultrasound, histology, and synchrotron X-ray imaging when adults.

Results: Approximately 60% of fbn2b-/- zebrafish embryos develop atrial endocardial detachment and pericardial edema starting at 2-3 dpf. A subset of these embryos progresses to vascular embolism, loss of blood flow, and death at 7-9 dpf. Interestingly, the remaining fbn2b-/- zebrafish survive normally, but during larval stages, they already develop dilation of the bulbus arteriosus, which persists into adulthood. Ex vivo histology and synchrotron imaging, as well as in vivo cardiac ultrasound, showed that adult fbn2b-/- zebrafish also have abnormalities in cardiac valves and general cardiac morphology.

Conclusion: Our fbn2b-/- zebrafish model recapitulates different aspects of the cardiovascular pathology observed in patients with MFS. This allows us to do compound screens on fbn2b-/- embryos, either with specific drug targets or in a high-throughput approach. Therefore, our zebrafish could be considered a relevant MFS animal model for finding much-needed disease-specific treatment options.

O11 - Methylation Biomarkers can Distinguish Pleural Mesothelioma from Healthy Pleura and other Pleural Pathologies

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Pleural mesothelioma (PM) is a rare and aggressive form of cancer, often requiring multiple diagnostic steps before a definitive conclusion can be reached. Given the emerging potential of DNA methylation as a biomarker, our goal was to develop a sensitive and specific methylation-based biomarker assay capable of differentiating PM from healthy pleura and other diagnostic confounders. We constructed a biomarker panel comparing Infinium EPIC array data of 134 PM samples to 22 healthy pleura samples and 143 healthy blood samples. We selected 744 hypermethylated CpG sites in PM as potential biomarkers. An initial validation of the individual CpG sites in external datasets showed high AUC values with a mean AUC of 0.936. Further validation was performed using IMPRESS, a novel methylation detection technique that allows bisulfite-free detection of thousands of CpG sites simultaneously. Our validation cohort included 29 PM, 31 healthy pleura, 11 healthy blood, 8 chronic pleuritis and 10 pleural metastasis samples. To differentiate PM from other conditions, we employed a stepwise approach with two models. The first model successfully differentiated tumoral from non-tumoral pleura samples, achieving a sensitivity of 88.9% and a specificity of 93.3%. The second model distinguished PM from pleural metastases with a sensitivity of 80.8% and a specificity of 100%. In conclusion, our methylation-based biomarker panel offers a promising approach to improve the diagnosis of pleural mesothelioma, providing high sensitivity and specificity across various diagnostic contexts.

O12 - BabyDetect project: interim results and some challenges in newborn screening.

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Newborn Screening (NBS) has come a long way since its first introduction to the public health system back in the 1960's. The advent of new technologies has paved the path for progressive inclusion of more metabolic, endocrine and rare disorders, resulting in saving thousands of children from severe disability and/or early death. In the context of NBS, 26 genomic newborn screening projects using tNGS, WES or WGS have been initiated worldwide.

In September 2022, BabyDetect project (ClinicalTrials.gov NCT05687474) started to explore feasibility and acceptability of first-tier genomic NBS using tNGS. BabyDetect panel consists of 405 genes responsible for 165 severe, pediatric, treatable diseases, including 14 genes for cardiac diseases (Table).

5000 newborns have been enrolled with a 90.5% consent-rate and 4814 assessed with a screen-positive rate of 2%. 97 positive cases have been identified, 29 of which were not detected by conventional NBS, with G6PD deficiency being the most frequent. Here we highlight challenges of NBS related to hereditary forms of cardiac diseases. Of 4814 neonates we have identified two with mutations in MYBPC3 and five with mutations in MYH7 genes. Both genes may have a phenotype with autosomal-dominant inheritance. Investigations of parents revealed that the father of neonate with a heterozygous variant MYH7:c.4498C>T had signs of undiagnosed cardiac hypertrophy. Child is under surveillance by cardiologist. Another child was reported to have the same mutation. Heterozygous variants MYH7:c.1750G>A, MYH7:c.2572C>T, MYH7:c.1370T>C also were communicated to specialist. Two cases with variants MYBPC3:c.3407_3409delACT and MYBPC3:c.3407_3409delACT were identified and reported.

The difficulties in management of these cases have led us to reconsider the inclusion of these genes after extensive discussion with expert panel of geneticists and pediatrician cardiologists. Detection of mild phenotypes goes beyond the scope of NBS and identifying "positive" cases with unclear penetrance increases the risk of overloading the healthcare system. These interim results demonstrate the feasibility of mid-scale targeted genomic NBS, highlighting the importance of combining biochemical and genomic methods in NBS.

O13 - Diagnosing HEreditary predisposition syndromes for Childhood cancer: Implementation in clinical PRactice (DHECIPR)– preliminary results

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Introduction:

Pediatric cancer is rare, with approximately 500 children under 19 years diagnosed annually in Belgium. Recent studies suggest that cancer predisposition syndromes (CPSs) are present in 10-30% of these patients. Despite their clinical relevance, CPSs are often underdiagnosed due to obstacles in referral and diagnosis. To aid clinicians, the MIPOGG referral tool has been developed. This study aims to prospectively validate MIPOGG in real-world clinical practice.

Methods:

This ongoing multicentric clinical validation study includes children under 18 years diagnosed with a (pre)malignant condition requiring treatment or follow-up, who have not previously been diagnosed with a CPS. Participants and their parents were referred for genetic counseling and testing. All participants received a WES-based pediatric onco-predisposition panel (in trio where possible). Results were communicated, and treatment and surveillance were adjusted for those with a CPS.

Results:

From June 2021 to November 2024, 288 patients with 298 (pre)malignancies were enrolled: 159 (55.2%) males and 129 (44.8%) females, with a median age at diagnosis of 6.5 years (IQR 2.6-12.0 years). Of all (pre)malignancies, 143 (48.3%) were hematologic, 104 (34.5%) were solid tumors and 51 (17.2%) CNS tumors. MIPOGG recommended genetic counseling for 127 (44.1%) patients, with referral rates of 34.5% for hematologic, 53.5% for solid and 56.8% for CNS conditions. Sequencing data was available for 241 (83.7%) patients, identifying CPSs in 20 patients (8.8%): 12 (5.0%) causal and 8 (3.3%) non-causal. MIPOGG demonstrated 75% sensitivity and 57% specificity for detecting causal CPSs. Sensitivity and specificity were 60%, 80% and 100%, and 63.8%, 48.1% and 47.5% for hematologic (pre)malignancies, solid tumors and CNS tumors, respectively.

Conclusions:

CPSs are detected in up to 8.8% of pediatric cancer patients without a prior CPS diagnosis. Although MIPOGG demonstrates moderate performance, current clinical referral tools are insufficient, highlighting the need for a genotype-first approach in routine practice.

O14 - Genetic background of patients with childhood-onset cardiomyopathy: results from a retrospective cohort study

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Background: Childhood-onset cardiomyopathy (CMP) is rare, affecting about 1 in 100,000 children. 40-50% of children have a familial history of cardiomyopathy or sudden cardiac death, with a genetic yielding of 50-60%. Dilated CMP (DCM) has the lowest yield (20-30%). Rare disease phenocopies, such as metabolic disorders and RASopathies are more common in early childhood (<10yrs).

Aim: To investigate genotype-phenotype correlations and cardiac outcomes.

Methods: Children under 18yrs diagnosed at our institution between 1990-2024 with any type of CMP, were included. Demographic, genetic, and cardiac outcome data were analyzed.

Results: A total of 157 children (63.1% male, age:5.3±5.8yrs) were diagnosed with CMP. The most frequent subtypes were DCM (49%) and hypertrophic CMP (HCM,47.1%) with fewer cases of restrictive CMP (RCM,5 patients) and arrhythmogenic CMP (ACM,1 patient). Nearly half of the patients (46.5%) were diagnosed during infancy. Genetic screening was performed in 68.8% of patients, most frequently in HCM (74.3%). Overall, a causative variant was identified in 56.5%. Genetic yield was higher in children with HCM compared to DCM (65.4%vs46.9%,p=0.067). Additionally, 15.7% of variants of unknown significance (VUS) were found. A trend of higher genetic yield was seen in older age groups. In infants (0-1yrs), variants in metabolic or RASopathy genes were found in 57.1%. Notably, sarcomere gene variants, traditionally associated with adult-onset CMP, contributed to 28.6% of infant cases. Major cardiac events occurred in 43.3%. Of all patients 25.5% died, 12.1% underwent a heart transplant and 7% received an implantable cardioverter-defibrillator. No significant differences in outcomes were observed across CMP subtypes.

Conclusion: Genetic testing identified the underlying etiology in over 50% of childhood-onset CMP. While rare disease phenocopies are highly prevalent in infants, sarcomere gene variants –once thought to be limited to adult-onset CMP– can also manifest in a very young age. This underscores the importance of early genetic testing to guide diagnosis and management.

O15 - Discovery, replication and characterization of protein, in vitro and iPSC-RPE stem cell models of a novel dominant RPE65-retinopathy, an actionable RPE disease

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Recessive RPE65-retinopathy is a well-known target for gene therapy (Luxturna). Dominant RPE65-retinopathy, however, due to an Irish founder variant is very rare. We present the discovery, replication and characterization of a novel dominant RPE65-retinopathy caused by c.1555G>A, p.(Glu519Lys) or p.(E519K).

This variant was found in genome (n=3), exome (n=28), or targeted sequencing data (index: n=14; segregation: n=30) from patients that underwent ophthalmological investigations. Haplotype phasing was based on long-read genome sequencing data (n=4) and microsatellite analysis (all probands). It was functionally assessed using enzymatic assays, western blotting, co-immunoprecipitation, CETSA, minigene assays, protein modelling (AlphaFold, crystal structure) and an electrostatic surface analysis (ChimeraX). Induced pluripotent stem cells (iPSCs) were reprogrammed from blood from three patients and differentiated to retinal pigment epithelium cells (iPSC-RPE).

Genomic profiling in Belgian inherited retinal disease (IRD) cases (discovery cohort, n=2,873) and interrogating genomic IRD databases from France, the Netherlands, Germany, UK, Ireland, and Canada (replication cohort, n=18,798) revealed 83 monoallelic p.(E519K)-IRD cases of Flemish ancestry. E519K-IRD is characterized by dominant inheritance with complete penetrance and phenotypic variability. It is hallmarked by a late-onset macula-predominant IRD, reminiscent of the common age-related macular degeneration (AMD). A shared haplotype of 464 kb in all probands supported a founder effect. Residue p.(E519K) affects a highly conserved amino acid and lowers RPE65 enzymatic activity and protein expression. While no increased interaction with wild type RPE65 or aberrant splicing could be demonstrated in vitro, a shift in protein stability was shown. Patient-derived iPSC-RPEs displayed changes in PEDF/VEGF secretion, morphology, phagocytosis and PMEL17 expression, like other RPE diseases such as AMD.

The discovery of the novel ‘Flemish’ dominant RPE65-IRD reduces the diagnostic gap in dominant IRD and highlights a novel target for intervention. Lastly, this rare retinopathy represents a promising model for the common AMD.

Van Vooren*, et al. Genome Medicine, under review.

O16 - Unravelling the proxisome of photoreceptor-specific nuclear receptor NR2E3 reveals a potential molecular link between transcriptional regulation and splicing in human retina

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Photoreceptor-specific nuclear receptor NR2E3 is a key transcription factor in the retinal transcriptional network, playing a key role in photoreceptor cell fate and maintenance. The dominant NR2E3 variant G56R is implicated in autosomal dominant retinitis pigmentosa (adRP), while biallelic loss-of-function variants lead to autosomal recessive inherited retinal diseases (IRD). Moreover, Nr2e3 has been shown to act as a modifier that rescues retinal degeneration and promotes homeostasis in RP models in mice. A comprehensive understanding of the interactome of NR2E3 remains elusive, however.

Here, we aimed to unravel the interactome of this orphan nuclear receptor through proximity labelling followed by mass spectrometry. We used the T2A split/link TurboID design to identify the proxisome of wild type NR2E3 (WT-NR2E3) and mutant G56R (G56R-NR2E3) in ARPE-19 cells. LC-MS/MS followed by label free quantification generated a dataset of over 2,000 quantified proteins. Principal component analysis of all samples confirmed clustering of the target and control conditions, but not discriminating WT-NR2E3 from G56R-NR2E3, suggesting similar interactomes in ARPE-19. Differential analysis revealed 51 significantly enriched proteins for WT-NR2E3 and 63 for G56R-NR2E3, respectively (FDR=0.05). Several protein complexes involved in transcriptional processes were identified, some of which interact with nuclear receptors and a subset of which shows a link with retinal development. Interestingly, a fraction of enriched proteins are components of the spliceosome. The retina is highly sensitive to spliceosomal dysfunction, which underlies several forms of IRD, adRP in particular. Our findings suggest that NR2E3 acts as a molecular link between transcriptional regulation and RNA splicing in the retina. Further validation is ongoing.

In conclusion, our integrative approach combining TurboID proximity labeling and quantitative proteomics has mapped the proxisome of NR2E3, for the first time. Our understanding of the interplay

between transcriptional regulation and splicing may reveal novel therapeutic targets for retinal disorders.

O17 - Structural variants disrupt a critical regulatory region downstream of FOXG1

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The forkhead box G1 (FOXG1) transcription factor is a crucial regulator during embryonic brain development. Pathogenic variants affecting FOXG1 cause FOXG1 syndrome, a congenital form of Rett syndrome. Interestingly, structural variants (SVs) disrupting the downstream region of FOXG1 have been identified in 40 reported individuals with features of FOXG1 syndrome. Yet, the regulatory mechanisms resulting in aberrant FOXG1 transcription have not been elucidated.

We identified a de novo non-coding deletion in a patient with FOXG1 syndrome features, allowing us to narrow down a ~100kb critical regulatory region affected in all patients with SVs 3' of FOXG1. By leveraging publicly available as well as in-house generated epigenomics data, Hi-C interaction maps and in vivo enhancer assays in zebrafish, we identified multiple regulatory elements in this region, including a cluster of neuronal enhancers and the distal boundary of the FOXG1-containing topologically associating domain (TAD). Subsequently, via a time series of UMI-4C, CUT&RUN and ATAC-seq at four timepoints (i.e. day 0, 4, 6 and 8) during differentiation of induced pluripotent stem cells towards neural progenitor cells, we were able to show that the enhancer cluster in this critical region shows dynamic activation and interaction with the FOXG1 promoter. In addition, Hi-C and UMI-4C on patient lymphoblastoid cells revealed that deletion of the critical regulatory region impacts FOXG1 interactions and TAD structure. Next, we assessed the impact of deleting these regulatory elements on FOXG1 transcription using CRISPR-Cas9 genome engineered iPSC lines differentiated towards NPCs. A noticeable decrease in FOXG1 expression was observed in both the enhancer cluster and TAD boundary KO models.

In conclusion, we have identified and characterized a critical regulatory region downstream of FOXG1 that is disrupted in a cohort of FOXG1 syndrome patients. This region contains both enhancer and architectural regulatory elements essential for proper FOXG1 transcription. Our findings provide valuable insights into the interplay between genome structure and function at the FOXG1 locus and highlight the consequences of non-coding SVs on chromatin architecture and gene regulation. These results significantly enhance the functional annotation of regulatory elements at the FOXG1 locus, improving SV interpretation in affected patients.

P1 - Development of a CRISPR/Cas9-engineered zebrafish model to investigate the role of tgfb2 in Loeys-Dietz syndrome.

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Thoracic aortic aneurysm and dissection (TAAD) is a major cause of morbidity and mortality in Western countries. One of the most extensively studied syndromes associated with TAAD, is Loeys-Dietz syndrome (LDS). It is an inherited connective tissue disorder caused by mutations in genes related to the transforming growth factor-β (TGFβ) signaling pathway. Interestingly, although these mutations are classified as loss-of-function mutations, they paradoxically result in an increase in TGFβ signaling, a phenomenon known as the TGFβ paradox.

In order to elucidate the molecular mechanisms underlying this apparent paradox, we developed a LDS zebrafish model by targeting the tgfb2 gene using CRISPR/Cas9. This gene is particularly interesting, as patients with loss-of-function mutations in TGFB2 often develop aortic root dilatation. Furthermore, since zebrafish possess only one orthologue for TGFB2, it serves as an ideal target for creating a LDS zebrafish model. Using CHOPCHOP and CRISPRscan, we selected three single guide RNAs (sgRNA) that were predicted to be the most efficient and ordered them from Synthego. A solution of 1 nl containing a mixture of 50 pg of sgRNAand 300 pg of Cas9 protein was injected in the one-cell stage zebrafish embryo for each guide. At 2 days post fertilization, DNA was isolated and Sanger sequences were analyzed using ICE. Initial findings demonstrate variable indel efficiencies among the three distinct sgRNAs, revealing one guide with a notably high efficiency of 78% (N=20), while the other two showed efficiencies of 41% (N=29) and 6% (N=32). Mosaic founder fish are currently being raised and will be outcrossed in order to obtain a stable mutant tg(kdrl:GFP)^(tgfb2+/-) zebrafish line. The progeny of this mutant line will be used for fluorescent in vivo imaging of the dorsal aorta and outflow tract to assess the aneurysm phenotype at different time points.

P2 - Differential impact of EPHB4 likely pathogenic variants between lymphatic and CM-AVM2-related phenotypes

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Introduction

Heterozygous EPHB4 mutations have been linked to capillary malformation-arteriovenous malformation 2 (CM-AVM2), as well as to lymphatic-related hydrops fetalis (LRHF), late-onset primary lymphedema (PL), and central conducting lymphatic anomaly (CCLA). However, the molecular mechanisms leading to these phenotypic differences remain unclear.

Methods

Using our Highlander software, we explored our large cohort of WES data of patients with lymphatic-related abnormalities and CM-related conditions for missense variants in EPHB4. Because the position of the variants in the protein does not discriminate the pathologies, we initiated in vitro molecular characterization by mutagenizing the variants in an expression vector and exploration of the transfected cells by western blot and flow-cytometry.

Results

We identified overall 73 amino acid substitutions, including 24 EPHB4 variants from lymphatic-related patients and 49 from CM-related patients. These variants were predicted as likely pathogenic by at least 5 out of 20 variant effect predictors used in Highlander. We have already characterized 25 of

them and demonstrate differential molecular defects for 9 found in CM-related patients, 5 in lymphatic-related patient and 1 in a patient with a combination of both phenotypes.

Conclusion

These findings underscore the importance to identify rare EPHB4 missense variants, generally classified as Variants of Unknown Significance (VUS) and to perform functional validation to discriminate between rare polymorphisms without a functional impact and pathogenic variants. Moreover, it enables to differentiate the molecular mechanisms between LRHF or CM-AVM2. This is an essential step to establish targeted therapeutic approaches for the two diseases with distinct etiopathogenic mechanisms.

P3 - Witdrawn

P4 - Proof-of-concept of DNA methylation-based multi-cancer detection in liquid biopsies using IMPRESS

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DNA methylation is a promising biomarker for cancer detection. Previously, our research group identified a common methylation pattern, shared between the eight most common cancer types (lung, colorectal (CRC), liver, breast, pancreas, head and neck, esophageal and prostate cancer). Moreover, we developed IMPRESS (Improved Methylation Profiling using Restriction Enzymes and smMIP sequencing), a cost-effective method for methylation detection without bisulfite treatment. Using methylation-sensitive restriction enzymes (MSREs) and single-molecule Molecular Inversion Probes (smMIPs), IMPRESS allows multiplex detection of thousands of differentially methylated CpG sites. Up to now, this multicancer IMPRESS assay was optimized for use in tissue samples.

Here, we optimized the IMPRESS protocol for use in liquid biopsies (LBs) and we present a proof-of-concept study showcasing its ability to differentiate LB samples from colorectal cancer patients and healthy controls. First, we optimized the protocol for a DNA input of 5 ng, compatible with a typical cfDNA yield from plasma samples. Next, we evaluated our multicancer assay on liquid biopsies from 16 metastatic CRC and 32 healthy control samples.

Normalized read counts, representing methylation levels, of CRC patients were compared to those of healthy controls. CRC results were validated with an NPY (neuropeptide Y) methylation ddPCR assay, a known marker for ctDNA-based CRC detection and used in our lab. NPY-positive samples showed significantly higher normalized counts compared to NPY-negative and healthy samples. Moreover, in two CRC patients, monitored from pre-treatment through stable and eventually progressive disease, normalized counts correlated with disease status. We noticed a decrease during treatment and an increase at progressive disease.

In conclusion, we successfully optimized the IMPRESS multi-cancer assay for LBs, enabling the distinction of CRC patients from healthy individuals. We will soon expand this test to cover the eight major cancer types, which can facilitate earlier and minimally invasive multi-cancer detection.

P5 - Family-level impact of extensive germline predisposition screening in childhood cancer: A multi family member interview analysis in parents.

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Objective: Genetic testing is increasingly integrated in pediatric oncology and a large number of families are interested. Research on the psychological impact of genetic testing is limited by a main focus on individual outcomes in parents or children and little is currently known about its impact on the family-level. Our study deals with that limitation by exploring parents' lived experiences of how their family -as a whole- is affected by genetic testing. Methods: In six families who opted for extensive germline sequencing for cancer predisposition, both parents were interviewed individually (N = 12). Their experiences were elicited through semi-structured interviews and the data were analyzed using Multi Family Member Interview Analysis. Results: Preliminary findings demonstrate that diagnostic genetic testing is perceived as a straightforward step in the child's oncology trajectory. When explicitly asked about its impact on the family, parents indicate that the challenges resulting from the cancer diagnosis and treatment are predominant, rather than the genetic testing. Yet several themes, for example, transmission and survivor's guilt, family communication, relational coping, mutual concern, and changes in family values and cohesion, emerged implicitly from the data. Conclusions and clinical implications: Clinicians need to be especially attentive to family-related themes during genetic counseling. Although reflected in their narratives, parents are not inclined to talk about these directly. Providing support in addressing these topics can be helpful for families.

P6 - Pathogenic variants in HGF give rise to childhood-to-late onset primary lymphoedema by loss of function

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Developmental and functional defects in the lymphatic system are responsible for primary lymphoedema (PL). PL is a chronic debilitating disease caused by increased accumulation of interstitial fluid, predisposing to inflammation, infections and fibrosis. There is no cure, only symptomatic treatment is available. Thirty-two genes or loci have been linked to PL, and another 22 genes are suggested to be linked to PL, including Hepatocyte Growth Factor (Brouillard et al, 2021).

We searched for hepatocyte growth factor (HGF) variants in 770 index patients from the Brussels PL cohort using whole-exome or targeted panel sequencing. We identified altogether ten variants predicted to cause HGF loss-of-function (six nonsense and two frameshift variants, and two splice-site changes; 1.3% of our cohort), and 14 missense variants predicted to be pathogenic in 17 families (2.21% of our cohort). We studied co-segregation with DNAs from available family members, mRNA stability for non-sense generating variants, and in vitro functional effects of the missense variants.

Analyses of the mRNA of patient cells harbouring variants generating premature stop codons revealed degradation of the mutant allele. Reduced protein secretion was detected for nine of the 14 missense variants expressed in COS-7 cells. Stimulation of lymphatic endothelial cells with these 14 HGF variant proteins resulted in decreased activation of the downstream targets AKT and ERK1/2 for three of them. Clinically, HGF-associated PL was diverse, but predominantly bilateral in the lower limbs with onset varying from early childhood to adulthood. Finally, aggregation studies in a second independent cohort (Genomics England's Whole Genome Sequencing project) underscored that rare likely pathogenic variants in HGF explain about 2% of primary lymphedema. Therefore, HGF signalling seems crucial for lymphatic development and/or maintenance in human beings.

P7 - Role of a rare variant in the NT5E gene in the pathogenesis of Systemic Sclerosis

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Background:

Systemic sclerosis (SSc) is a rare autoimmune disease characterized by vascular, immunological and connective tissue abnormalities. Current therapies are only marginally effective. While typically sporadic, familial clustering is observed in rare cases (≤1.6%). We apply a family-based rare disease genetics approach to SSc, in order to identify central disease mechanisms and potential therapeutic targets.

Methods:

Whole exome sequencing (WES) performed on six SSc families identified a heterozygous rare variant in NT5E in one family with extensive skin and internal organ involvement. We used lentivirally transduced HEK293T cells, as well as CRISPR/Cas9-engineered NT5E knock-out (KO) and heterozygous knock-in (HET KI) cell lines, in order to assess the impact of this new variant on protein function, and on SSc-pertinent cellular phenotypes.

Results:

NT5E codes for CD73, an ecto-5'-nucleotidase that converts extracellular AMP to adenosine: a major modulator of immune response, angiogenesis and fibrosis. Loss-of-function mutations in NT5E cause CALJA (calcification in joints and arteries), an autosomal recessive disorder with phenotypic overlap with SSc. A colorimetric assay measuring the level of inorganic phosphate produced by degradation of AMP showed that the variant results in a loss of CD73 enzymatic activity, and interestingly, seems to exert a dominant negative effect. HET KI fibroblasts cultured in pro-calcification medium showed a strong increase in calcium phosphate crystal production, in comparison to WT cells: a phenotype also described in CALJA patient fibroblasts.

Conclusion:

We identified a heterozygous rare variant in the NT5E gene in an SSc family with extensive skin fibrosis, and demonstrated that it exerts a dominant negative effect on the enzyme function of this dimeric, cell surface bound ectonucleotidase. This results in a strong calcification phenotype in engineered fibroblasts. Functional assays are ongoing in order to test for the impact of this new variant on cGAS/STING-mediated inflammation, fibrosis and angiogenesis: the hallmarks of SSc.

P8 - Cell-free DNA methylation analysis reveals tissue of origin dynamics in health

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Cell-free DNA (cfDNA), fragmented DNA found in blood plasma and other bodily fluids, serves as a minimally invasive biomarker for monitoring physiological and pathological conditions, including pregnancy, transplantation, and cancer. Despite the rapid advancement of liquid biopsy techniques, knowledge gaps, particularly in biological variation, hinder clinical implementation. While the hematopoietic system is the primary contributor to plasma cfDNA, factors such as sex, age, and intra-individual variability in immune cell contributions remain poorly understood. Addressing these gaps could improve the sensitivity and specificity of cfDNA-based diagnostics.

To explore cfDNA dynamics in health, two cohorts of healthy individuals were recruited and sampled. A longitudinal cohort (n = 150; 20–88 years) was sampled at baseline and one year later, with balanced age and sex distribution. A diurnal cohort (n = 16; 20–30 years) was sampled over three days at morning, afternoon, and evening intervals. Participants reported no acute or chronic diseases, and biological and lifestyle data were collected. cfDNA libraries were constructed using enzymatic methyl-seq, with targeted sequencing of 4,989 tissue- and age-specific DNA methylation markers.

Deconvolution results from the longitudinal cohort (n = 144) showed that cfDNA is primarily of hematopoietic origin, with granulocytes (37.4%), monocytes (16.8%), megakaryocytes (11.1%), erythrocyte progenitors (8.8%), and natural killer cells (7.9%) as main contributors. Additional tissues with substantial turnover, such as liver, vascular endothelium, and intestine, each contributed approximately 3%. Future analyses highlighted sex-, age-, and diurnal-related differences.

This study underscores the importance of understanding cfDNA variability to optimize cfDNA-based tests. The cohorts' diverse yet healthy composition offers insights into baseline cfDNA characteristics, laying the groundwork for advancements in liquid biopsy-based applications.

P9 - Constitutional Mismatch Repair Deficiency (CMMRD) syndrome: A Case Report of a Patient with biallelic germline PMS2 Mutations

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Constitutional mismatch repair deficiency (CMMRD) is a rare autosomal recessive cancer syndrome caused by pathogenic variants in both alleles of one of the four mismatch repair (MMR) genes: MSH2, MLH1, MSH6, and PMS2. The defective MMR system results in the accumulation of frequent somatic mutations and microsatellite instability (MSI). Among these, PMS2 is the most frequently mutated gene, accounting for 60% of CMMRD cases. In contrast to Lynch syndrome, which is caused by a heterozygous mutation in MMR genes and primarily leads to colorectal and endometrial cancer in adults, CMMRD predisposes individuals to a broad spectrum of childhood tumors. These include, most commonly, hematological malignancies, brain tumors, and gastrointestinal tract neoplasms. Our case report presents a 17-year-old patient admitted to our hospital with pancytopenia and blastosis, accompanied by rectorrhagia, with no relevant family history. He was diagnosed with B-cell acute lymphoblastic leukemia. One year later, a colonoscopy revealed multiple colorectal adenocarcinomas with loss of PMS2 expression and MSI, along with metastases to the bone, liver, and abdominal lymph nodes. Due to the suspicion of CMMRD syndrome, genetic testing was proposed. We identified biallelic germline PMS2 pathogenic variants in our patient using NGS (NM_000535.6) : deletion of exon 10-15 and c.137G>T, p.(Ser46Ile). Three months later, a brain MRI revealed a low-grade glial tumor and the patient passed away a few months later. CMMRD remains an underdiagnosed syndrome. Therefore, genetic testing is crucial for identifying CMMRD. It is also essential to provide personalized and close surveillance for both patients and their relatives.

P10 - UMOD Genotype and Determinants of Urinary Uromodulin in African Populations

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Introduction: Single-nucleotide polymorphisms (SNPs) in the UMOD-PDILT genetic locus are associated with chronic kidney disease (CKD) in European populations, through their effect on urinary uromodulin (uUMOD) levels. The genetic and nongenetic factors associated with uUMOD in African populations remain unknown.

Methods: Clinical parameters, 3 selected UMOD-PDILT SNPs and uUMOD levels were obtained in 1202 young Black and White adults from the African-PREDICT study and 1943 middle aged Black adults from the PURE-NWP-SA study, 2 cross-sectional, observational studies.

Results: Absolute uUMOD and uUMOD/creatinine levels were lower in Black participants compared to White participants. The prime CKD-risk allele at rs12917707 was more prevalent in Black individuals, with strikingly more risk allele homozygotes compared to White individuals. Haplotype analysis of the UMOD-PDILT locus predicted more recombination events and linkage disequilibrium (LD) fragmentation in Black individuals. Multivariate testing and sensitivity analysis showed that higher uUMOD/creatinine associated specifically with risk alleles at rs12917707 and rs12446492 in White participants and with higher serum renin and lower urine albumin-to-creatinine ratio in Black participants, with a significant interaction of ethnicity on the relationship between all 3 SNPs and uUMOD/creatinine. The multiple regression model explained a greater percentage of the variance of uUMOD/creatinine in White adults compared to Black adults (23% vs. 8%).

Conclusion: We evidenced ethnic differences in clinical and genetic determinants of uUMOD levels, in particular an interaction of ethnicity on the relationship between CKD-risk SNPs and uUMOD. These differences should be considered when analyzing the role of uromodulin in kidney function, interpreting genome-wide association studies (GWAS), and precision medicine recommendations.

P11 - Towards patient-specific aorta-on-a-chip models for thoracic aortic aneurysm and dissection

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Thoracic aortic aneurysm and dissection (TAAD) is a severe complication of heritable connective tissue disorders such as Marfan syndrome (MFS) or Loeys-Dietz syndrome (LDS). Mice are frequently used to investigate the molecular mechanisms underlying syndromic TAAD, as obtaining native aortic samples of patients and control individuals is challenging. However, in vivo studies are lengthy, and their drug testing results often fail to translate effectively to patients. Thus, robust disease models that faithfully capture patients' genetic and physiological context are urgently needed to improve our understanding of TAAD and to develop effective treatments.

To address this, we created the three cell types populating the aorta — endothelial cells (EC) and vascular smooth muscle cells (VSMCs) of neural crest and lateral mesoderm origin — using iPSCs of syndromic TAAD patients and CRISPR-Cas9-corrected isogenic controls. While iPSC-VSMCs express early VSMC markers, late-stage marker expression remains limited in 2D cultures. Efforts to enhance maturation using heparin, cyclic stretching or serum starvation showed limited efficacy, though patient-derived VSMCs demonstrated a mildly attenuated contractile response compared to controls. To overcome the immaturity of 2D cultures and extend pathological readout windows, we aim to develop the first iPSC-derived MFS and LDS aorta-on-a-chip (AoC) model. These 3D models will combine iPSC-VSMCs and ECs seeded in concentric layers, subjected to (patho)physiological mechanical cues, such as pressure and shear stress, to mimic the native aortic environment. This setup is expected to significantly improve cell maturity and provide a platform for studying established pathomechanisms and evaluating drug responses.

Overall, these models enable the comparison of EC and VSMC phenotypes, revealing how FBN1 mutations disrupt cellular integrity and contribute to TAAD pathogenesis. By developing these novel MFS and LDS models, we aim to enhance the relevance of iPSC-based models, reduce reliance on animal studies, accelerate TAAD research and drug discovery, paving the way for patient-specific therapies.

P12 - Finding the missing piece: Cowden syndrome - a case report

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Cowden syndrome (CS) is an autosomal dominant disorder with an estimated prevalence of 1/200000. CS patients manifest commonly, but not uniformly, mucocutaneous lesions, macrocephaly and ganglioneuromatous gastrointestinal polyps. They have a high lifetime risk of developing breast, thyroid, endometrial, and renal cancer. Hamartomatous polyposis is frequent and increase slightly the risk of colorectal cancer. The diagnosis of CS is nowadays easier with the quantitative scoring system established by the International Cowden Consortium for CS, however it remains challenging due to the diversity of symptoms. Therefore, genetic testing for germline variants is highly recommended since many genes have shown their implication in the disease like PTEN (25% cases), KLLN (30%), SDHB-D (10%), AKT1 and PIK3CA (10%). Our case report presents a patient referred to our clinic for a suspicion of CS. His clinical phenotype included multiple facial trichilemmomas, gingival papillomatosis and palmoplantar hyperkeratosis as well as a family history of breast cancer. We identified a pathogenic nonsense variant in the exon 5 of PTEN (NM_000314.6): c.388C>T, p.(Arg130*). It has been widely reported in the literature in CS patients and is predicted to lead to a truncated non-functional or absent protein affecting the two main PTEN domains. This allowed us to confirm the CS diagnosis in our patient and advise other family members. Preventive surveillance guidelines were issued including routine ultrasounds and colonoscopies.

P13 - Investigation of ROS blocking strategy to prevent aneurysm formation in Marfan Syndrome

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The prognosis of Marfan Syndrome (MFS) largely depends on aortic aneurysm formation, which may lead to dissection. While angiotensin II receptor blockers (ARBs) like losartan prevent aneurysms in MFS mouse models, they only slow progression in patients, partly due to tolerable lower doses. A key feature of aneurysm is increased reactive oxygen species (ROS) production, with xanthine oxidoreductase (XOR) playing a crucial role in this process.

In this preclinical study, we evaluated the individual and combined therapeutic effects of the ARB losartan (300 mg/l) and the XOR-inhibitor allopurinol (70 mg/l) in the MFS mouse model Fbn1C1041G/+. Oral treatments were initiated at 4 weeks of age and continued until 12 weeks, with aortic diameters measured every four weeks by echocardiography.

Wild-type (WT) and Fbn1C1041G/+ mice already demonstrated significant difference (padj<0.0001) of aortic root diameter at four weeks, at the start of treatments. By eight weeks, untreated mutant mice exhibited significantly larger aortic diameters compared to WT, whereas no statistically significant difference was observed between the treated Fbn1C1041G/+ groups and the control WT. At 12 weeks, losartan-treated Fbn1C1041G/+ mice displayed no significant difference in aortic diameter compared to WT controls, whereas allopurinol-only and combination-treated groups showed significant differences. These findings suggest losartan's greater efficacy in maintaining aortic root diameter relative to allopurinol itself or their combination. However, analysis of absolute aortic growth revealed no significant difference between both losartan- and allopurinol-treated groups when compared to the control WT. Notably, allopurinol treatment significantly reduced aortic growth compared to untreated mutant mice (padj=0.0357), an effect not observed with losartan.

Our findings indicate that both losartan and allopurinol exert therapeutic effects on aneurysm development in Fbn1C1041G/+ mice, though no significant synergistic benefit was observed when combined. Limitations of this pilot study include a small sample size (N=8 per group) and a relatively short follow-up period.

P14 - Variant of uncertain significance testing in zebrafish: a proof of concept using a COL1A2 variant as example

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Genomic sequencing has led to the identification of numerous variants of uncertain significance (VUS), posing challenges for clinical decision-making. While segregation studies are helpful in VUS classification, they are not always feasible, emphasizing the need for functional testing to accurately interpret these variants. In this study, we investigated the pathogenicity of the COL1A2 c.2123G>A VUS, identified in a 59-year-old female with recurrent fractures, using zebrafish as a model organism. Using prime-editing, we previously introduced the VUS into the zebrafish genome, alongside a benign missense variant as negative control and a known pathogenic missense variant as positive control. Comprehensive skeletal phenotyping revealed no significant abnormalities in the VUS zebrafish model, suggesting the variant is likely benign. Our findings highlight the effectiveness of zebrafish models for functional validation of VUS in COL1A2 and underscore their potential to elucidate pathogenic mechanisms. This approach could be adapted to study VUS in other genes, offering a valuable tool for genomic screening.

P15 - Elucidating the pathomechanisms of Meester-Loeys syndrome: Insights from a Bgn-/0 mouse model

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Pathogenic loss-of-function variants in biglycan (BGN) are associated with Meester-Loeys syndrome, a thoracic aortic aneurysm and dissection syndrome. To study the pathomechanisms underlying biglycan-related aortopathy, a Bgn-/0 mouse model was developed. Aortic dissection/rupture was induced in this model using angiotensin-II (AngII) infusion via minipumps. A dose-dependent survival study in male mutant mice determined an optimal, non-hypertensive AngII dose of 400 ng/kg/min. This dose was infused for five days prior to tissue collection.

Aortic wall composition was examined using Verhoeff-Van Gieson and Masson's Trichrome stainings of transverse descending aorta sections. Elastin staining showed no consistent differences in fiber breaks or organization between AngII-challenged Bgn-/0 mice, AngII-challenged wildtype mice, and vehicle-treated controls. Similarly, collagen content in the aortic media was not significantly increased in AngII-challenged Bgn-/0 mice. Exploratory assessment of descending aorta tissues by transmission electron microscopy revealed variability in collagen fibril diameter, with an overall smaller fibril diameter in Bgn-/0 mice relative to wildtype mice, regardless of the treatment. This suggests that reduced fibril diameter may reflect a genetic predisposition caused by the pathogenic biglycan variant, but it does not explain the dissection/rupture phenotype.

Macroscopic and histological examination of descending aortic tissue of AngII-challenged Bgn-/0 mice revealed red blood cell accumulation within the aortic wall, indicating aortic damage. To further explore the underlying pathomechanisms, bulk and single-cell RNA sequencing have recently been performed on descending aortic tissues from AngII- and vehicle-treated wildtype and Bgn-/0 mice. Principal component analysis highlighted AngII-challenge as the greatest source of variation between samples, suggesting a more heterogeneous response to the environmental challenge compared to the relatively modest effect of the biglycan deficiency. Differential expression analysis of the sequencing data is ongoing. The (preliminary) findings obtained from this Bgn-/0 mouse model provide a valuable foundation for further mechanistic studies to unravel the drivers of biglycan-related aortopathy.

P16 - Zebrafish as a model for Myhre syndrome: growth deficits and vascular narrowing

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Myhre syndrome (MS, MIM 139210) is a rare multisystemic disorder caused by recurrent pathogenic missense variants in SMAD4. The clinical features comprise variable neurocognitive development, autism, recognizable craniofacial features, short stature, hearing loss, a thickened skin, joint limitations, diverse cardiovascular and airway manifestations including arterial stenosis, and increased fibrosis often following trauma or surgery.

We developed a zebrafish model for Myhre syndrome. Using CRISPR technology, we generated heterozygous and homozygous smad4a p.Ile495Thr knock-in (KI) zebrafish, which correspond to the known human SMAD4 p.Ile500Thr variant. The knock-in was crossed with the transgenic Tg(kdrl:EGFP) zebrafish line to visualize the ventral aorta and aortic branches. At 5 days post-fertilization (dpf), vasculogenesis was not different in p.Ile495Thr KI versus wild type controls. At 10 dpf, heterozygous and homozygous KI zebrafish exhibited a significant reduced diameter of the ventral aorta between aortic arch two and three, as well as a decreased aorta diameter adjacent to the bulbus arteriosus compared to wild-type controls. Cardiac ultrasounds of 6-month-old zebrafish showed no significant abnormalities. Measurements of 4-month-old zebrafish revealed decreased body length in homozygous mutants compared to wild type controls. Mortality at three months post fertilization seems higher in homozygous KI zebrafish (up to 50%).

In conclusion, our zebrafish model for MS exhibits reduced body length, narrowing of the ventral aorta, and potentially increased mortality. Further phenotypic assessment and multi-omic analyses are underway to elucidate the underlying disease mechanisms.

P17 - In Vivo Functional Evaluation of BRCA2 Variants via CRISPR-mediated Knock-In in Zebrafish

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BRCA2 is one of the most frequently analyzed genes worldwide, and numerous variants of unknown significance (VUS) have been identified, complicating cancer risk interpretation, counseling and treatment. Functional data provide moderate to strong evidence for variant classification, but current in vitro assays often lack biological complexity. To further improve classification, we aim to develop in vivo functional assays for BRCA2 variants using zebrafish genome editing. Zebrafish offer strong conservation of DNA damage response components, high fecundity, rapid external development, and a short reproductive cycle, making them an ideal model.

First, we optimized CRISPR/Cas9-mediated homology-directed repair (HDR) for precise knock-in (KI) of genetic variants and compared its efficacy with the newer prime editing (PE) technique. For HDR, higher Cas9 levels improved KI efficiency, as did using Alt-R HDR templates, while guide-blocking modifications had no effect. Micro-injection into the cell offered no advantage over yolk injection. PE, however, increased KI efficiency up to fourfold, expanded the founder pool, and reduced off-target effects compared to HDR.

Next, we employed PE to generate a stable zebrafish line harboring the brca2 p.(Asp2268Asn) variant, corresponding to the human BRCA2 p.(Asp2723Asn) variant. This variant, affecting a conserved residue of the DNA binding domain, has been reported as functionally damaging (PMID:33609447). We assessed Brca2 functionality in homozygous p.(Asp2268Asn) embryos using our previously published functional assays based on the concept of synthetic lethality between Brca2 and Parp (PMID:33341473).

brca2 p.(Asp2268Asn) homozygous embryos developed as sterile males and PARP inhibition led to smaller eyes and increased acridine orange staining, resembling our previously published brca2 knockout embryos and confirming the variant's damaging effects observed in prior assays.

To validate this pipeline, we are generating additional lines with known benign and pathogenic variants to define assay thresholds that differentiate damaging variants from neutral ones, supporting future VUS classification for BRCA2.

P18 - Biases in the Parsortix® system observed with pancreatic cancer cell lines

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Pancreatic cancer has a 5-year survival rate of 12%, highlighting the need for reliable biomarkers for early detection and disease monitoring. Circulating tumor cells (CTCs) have emerged as a promising biomarker, yet their detection remains challenging. This study evaluates the Parsortix® system, a microfluidic device that enriches CTCs based on size and deformability, using pancreatic cancer cell lines. As increasing evidence indicates that during epithelial to mesenchymal transition (EMT) a cell's deformability increases, we evaluated possible biases by the device. The EMT stage of three pancreatic cancer cell lines, CAPAN-1, MIA PaCa-2 and PANC-1, was assessed, to classify them as epithelial, mesenchymal-like, and hybrid, respectively. Spike-in experiments showed that epithelial and hybrid phenotypes were more efficiently captured (62.6 ± 18.5% and 65.4 ± 11.1%) than mesenchymal-like cancer cells (32.8 ± 10.2%). These results were confirmed using an EMT inducible breast cancer cell line. Lower recovery rates were found for the cells in a mesenchymal-like state (31.5 ± 6.4%) compared to those in an epithelial state (47.56 ± 7.2%). In conclusion, the Parsortix® device may underestimate the number of mesenchymal CTCs.

P19 - Diagnostic Yield and Clinical Impact of Prenatal Whole-Exome Sequencing (WES) – Four-Year Single Center Experience

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Background: Whole-Exome Sequencing (WES) is increasingly utilized in the prenatal setting, specifically in fetuses with ultrasound anomalies. This study evaluates the diagnostic yield and impact of prenatal WES on outcome, such as termination of pregnancy (TOP) or neonatal management.

Methods: We conducted a retrospective analysis of the first ≥4 years of prenatal WES at the Center for Medical Genetics of the Antwerp University Hospital (Belgium). Inclusion criteria were negative QF-PCR and genome wide deletion/duplication analysis, presence of at least one ultrasound anomaly and availability of parental DNA. Trio analysis was performed, filtering for de novo, compound heterozygous, homozygous, and X-linked variants. In most cases, additional HPO-based filtering was applied using AI technology.

Results: Pathogenic or likely pathogenic variants were identified in 36 of 171 cases (21.1%), including 19 de novo, 14 autosomal recessive, 1 inherited autosomal dominant, and 2 X-linked dominant variants. The median turnaround time was 16 days. Of 36 cases in which a (likely) pathogenic variant was identified, the parents opted for TOP in 21 cases (58.3%), in 3 cases there was spontaneous intrauterine death (8.3%), one case was lost to follow-up (2.8%) and in 11 cases the pregnancy was carried to term (30.6%). Among the latter group, this led to optimal postnatal management in 70%, the decision to abstain care in 20% and the exclusion of a syndromic cause in 10%.

Conclusions: Our findings indicate that in 1 out of 5 pregnancies with ultrasound anomalies and normal deletion/duplication analysis, a (likely) pathogenic variant explaining the phenotype can be identified. The short turnaround time allows for timely decisions regarding the ongoing pregnancy or neonatal management. The fact that 47.2% (17/36) of the identified variants were inherited highlights the importance of this analysis, as the recurrence risk in future pregnancies can be as high as 50%.

P20 - Cancer risk in RASopathies

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Introduction

Patients with RASopathies have an increased cancer risk, including juvenile myelomonocytic leukemia and neuroblastoma. German and Dutch studies show significantly higher childhood cancer risks in these patients (Kratz 2015 and Jongmans 2011). However, data remains scarce, and genotype-associated risks are unclear, warranting further research in the Belgian population.

Methodology

We conducted a retrospective cohort study on patients with RASopathies in Belgium. So far, we have collected data from patients recruited from the databases of the Antwerp University Hospital (UZA) and Ghent (UZGent). This includes information on demographics, genotype, phenotype, and details of any malignancy developed during their follow-up period. To assess the underlying risks of malignancy, we compared our study population with the general Belgian population using data from the Belgian Cancer Registry.

Results

Our study population consists of 101 RASopathy patients, most of them had a (likely) pathogenic germline mutation in PTPN11 (62/101) and SOS1 (9/101) or RIT1 (9/101). The study population included 56 males and 45 females. Their ages ranged from 0 through 76 years old. In this population, two patients developed a malignancy. One case describes a juvenile myelomonocytic leukaemia in a patient with Noonan syndrome based on a PTPN11 mutation, diagnosed at the age of 3,5 months old. The second case describes alveolar rhabdomyosarcoma in a patient with Noonan syndrome based on a SOS1 mutation, diagnosed at age 5. The data of UZA and UZGent leads to a standardized incidence ratio (SIR, 95% CI) of 11,46 (0,00– 44,95) for all patients with a RASopathy aged 0-5 years and 26,03 (0,01 – 102,05) for patients aged 5-10 years.

Conclusion

This study highlights the increased cancer risk associated with RASopathies. More data from a larger cohort is needed to fully understand the genotype-phenotype correlations and improve risk assessment. Additional data will be collected through a Belgian multicenter study.

P21 - A large deletion responsible for ADTKD-UMOD

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ULB

Introduction

Autosomal Dominant Tubulointerstitial Kidney Disease-Uromodulin (ADTKD-UMOD) is an autosomal dominant renal disease classically due to missense variants displaying a dominant negative effect.

Here we describe a family with high clinical suspicion of ADTKD-UMOD for which no molecular diagnosis was obtained through standard techniques including extensive panel sequencing.

Methods and Results

Whole genome and long read sequencing in multiple affected family members uncovered a large deletion (1313 bp) encompassing part of exon 3, intron 3, the entire exon 4 and part of intron 4, a conserved region of UMOD where most known pathogenic variants are found. The pathogenic effect of the variant was validated through multimodal analysis of the urine protein composition: by ELISA assay, Western blotting and mass spectrometry.

Conclusion

This is the first large deletion reported to be responsible for ADTKD-UMOD, and importantly it was undetected by conventional variant calling on short read extensive panel sequencing and underestimated by copy number variant analysis. Although this type of variant is expected to cause loss-of-function, our data suggest that it may result in an altered protein with dominant negative effect. Our result show that copy number variant may also be responsible of ADTKD-UMOD. We propose that when ADTKD is suspected but not found by massive parallel sequencing and MUC-1 analysis, additional sequencing techniques might unravel other types of variants.

NB: a whole exome sequencing and RNA sequencing on urine samples are undergoing and will hopefully complete the case before the congress.

P22 - ATRX syndrome diagnosed through methylation and RNA analysis: A case highlighting the benefits of an integromic approach

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Background: More than half of neurodevelopmental disease (NDD) cases remain unexplained despite the current standard of care of whole-exome sequencing (WES). Some unresolved cases may result from non-coding variants, which are often challenging to interpret. New complementary omics technologies, including methylomics and transcriptomics, may enable improved interpretation of non-coding variants and enhance diagnostic capabilities.

Clinical Case: The patient was seen at age 4, presenting with axial hypotonia, global developmental delay and dysmorphic facial features such as up-slanting palpebral fissures, a small and upturned nose, retrognathia, a large mouth with a short philtrum and tented upper lip as well as underfolded helices. Independent sitting and standing were delayed (at 12 and 20 months respectively), and he had not achieved independent walking or expressive language. He also had multiple congenital abnormalities such as ear malformations, gallbladder aplasia and an atrial septal defect. He presented feeding difficulties requiring gastrostomy, esophagitis, chronic constipation as well as kyphoscoliosis. Dysmorphic features were suggestive of ATRX syndrome, but initial genetic testing with array comparative genomic hybridization (aCGH) and WES was inconclusive.

Methylation analysis was performed using an Infinium MethylationEPIC v1.0 array and an in-house designed tool to visualize publicly available methylation signatures, revealing an ATRX-specific methylation signature. RNA sequencing of patient derived EBV transformed B-lymphoblastoid cell lines identified a 201 base pair pseudo-exon inclusion between exons 15 and 16 of ATRX. Targeted analysis of Whole-genome sequencing (WGS) data detected a hemizygous deep intronic variant, ATRX c.4558-2416G>C. The variant was inherited from his unaffected mother which showed completely skewed X-inactivation. Together these results confirmed the diagnosis of ATRX syndrome.

Conclusions: This case illustrates how integrating methylomics and transcriptomics can resolve diagnoses elusive to WES. It also emphasizes the value of in-house methylation analysis, advancing precision diagnostics in unexplained NDDs.

P23 - Uncovering transcriptomic signatures associated with resistance to anti-EGFR therapy in colorectal cancer

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Background: Although anti-epidermal growth factor receptor (EGFR) therapy has proven efficacy in treating RAS/BRAF wild-type (WT) metastatic colorectal cancer (mCRC), primary resistance to this treatment is observed in 20–30% of WT patients. Variations in gene expression hold great promise for discovering resistance mechanisms as RNA levels have already been associated with resistance to anti-EGFR therapy in CRC. To confirm and build upon these findings, we examined the gene expression profiles of primary tumors with resistant and responsive phenotypes.

Material and Methods: We isolated RNA from FFPE tissue of twelve primary CRC tumors, comprising six with progressive disease and six with a partial response to anti-EGFR treatment. Using the NanoString PanCancer IO360 panel, we profiled the expression of 770 genes related to the tumor, the cancer microenvironment, and immune responses to create a predictive transcriptomic signature. The data was normalized and analyzed using the nSolver 4.0 Advanced Analysis module to derive differentially expressed genes. Treatment-associated changes in gene expression were corrected for multiple testing using the Benjamini-Hochberg method.

Results: Thirteen differentially expressed genes (DEGs) (FDR ≤ 0.05) were found between anti-EGFR resistant and responsive patients. One gene is upregulated, and twelve genes are downregulated in the resistant group as opposed to the responsive group. These genes are involved in the MAPK pathway, matrix remodeling and metastasis, TGFβ signaling, cell proliferation, metabolic stress, angiogenesis, Notch signaling, cytotoxicity, interferon signaling, apoptosis, and the myeloid compartment.

Conclusion and future work: The DEGs are involved in several biological pathways known to play pivotal roles in cancer progression and resistance mechanisms. This suggests that dysregulation of these pathways could be responsible for innate resistance to anti-EGFR treatment. Furthermore, differentially expressed genes related to matrix remodeling and metastasis, angiogenesis, cytotoxicity, interferon signaling, and the myeloid compartment demonstrate the relevance of the tumor microenvironment and immune response in anti-EGFR therapeutic efficacy.

We propose a potential predictive gene expression signature that could eventually lead to more tailored therapeutic strategies to improve treatment outcomes. Next, the DEGs will be validated on

cetuximab-sensitive and in-house created cetuximab-resistant cell lines, as well as a larger cohort of resistant and responsive patients.

P24 - Shared genetic architecture of brain shape and IBD is specifically enriched for inflammatory response genes

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The gut-brain axis (GBA), a bidirectional communication network that connects the central nervous system and the enteric nervous system of the gastrointestinal tract, is central to human health and disease. Its dysregulation plays a significant role in the pathophysiology of inflammatory bowel disease (IBD) and several psychiatric disorders. Therefore, a better genetic understanding of the GBA is key to understanding disease mechanisms and developing targeted therapies. Here, we conduct a genome-wide association study (GWAS) on multivariate cortical brain shape from 48,564 participants of the UK Biobank, yielding 1,018 independent genomic loci. Using the conjunctional false discovery rate (FDR), the resulting summary statistics were subsequently leveraged to investigate shared genetic signals with IBD based on previously published summary statistics on 25,042 cases and 34,915 controls. This yielded 193 shared genomic loci at 1% FDR, many of which harbor genes related to immune response, including IL23R, IL1R2, IL10/27, SLC22A4, CARD9. Immune-related biological processes were also specifically overrepresented among the shared loci versus all brain-associated loci, suggesting that they specifically underlie the GBA. Together, the shared loci disproportionately explained brain shape variation in regions previously linked to major depressive disorder. PheWAS analysis of the shared loci highlighted pleiotropy with immunological, autoimmune, and psychiatric traits such as schizophrenia. Together, these results align with existing knowledge on IBD pathophysiology and symptomology and suggest that a shared genetic basis of the brain and gut is based in the immune system. The specific enrichment of shared loci for inflammatory response genes therefore also supports the relationship between neuroinflammation and psychiatric conditions such as depression and schizophrenia and lays a foundation for further follow-up regarding the conditions affected by the GBA.

P25 - First report of a homozygous SYCP2 variant implicated in female infertility due to embryo developmental arrest

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The synaptonemal complex (SC) is a zipper-like protein structure essential for the alignment, pairing, and recombination of homologous chromosomes during meiosis. It holds homologous chromosomes together by anchoring their chromatin loops to its lateral elements, with SYCP2 acting as a key component. In mice, a truncated SYCP2 protein disrupts synapsis formation, leading to a sexually dimorphic phenotype: male mice are completely sterile, while Sycp2 mutant females are subfertile with significantly reduced litter sizes. In humans, loss-of-function variants in SYCP2 have been implicated in spermatogenic failure; however, no cases of female infertility linked to SYCP2 have been reported until now.

Here, we report a consanguineous family consisting of six sisters and seven brothers, where three sisters presented with embryo developmental arrest, a rare infertility phenotype known as oocyte, zygote, or embryo maturation arrest (OZEMA). OZEMA has been linked to pathogenic variants in other meiosis-related genes, including CDC20, TUBB8, and WEE2. Whole-exome sequencing in the affected sisters identified a homozygous, likely pathogenic variant in SYCP2 [NM_014258.4:c.186dup; p.(Ile63Tyrfs*43)]. This frameshift variant is predicted to result in complete loss-of-function through nonsense-mediated mRNA decay. A fertile sister and their mother were heterozygous carriers, while a fertile brother did not carry the variant. No other family members were available for genetic testing, and there was no reported history of male infertility in the family.

This study identifies a homozygous loss-of-function variant in SYCP2 as a potential novel genetic cause of OZEMA, extending the phenotypic spectrum of SYCP2-related reproductive disorders to encompass female infertility. These findings highlight the need for further research to elucidate the underlying mechanisms of SYCP2 loss-of-function mutations in female meiosis and fertility.

P26 - Assessment of gene–disease associations and recommendations for genetic testing for somatic variants in vascular anomalies by VASCERN-VASCA

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Introduction

Vascular anomalies caused by somatic (postzygotic) variants are clinically and genetically heterogeneous diseases with overlapping or distinct entities. The genetic knowledge in this field is rapidly growing, and genetic testing is now part of the diagnostic workup alongside the clinical, radiological and histopathological data. Nonetheless, access to genetic testing is still limited, and there is significant heterogeneity across the approaches used by the diagnostic laboratories, with direct consequences on test sensitivity and accuracy. The clinical utility of genetic testing is expected to increase progressively with improved theragnostics, which will be based on information about the efficacy and safety of the emerging drugs and future molecules. The aim of this study was to make recommendations for optimising and guiding the diagnostic genetic testing for somatic variants in patients with vascular malformations.

Results

Physicians and lab specialists from 11 multidisciplinary European centres for vascular anomalies reviewed the genes identified to date as being involved in non-hereditary vascular malformations, evaluated gene–disease associations, and made recommendations about the technical aspects for identification of low-level mosaicism and variant interpretation. A core list of 24 genes were selected based on the current practices in the participating laboratories, the ISSVA classification and the literature. In total 45 gene–phenotype associations were evaluated: 16 were considered definitive, 16 strong, 3 moderate, 7 limited and 3 with no evidence.

Discussion and Conclusions

This work provides a detailed evidence-based view of the gene–disease associations in the field of vascular malformations caused by somatic variants. Knowing both the gene–phenotype relationships and the strength of the associations greatly help laboratories in data interpretation and eventually in the clinical diagnosis. This study reflects the state of knowledge as of mid-2023 and will be regularly updated on the VASCERN-VASCA website (VASCERN-VASCA, https://vascern.eu/groupe/vascular-anomalies/).

P27 - Altered collagen receptors and ECM organisation in dermal fibroblast cultures from patients with classical Ehlers-Danlos Syndrome

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The Ehlers-Danlos Syndromes (EDS) are a heterogeneous group of heritable connective tissue disorders caused by genetic defects disrupting collagen biosynthesis and/or fibrillogenesis, resulting in a disorganized extracellular matrix (ECM). EDS is characterized by soft and hyperextensible skin, joint hypermobility, generalized tissue fragility and chronic pain. Previous research from one research group on a limited number of dermal fibroblast cultures of classical EDS patients (cEDS) carrying defects in type V collagen (glycine substitutions and frameshift variants in the pro-α1 chain and in-frame deletion in the pro-α2 chain), revealed altered collagen and fibronectin organization in the ECM, as well as altered integrin expression. Our aim is to confirm these findings in multiple patient-derived fibroblast cultures with similar defects and cultures carrying a different genetic defect (i.e. in-frame exon skips in the pro-α1 chain), and to investigate alterations in three other collagen receptors. With this, we will gain insight into alterations in the ECM and the receptor organisation, which we can then employ to further explore the molecular mechanisms of cEDS pathogenesis. Preliminary immunocytochemistry observations revealed altered organisation of type I collagen deposited by patient-derived dermal fibroblast cultures. We could not confirm alterations in integrin α2β1 (collagen receptor), integrin α5β1 (major fibronectin receptor), integrin αvβ3 (minor fibronectin receptor) and no significant differences were found between patient-derived and control fibroblasts in average fluorescence per cell surface for collagen receptors syndecan-1 (SDC1) and discoidin domain-containing receptor 2 (DDR2). This finding possibly indicates that alterations in type V collagen may lead to alterations in type I collagen organisation, potentially due to the role of type V collagen as a nucleating factor for type I collagen fibril formation. Analyses of fibroblast-derived RNA and protein to evaluate the mRNA expression levels and protein levels of these targets via RT-qPCR and western blot are ongoing.

P28 - A PDGFRB splice site variant associated with familial infantile myofibromatosis and resistance to imatinib

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Infantile myofibromatosis involves the formation of myofibroblastic tumors during early childhood. In most cases, the disease is caused by somatic or germline gain-of-function mutations in PDGFRB, which encodes platelet-derived growth factor receptor beta. Here, we report a novel germline intronic PDGFRB variant, c.2905-8G>A, in six unrelated infants with multifocal myofibromatosis. All patients had multiple skin nodules and four had bone lesions. Two patients had aggressive disease with bowel obstruction, which was treated by surgery, chemotherapy and tyrosine kinase inhibitors. The variant was also found in one affected parent and two healthy relatives, suggesting incomplete penetrance. The c.2905-8G>A substitution creates an alternative acceptor splice site in intron 21, inserting six nucleotides in the PDGFRB transcript and two residues in frame at the end of the kinase domain. Functional studies revealed that the splice change induced a partial loss of function, contrasting with previously described variants. In four out of five analyzed tumor samples, we identified a second somatic hit in PDGFRB exon 18, which led to a constitutive receptor activation and resistance to imatinib in a cellular assay. Two of these patients had received imatinib without objective response.

One of them switched to dasatinib with concomitant improvement. In conclusion, we describe a new type of PDGFRB variant associated with multicentric infantile myofibromatosis, which accounts for a significant proportion of reported families. We show that this germline variant favors the development of tumors featuring an acquired oncogenic mutation in the same gene and resistance to targeted therapy.

P29 - Decreased local TGF-beta sequestration in a mutant fibrillin-1 mouse model leads to thoracic aortic wall damage

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Background: Marfan syndrome (MFS) is caused by a defect in the gene coding for fibrillin-1 (Fbn1), a building block for microfibrils which binds transforming growth factor beta (TGF-beta) via interaction with latent TGF-beta binding proteins (LTBPs). Nevertheless, the role of TGF-beta in MFS remains controversial.

Methods: We studied a mouse model with a deletion of the first hybrid domain of fibrillin-1 (Fbn1H1Δ), which includes the binding site for LTBPs, to investigate the role of TGF-beta/fibrillin-1 interaction in vivo. We used standard histology as well as propagation-based phase-contrast synchrotron X-ray imaging to assess aortic wall damage, together with proteomics and immunohistochemical staining to elucidate the signaling mechanisms involved.

Results: Both Fbn1H1Δ/+ and Fbn1H1Δ/ H1Δ mice did not develop aortic aneurysms, but ex vivo synchrotron X-ray imaging revealed distinct 'microdissections' –very localized major breaks in elastic lamellae– in the ascending thoracic aorta, which did not progress to fatal rupture. Mass spectrometry analysis of the ascending thoracic aorta of Fbn1H1Δ/+ and Fbn1H1Δ/ H1Δ mice showed a signature of reduced TGF-beta signaling. Mast cell proteases were strongly enriched in the mutant mice. Immunohistological analysis confirmed the presence of mast cells at the location of aortic microdissections.

Conclusions: Our data indicates that decreased local sequestration of TGF-beta predisposes the thoracic aorta of Fbn1H1Δ/+ and Fbn1H1Δ/ H1Δ mice to increased wall damage without aneurysm. We hypothesize that the local environment of decreased TGF-beta signaling might be permissive for the expansion of mast cells and release of mast cell proteases, resulting in degradation of elastic lamellae.

P30 - Understanding human linkeropathies: generation of knock-in models to study of the consequences of defective proteoglycan biosynthesis

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Proteoglycans (PGs) are vital molecules in the extracellular matrix and cell membranes where they contribute to structural integrity of tissues, cell adhesion, and signal transmission. They consist of core proteins linked to glycosaminoglycan (GAG) chains through a common PG linker region. Pathogenic variants in genes encoding enzymes involved in the linker region formation (XYLT1, XYLT2, B4GALT7, B3GALT6 and B3GAT3) cause a group of rare heritable connective tissue disorders, collectively coined the "linkeropathies". Despite the shared biosynthetic pathway, linkeropathies display a variable phenotype with skeletal dysplasia, joint laxity/dislocations, short stature, muscle hypotonia, and developmental delay. The underlying pathogenic mechanisms connecting the genetic defects to the clinical spectrum remain poorly understood, partly due to the rarity of these diseases and the limited availability of relevant tissues for studies.

Given the predominance of missense mutations in human patients, we aim to generate two different knock-in (KI) zebrafish models for each of the five linkeropathy genes. Generating these KI models is challenging due to the early lethality of some mosaic knock-out zebrafish and suboptimal CRISPR-Cas9 cut-and-repair site distances. To address this, we are optimizing the injection protocols by adjusting the Cas9 and homology directed repair template concentrations and, alternatively, trying a prime editing-based approach. Using these techniques, we generated the first KI zebrafish model carrying a specific b3galt6 p.(T79A) mutation, found in human patients with spondyloepimetaphyseal dysplasia with joint laxity, type 1. Generation of additional linkeropathy models is currently ongoing.

These models will enable to investigate the impact of enzyme defects, particularly focusing on musculoskeletal, cardiovascular, ophthalmological, and integumentary structures and functions, along with the consequences for PG and GAG biosynthesis and collagen fibril organization in different tissues. This strategy ultimately aims to explore the molecular mechanisms that contribute to the variable clinical manifestations observed both between and within different linkeropathies.

P31 - Characterization of endothelial cell subtypes in Venous Malformations including the Blue Rubber Bleb Nevus syndrome by snRNA-seq.

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Venous malformations (VMs) are slow-flow lesions caused by aberrant angiogenesis and characterized by dysplastic vessels. Venous lesions are also observed in Blue Rubber Bleb Nevus (BRBN) syndrome, a less common and more severe vascular disorder. BRBN phenotype is defined by numerous cutaneous and internal VMs, which can lead to significant morbidity due to complications such as bleeding, anemia, and organ dysfunction.

Our previous research demonstrated that somatic mutations in the TEK gene, encoding the TIE2 receptor tyrosine kinase, result in ligand-independent activation of TIE2 in both VMs and BRBN. However, these mutations are mutually exclusive, with the most common mutation in VMs being the somatic L914F variant, while T1105N-T1106P double-variant is characteristic of BRBN. Additionally, VMs can sometimes be caused by less frequent TEK mutations or oncogenic mutations in PIK3CA. Aberrant TIE2/PI3K signaling promotes increased survival, invasion, and abnormal behavior of endothelial cells (ECs), contributing to lesion formation and progression.

To further elucidate the mechanisms underlying VMs and BRBN syndrome, we use single nuclei transcriptomics (snRNA-seq). To date, we have snRNA-seq data from seven lesions. These include three VMs with mutations in PIK3CA (H1047R), TEK (G1115*), and one with an uncharacterized mutation, as well as four BRBN lesions, including two with TEK mutations T1105N-T1106P and two with Y897F-R915L. The number of single nuclei sequenced varies from 4.000 to 14.000 cells, with 5 to 60% of ECs per sample. Using markers for different EC types (e.g., lymphatic, arterial, capillary, venous), we have clustered them in both VM and BRBN samples. We continue to characterize these subpopulations to explore cellular and molecular heterogeneity within the lesions and delineate transcriptional signatures and pathways unique to mutant venous ECs. By comparing venous ECs to other endothelial subtypes in the resected lesions, we aim to uncover key molecular drivers of venous EC dysfunction in VMs and BRBN for therapeutic approaches.

P32 - Assessing the effect of aberrant FOXG1 expression in neural organoids

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FOXG1 is a transcription factor specifically expressed in the developing brain. Heterozygous loss-of-function (LoF) causes the severe neurodevelopmental disorder FOXG1 syndrome. FOXG1 is involved in regulating the proliferation of neural progenitor cells (NPCs) and reduced dosage is associated with NPCs prematurely exiting the cell cycle and starting neuronal differentiation. Our goal is to thoroughly study its function and characterise the effects of FOXG1 LoF.

We developed several FOXG1 LoF iPSC lines and differentiated them towards minimally guided cerebral organoids. These organoids were compared with isogenic controls using immunostainings, qPCR and single cell RNA sequencing (scRNA-seq) techniques.

First of all, the variability in the control organoids was apparent. Immunostainings and qPCR results of batch one showed that not all control organoids were able to induce FOXG1 expression, pointing to the limited reproducibility of forebrain formation using the minimally guided protocol. Further, scRNA-seq revealed a disproportionally bigger cluster of choroid plexus cells in control organoids which failed to induce FOXG1 expression, while radial glial cells and neurons were almost absent in these organoids.

Preliminary results of batch two show that compound heterozygous frameshift mutations in FOXG1 lead to the absence of forebrain radial glia and intermediate progenitor cells. Differential expression analysis revealed a list of genes significantly upregulated in radial glia cells with a heterozygous frameshift mutation in FOXG1 and gene set enrichment analysis showed that this gene list is enriched for terms related to neuronal development. Since FOXG1 is mainly known as a transcriptional repressor, these genes are potential targets repressed by functional FOXG1.

Further research towards the downstream targets of FOXG1 as well as the regulation of FOXG1 expression itself is necessary in order to improve diagnostics and develop treatments for patients with FOXG1 syndrome.

P33 - Incidental finding of X-linked periventricular heterotopia in a male presenting joint hypermobility

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Background:

Filamin A (FLNA) is an actin-binding protein encoded by the X-linked FLNA gene, involved in elastic properties of the cytoskeleton, in cell remodelling and migration. FLNA deficiency, caused by heterozygous/hemizygous FLNA pathogenic variants, is responsible of filopathies presenting as periventricular heterotopia and other symptoms such as joint hypermobility. Most of patients with loss-of-function mutations are females. Male lethality is frequent but some cases of male carriers of a FLNA hypomorphic variant have been described.

Methods:

We report a 33-year-old male presenting generalized joint hypermobility, premature loss of teeth and gingival recession. His mother, sisters and brother also had joint hypermobility and other features such as soft skin with atrophic scars, premature loss of teeth and umbilical hernia. In the maternal branch, half of the siblings had teeth anomalies like premature loss and dental agenesia. Ehlers-Danlos syndrome (EDS) panel (UZ Gent, version 2) was tested under suspicion of periodontal EDS.

Results:

Genetic analysis identified c.6202T>C,p.(Phe2068Leu) hemizygous FLNA missense class 3 variant. It concerned a highly conserved nucleotide and protein, had a predicted damaging effect on protein function, was not found on control population and not described in the literature. It was inherited from his mother and also present in his siblings. Brain MRI demonstrated periventricular nodular heterotopia in our patient but not in females carrying the variant. The exam has yet to be performed in his brother.

Conclusion:

FLNA c.6202T>C,p.(Phe2068Leu) missense variant could be a periventricular heterotopia causative variant with a variable expressivity, a hypomorphic variant with partial loss-of-function of the protein allowing males survival. The presence of periventricular heterotopia in the index case brother would support this hypothesis. Functional studies will still be necessary to confirm the pathogenic effect of the variant.

P34 - A first case of desmin-related myofibrillar myopathy due to inheritance from a confirmed mosaic asymptomatic carrier

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Introduction:

Desmin-related myofibrillar myopathy is a hereditary disorder caused by pathogenic variants in the DES gene (MIM#125660), altering desmin, a muscle-specific intermediate filament which is crucial for sarcomere integrity. This condition presents with skeletal myopathy, cardiomyopathy, and conduction abnormalities. The genetic diagnosis may be complicated by incomplete penetrance, variable expressivity, and de novo occurrence. Mosaicism in asymptomatic parents can obscure inheritance patterns, particularly when low-grade mosaic variants in blood are misinterpreted as sequencing artefacts.

Patients and Methods:

The proband initially presented with muscle weakness, cardiac conduction abnormalities, namely atrioventricular conduction disorder and fasciculoventricular bypass tracts, due to hypertrophic cardiomyopathy. Whole exome sequencing (WES) was performed on leukocyte DNA from the proband and her mother, in the latter after previous Sanger sequencing. Cascade testing of the proband's siblings was additionally performed.

Results:

A heterozygous NM_001927.4:c.1216C>T, p.(Arg406Trp) (R406W) variant in the DES gene was identified in the proband and classified as pathogenic (ACMG guidelines). While Sanger sequencing of the mother's DNA was inconclusive, WES revealed low-level mosaicism with a variant allele frequency of 12.3% in blood. The proband's siblings did not carry the variant. The mother, asymptomatic for myopathy or cardiomyopathy, was counselled as a mosaic carrier, while the proband was diagnosed with desmin-related myopathy.

Discussion:

This case illustrates the diagnostic challenges posed by low-level mosaicism. Initial Sanger sequencing was inconclusive for the variant in the mother, while subsequent WES confirmed mosaicism. The presence of a pathogenic DES variant in mosaic form highlights the importance of advanced techniques to ensure accurate diagnostics, inheritance pattern identification, and genetic counselling.

Conclusion:

This is the first reported case of desmin-related myopathy due to inheritance of a mosaic DES R406W variant. In heriditary myopathies, detailed genetic analysis, including WES, is vital for accurate diagnosis, counselling, and risk assessment.

P35 - PDGFRB Variants in Hereditary Progressive Mucinous Histiocytosis

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Histiocytoses constitute a heterogenous group of hematopoietic neoplasms from the macrophages/dendritic cell lineage. Hereditary Progressive Mucinous Histiocytosis (HPMH) is a rare, benign, non-Langerhans form of histiocytosis limited to skin. The disease typically manifests in childhood or adolescence with red-brown papules that persist without spontaneous resolution. Currently, there is a lack of specific treatments for HPMH. The diagnosis is challenging and would be greatly facilitated by genetic biomarkers.

Recently, our team has reported the first gene alteration associated with HPMH: a gain-of-function missense mutation (p.R853W) in the PDGFRB gene in a family comprising two affected individuals. Through collaborations, we identified two new families with HPMH, carrying PDGFRB variants. These findings confirm PDGFRB as a causal gene.

The aim of this project is to characterize the functional impact of these variants. We characterized PDGFRB variants by performing luciferase reporter assays to assess PDGFRB signalling. One variant reveals significantly increased activity compared to the wild-type receptor while another demonstrated a partial loss of function in luciferase assays, contrasting with the previously described p.R853W variant. To further analyse these variants, we introduced the identified changes into the genome of human fibroblasts (HFF2 cells) using CRISPR Cas9 technology and studied downstream signalling pathway by western blot. Moreover, PDGFRB expression in macrophages is unclear in the literature. Therefore, we examined its expression and investigated the impact of these mutations in macrophage cell lines. In conclusion, this project confirmed PDGFRB variants as a cause of HPMH and sheds light on the pathophysiology of the disease, providing insight to improve diagnosis and treatment.

P36 - RNA-sequencing unveils novel FLT4 splice site variants in isolated CHD

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BACKGROUND AND AIM:Congenital heart disease (CHD) affects more than 8 in 1000 live-born babies and may occur isolated or in the context of a syndromic constellation. The pathophysiology of CHD is complex with both genetic and environmental contributions, and the etiology remains unknown in the majority of the patients. Heterozygous loss-of-function (LOF) pathogenic variants (PVs) in FLT4 have recently been associated with Tetralogy of Fallot (TOF). Here we report on two novel families with LOF PVs in FLT4, further contributing to the molecular etiology of isolated CHD.

METHOD:In 318 probands with isolated CHD and normal copy number variant analysis, we performed trio exome sequencing (ES) on genomic DNA followed by analysis of a virtual congenital heart disease gene panel including 471 genes. FLT4 (NM_182925.5) variants were classified according to the American College of Genetic and Genomic Medicine (ACMG) guidelines. We applied RNA-sequencing on lymphocytes to evaluate the consequences of variants predicted to affect splicing of FLT4.

RESULTS:We identified novel PVs in FLT4 in two families with non-syndromic CHD. In family one, two siblings diagnosed with TOF and major aortopulmonary collateral arteries (MAPCAs) harbored a heterozygous c.985+1G>A FLT4 variant, inherited from their asymptomatic father. In family two, the proband presented with bicuspid aortic valve and aortic coarctation and harbored a heterozygous c.1657+6T>C variant in FLT4. Of note, the variant was inherited form her asymptomatic mother, but occurred de novo in the maternal grandmother who was diagnosed with TOF. For both variants RNA-sequencing revealed skipping of exon 7 and 12 in the c.985+1G>A and c.1657+6T>C variant respectively, resulting in a premature termination codon, reclassifying both variants as class 5 (pathogenic). Notably, previous targeted RT-PCR analysis for the c.1657+6T>C variant revealed normally.

CONCLUSIONS:Our report widens the clinical presentation of heterozygous splice site variants in FLT4, that further show reduced penetrance. Reanalysis of patients with recent diagnostic techniques such RNA-sequencing are powerful to elucidate some of the missing heritability in isolated CHD.

P37 - Distinct genetic patterns involving multilocus inheritance and unique and novel variants drive a high diagnostic yield in an understudied consanguineous retinopathy cohort

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Inherited retinal diseases (IRDs) are genetically heterogeneous blinding disorders that are underrepresented in large genomic studies from Middle Eastern populations. In this study, we leveraged an integrated approach combining whole exome sequencing (WES) and autozygosity mapping to investigate the genetic underpinnings of IRDs in a cohort of 192 unrelated Iranian families, predominantly from consanguineous backgrounds (76%).

This strategy provided a diagnostic yield of 73%, significantly surpassing global detection rates. Out of 209 identified causative variants, 82% were unique to single families and 53% were novel, highlighting the heterogeneous landscape of variants in this cohort and a paucity of founder variants. The most frequently involved IRD genes were ABCA4 (19%), CRB1 (6%) and EYS (6%). Strikingly, the usually prevalent USH2A gene was implicated at a much lower frequency than expected (1%). Multilocus inheritance was observed in two families (TMEM126A/GUCY2D and CNGB1/REEP6), showcasing the complex segregation that can be associated with consanguinity and the necessity for deep phenotyping to disentangle different phenotypic consequences of multiple genetic diagnoses

such as distinct or blended phenotypes. The identification of a novel CEP78 variant in a patient with IRD but without hearing impairment uncovered a novel genotype-phenotype association.

These findings not only expand the mutational spectrum of IRD-associated genes but also highlight population-specific trends, such as the underrepresentation of USH2A variants in the studied Iranian cohort. Novel insights into genotype-phenotype correlations were uncovered, demonstrating the critical role of comprehensive genomic analyses. Overall, mapping these distinct genetic patterns using autozygosity-guided WES in understudied consanguineous populations results in a high diagnostic yield and offers direct implications for genetic counseling, precision diagnostics, and eligibility for emerging gene therapies.

P38 - Unexpected STK11 Gene Deletion in a Breast Cancer Patient: Implications for Cancer Predisposition Panels

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Introduction: Recent discussions at Belgian genetic oncomeetings have focused on which genes should be included in gene panels for breast cancer predisposition screening. Although the addition of STK11, CDH1 and PTEN genes was recently decided, this decision is controversial because (likely) pathogenic variants in theses genes are usually associated with typical clinical features. This is why NF1 was not included.

Case Report: We report a case of STK11 gene deletion associated with Peutz-Jeghers syndrome in a patient with isolated breast cancer. The patient was referred to genetic counselling after a diagnosis of breast cancer at the age of 67. Systematic anamnesis and clinical examination revealed no particularities. The family history was marked by prostate cancer at age 76 in one of her four brothers and aggressive breast cancer at age 55 in one of her two sisters, who has since deceased. The standard panel for Hereditary Breast and Ovarian Cancer (HBOC) of 13 genes (ATM, BARD1, BRCA1/2, BRIP1, CHEK2, MLH1, MSH2, MSH6, PALB2, TP53, RAD51C/D) returned negative results. However, a complete deletion of the STK11 gene was incidentally discovered through our sequencing depth-based method for copy number variation detection in a research approach. Thanks to this unexpected diagnosis, the patient has since benefited from specific monitoring following recommendations for Peutz-Jeghers syndrome. Gastroscopy and colonoscopy led to the identification of gastric and colonic polyposis. About ten hyperplasic polyps were already resected. Pathological examination revealed hamartomatous polyps. Additional tests such as videocapsule and pancreatic MRI are still ongoing.

Conclusion: This case highlights the importance of including the STK11 gene in systematic gene panels for HBOC screening, even in the absence of distinctive clinical features. A comprehensive genetic evaluation can identify significant variants and enable personalized clinical care, thereby optimizing the management of at-risk patients.

P39 - Germline mutational landscape in pediatric cancers and disease relevance: : Insights from a Canadian Experience

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Background and aims: The prevalence of children with germline variants predisposing to pediatric cancer varies greatly across studies. This variation could be explained by factors like cohort selection criteria, number of genes tested, and degree of causality. This study aims to map the germline mutational landscape of an unselected pediatric cancer cohort and clarify the disease relevance of findings.

Method: Sequencing (WES and transcriptome) data from prospectively enrolled 484 pediatric cancer Quebec patients were analyzed. Comprehensive integration of germline findings with tumor features and clinical genetic assessments was performed.

Results: A total of 153 germline alterations were identified in 133 patients (27%). These anomalies spread across 76 cancer-related genes. Germline (likely) pathogenic (P/LP) variants in cancer predisposition genes (CPGs) related to pediatric-onset cancer predisposition syndromes (pCPS), considered in established association (EA), were identified in 40 patients (8%). Among these patients, 68% (27/40) had solid tumors, while 32% (13/40) had hematological cancer, representing 11% (27/244) and 5% (13/240) of each population, respectively. EA variant was known prior to cancer onset in only 30% of patients (12/40). Thirteen percent (5/40) of EA were in mosaic (PTPN11 [n=1], SMARCB1 [n=2], T21 [n=1], TP53 [n=1]). Somatic data identified second hits in 93% (26/28) of tumor suppressor genes. Somatic RNA-sequencing confirmed aberrant splicing in all splice-site variants, upgrading a deep intronic SDHB variant from VUS to LP. No patient carried two distinct EA variants. Eleven alterations (2% of patients) were classified as in suspicious association and fell into four categories: 1) P/LP variant in genes not classically associated with the cancer type, 2) heterozygous P/LP variant in autosomal recessive genes but concordant cancer type, 3) variants not respecting classical age of onset and 4) highly suspect VUS in a gene concordant with cancer type and mode of transmission. Remaining alterations are secondary findings, unlikely related to cancer occurrence. Eight pCPS could have been missed by clinical approach (DICER1 [n=3], TP53 [n=1], FBXW7 [n=1], TRIM28 [n=1], CDC73 [n=1], RUNX1 [n=1]) and 8 by exome-based SNV analysis (constitutional T21

[n=5] and microdeletions in BRCA2 [n=1], CREBBP [n=1] and SMARCB1 [n=1]). One Beckwith-Wiedemann case was diagnosed solely based on clinical features.

Conclusion: These results highlight the complementary nature of conventional and NGS approach in pediatric cancer patients and the importance of considering variant causality within clinical context, necessitating multidisciplinary expert discussions. These findings underscore remaining uncertainties in pediatric cancer predisposition and the need for functional analyses.

P40 - Cutis Laxa type 2E: report of a new case highlighting hypotonia as a major feature

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Cutis laxa syndrome type 2E (CL2E) is a very rare disorder caused by pathogenic variants in the latent transforming binding growth factor β1 (LTBP1) gene. LTBP1 truncating variants may induce a defective binding of LTBP1 to the extracellular matrix components (ECM) and dysregulate the transforming growth factor β (TGFβ) signalling. CL2E patients share some common features to all cutis laxa syndromes. However, they are characterised by short stature and skeletal abnormalities. Here we report a new patient with a novel homozygous c.3731-1G>A variant in the LTBP1 gene. Congenital hypotonia was the first symptom. The clinical features of our patient were compared to the eight patients previously reported in the literature. A transmission electronic microscopy (TEM) on proband-derived dermal fibroblasts was performed to improve the phenotype-genotype correlations. Our patient presents skin and joint hyperlaxity as others CL2E patients, but a late onset of a very mild cutis laxa appeared only since one year of age. He showed no short stature, no typical skeletal abnormalities such as brachydactyly, syndactyly or clinodactyly. Cardiac malformations, learning difficulties, or hearing loss were also absent. The phenotype seems milder compared to previous descriptions. However, the TEM analysis supports the pathogenicity of the variant because of a result compatible with a cutis laxa. In conclusion, our case extends the phenotype of CL2E and highlights a new phenotype considering hypotonia as the main clinical sign.

P41 - Atypical glutamic acid to lysine substitution in the triple helix of type III collagen manifests as overlap between classical and vascular Ehlers-Danlos syndrome

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Vascular Ehlers-Danlos syndrome (vEDS) is caused by pathogenic variants in the COL3A1 gene. The most frequently reported pathogenic COL3A1 variants are glycine substitutions within the triple-helical domain of type III procollagen and splice site variants leading to an exon skip.

A 34-year-old man was referred because of recurrent shoulder dislocations in the context of a suspected connective tissue disorder. At birth, bilateral congenital clubfeet were observed. As a child, joint hypermobility was present, and recurrent shoulder dislocations began at the age of 29 after a fall and persisted. There was a history of delayed wound healing and wound dehiscence, but no signs suggestive for vascular fragility.

On clinical examination, hyperelastic, translucent, doughy skin and generalized joint hypermobility (Beighton score 9/9) were observed. Facial features included hypotelorism, deep set eyes and epicanthal folds. At the skeletal level, a flattened back and mild pedes plani were noted. There was an atrophic scar with deposition of hemosiderin on his left leg.

Due to the combination of skin fragility and joint hypermobility, classical Ehlers-Danlos syndrome (EDS) was suspected. Whole exome sequencing of known EDS-associated genes, revealed a novel glutamic acid to lysine (Glu>Lys) substitution in the triple helix of COL3A1 (p.(Glu730Lys). Segregation analysis in the family showed the younger brother (33y) also carried the variant and exhibited similar phenotypic features. Hence the variant was classified as likely pathogenic.

The clinical presentation of our patients shows significant overlap with previously reported cases carrying a COL3A1 Glu>Lys substitution, often initially suspected as classical EDS, with some exhibiting vEDS-like features (e.g., arterial aneurysm, dissection/rupture, gastrointestinal rupture, pneumothorax, premature varicose veins). To date, our patients have not developed severe complications related to tissue fragility. Given the limited number of reported cases, additional patients are necessary to assess the risk of these complications and further elucidate the underlying pathophysiology.

P42 - Mutant zebrafish model in the search for new therapies for childhood chronic kidney disease

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Children with chronic kidney disease (CKD) face a reduced life-expectancy due to an accelerated cardiovascular disease (CVD). Indeed, histological signs of CVD are already present prior to dialysis initiation in even young children with CKD. Protein-bound uremic toxins (PBUT), i.e. toxic organic metabolites that accumulate with deterioration of kidney function, are recognized as key players in the pathophysiology of accelerated CVD, by contributing to the chronic pro-inflammatory state. Treatment options that can mitigate PBUT-driven inflammation and CVD are lacking. We hypothesize that a CKD zebrafish model, created by selective knock-out of genes involved in kidney development (wt1a, nup107, eya1, myo1e, hnf1β-α, pax2a) by CRISPR-Cas9 technology, can be used for the discovery of new therapeutic treatments specifically targeting accelerated CVD in children. Zebrafish (Danio Rerio) offers the complexity of an intact in vivo model, and experiments in larval stages can be performed at scale, allowing high throughput applications such as unbiased in vivo drug screening. We found in wild type (WT) zebrafish the presence of PBUTs, indicative for orthogonal pathways and metabolites in zebrafish underscoring its suitability for PBUT research. Also, PBUT administration to WT larvae resulted in a decrease in cardiac function and an increase in oxidative stress. The transgenic Tg(kdrl:GFP) and Tg(acta2:GFP) reporter line which visualize the vascular endothelial and smooth muscle cells, was incorporated into the CKD zebrafish model, allowing visualization of the vascular abnormalities in the CKD model. The clearance of fluorescently labeled dextran and a proteinuria nanoluciferase reporter line was used for description of the kidney phenotype. Subsequently, the in vivo efficacy of drugs targeting PBUT-driven inflammatory pathways will be performed with the established CKD-zebrafish models. Altogether, by offering a widely applicable CKD zebrafish model, we aim to positively impact the currently unacceptably high CVD mortality present in the pediatric CKD population.

P43 - Insights in COL4A1 and COL4A2 Variants through Comprehensive Genotype-Phenotype Mapping

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COL4A1 and COL4A2 encode the collagen IV alpha 1 and alpha 2 chains, essential for basement membrane integrity. Pathogenic variants in these genes lead to a broad and variable spectrum of phenotypes, including neurological, ocular, renal, cardiovascular, and muscular disorders. This study investigates associations between the type and location of variants in COL4A1 and COL4A2 and the frequency of specific clinical features.

By reviewing published patient cases in the PubMed database and reevaluating all described variants using the ACMG criteria, we collected a cohort of 705 cases with COL4A1 (N = 613) or COL4A2 (N = 103) variants classified as pathogenic, likely pathogenic, or uncertain significance and their respective phenotype. Correspondence analysis was used to explore genotype-phenotype correlations.

The distribution of missense variants in major domains was similar in COL4A1 and COL4A2, with the majority in the triple helix domain (COL4A1: 84,1%; COL4A2: 88,4%) and fewer variants observed in the NC1 (COL4A1: 9,4%; COL4A2: 7,7%) and 7S (COL4A1: 6,3%; COL4A2: 3,8%) domains. Glycine substitutions in the Gly-Xaa-Yaa repeat in the triple helix domain accounted for ~75% of all missense variants and ~57% of the total number of variants in both genes. Other frequently described variants were miRNA-29 binding site variants (COL4A1: 12%), splice site variants (COL4A1: 9,5%; COL4A2: 12%), and frameshift or stop-gain variants (COL4A1: 5,7%; COL4A2: 5,8%).

Correspondence analysis confirmed the association of miRNA-29 binding site variants with ischemic features and cerebral small vessel disease (cSVD), as described in literature. We also identified a potential link between COL4A2 NC1 domain variants and cSVD, a pattern not observed for COL4A1. Previously suggested correlations between integrin binding sites and muscle and kidney phenotypes were absent in our analysis. These findings require further statistical and experimental validation.

P44 - Filamin C associated cardiomyopathy in pediatric patients: a Belgian case series and literature review

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Background: Recently, there is increasing interest in the role of the Filamin C (FLNC) in cardiomyopathy. FLNC, a member of the filamin family of actin-binding proteins, plays a vital role in maintaining the structural integrity, signaling and mechanotransduction of sarcomeres in cardiac and skeletal muscles. Variants in the FLNC gene are extensively described in skeletal myopathies and all types of cardiomyopathies, mostly in adults. There is increasing literature discussing an early-onset form of cardiomyopathy. FLNC variant contribution of1-8% in different CMP is reported.

Aim: To study the cardiovascular outcome of children carrying a (likely) pathogenic variant in FLNC and evaluate genotype-phenotype correlations.

Methodology: Children up to 18yrs, found via literature search or under follow-up in one of the participating Belgian centers. The NM_001458.5 FLNC-transcript was used as reference. Demographic, genetic and cardiovascular data were collected.

Results: 70 children (61.4% male, mean age 7.6±6.1 yrs) were included (8 in Belgium). A summary of FLNC variants is shown in the figure. 31.4% of patients presented with associated extracardiac manifestations. Missense variants were most frequent (65.7%), followed by nonsense variants (15.7%). Truncating variants –caused by nonsense, frameshift or splice-site mutations– were mostly associated with DCM and ACM ($p<0.001$). Missense variants can result in all CMP subtypes, with an important proportion of RCM (60.4%). 40% of the children suffered a major cardiac event (mean age 10.5±8.6 yrs, time to event 3±4.2 yrs): 18 patients (25.7%) underwent a heart transplantation, 4 received an ICD (5.7%), and 4 patients (5.7%) suffered from sudden cardiac death (SCD) at presentation. RCM had the worst outcome (>45% of cardiac events).

Conclusion: FLNC-related cardiovascular phenotype can present at an early age and is associated with important morbidity and mortality. Truncating variants are associated with DCM and ACM, while missense variants with RCM. Early screening and follow-up are necessary to identify children at risk.

P45 - Immunogenomics solves missing heritability and ends the diagnostic odyssey in RAG1-SCID

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Background: Severe combined immunodeficiency (SCID) is a life-threatening Inborn Error of Immunity (IEI) due to defective T-cell maturation. The diagnosis is usually established by rapid and highly sensitive immunophenotyping, directing downstream genetic testing. A genetic diagnosis is needed for personalized treatment and genetic counseling. Despite genomic advances, 15% of SCID remains unsolved. We report a consanguineous family with three relatives displaying T-B-NK+SCID, supporting RAG1/RAG2 deficiency but molecularly undiagnosed for several years.

Methods: Flow cytometric immunophenotyping (IF), targeted next-generation sequencing (tNGS) of RAG1 and RAG2 and IEI gene panel-based clinical exome sequencing (CES) was performed in two affected cousins. AutoMap revealed regions of homozygosity (ROH) in the exome data. Copy number variants were evaluated using ExomeDepth. Reads of RAG1 and RAG2 were manually inspected in Integrative Genomics Viewer. Segregation analysis in parents was done using tNGS. Optical Genome Mapping (OGM) and long-read sequencing (LRS) using adaptive sampling were performed to delineate and characterize a putative complex structural variant (SV).

Results: Pre-transplant IF showed T-B-NK+SCID with absence of naïve T-cells, B-cells and recent thymic emigrants (RTE) and presence of NK-cells, supporting RAG1/RAG2 deficiency. tNGS and IEI-CES did not reveal pathogenic variants in known SCID genes nor in other IEI genes. AutoMap detected several ROHs, containing the RAG1 and RAG2 genes. Manual inspection of their respective reads in IGV showed a putative complex SV impairing RAG1, homozygous in the cousins and heterozygous in the parents. OGM suggested a ~340 bp insertion. LRS revealed a homozygous insertion of ~320 bp including an AluYb8 element. Further characterization of this genomic event is currently ongoing.

Conclusion: Identification of a novel Alu insertion in the RAG1 gene ends the diagnostic odyssey in a SCID family, expands the genetic architecture of RAG1-SCID and emphasizes the value of immunogenomics combining flow cytometric immunophenotyping and genomics in standard-of-care diagnostics of SCID.

P46 - Prevalence of intronic AAGGG repeat expansion in RFC1 in Belgian patients

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CME - UZ Leuven

Background: Cerebellar Ataxia with Neuropathy and Vestibular Areflexion Syndrome (CANVAS) is an adult-onset slowly progressive neurodegenerative condition characterized by the impairment of three neurological pathways responsible for balance: the cerebellar, sensory and vestibular system. In April 2019, a biallelic expansion of an intronic AAGGG repeat in RFC1 was identified as the genetic cause of CANVAS by Cortese et al.

Methods: Screening of the RFC1 repeat expansion is performed on genomic DNA by short-range flanking PCR and repeat-primed polymerase chain reaction (RP-PCR) using primers targeting the three conformations separately ((AAAAG)exp, (AAAGG)exp and (AAGGG)exp). In case of a positive RP-PCR result, the size of AAGGG repeat expansion is further determined using optical genome mapping (OGM).

Results: Since the introduction of this diagnostic test in UZ Leuven in October 2019, 378 patients have been analyzed. Using PCR and RP-PCR, we were able to identify a biallelic AAGGG expansion in 15% of the patients. 8% of the patients carried a heterozygous AAGGG expansion. A biallelic AAGGG expansion (>400 repeats) has been confirmed in 26 patients by OGM. In the remaining RP-PCR positive patients the length of the expansion could not be confirmed by OGM due to the lack of a fresh blood sample. In 2 carrier patients WES was performed to screen for a second defect, but none was found. Unfortunately, clinical information was insufficient or absent in most cases, making it difficult to determine a genotype-phenotype correlation.

Conclusion: We have shown that the genetic test to identify the presence of a recessive AAGGG repeat works well in our laboratory. Our diagnostic yield is in agreement with the data from literature. There are a few limitations with our current flow: (1) the lack of detection of other possible pathogenic conformations; (2) in some cases several tests ((RP-)PCR/OGM/WES) are required, making testing laborious and costly.

P47 - Introduction of a new diagnostic test for the detection of a GAA expansion in the FGF14 gene

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CME - UZ Leuven

Background: SCA27B is one of the most common forms of late-onset cerebellar ataxia and is characterized by slowly progressive cerebellar ataxia, early episodic symptoms, downbeat nystagmus, diplopia and vertigo. Recently, a non-coding GAA repeat expansion in intron 1 of the fibroblast growth factor 14 (FGF14) gene has been identified as a common cause of autosomal dominant SCA27B. Current data suggests a pathogenic threshold of (GAA)250-300 , with possible incomplete penetrance, and (GAA)>300 with full penetrance.

Methods: In first instance, 33 previously unsolved adult ataxia patients were selected for optimization and validation of the diagnostic test.

Screening of the FGF14 repeat expansion was performed on genomic DNA by short-range flanking PCR and bidirectional repeat-primed polymerase chain reaction (RP-PCR) using primers designed by Bonnet et al (2023).

Since the introduction of the genetic test, 42 ataxia patients have been tested for GAA repeat expansions in FGF14.

Results: Among the 42 patients that were tested for FGF14 GAA repeat expansions in a diagnostic setting, 17 (41%) carried a (GAA)>250 repeat expansion. However, the exact length of the repeat expansion could not be determined by PCR/RP-PCR. All patients presented with adult-onset ataxia, but unfortunately for most patients no further clinical information was available.

Conclusion: We have shown that the genetic test to identify the presence of a GAA repeat in FGF14 works well in our laboratory. Further analysis is required to determine the exact size of the expansion and to confirm the diagnosis in these patients (data in progress).

Our diagnostic yield (41%) is in accordance with the yield described in similar cohort studies, ranging from 9% to 61%. The high variability is presumably mostly due to geographic variation. These results confirm GAA-FGF14-related ataxia to be among the most common causes of inherited adult-onset ataxia and encourages us to redesign our diagnostic ataxia flow.

P48 - Nuclear metabolism in the control of DNA methylation in tumour hypoxia.

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Hypoxia induces both metabolic and epigenetic remodelling in cancer cells. Epigenetic reactions are directly regulated by the nuclear bioavailability of key metabolites, reflecting environmental changes. Yet, how hypoxic-induced metabolic changes concertedly translate to specific epigenetic profiles remains to be determined. Recently, an exciting body of literature has demonstrated that multiple metabolic enzymes, including those providing metabolites involved in DNA methylation, translocate to nuclei where they produce local metabolic fuels for epigenetic reactions.

We show that early upon hypoxia, the translocation dynamics of DNA methylation-relevant metabolic enzymes are impacted. We further demonstrate the implication of subcellular localization of metabolic enzymes on the nuclear production of key metabolites in hypoxic cancer cells. Finally, we are currently characterizing in more depth how changes in DNA methylation-relevant metabolites control oncogenic programs under hypoxia.

In conclusion, tumour hypoxia is associated with both metabolic and epigenetic remodelling. Here, we reveal how the nuclear presence of metabolic enzymes that synthesise important metabolites for epigenetic reactions provides a rationale for cancer cell adaption to low oxygen conditions.

P49 - Unraveling the enhancer - promoter interaction networks that drive craniofacial development

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Craniofacial development is a complex process that occurs at the early stages of embryonic life. During this period, multipotent cranial neural crest cells (CNCCs) migrate from the neural tube towards the branchial arches, and differentiate into the cartilage and bone structures that form the human face. Non-coding cis-regulatory elements, like enhancers, fine-tune this process by controlling the spatiotemporal expression of often distal genes, through the formation of chromatin loops. Perturbations in enhancer sequences can alter these interactions and thus disrupt gene expression and cause craniofacial birth defects. Identifying the enhancers active during craniofacial development and their target genes is essential for unraveling the regulatory networks driving face formation and for identifying candidate non-coding regions implicated in craniofacial defects. To address this, we are generating cell-type specific 3D chromatin interaction maps. Specifically, we are in vitro differentiating H9 human embryonic stem cells into CNCCs and chondrocytes. The differentiated cells express cell-type specific markers indicating successful differentiation. To detect promoter-enhancer interactions we applied promoter capture hi-c (PCHiC) at the different time points of cell differentiation. In CNCCs, PCHi-C revealed 101,520 significant interactions, among which 65,012 are long-range (distance >100kb). 57% of interactions are between promoters and promoter interacting regions (PIRs). PIRs are enriched for marks of active enhancers (H3K27ac and ATAC-seq peaks) and for CNCC transcription factor binding sites. Our chromatin interaction maps can also link known craniofacial enhancers to target genes. By generating temporal-specific chromatin interaction maps in progenitor cells of the human face we are gaining knowledge into the spatiotemporal specific gene regulatory signals that control human face development. Such datasets, when complimented with other -omics data are powerful tools in detecting putative enhancers and linking these regulatory elements to target genes, a critical step in assigning function to the non-coding genome.

P50 - Nanopore long read whole genome sequencing in developmental disorders

Mathilde Geysens, Benjamin Huremagic, Erika Souche, Greet Peeters, Joris Robert Vermeesch & Kris Van Den Bogaert

KU Leuven

Despite the implementation of massive parallel sequencing as standard of care, about half of patients with developmental disorders (DD) remain without genetic diagnosis. Structural and epigenetic variants have long been known to be involved in the pathogenesis of DD but remain challenging to map due to technological limitations. However, with dropping costs and increasing accuracy of long read sequencing (LRS) platforms, the concomitant assessment of single nucleotide, structural and epigenetic variation becomes feasible.

To evaluate the relevance of LRS clinical implementation for unsolved DD we performed whole genome nanopore sequencing in 50 patient-parent trios with intellectual disability and/or multiple congenital anomalies without molecular diagnosis after short read exome or genome sequencing. We optimized the nanopore sequencing technical workflow, developed an analytical pipeline and built a population reference set to assess structural variants (SVs). In parallel, we implemented the concurrent detection of targeted disease- associated methylation disturbances such as imprinted defects and skewed X-inactivation, as well as genome-wide episignatures.

We identified 25000 SVs in each individual. Upon filtering, only 0.6 are true de novo SVs and 2.1 SVs are X-linked inherited SVs in males. An average of 30 inherited SVs are assessed for each individual to rule out a potential contribution to a recessive disease. Skewed XCI was identified in 2 female patients as well as 1 mother, enabling to direct further genetic investigations. The presence of an imprinting disorder or episignature is currently being investigated in all individuals.

Our study demonstrates that LRS enables concurrent haplotyped genomic and epigenomic analyses, leveraging simultaneous detection of single nucleotide and structural variants, X-inactivation, imprinting, as well as episignatures, consolidating a multi-step sequential process into a single diagnostic assay.

P51 - GIPXplorePLUZ: a comprehensive ctDNA test using multi-omics to enhance cancer detection and diagnosis

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GIPXplorePLUZ is an liquid biopsy profiling tool that integrates multi-omics layers to enhance the detection and analysis of cancer biomarkers. This test uses different information in circulating cell-free DNA (cfDNA) to enhance diagnosis. As such it detects mutations, fusions, microsatellite instability, tumor mutational burden, genome-wide copy number alterations (CNAs), and cfDNA fragmentome profiles. By integrating these data layers, GIPXplorePLUZ achieves higher sensitivity and specificity, supporting personalized decision-making and pan-cancer analysis.

The platform offers a scalable and dynamic solution with features such as sensitive variant detection, robust data processing in a cloud-based environment, and user-friendly clinical tools for variant analysis and reporting. It is a cost-effective and rapid (4-7 working days) non-invasive diagnostic solutions for solid and hematological tumors.

Key innovations include wet-lab protocols, highly sensitive and specific variant calling pipelines, genome-wide imbalance profiling, and machine learning algorithms for fragmentome analysis. These tools enable simultaneous multi-cancer detection with 81.62% accuracy and 95% specificity, offering potential applications in early cancer detection, therapy monitoring, and prenatal diagnostics. The platform incorporates advanced data visualization, cloud scalability, and a federated AI system for secure, collaborative genomic analysis across multiple sites.

We thus provide a comprehensive framework for pan-cancer ctDNA screening, diagnosis and follow-up, with increased sensitivity and specificity due to the combination of different information sources extracted from cfDNA.

P52 - Unraveling syndromic traits in dual genetic diagnoses with 3D facial phenotyping

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Co-existing Mendelian disorders can cause atypical phenotypes, challenging genetic diagnostics and clinical management. Deep familial phenotyping contributes to matching genomic and phenotypic data, but is particularly challenging for facial features. Here, we report an individual with a dual diagnosis of paternally inherited KBG syndrome and a maternally inherited 3q26 deletion. We performed three-dimensional (3D) facial phenotyping to investigate the presence of a subclinical dual phenotype.

We collected 3D facial photos of the proband and their nuclear family, eight individuals with KBG syndrome and a large control cohort. Using craniofacial growth curves, we accounted for age- and sex-related variation in facial shape. We corrected the probands facial shape for the maternal (3q26del) and paternal (KBG) shape effects to isolate the syndromic shape effects, and assessed the contributions of both syndromic traits to their facial features using principal component analysis and cosine-distance based analysis.

Clinically, the probands facial gestalt is dominated by features associated with the 3q26 deletion. However, the cosine similarity to KBG syndrome is high, which is an objective indication of the presence of the KBG gestalt. Principal component analysis shows separate clustering of controls, KBG syndrome and both 3q26del heterozygotes. After correcting the proband for maternal shape effects, their corrected shape clusters with KBG syndrome, again unveiling hidden KBG-like facial features.

We introduce objective 3D facial phenotyping to deconstruct facial features of major gene effects, unveiling subclinical facial features of KBG syndrome in an individual with a 3q26 deletion and KBG syndrome. This innovative approach provides a powerful tool for assessing and understanding atypical syndromic presentations and interpreting variants in individuals with multiple genetic diagnoses.

P53 - Genetic yield in a large ataxia and spastic paraplegia cohort in Flanders

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BACKGROUND

Next generation sequencing has revolutionized the diagnostic possibilities for phenotypic and genetic heterogeneous disorders as hereditary ataxia and spastic paraplegia (SPG), with reported yields up to 50%. However, most studies use stringent inclusion criteria, making it difficult to estimate the yield in an unselected population presenting in real-life clinical practice. Also, a considerable number of cases remain unexplained and it is not sure whether Mendelian mutations can close the diagnostic gap.

METHODS

We retrospectively studied a large cohort of 262 ataxia and 172 SPG patients presenting at the tertiary neuromuscular reference center in University Hospitals Leuven, Flanders, Belgium. For a subset of patients unexplained after gene-by-gene testing we performed gene panel analysis. We defined the yield of gene-by-gene testing and gene panel analysis. Additionally, we looked for heterozygous variants in genes linked with autosomal recessive ataxia or SPG.

RESULTS

With gene-by-gene testing followed by gene panel analysis, we can explain 54.6% of cases. The yield of gene-by-gene testing was 36.2% and of a subsequent gene panel 28.9%. The latter revealed 44 novel variants. Friedreich ataxia was the most common diagnosis for ataxia patients, followed by SCA6 and RFC1-linked CANVAS. For SPG, the most common subtype was SPG4, followed by SPG7. We identified 31 heterozygous (likely) pathogenic variants in genes linked with autosomal recessive ataxia or SPG, and a combination of variants in eight patients.

CONCLUSIONS

Combined gene-by-gene testing and gene panel analysis provides a high yield for an unselected cohort of patients presenting with ataxia and/or SPG.

P54 - GENETIC RISK STRATIFICATION IN THE LEUVEN FAMILIAL PULMONARY FIBROSIS COHORT

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Background

Familial pulmonary fibrosis (FPF), defined as the presence of fibrotic interstitial lung disease (ILD) in at least two first or second degree relatives, makes up about 20% of all idiopathic ILDs. Both rare and common variants contribute to the risk of FPF, but true genetic risk stratification accounting for both types of variants is lacking.

Objective

We aim to offer accurate risk prediction for family members of FPF patients and personalize the screening approach.

Methods

We assembled a cohort of 360 individuals: 154 patients and 206 relatives. Patients diagnosed with idiopathic pulmonary fibrosis (IPF) and a positive family history (at least one additional first degree relative) were asked to participate, and their first degree relatives were recruited. Gene panel analysis, genotyping of the MUC5B promoter polymorphism (rs35705950) and telomere length assessment using Flow-FISH was performed for FPF patients. Relatives took part in a multimodal screening program (pulmonary function test, high-resolution CT-scan, genetic testing, genotyping of the MUC5B promoter polymorphism (rs35705950) and telomere length assessment.

Furthermore, the cohort was genotyped using the Infinium Global Screening Array (Illumina) to perform polygenic risk score (PRS) analysis. Quality control of the SNP array data was carried out according to Marees et al. (2018). Imputation was performed through the TOPMed Imputation Server. The PRSice tool was used for polygenic risk score analysis, using summary statistics from Allen et al. (2022) as base data.

Results

The Leuven FPF cohort consists of 130 families. The size of the families ranges from 1-18 individuals included per family. Gene panel analysis was negative in 78/130 families, detected a variant of unknown significance in 28/130 families and found a (likely) pathogenic (LP/P) variant in 22/130 families. LP/P variants were mostly detected in telomere-related genes (RTEL1, TERT, PARN, TERC), in 2 families we found a LP variant in a surfactant-related gene (SFTPA2, SFTPC). The minor allele frequency of the MUC5B risk allele was 29.2%. In about 13% of relatives we observed signs of preclinical ILD on CT-scan as part of the screening program. Strikingly, in 3 families where a variant in a telomere-related gene was found, (preclinical) ILD was seen in non-carriers. The best PRS model

using the PRSice tool includes 53 SNPs below a significance threshold of 5e-08 and has an R squared of 0.05.

P55 - Breaking the mold: exploring the impact of sex on phenotypical features and challenges in Marfan Syndrome

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Marfan syndrome (MFS), an inherited connective tissue disorder associated with variants in the FBN1 gene, presents a wide array of clinical features impacting cardiovascular, skeletal, and ocular systems. Despite equal prevalence among sexes, symptom severity varies significantly within and between sexes. Females generally exhibit lower rates of aortic root dilation but experience poorer outcomes post-aortic complications compared to males, who often present with more severe aortic pathology. Skeletal (e.g., scoliosis) and ocular (e.g., ectopia lentis) manifestations may also differ between sexes. These phenotypic traits are crucial for MFS diagnosis according to the revised Ghent nosology criteria, which remain consistent across sexes despite confirmed cardiovascular variations. This study aims to explore sex-related disparities, vital for tailoring diagnostic criteria and treatments to optimize patient care. Objectives include validating previous findings on aortic pathology in MFS males and investigating differences in skeletal and ocular features listed in the MFS systemic scoring criteria.

Phenotypical data were collected from 471 MFS patients, including skeletal and ocular features alongside aortic measurements and events. The cohort was stratified into pediatric (0 – 18 yrs; n= 65) and adult (18+; n = 406) groups.

Results confirm earlier findings on aortic prevalence. Within both groups there were no significant age differences. In the pediatric group, no significant sex differences were found in aortic aneurysm prevalence, skeletal and ocular features, but the rate of Z-score increase per year differed between sexes, while aortic growth rate (mm/year) did not. The adult cohort exhibited significant sex differences in armspan/height ratio and pectus deformity. Additionally, our data show significant sex discrepancies in the use of aortic medication, aortic growth, and aortic surgery/dissection. Armspan/height ratio and pectus deformity are criteria for the systemic MFS score, but our research suggests they, alongside aortic aneurysm prevalence, are sex-specific and vary between sexes. Establishing sex-specific diagnostic criteria for MFS may enhance diagnostic accuracy and treatment efficacy.

P57 - Withdrawn

P57 - Implication of large heterozygous deletions in the GBA gene in Parkinson disease

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Parkinson disease (PD) is a neurodegenerative disorder mainly characterized by rest tremor, muscle rigidity, bradykinesia and postural instability. Onset of PD is most commonly around age 60 years with description of early-onset adult PD at age 20-50 years and late-onset adult PD above 50 years. It is estimated that around 5-10% of PD cases are due to pathogenic variants in single genes, including the glucocerebrosidase (GBA) gene. This gene is associated with an autosomal dominant, often early-onset and severe form of the disease. The penetrance is variable and ranges from 10 to 30%. In contrast to heterozygous pathogenic variants in the GBA gene, biallelic mutations are responsible for Gaucher disease, an autosomal recessive metabolic disorder characterized by deficient GBA enzyme activity.

We present three patients in whom specific analysis of the GBA gene by Next Generation Sequencing revealed large deletion in the GBA gene. The first patient developed at 57 years-old a severe form of Parkinson disease. Patient's phenotype included cognitive and autonomic dysfunctions that rapidly progressed to a fatal issue within 6 years. Analysis revealed heterozygous deletion of the exons 3 to 11 of the GBA gene. Splenectomy was performed in the patient's mother who was diagnosed with Gaucher disease type 1. However, she never developed signs of PD. The second patient carried heterozygous deletion of the exons 9 to 11 of the GBA gene and was presented with extrapyramidal signs and Parkinson disease. Unlike the two first patients, the third one didn't show any signs of Parkinson disease. Analysis revealed, in addition to deletion of the exons 10 and 11 of the GBA gene, the c.1093G>A p.(Glu365Lys) variant in the GBA gene. Patient's diagnosis has not been clearly establised, but would be suggestive of Gaucher disease.

Our data support that, in addition to the autosomal recessive disorder associated with biallelic pathogenic variants in the GBA gene, heterozygous carrier may develop symptoms of Parkinson disease. This strongly points to the importance of (1) GBA gene screening; (2) reporting specific variants in GBA Parkinson's disease to help appreciation of penetrance of specific GBA variant or deletion and (3) performing appropriate genetic counseling in the management of patients and family with history of Gaucher disease.

P58 - Residual Protein Function Shapes Immune Dysregulation in Loss-of-Function RC3H1 Mutations

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The genetic landscape and biological mechanisms underlying immune dysregulation remain incompletely understood. Recently, we identified a homozygous truncating RC3H1 mutation in a patient with intellectual disability and relapsing hemophagocytic lymphohistiocytosis. Here, we present a larger cohort of biallelic and heterozygous truncating RC3H1 variants in five unrelated families, grappling with pleiotropic immune disease ranging from lymfoproliferation, autoinflammation and systemic autoimmunity. RC3H1 encodes for ROQUIN1, a posttranscriptional regulator of immunological checkpoint molecules such as ICOS, CTLA4 and OX40. ROQUIN1 is indispensable for immune homeostasis, as mice bearing homozygous missense Rc3h1 mutations manifest a systemic lupus erythematosus (SLE)-like phenotype.

Genetic analyses were performed using whole exome sequencing (WES). Patients were immunologically characterized using flow cytometry and electrochemiluminescence. The pathogenic nature of RC3H1 mutations was studied by analyzing expression of ROQUIN1 targets in murine Rc3h1/Rc3h2 knock-out T cells, retrovirally complemented with Rc3h1 constructs harboring patient mutations.

All families presented with immune dysregulation with variable degree of lymfoproliferation, inflammation or autoimmunity, resembling SLE and Sjögren syndrome. Using WES, we identified both biallelic and heterozygous truncating mutations in RC3H1 in all afflicted family members. Flow cytometry performed on peripheral blood mononuclear cells unveiled increased activated T cells numbers and elevated expression levels of the regulatory proteins ICOS and OX40. We retrovirally transduced primary murine T cells with the identified RC3H1 mutations and noted ICOS and OX40 dysregulation, corroborating the deleterious nature of these mutations. The extent of immune dysregulation was associated to both zygosity and length of the residual truncated ROQUIN1, suggesting a potential phenotype-genotype correlation.

In summary, our dataset reveal an unappreciated role for RC3H1 mutations in the realm of human autoimmune disorders and highlights the potential for genetic studies in families with both autosomal recessive and dominant immune disease.

P59 - The role of the meiotic chromosome axis in the assembly of the DNA double-strand break machinery

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UCLouvain

The formation of haploid gametes depends on the programmed induction of DNA double-strand breaks (DSBs) by Spo11, which are repaired by a mechanism of DNA recombination that promotes the pairing and subsequent segregation of homologous chromosomes. DSB formation is intrinsically tied to the chromosome axis, a meiosis-specific proteinaceous structure that provides the anchor for a linear array of chromatin loops. In S. cerevisiae, Hop1, Red1 and Rec8 constitute the key components of the axis and are responsible for recruiting Spo11's partners, RMM (Rec114, Mei4, Mer2). However, the mechanism whereby the RMM proteins are recruited to the chromosome axis remain poorly understood. Here, I propose to characterize a series of uncharted interactions between axis proteins and DSB proteins, namely interactions between Hop1 and Mer2, between Hop1 and Rec114, and between Mer2 and Red1. For each of these, I will use in vitro assays to validate the respective interaction and will use AlphaFold-guided mutagenesis to identify mutations that specifically compromise them. Finally, I will use yeast molecular genetics to test the importance of these interactions for the assembly of the meiotic chromosome axis and for DSB formation in vivo. This work will provide important insights into the role of the chromosome axis in the initiation of meiotic recombination, which is essential to promote genetic diversity and evolution.

P60 - Insights in underlying pathophysiology of microcephaly with simplified gyral pattern associated with VRK1 pathogenic variants derived from fetal neuropathology.

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Biallelic variants in VRK1 have been described in patients with heterogeneous phenotypes with childhood or adult-onset of progressive upper and lower motor neuron disease. Prenatal onset and neurodevelopmental disorders associated with VRK1 have rarely been described in the literature. Here, we report on the first fetal cases and first neuropathological examination of the brain in patients with pathogenic variants in VRK1. Two fetuses from consecutive pregnancies of second-degree consanguineous parents presented with microcephaly prenatally during the early second trimester. Termination of pregnancy was performed for both cases. Post-mortem examination showed overlapping features including facial dysmorphisms, microencephaly, agenesis of the corpus callosum, absent gyration and reduced neuronal density of the cortex, suggesting a simplified gyral pattern. One of the two fetuses showed an unusual macroscopic appearance of muscle hypertrophy that could underlie a neuromuscular disease, in the absence of histological anomalies. No visceral malformations were observed. Whole exome sequencing identified a homozygous likely pathogenic variant in VRK1 (NM_003384.3, GRCh38):c.238C>G p.(Leu80Val) in both fetuses. The variant is located in a previously reported cluster close to the ATP-binding site. The parents were heterozygous carriers of the variant. Neuropathological examination in these cases provides first insights in the underlying pathophysiological process of biallelic pathogenic variants in VRK1 in humans. Our findings are evocative of a combination of impaired neuronal proliferation, of a neuronal migration deficit, and abnormal axon guidance.