P1. Results From A Large Prospective Genetic Study In Children With Brugada Syndrome

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Brugada syndrome (BrS) is an inherited arrhythmia syndrome that is associated with sudden cardiac death (SCD) and ventricular arrhythmias (VA). Pathogenic or likely pathogenic (P/LP) variants are found in 20-25% of BrS patients. P/LP variants in SCN5A indicate in the worse outcome. Nonetheless, the diagnostic yield and the prognosis of large gene panel testing in pediatric BrS has not been established. This study aims to define the diagnostic yield of a wide gene panel on a large cohort of pediatric BrS patients, based on the ACMG guidelines. An additional aim is to investigate the correlation between the clinical outcome and the genetic background in children with BrS as well as provide a comparison with adult BrS patients.

A total of 452 patients, including 38 children and 414 adults, were enrolled in the UZ Brussel monocentric BrS registry, between 1992 and 2022. Inclusion criteria: BrS diagnosis, genetic analysis for BrS performed with a next generation sequencing-based large gene panel and recent re-classification of gene variants following current ACMG guidelines. Pediatric patients were defined as ≤ 12 years. The primary endpoint was VA occurrence, defined as documented SCD, aborted SCD, ventricular tachycardia or ventricular fibrillation or appropriate implanted cardioverter defibrillator (ICD) intervention.

The diagnostic yield for P/LP variants in the pediatric population is 44.7% (P+), all present in the SCN5A gene, non in other BrS associated genes. After a mean follow-up of 142.7 months, 3 children (7.9%) experienced a VA, with appropriate ICD shocks. Inappropriate shocks occurred in 3 pediatric patients (7.9%). Pediatric patients without P/LP variants (P-) (55.3%) had higher VA free survival during the follow-up, compared with P+ pediatric patients. There was no difference in VA free survival between pediatric and adult BrS patients in both P- and P+ groups. P+ children with BrS have a worse arrhythmic prognosis.

P2. The prognostic role of (predicted) null variants in Brugada syndrome

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Background: A pathogenic/likely pathogenic (P/LP) variant can be found in 20-25% of patients with Brugada syndrome (BrS) and a P/LP variant in SCN5A is associated with a worse prognosis. However, the prognosis of variants of unknown significance (VUS) and non SCN5A variants is unclear. The aim of this study is to define the diagnostic yield of a large primary and secondary cardiac arrhythmia gene panel with ACMG variant classification; furthermore, a clinical assessment of both P/LP and VUS variants in SCN5A and non SCN5A genes is aimed.
Methods: All BrS patients, were prospectively enrolled in the UZB registry between 1992 and 2022. Inclusion criteria for the study were: 1) clinical BrS diagnosis based on current recommendations; 2) Genetic analysis performed with a large primary and/or secondary cardiac arrhythmia gene panel; 3) Classification of variants following ACMG guidelines. Patients with a P/LP variant were defined as (P+); patients with a P/LP or VUS were classified as P+/VUS. All other patients were classified as No-variants carriers (No-V). Furthermore, P/LP and VUS variants were classified into missense and (predicted) null variants.
Results: A total of 452 BrS patients were analyzed. A P/LP variant was found in 96 patients (21.2%), 91 patients (20.1%) in SCN5A gene and 5 patients (1.1%) in a gene different from SCN5A. A VUS variant was present in 54 patients (11.9%).

After a mean follow-up of 116.7 months, 47 patients (10.4%) experienced a ventricular arrhythmia (VA). At survival analysis, No-V carriers had higher VA free survival, compared with P+/VUS (predicted) null variant carriers. P+/VUS missense variant carriers had higher VA free survival, compared with P+/VUS (predicted) null variant carriers. There was no difference in VA free survival between No-V carriers and P+/VUS Missense variant carriers. There was no difference in VA free survival between P+/VUS (predicted) null variant carriers in SCN5A and non SCN5A genes and both had a lower VA free survival compared with No-V. At Cox multivariate analysis, P+/VUS (predicted) null variant carrier status was an independent predictor of VA occurrence (HR= 1.64, CI 95% 1.10-2.48, p=0.018).
Conclusions: In a large BrS cohort, the diagnostic yield for P/LP mutations is 21.2%. P+/VUS (predicted) null variant carrier status is an independent predictor of VA.

P3. Assessing univariate facial phenotyping approaches in GWAS

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While human facial shape is highly heritable, it is also complex, which makes elucidating its genetic underpinnings heavily dependent on a genetically relevant phenotypic description. Simplifying

complex 3D facial features into biologically meaningful univariate traits is a frequently used strategy to increase power. Common univariate traits include inter-landmark measurements (i.e., linear distances) and scores resulting from dimension reduction techniques (e.g., principal component analysis or via deep learning approaches). However, no studies have formally investigated the biological value of these alternative phenotypic descriptions. Here, we compare SNP-based heritability and GWAS results of different univariate facial traits representing the nose in a sample of 8,426 individuals. Additionally, we evaluate the performance of the proposed novel phenotyping method, where each face in the dataset is scored with respect to the direction to 1) randomly selected faces, 2) extreme faces, or 3) syndrome average faces. Distances between sparse landmarks demonstrated the highest mean heritability, followed by the latent dimensions of an autoencoder; which is a modern dimension reduction technique using deep learning. Principal component scores and likeness to randomly selected faces were similar and significantly less heritable than the above-mentioned phenotypes. Interestingly, likeness to extreme and syndromic faces exhibited similar distributions of heritability and were overall least heritable, suggesting that extreme and syndromic facial traits have a lower relative contribution of common genetic variants. Furthermore, more independent genetic loci were revealed when multiple GWASs of likeness traits based on randomly selected faces were aggregated. Our results suggest that likeness to randomly selected faces, which is a simple to apply phenotyping method, shows the potential to capture genetically relevant shape variation in faces.

P4. Case report: Missense variant c.593A>G p.(Lys198Arg) in CSNK2A1, a recurrent variant in Okur-Chung neurodevelopmental syndrome.

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Background
Okur-Chung neurodevelopmental syndrome (OCNDS), caused by CSNK2A1 pathogenic variants, is characterized by a developmental delay (DD), primarily affecting language, accompanied by intellectual disability (ID) and behavioral issues. To date, 51 patients have been described and the missense c.593A>G p.(Lys198Arg) variant was found in 20% of them.

Method
We report a new case and compare the phenotypic features presented by the patients with the missense c.593A>G p.(Lys198Arg) variant (N=10 including our patient) (Group 1) and those affected by other CSNK2A1 variants (Group 2).

Case report
A 15-year-old Moroccan girl presented with an ID and a motor DD. Clinical examination revealed a short stature (at -2,2SD), a bilateral enophthalmos, a bulbous nose, a thin upper lip and hirsutism. She walked at three year. At 13 years, she presented recurrent episodes of vomiting finally attributed to an epilepsy. Currently, she presents persistent fine psychomotor motor difficulties, a language delay and major difficulties for the daily life. The brain MRI showed a discrete lateral ventricular colpocephaly, an arachnoid cyst and a decreased pituitary size.

Results
All the patients encompassed the ID, DD and behavioral troubles. The dysmorphic features were not discriminant. Interestingly, cerebral MRI showed not only a delayed of myelination but also pituitary abnormalities in the two groups (Group 1: 3/8 and Group 2: 4/26). Moreover, cardiac abnormalities were only described in the group 2 (5/26).

Conclusion
Performing genotype-phenotype correlations in OCNDS is still challenging, mainly due to the limited available data. However, our comparative review highlights the first significative difference regarding the cardiac abnormalities, which are never reported for the patients with the missense c.593A>G p.(Lys198Arg) variant.

P5. Report of a novel familial DNMT3A variant causing a variable clinical phenotype of Tatton-Brown-Rahman syndrome

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Introduction. Tatton-Brown-Rahman syndrome (TBRS) is a rare overgrowth and intellectual disability syndrome caused by pathogenic variants in the DNMT3A gene, a DNA methyltransferase.
We here report a family with a new DNMT3A variant.

Results: The proband was born with birth parameters at the upper centiles (p75-p97). He had a developmental delay and intellectual disability. He experienced rapid growth and advanced bone age. We noted pneumothorax, multiple fractures, easy bruising, moderate mitral valve prolapse and cardiomyopathy. His adult height was 210 cm (+4.5SD) and his head circumference was at +4.2SD. He has a dolichocephalic skull and elongated face, full eyebrows with synophris, downslanting of the palpebral fissures, prominent upper central incisors, a high and narrow palate, large extremities, finger contracture and scoliosis. He comes from a family with tall stature (3 sibs and parents) but there are no other family members with intellectual disability. Exome-based analysis showed a heterozygous DNMT3A variant (c.2206C>T, p.(Arg736Cys)) inherited from the father (slightly skewed 40% in blood, buccal mucosa and eyebrows), who has normal development and intelligence, a cardiovascular phenotype (aortic root dilatation, mitral valve bulging and atrial and right ventricle dilatation) and lymphocytosis. Episign analysis on a blood sample of both the proband and his father showed a methylation signature characteristic for Tatton-Brown-Rahman syndrome, underscoring pathogenicity of this variant.

Conclusion. Pathogenic loss of function DNMT3A variants cause an overgrowth and intellectual disability syndrome, coined Tatton-Brown-Rahman syndrome. Mostly, pathogenic DNMT3A variants are found de novo, although rare familial cases have been reported, including inheritance from an unaffected mosaic or an affected parent. We here further expand the spectrum of TBRS, reporting a novel familial mutation and further elaborating on the clinical phenotype with, in our family, the presence of cardiovascular problems and incomplete penetrance of the intellectual disability phenotype.

P6. Immunomodulatory therapy helps patient with biallelic FLG variants become less itchy

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A 30-year-old woman was seen at the genetics department for preconceptional counselling because she suffers from severe atopic dermatitis. She has a lifelong history of erythematosquamous plaques and lichenification on the scalp, the neck, elbows, knees, and back.
Targeted testing of the FLG gene was done by Sanger sequencing of all coding exons and the flanking intronic regions. Two heterozygous pathogenic variants were detected: GRCh37 (hg19): NM_002016.1(FLG): c.1501C>T, p.(Arg501*) and c.2282_2285delCAGT, p.(Ser761Cysfs*36). Both variants result in a premature stop codon. Segregation analysis confirms the trans-position of the variants.
Pathogenic variants in the FLG gene result in ichtyosis vulgaris and a susceptibility to atopic dermatitis with an autosomal dominant inheritance pattern. Due to incomplete penetrance, variable expression and the high incidence of these skin problems, heterozygous variants in the FLG gene are often discovered as an incidental finding.
Nevertheless, diagnostic testing of the FLG gene is possible, as there are patients reported in literature with homozygous or compound heterozygous mutations, causing moderate to severe ichtyosis vulgaris or atopic dermatitis, suggesting a semi dominant inheritance pattern. There is a distinction in severity of the clinical picture between heterozygous and compound heterozygous or homozygous patients, as seen in this family. The patient has a severe phenotype and is compound heterozygous; her parents had paediatric eczema and are heterozygous. Her brother, with paediatric eczema and sister, with severe atopic dermatitis, are currently being tested.
Immunomodulatory therapy by intravenous injections of dupilumab is now available for patients with no sufficient improvement after intensive topical treatment and at least one systemic immunosuppressive medicine. Our patient, currently treated with this therapy, has a remarkable decrease in the extension and severity of the skin lesions, as well in symptoms due to these lesions with less itchiness and improvement of sleep.

P7. A Case of Microphthalmia and Bilateral Papillary Coloboma Associated With a New Gain-of-Function Mutation of RBP4

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Microphthalmia, anophthalmia, and coloboma (MAC) are related structural, congenital eye malformations that display a spectrum of severity and can occur in isolation or as part of a syndrome. Recently, Chou and colleagues identified missense mutations in RBP4, encoding serum retinol binding protein (RBP), causing autosomal dominant MAC with reduced penetrance and a maternal parent-of-origin effect.
We identified a novel, maternally inherited, missense variant in RBP4 (p.G34V) trough clinical trio-exome sequencing in a boy with non-syndromic MAC possible encoding a dominant-negative RBP blocking transmembrane transport of vitamin A. Our findings might provide new knowledge of the variant spectrum of RBP4 gene.

P8. Expanding genotype-phenotype associations in BGN-related Meester-Loeys syndrome

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Mutations in BGN, an X-linked gene coding for biglycan, are associated with two divergent phenotypes: Meester-Loeys syndrome (MRLS), a syndromic form of aortic aneurysm/dissection, and spondylo-epimetaphyseal dysplasia. For the latter, two missense BGN variants are described, whereas only loss-of-function variants are considered causal for MRLS. Since the initial publication of five families in 2017 [1], we identified eleven additional MRLS families. All sixteen probands, except one, are male with an average age of presentation of 36 years. Thirteen male probands presented with aortic and/or widespread arterial aneurysms/dissections, one male (11y) presented with syndromic features without cardiovascular symptoms (yet), another male (unknown age) had a family history of aortic aneurysm/dissection, and one female proband (42y) was detected as part of a comprehensive prenatal testing study. Segregation analysis revealed an additional 24 BGN variant-harbouring family members (4 males and 20 females). Their phenotype ranged from no cardiovascular or connective tissue phenotype to death due to aortic dissection. Identified BGN mutations causing a stop codon insertion, frameshift, or splicing defect were shown to lead to loss-of-function by cDNA and Western Blot analysis of skin fibroblasts of seven probands or were strongly predicted to lead to loss-of-function based on the nature of the variant. No pathogenic missense variants without additional (predicted) splice effect were identified. Interestingly, a male proband with a coding BGN deletion presented with a more severe skeletal phenotype. This may possibly be explained by expressional activation of a downstream ATPase (ATP2B3; normally repressed in skin fibroblasts) driven by the remnant BGN promotor in the 5’ untranslated region. In summary, extensive analysis at RNA, cDNA and protein level is necessary before concluding on the pathogenicity of identified BGN variants, and distinct mutational mechanisms may underlie the wide phenotypic spectrum of MRLS patients carrying loss-of-function variants in BGN.

[1] Meester JA, Vandeweyer G, Pintelon I, et al. Loss-of-function mutations in the X-linked biglycan gene cause a severe syndromic form of thoracic aortic aneurysms and dissections. Genet Med. 2017;19(4):386-395. doi:10.1038/gim.2016.126

P9. Update on the allelic heterogeneity and phenotypic diversity in CBFB-related cleidocranial dysplasia

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Background: Cleidocranial dysplasia (CCD) is a rare skeletal dysplasia with significant clinical variability. Patients with CCD typically present with delayed closure of fontanels and cranial sutures, dental and clavicular anomalies and short stature. RUNX2 has been the only known disease-causing gene for CCD for several decades until we recently identified pathogenic variants in CBFB in eight individuals from five families with a CCD-like phenotype. CBFB encodes the core-binding factor β subunit (CBFβ) that interacts with all RUNX proteins (RUNX1,RUNX2,RUNX3) to form heterodimeric transcription factors, which may explain phenotypic differences between CBFB-related and RUNX2-related CCD.

Methods: We provide an update on the genotypic and phenotypic data of our current series of individuals with CBFB-related CCD.

Results: We had previously reported five pathogenic CBFB variants, all located in the RUNX-binding domain of CBFβ, resulting in either protein truncation or deletion of exon 4. We have now ascertained a sixth family with a sporadic case presenting with a CCD-like phenotype, including clavicula bipartita, pseudo-epiphyses of the 2nd metacarpal, shortening of distal phalanges, delayed carpal ossification and normal stature. In this girl we identified a novel heterozygous variant (c.314G>A, p.(Gly105Glu)) in exon 4 of CBFB. This missense variant is also located in the RUNX-binding domain of CBFB and in silico programs uniformly predict high pathogenicity. The variant is

absent in public databases. Functional studies are currently performed to investigate how these 6 different CBFB variants affect the function of CBFβ-RUNX complexes, and how this may contribute to the development of CBFB-related CCD.

Conclusion: We now added a sixth family with CBFB-related CCD to our cohort, the first with a missense CBFB variant, herewith expanding the genotypic spectrum of this novel rare bone disease. By including this variant in our ongoing functional studies, we aim to improve our knowledge on genotype-phenotype correlations in CBFB-related CCD.

P10. Evolution of Ehlers-Danlos Syndrome consultations in the university hospital of Liege

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Introduction :
In 2018, we evaluated the number of appointment requests for suspected Ehlers Danlos syndrome (EDS) and related collagenopathies, as well as the indication to receive these patients in the genetics department of the University Hospital of Liege. We found that in 97.8% of the cases, their diagnosis had been made (or excluded) clinically and no genetic consultation was necessary. Therefore, we tried to better screen the patients we received by sending them an explanatory letter regarding the criteria for a genetic consultation and a clinical form to fill in to apply for an appointment, (if one is needed). Three years after the implementation of this system, we wanted to evaluate it.

Methods :
We performed a retrospective observational study of the patients from the University Hospital of Liège who had come to a genetic consultation for EDS suspicion and related collagenopathies since the implementation of our pre-screening system in June 2018 and compared the data to those we had collected before.

Results :
We received 634 appointment requests: 149 (23.5%) were suggestive so a consultation was directly scheduled. We sent our questionnaire to the remaining 485 requests: only 99 patients (20.4%) completed it. A total of 248 patients (39.11%) were received : the percentage of patients without signs of collagenopathy was lower (16.5% vs 37.3%), as was the number of hypermobile EDS (46.8% vs 54.2%) and the percentage of molecular diagnosis was higher (2.4% vs 1.4%) compared to 2018.

Conclusion :
After having been informed via our letter, 79.6% of patients did not continue their request. The proportion of patients without signs of collagenopathy has decreased but the rate of hypermobile EDS is still very high. Introducing guidelines for referral to genetic consultation among Physicians of EDS patients remains a challenge.

P11. MIRAGE Syndrome caused by a pathogenic variant c.1376G>A (p.Arg459Gln) in the SAMD9 gene with presumed maternal gonadal mosaicism : report of a family with two affected sisters.

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MIRAGE (Myelodysplasia, Infection, growth Restriction, Adrenal hypoplasia, Genital phenotypes, Enteropathy) Syndrome is a rare autosomal dominant and complex multisystemic disorder. Other clinical features are prematurity, failure to thrive, cerebral and renal malformations, variable dysmorphism, chronic lung disease, and high early mortality rate. Heterozygous gain-of-function variants in SAMD9 gene are currently reported in ~52 children with a broad phenotype of MIRAGE Syndrome. The majority are missense variants frequently affecting Arginine residue, are located in the P-loop NTPase domain and occur de novo. Nevertheless, SAMD9 c.2305G>A (p.Arg769Gln) variant in two affected siblings was reported in a study as the only family presumed with gonadal mosaicism in one of the unaffected parents.
Here, we describe two sisters, who survived only few days after birth, with clinical characteristics of MIRAGE syndrome respectively born at 37 and 28 weeks of gestation by emergency caesarean for IUGR and foetal distress. Both had intrauterine growth retardation, respiratory distress, pulmonary arterial hypertension, circulatory instability, thrombopenia and, glycaemia and ionic troubles. They were dysmorphic and had cerebral anomalies. The first girl had a necrotizing enterocolitis and died at 70 days of life from septic shock while the second, who died at 5 days of life, had renal malformations, skin pigmentation, anaemia, and presumed lung hypoplasia. Parents and their other child were asymptomatic. Whole exome sequencing exhibited the presence, in the two symptomatic children DNAs, of the pathogenic variant c.1376G>A (p.Arg459Gln) located in the SIR2_2 domain of the SAMD9 gene. This variant, previously reported in four patients with similar features, was not present in neither of parents. Furthermore, raw data showed inherited paternal polymorphisms on the wildtype allele supporting a maternal germline mosaicism.
In conclusion, we report the MIRAGE syndrome phenotype probably caused by maternal germline mosaicism in two sisters.

P12. How to adapt clinical practice for genetic testing when the index case is negative: a case report.

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Genetic testing for cancer susceptibility is performed in patients with personal and family history of breast cancer. Worldwide, multiple guidelines for genetic testing are available. Recently, an international group of experts defined and updated the guidelines for BRCA testing practices (1). These guidelines had helped us in defining which patient is more informative across a pedigree. Here, we report a family with clinical history of breast cancer. This family is composed by three sisters, aged 46, 51 and 54. The elder sister had developed breast cancer at the age of 53. Six months later, the mother developed breast cancer at 73 years old. The other two sisters were healthy at that time. A genetic testing for breast and ovarian cancer predisposition was proposed to the elder sister but she declined. No tests were carried out in the mother. One year after, the younger sister developed a bifocal lobular breast carcinoma. Therefore, we proposed to perform the genetic testing and she agreed. We identified a BRCA2 pathogenic variant. Consequently, the mother performed genetic test and finally her elder sister also. The mother was also carrying the BRCA2 pathogenic variant but surprisingly it was not found in the elder sister. The last sister didn't wish to perform the test. Our case report illustrates the importance in genetic counseling to consider each member of the family as phenocopies may be possible.

Ref: (1) Pujol P et al. Clinical practice guidelines for BRCA1 and BRCA2 genetic testing. European Journal of Cancer. 2021 (146) 30-47.

P13. Characterization of a Belgian pathogenic founder variant in MYBPC3

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Background Variants in the MYBPC3 gene are a frequent cause of hypertrophic cardiomyopathy (HCM). Founder mutations are believed to be more benign as they prevailed despite negative selection pressure.
Methods A pathogenic deletion of exon 23-26 of MYBPC3 (ENST00000545968) was identified in probands with HCM through Copy Number Variant analysis and confirmed with a specific MAQ assay. Cascade-screening of first-degree relatives was performed.
Haplotype analysis, using microsatellite markers, was performed to identify a common haplotype, which was used to estimate the age of the founder event the using a linkage-disequilibrium based approach.
Results We identified 19 probands carrying the MYBPC3 exon 23-26 deletion. 54 first-degree relatives were genetically tested, of whom 35 (64.8%) were genotype positive (G+) and 19 (35.2%) genotype negative (G-). Subsequent clinical assessment revealed a HCM phenotype in 19 (54.3%) G+ relatives, of whom only 5 (14.3%) were symptomatic at the time of diagnosis.
Probands had more severe phenotypes than G+ family members (Table 1). This may be in part explained by age, as probands were older than G+ relatives. At the age of 50, a penetrance of 78.6% was observed (HCM in 11/14 G+ relatives with age ≥50 years). Overall, 24.1% of all G+ patients have reached the composite endpoint after a median follow-up of 8 years with an average age of 50 years. Of G+ patients ≥50 years of age, 33.3% had reached the composite endpoint.
A common haplotype of 1.19 Mb was identified in all 19 probands (Figure 1). The founder event was estimated to have happened 6 generations ago and the founder event presumably took place 200-225 years ago (around the year 1800).
Conclusion: The deletion of exon23-26 of MYBPC3 is a novel Belgian pathogenic founder variant with a significant penetrance of >75% in G+ relatives at the age of 50 years.

P14. Genetic variability in known ALS genes in sporadic ALS patients

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BACKGROUND
With the advent of gene therapies for amyotrophic lateral sclerosis, there is a surge in gene testing for ALS. Although there is ample experience with gene testing for C9orf72, SOD1, FUS and TARDBP in familial ALS, large studies exploring genetic variation in all ALS-associated genes in sporadic ALS (sALS) are scarce. Gene testing in a diagnostic setting is challenging given the complex genetic architecture of sALS.
METHODS
We characterized genetic variability in ALS by studying genetic variation in 90 ALS-associated genes on whole genome sequencing data from a cohort of 6013 sporadic ALS patients and 2411 matched controls from Project MinE. We applied customized ACMG-criteria to identify pathogenic and likely pathogenic variants. We determined the length of repeat expansions in C9orf72, ATXN1, ATXN2 and NIPA1 using the ExpansionHunter tool.
RESULTS
We found C9orf72 repeat expansions in 5.21% of sALS patients. In 50 ALS-associated genes, we did not identify any pathogenic or likely pathogenic variants. In 5.89%, a pathogenic or likely pathogenic variant was found, most commonly in SOD1, TARDBP, FUS, NEK1, OPTN or TBK1. Significantly more cases carried at least one pathogenic or likely pathogenic variant compared to controls (OR 1.75; p-value 1.64x10-5). Isolated risk factors in ATXN1, ATXN2, NIPA1 and/or UNC13A were detected in 17.33% of cases. In 71.83%, we did not find any genetic clues. A combination of variants was found in 2.88%.
CONCLUSION
This study provides an inventory of pathogenic and likely pathogenic genetic variation in a large cohort of sALS. Overall, we identified pathogenic and likely pathogenic variants in 11.13% of ALS patients in 39 known ALS genes. In line with the oligogenic hypothesis, we found significantly more combinations of variants in cases compared to controls. Further characterization of the genetic architecture of sALS is necessary given the growing interest in gene testing in ALS.

P15. Familial Mediterranean fever in Moroccan population

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Background: Familial Mediterranean fever (FMF) is a systemic auto-inflammatory disease with a high prevalence in individuals from the Mediterranean region. The main factor of FMF disease is a mutation in the MEFV (MEditerranean FeVer) gene located on the short arm of chromosome16. This gene has undergone various mutations, which are transmitted in an autosomal recessive pattern, but in rare cases it can be transmitted in an autosomal dominant pattern. Clinically, FMF is manifested in childhood with several symptoms, such as recurrent febrile attacks, abdominal and articular pain. In severe cases, the evolution of the pathology leads to renal Amyloidosis. As a treatment, Colchicine is prescribed for FMF patients to reduce the frequency and severity of crises and prevent renal failure.
Methods and materials: This is a retrospective study that was conducted at Hassan II University Hospital Fez from January 2014 to august 2022. A total of 55 patients presenting symptoms of FMF disease (from 50 families) were screened by Sanger sequencing of the both exons 2 and 10 of the MEFV gene which are considered as mutation hot spots in our country.
Results: Totally, pathogenic variants were detected in 23 patients belonging to 20 families (40%) with a sex ratio of 1,3. Among these families, 15 (75%) had biallelic variants (homozygous and compound heterozygous), and 5 (25%) had monoallelic variants. The majority of these patients had onset of symptoms before 20 years.
Conclusion: In conclusion, the use of molecular genetic analysis and the diagnostic criteria of our patients helped to confirm the diagnosis of FMF and institute therapy. The complete analysis by whole-exome sequencing is highly recommended for patients with heterozygous or no mutations to better understand the molecular basis of FMF Moroccan patients.
Keys words: Auto-inflammatory, MEFV gene, Molecular genetic analysis.

P16. The clinical use of exome sequencing to diagnose PCD patients

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Background: Primary ciliary dyskinesia (PCD) is a heterogeneous disorder of dysfunctional motile cilia. Motile cilia generate a monodirectional flow for mucociliary clearance in the epithelium of the respiratory tract, sinuses, and middle ear. In addition, motile cilia drive the left-right patterning during embryonic development. PCD is characterized by severe recurrent otosinopulmonary infections, male infertility, situs inversus totalis, situs ambiguous (heterotaxy) and associated congenital heart disease. Identification of ultrastructural defects in the cilia based on electron microscopy has been the traditional test to confirm the diagnosis, but this approach is no longer the sole “gold standard”. The clinical use of exome sequencing (ES) has increased the diagnostic yield in patients with heterotaxy PCD.

Methods: ES data of > 146 patients (and when available their parents) were analyzed for a panel of 148 heterotaxy PCD related genes from 2019 until now at the Center of Medical Genetics in Ghent. Variant classification was based on ACMG/AMP and ACGS guidelines.

Results: We identified multiple class 4 or 5 variants explaining the clinical presentation in 21% of the cases. The majority (97%) of the cases have an autosomal recessive inheritance. In our cohort, 70% of the class 4 or 5 variants were loss-of-function variants and DNAH11 was the most affected gene. Furthermore, we reported variants of unknown significance (class 3) in 6% of the patients. Hence, more studies are required to solve these cases (coverage completion, segregation analysis or functional studies).

Conclusion: With a diagnostic yield between 21-27%, we confirm that ES can be used as a second “golden standard” for the diagnosis of heterotaxy PCD.

P17. PDGFC : new candidate gene for cleft lip and palate

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Introduction:

Cleft lip and/or palate (CL/P) is a common congenital birth defect (1/700 live births). The etiology CL/P is complex and multifactorial, involving both genetic and environmental factors. Recent studies have suggested that the PDGF signaling pathway, in which PDGFC plays a key role, is involved in the development of the craniofacial region.

Methods:

From a cohort of >1400 CL/P families, 358 index cases and some of their family members have been exomed by whole exome sequencing (WES). Filtering of variants was retained for variants that satisfied the following criteria: (i) pass GATK standard quality-control filters, (ii) within a list of 459 candidate genes for oral clefts; (iii) missense, nonsense, frameshift and splice-site changes; (iv) <1% allele frequency in the ExAC database of WES from 60,706 unrelated individuals; (v) not detected in samples from individuals with unrelated pathologies (or unaffected controls) in the in-house database of 1800 WES and (vi) for missense variants, predicted to affect protein function by at least 6 out of 20 in silico prediction tools.

Results:

Five patients with cleft lip and palate from 3 different families showed 3 different variants around the hinge site in PDGFC. Another patient identified through GeneMatcher with a cleft lip and palate was also found to have another variant in the hinge region of PDGFC.

Conclusion:

Our study shows that variants in PDGFC may be associated with an increased susceptibility to cleft lip and palate. Further studies by mutagenesis and western blot analysis are ongoing to understand the mechanisms by which PDGFC gene variations contribute to cleft lip and palate. PDGFC is a new candidate gene for non-syndromic cleft lip and palate.

P18. Population screening for 15q11-q13 duplications: corroboration of the difference in impact between maternally and paternally inherited alleles

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Maternally inherited 15q11-q13 duplications are generally found to cause more severe neurodevelopmental anomalies compared to paternally inherited duplications. However, this assessment is mainly inferred from the study of patient populations, causing an ascertainment bias. Here, we analyze the low coverage genome-wide cell-free DNA sequencing data obtained from pregnant women during non-invasive prenatal screening (NIPS). We detect 23 15q11-q13 duplications in 333,187 pregnant women (0.0069%), with an approximately equal distribution between maternal and paternal duplications. Maternally inherited duplications are always associated with a clinical phenotype (ranging from mild learning difficulties to intellectual impairment, epilepsy and psychiatric disorders), while paternal duplications are associated with milder phenotypes (from normal to learning difficulties and dyslexia). This data corroborates the difference in impact between paternally and maternally inherited 15q11-q13 duplications, contributing to the improvement of genetic counselling. We recommend reporting 15q11-q13 duplications identified during genome-wide NIPS with appropriate genetic counselling for these pregnant women in the interest of both mothers and future children.

P19. TWO MOSAIC ENG MUTATIONS IN A PATIENT WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Objective
Mosaic variants have rarely been reported in patients with hereditary hemorrhagic telangiectasia. Here we report two sisters with different germline ENG pathogenic variants, whose father has the 2 variants in a mosaic state.

Methods
DNA was extracted from peripheral blood lymphocytes. Sanger sequencing and custom gene panel sequencing were used.

Results
The ENG and ACVRL1 genes were analyzed by Sanger sequencing, in 2015, in an 8-year-old girl with multiple pulmonary arteriovenous fistulas. A heterozygous deletion was identified in the ENG gene: c.685del; p.(Ala229Profs*6). The variant was absent in her asymptomatic parents. As her older sister presented frequent epistaxis, the investigation of this specific variant was requested. Surprisingly,

the analysis showed the deletion of the same nucleotide plus the adjacent nucleotide (c.684_685del; p.(Ala229Argfs*104). Recently, the father consulted because of dyspnea with hypoxemia. The work-up showed multiple pulmonary arteriovenous fistulas. He also reported occasional epistaxis and the clinical examination revealed rare, discrete telangiectasias on the lower lip. Genetic investigation was performed by custom gene panel sequencing (ENG, ACVRL1, SMAD4). The 2 ENG variants were identified, both as mosaic changes. The allelic fraction was around 1.3% for each of them.

Conclusion

This study shows that the 2.6% mosaicism in the father was associated with HHT phenotype of later onset but with high risk to offspring. Similar patients with unknown genetic bases may also be mosaic. Deep sequencing may be needed to identify the mutation. This study also demonstrates how Sanger sequencing-based diagnostic testing may lead to wrong conclusions in rare cases.

P20. Genetics in Sunflower syndrome: GABRG2 as a culprit gene?

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Rationale:
Sunflower syndrome is a unique photosensitive epilepsy, characterized by an attraction to light and stereotyped seizures associated with handwaving. These handwaving events (HWE) are thought to be an ictal phenomenon, rather than being “self-induced” although current data are contrasting. Photosensitive epilepsy occurs in 2 to 5% of the epilepsy forms and several gene mutations have been associated with photosensitive epilepsy. However, the genetic etiology of Sunflower syndrome remains unknown.

Methods and results:
Here, we report an 8-year-old boy with episodes of nose-rubbing and HWE that were initially categorized as behavioral problems for which risperidone was started. After a few years, a video EEG was performed to exclude an epileptiform origin. This EEG showed focal mostly temporal and fronto-temporal (right and left) epileptiform activity coincident with the HWE. Subsequently, valproic acid (VPA) was started, which led to a satisfactory seizure frequency reduction (SFR).
Molecular analyses showed a pathogenic variant in GABRG2 (c.1431G>A p.(Trp477Ter)). Pathogenic GABRG2 variants have been associated with photosensitive epilepsy. Moreover, a recent zebrafish study showed that gabrg2-/- zebrafish mutants are prone to light-induced seizures and respond well to VPA; mimicking the phenotype and drug response of our patient.

Conclusions:
Overall, clinicians worldwide should be cautious by interpreting HWE and/or other tic-like movements as a behavioral problem, since an epileptic origin cannot be ruled out. A prompt and correct diagnosis can be made by performing a video EEG early on in the diagnostic process when epileptic seizures are part of the differential diagnosis. Even though the genetic etiology of Sunflower syndrome remains poorly understood, this constellation should not impede a clinician for further genetic testing.
While future genetic studies in Sunflower syndrome remain necessary to unravel the genetic etiologies of Sunflower syndrome, our case report suggests GABRG2 as a likely culprit gene for Sunflower syndrome.

P21. Discordancy in interpretation of a BRCA1 variant in the Belgian population.

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Pathogenic variants of BRCA1 gene predispose to hereditary breast and ovary cancer . A correct classification of these variants is essential for an accurate genetic counselling and management of the patient in order to prevent over or undertreatment.
However, the classification of BRCA1 c.5071A>G or p.(Thr1691Ala) remains unclear.
This variant is absent from public databases and is predicted to be deleterious to protein function by in silico analyses. Functional studies are contradictory. Bowman and al. (2020) suggest a deleterious effect in DR-GFP assay, but a neutral and intermediate one in Cisplatin and Olaparib assays respectively. Findlay and al. (2018) report an intermediate impact on cell survival. Petitalot and al. (2019) report four different assays (HR, localization, solubility, phosphopeptide-binding assay) in which this variant does not impact protein function. Therefore, we classified this variant as a variant of unknown significance following the ACMG classification.
We then reviewed all personal and familial history of the five cases included in CHU de Liège database. Familial history of breast or other BRCA1 related cancers was not informative in all the pedigrees. Some presented with early breast cancer others with late onset breast cancer. Two patients presented this variant in co-occurrence with another pathogenic variant of a different breast cancer predisposition gene.
In conclusion, despite the rarity of this variant in databases, its frequency seems to be higher than expected in our region questioning a potential founder effect. Clinical data from our own database are not in favor of pathogenicity, although it is not excluded. We hope that future data will help to classify this variant more precisely in order to facilitate the management and the genetic counselling for patients.

P22. TRIPLICATIONS OF CHROMOSOME 1P36.3, INCLUDING THE GENES GABRD AND SKI, ARE ASSOCIATED WITH A DEVELOPMENTAL DISORDER AND RECURRENT FACIAL FEATURES

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We report on a series of four cases with a de novo triplication on 1p36.3 in which we identified a novel chromosomal disorder with similar phenotypic features. It is associated with a distinct phenotype, characterized by global developmental delay, moderate intellectual disability, seizures, feeding difficulties in the neonatal period, behavioral problems and specific facial dysmorphic features, including ptosis, hypertelorism and arched eyebrows. We compared the four patients to previously described patients with an isolated triplication or duplication of this region. The de novo occurrence of these triplications demonstrates the reduced reproductive fitness associated with this genotype, in contrast to 1p36.3 duplications which are mostly inherited and which can be associated with similar facial features but with a less severe developmental phenotype. The triplicated region in our four patients varies in size. The minimal overlapping region of 508kb encompasses 12 genes, including two disease-related genes (SKI an GABRD), which are most likely to contribute to the phenotype. SKI is a negative regulator of the transforming growth factor-beta signaling pathway (TGF-β). When SKI interacts with SMAD binding elements, it is degraded upon TGF-β stimulation. In patients with Shprintzen-Goldberg syndrome, de novo heterozygous missense variants prevents SKI degradation, resulting in attenuation of TGF-β signaling. Mild-to-moderate intellectual disability and hypertelorism are shared symptoms between patients with SGS and patients with 1p36.3 triplications. Heterozygous gain-of-function missense variants in GABRD were recently associated with neurodevelopmental disorders with behavioral issues, intellectual disability ranging from mild to severe and generalized epilepsy. Further studies are required to explore whether copy number gain of these genes are causing the phenotype in 1p36.3 triplication syndrome.

P23. Characterization of the genetic architecture of inherited retinal disease in a consanguineous Iranian cohort, an understudied population

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Aim: To uncover the genetic basis of inherited retinal disease (IRD) in 190 unrelated Iranian families, using an integrated approach of autozygome-guided whole exome sequencing (WES).

Methods: WES was performed in 190 Iranian IRD families, predominantly from a consanguineous background (74%). A panel of 290 known IRD genes was analyzed using the in-house Seqplorer tool and ExomeDepth. AutoMap was used to determine runs of homozygosity (ROHs). To identify variants in novel candidate IRD genes, additional platforms such as Franklin and QCI Interpret Translational were used, while expression was assessed in retinal single-cell transcriptomes (Retinal IOB Atlas, Spectacle). Variants were validated and classified (ACMG/ACGS guidelines). Segregation analysis was performed if possible.

Results: Using a WES-based analysis, we obtained a molecular diagnosis for 79.6% of the IRD cohort. In total, 161 (likely) disease-associated variants (69 novel) were identified in 63 genes with variants in ABCA4, EYS, and CRB1 being the most prevalent. In addition, the importance of copy number variations (CNVs) in IRD was demonstrated (5%), with novel CNVs found in CDH3, CDHR1, CHM and RD3. Moreover, variants were identified in novel retina-expressed candidate IRD genes, including COBL, FRMPD2, SLC26A7, TRAPPC14 and TRPM2.

Conclusion: This integrated study using WES and in-depth variant assessment provides insight into the genetic architecture of IRD in Iran, an underrepresented population. We provided 79.6% of patients with a genetic diagnosis and expanded the molecular spectrum of IRD in Iran by the identification of known and novel variants, offering perspectives for family counseling. Finally, autozygome-guided exome sequencing revealed several novel candidate genes in unsolved cases.

P24. The psychological impact of genetic screening in childhood cancer: A systematic review.

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Introduction: Genetic screening for Cancer Predisposition Syndromes (CPSs) in pediatric cancer patients is medically highly relevant in terms of optimizing risk-detection and treatment. However, genetic screening in CPS does raise psychological concerns in both clinicians and parents. More specifically, as the patient is too young to give consent, parents have to determine what is in the best interest of their child. To allow an informed parental decision counselling about the consequences of a genetic test result for their child and themselves is needed. As the impact of genetic testing in CPS is not yet systematically documented, we performed a systematic review to critically assess the available empirical evidence on the short/long-term psychological impact of genetic screening in childhood cancer for children and parents.

Methods: Searches in Web of Science, Pubmed and Embase were performed to identify empirical studies that (a) assessed the psychological impact or experience of genetic screening in childhood cancer, and (b) included a pediatric sample (≤ 18 years of age) in which both the impact on parents and child were eligible. After screening 3838 articles (cf. Cochrane guidelines) 17 studies were included.

Results and conclusions: Preliminary findings are the following: in both carriers as non-carriers, no elevated levels of negative emotions and distress in parents and children as a result of genetic screening are found; negative emotions (depression, anxiety, uncertainty, guilt) are were found to be limited in time and to individuals with a predisposition to worry. Positive emotions (hopefulness, relief and peace of mind) after genetic counseling were also reported. Finally, knowing the risk status, regardless of the result and the emotions associated with it, was found to foster

empowerment to deal with the future. The current findings might be informative to genetic counselors working in the context of pediatric cancer.

P25. Unravelling a familial case of DOK7 congenital myasthenic syndrome by analyzing RNA in patient's cultured cells

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Congenital myasthenic syndromes are disorders characterized by impaired neuromuscular transmssion inducing muscle weakness. DOK7 encodes for an adaptor protein that is crucial for forming and maintaining neuromuscular synapses and bi-allelic DOK7 mutations are found in 10-15% of congenital myasthenic syndromes. Here, we present a family with two brothers (6 and 3-year-old) diagnosed with congenital bilateral vocal cord palsy. During the first weeks after birth, they both experienced stridor and repiratory distress. At the age of 3, muscle weakness was also observed in the oldest brother. Quatuor-based whole exome sequencing (WES) allowed us to identify a pathogenic (class 5) heterozygous variant in the DOK7 gene in the two brothers (c.1263dup p.(Ser422Leufs*97), NM_173660.5). This variant was inherited from the father. No other variant in the DOK7 gene was found. We then analyzed the RNA in EBV-transformed cells originating from patients' blood and identified, in the two brothers, a 88 bp-insertion between exons 3 and 4 corresponding to a sequence within intron 3 (from c.331+258 to c.331+345) and predicted to induce a frameshift. This insertion was inherited from the mother. A long-range PCR of intron 3 on genomic DNA followed by Sanger sequencing revealed the presence of the substution c.331+347A>T in the two brothers and the mother. This variant was predicted to introduce a cryptic donnor splice site between c.331+345 and c.331+346 which could trigger the retention of a part of intron 3 in the mRNA. This rare variant (frequency around 0.0007% in gnomAD v3.1.2) was classified as a potentially pathogenic variant (class 4). In conclusion, the use of cultured cells originating from patients' blood to study RNA allowed us to solve a familial case of DOK7 congenital myasthenic syndrome for which only one pathogenic variant had been identified by WES.

P26. The neurodevelopmental and facial phenotype in individuals with a TRIP12 variant

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Haploinsufficiency of TRIP12 causes a neurodevelopmental disorder characterized by intellectual disability associated with epilepsy, autism spectrum disorder and dysmorphic features, also named Clark-Baraitser syndrome. Only a limited number of cases have been reported to date. We aimed to further delineate the TRIP12-associated phenotype and objectify characteristic facial traits through GestaltMatcher image analysis based on deep-learning algorithms in order to establish a TRIP12 gestalt. 38 individuals between 3 and 66 years (F = 20, M = 18) - 1 previously published and 37 novel individuals - were recruited through an ERN ITHACA call for collaboration. 35 TRIP12 variants were identified, including frameshift (n = 15) and nonsense (n = 6) variants, as well as intragenic deletions (n = 5), missense (n = 5) and splice (n = 3) variants and a multigene deletion disrupting TRIP12. Though variable in severity, global developmental delay was noted in all individuals, with language deficit most pronounced. About half showed autistic features and susceptibility to obesity seemed inherent to this disorder. A more severe expression was noted in individuals with a missense variant.

Facial analysis showed a clear gestalt including deep-set eyes with narrow palpebral fissures and fullness of the upper eyelids, downturned corners of the mouth and large, often low-set ears with prominent earlobes. We report the largest cohort to date of individuals with TRIP12 variants, further delineating the associated phenotype and introducing a facial gestalt. These findings will improve future counseling and patient guidance.

P27. Coinheritance of pathogenic variants in ATM and BRCA2 in families with multiple cancers: a case series

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Background. Pathogenic variants (PVs) in BRCA2 and ATM genes have been linked to an increased lifetime risk of various cancers. BRCA2 and ATM are part of the homologous recombination pathway, but the tumor risk in patients with simultaneous PVs in them remains largely unknown.
Aim.The aim of this study was to describe four patients from three families with multiple cancers who coinherited PVs in BRCA2 and ATM genes.
Methods. PVs in the patients were identified using NGS sequencing of the DNA and were confirmed by Sanger sequencing.
Results. The first family included a 67-year-old male with kidney, prostate, and pancreatic adenocarcinomas, and his 35-year-old daughter diagnosed with breast cancer at 29 years. In the second family, a 28-year-old female had breast cancer, while a 65-year-old male from the third family was diagnosed with prostate cancer at the age of 49, gastric cancer one year later and pancreatic cancer recently. The three identified BRCA2 PVs were nonsense variants previously described as pathogenic, leading to a severely truncated or absent protein due to nonsense-mediated mRNA decay (NMD). Two of the ATM variants were previously reported as pathogenic, while the third one was affecting a conserved splice site.
Conclusion. The early age of diagnosis and the development of multiple cancers in the reported patients indicate a very high risk of cancer in double heterozygous patients, carrying PVs in the BRCA2 and ATM genes.

P28. Bi-allelic variations in CRB2, encoding the Crumbs Cell Polarity Complex Component 2, lead to non-communicating hydrocephaly due to atresia of the Aqueduct of Sylvius and central canal.

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Congenital Hydrocephaly is a common condition caused by the accumulation of cerebrospinal fluid in the ventricular system. Four major genes are currently known to be causally involved in hydrocephaly, either isolated or as a common clinical feature: L1CAM, AP1S2, MPDZ and CCDC88C. Here, we report 3 cases from 2 families with congenital hydrocephaly due to bi-allelic variations in CRB2, a gene previously reported to cause nephrotic syndrome associated or not to hydrocephaly. While 2 cases presented with renal cysts, one case presented with isolated hydrocephaly. Neurohistopathological analysis allowed us to demonstrate that, contrary to what was previously proposed, the pathological mechanisms underlying hydrocephaly secondary to CRB2 variations are not due to stenosis but to atresia of both Sylvius Aqueduct and central medullar canal. While CRB2 has been largely shown crucial for apico-basal polarity, immunolabelling experiments in our fetal cases showed normal localization and level of PAR complex components (PKC and PKC) as well as of tight (ZO-1) and adherens (-catenin and N-Cadherin) junction molecules indicating a priori normal apicobasal polarity and cell-cell adhesion of the ventricular epithelium suggesting another pathological mechanism. Interestingly, atresia but not stenosis of Sylvius aqueduct was also described in cases with variations in MPDZ and CCDC88C encoding proteins previously linked functionally to the Crumbs (CRB) polarity complex, and all 3 being more recently involved in apical constriction, a process crucial for the formation of the central medullar canal. Overall, our findings argue for a common mechanism of CRB2, MPDZ and CCDC88C variations leading to abnormal apical constriction of the ventricular cells of the neural tube that will form the ependymal cells lining the definitive central canal. Our study thus highlights that hydrocephaly related to CRB2, MPDZ and CCDC88C constitutes a separate pathogenic group of congenital non-communicating hydrocephaly with Sylvius aqueduct and central medullar canal atresia.

P29. Pre and postnatal follow-up of a boy affected with 3M syndrome diagnosed in utero by trio exome analysis.

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The Miller-McKusick-Malvaux syndrome (3M syndrome) is a rare autosomal recessive disorder that can lead to intra-uterine and postnatal severe growth retardation and dysmorphic features, without intellectual disability. For most patients, diagnosis is based on clinical and radiological observations during neonatal period or infancy and is confirmed with genetic analysis. Three genes have been implicated so far in this syndrome: CUL7, OBSL1 and CCDC8.
We report here a patient for which the diagnosis was established in prenatal period. Ultrasounds examination at 28 weeks revealed short (under percentile 1) and slender long bones without bone structural abnormalities, relative macrocephaly with frontal bossing, and facial dysmorphism with anteverted nostrils and low nasal bridge. Genetic analysis on amniotic fluid disclosed two heterozygous truncating pathogenic variants in OBSL1 (one inherited from each parent), responsible for 3M syndrome. Parents opted for continuation of the pregnancy so we had the opportunity to compare the pre and postnatal phenotype of the boy.
No fetal description has been established in 3M syndrome patients so far. Nevertheless since the development of high resolution ultrasound, morphological exams are more efficient and more clinical findings can be viewed antenatally. The association of intra-uterine growth retardation (IUGR), slender and short long bones with relative macrocephaly and facial dysmorphism observed antenatally constitue indications for 3M syndrome. Differential diagnosis should include Silver-Russel syndrome, Dubowitz syndrome, Mulibrey nanism, and fetal alcohol syndrome. Genetic diagnosis can be achieved in utero by NGS analysis and can guide genetic counselling to the couple.

P30. Biallelic ANGPT2 loss-of-function causes severe early onset non-immune hydrops fetalis

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Background
Primary lymphedema (PLE) and hydrops fetalis are associated with mutations in >40 genes. They explain <40% of cases. Hydrops fetalis, a pathological fluid accumulation in two or more body compartments, is etiologically heterogeneous. We investigated a consanguineous family with recurrent pregnancy loss due to severe early onset non-immune hydrops fetalis.

Methods and results
Whole exome sequencing in four fetuses with hydrops fetalis revealed that they were homozygous for the angiopoietin 2 (ANGPT2) variant Chr8 (GRCh37/Hg19): 6385085T>C, NM_001147.2:c.557A>G. Instead of purely a p.D186G missense mutation, the substitution introduces a cryptic, exonic splice site predicted to result in loss of ten nucleotides, with subsequent shift in reading frame, leading to a premature stop codon. RNA analysis in the heterozygous parents demonstrated loss of detectable mutant allele, indicative of loss-of-function via nonsense-mediated mRNA decay. Serum ANGPT2 levels were reduced in the parents. In a pregnancy with a healthy, heterozygous child, transiently increased fetal nuchal translucency was noted.

Conclusion
In 2020, we showed that pathogenic heterozygous ANGPT2 missense variants cause autosomal dominant primary lymphedema, including one whole-gene deletion, three dominant-negative and one gain-of-function mutations. Angiopoietin-2, is a ligand of the TIE1-TIE2 (tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains 1 and 2) pathway. It is critical for

formation and remodeling of blood and lymphatic vessels, and is involved in vessel maintenance. Indeed, ANGPT2 knock-out mice die from generalized lymphatic dysfunction. Here, we show that a homozygous pathogenic variant causes loss-of-function and results in severe early onset hydrops fetalis. This is the first report of an autosomal recessive ANGPT2-related disorder in humans.

P31. Discordant fetal sex on NIPT and ultrasound. Monocentric retrospective study.

Julie Désir, Jean-Martin Billard, Céline Evrard, Axel Marichal, Colombine Meunier, Aude Tessier & Sonia Rombout

IPG

With the availability of noninvasive prenatal testing (NIPT), and high-resolution ultrasound, for all pregnant women in Belgium, more cases of sex discordance are now being identified in routine clinical practice. To assess the causes of reported discordance between NIPT and ultrasound, we did retrospective, observational study of all cases with genome-wide NIPT reported normal sex chromosomes and notification by the healthcare provider of discordance between NIPT and observed fetal sex. When discordances were unresolved after laboratory data review and repeat ultrasound imaging, genetic invasive testing results and pregnancy outcomes were reviewed. 11 cases of confirmed discordance were detected on 140.760 NIPT analyzed at Institute of Pathology and Genetics (IPG) since the reimbursement of the NIPT for all women in Belgium (1st July 2017), highlighting a very rare incidence of 0.0078% for these confirmed cases. The follow-up is detailed for these 11 cases.

P32. Prenatal diagnosis of de novo RAC3 variant associated to severe cerebral malformations and bilateral clubfoot

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RAC3 pathogenic variants have been associated to neurodevelopmental disorder with structural brain anomalies and dysmorphic facies (NEDBAF). So far, few cases have been described. Cerebral malformations consist in ventriculomegaly, corpus callosum anomalies, gyration anomalies and dysgenesis of brainstem. Additional features are notably severe intellectual disability, epilepsy, aspecific craniofacial dysmorphism and osteoarticular anomalies. We report here a prenatal case of this condition. The secund trimester ultrasound follow-up showed bilateral ventriculomegaly with square frontal horns, slightly widened third ventricle, frontal lobes hypoplasia with enlarged sylvian fissure, thin and short corpus callosum, polyhydramnios and bilateral clubofoot. The prenatal MRI performed at 31 weeks confirmed all fetal malformations except corpus callosum anomalies. Additional cerebral anomalies were identified : expanded subarachnoid spaces, polymicrogyria and atypical morphology of cerebellar vermis and brainstem. Regarding the expected poor prognosis, parents decided to interrupt pregnancy at 32 weeks of gestation. Macroscopic examination showed craniofacial dysmorphism (large forehead, hypertelorism, downslanting palpebral fissures, bilateral epicanthus, short nose with anteverted nostrils, small mouth, thin upper lip, microretrognathia and short neck) and bilateral congenital pes talipes equinovarus. Shallow Whole Genome Sequencing (SWGS) performed on fetal cells from amniotic fluid was normal. Trio Whole Exome Sequencing hightlighted in the fetus a novel de novo heterozygous variant (c.276T>A p.(Asn92Lys)) in the gene RAC3 (NM_005052.3, GRCh38), classified as class 4 variant.

P33. Unexpected 6 Mb terminal 10p balanced rearrangement in a man revealed by an abnormal Non Invasive Prenatal Testing (NIPT)

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IPG

Low coverage whole genome sequencing of maternal plasma DNA (NIPT) is typically used in our lab not only to detect common trisomies, but also to screen for aneuploidies and large structural chromosomal abnormalities. We report a case of a 6Mb sub-chromosomal 10p terminal duplication detected by NIPT in a 15 weeks pregnant woman. This unbalanced fetal rearrangement originates from a paternal cryptic terminal 10p translocation/insertion on the short arms of an acrocentric chromosome 14.
Association with disease risk and significance are unclear and this derivative 14p (duplication 10 pter) was considered as a variant of unknown significance and the couple was reassured for the current pregnancy.
But at the other hand, the characterisation of this rearrangement is important for a further pregnancy given the associated risk for the derivative 10p deletion. Indeed, 10p terminal deletion is pathogenic and clearly associated with a clinical syndrome.
In conclusion, our observation highlights that a single terminal rearrangement detected by NIPT may hide a relevant parental submicroscopic balanced rearrangement.

P 34. A ticking biological clock in men: age-related alterations in the human sperm methylome and risk for diseases in offspring.

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Like women, men have a “biological clock” when it concerns fertility. Reports show that the decline in fertility begins after the age of 30. Besides age-associated decrease in sperm quality, ageing also generates de novo DNA mutations, and failure of DNA repair increases the risk for chronic disorders. Additionally, the sperm epigenome is sensitive to various exposures, including ageing and age-associated chronic exposures to harmful lifestyle factors and environmental pollution. If advanced paternal age is a trigger for inducing diseases in offspring -and the current trend towards a delay in fatherhood continues to grow- it is essential to understand age-related changes in sperm cells. We used the Infinium HumanMethylation450 array to examine associations between age and DNA methylation at 485,512 CpG sites in sperm from 63 men, aged 18 to 35 years. Linear regression models were used; age was included as a continuous variable, and we controlled for obesity and patient status. We corrected for multiple testing using the Benjamini-Hochberg method. We found 14,622 differentially methylated (dm) CpGs correlated with age. At nearly 70% of the identified dm sites, methylation percentage was inversely correlated with age. Most significant associations were enriched for genes previously implicated in sperm motility, spermatogenesis and morphology, embryo growth/development and implantation. We further identified 231 dm CpGs mapping 88 (putative) imprinted genes. Due to resistance to postfertilization reprogramming, such age-related changes at imprinted loci could be carried forward to the next generation, increasing chronic disease risk for children of older fathers. Screening for biological relevance of these CpGs revealed roles in development of cardiomyopathy, childhood retinoblastoma, skin disorders, arthritis, and brain/sensory development or epilepsy. In conclusion, if age of first fatherhood continues to increase in our society and acquired methylation “signatures” in sperm of older men are heritable, more children may develop chronic diseases.

P35. A systematic review of monogenic gene-disease relationships in human female infertility and differences in sex development

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Background

High-throughput sequencing methods have led to the identification of an ever-increasing number of gene variants in the fields of both male and female infertility. The increasing number of identified genes allows an accurate diagnosis for previously idiopathic cases of female infertility and more appropriate patient care. However, robust evidence of the gene-disease relationships (GDR) allowing the proper translation to clinical application is still missing.

Objective and rationale

An evidence-based curation of genes involved in female infertility and differences in sex development (DSD) would significantly improve diagnostic performance and genetic research. We therefore performed a systematic review to summarize current knowledge and assess the available GDR.

Search methods

PRISMA guidelines were applied to curate all available information from PubMed and Web of Science on genetics of human female infertility and DSD up to 01/11/2021. The evidence that an identified phenotype is caused by a specific gene was assessed according to a standardized scoring system. A final score (no evidence, limited, moderate, strong, or definitive) was assigned to every GDR.

Outcomes

A total of 45,271 publications were identified, of which 1,078 were selected for gene and variant extraction. We have identified 395 genes and validated 466 GDRs covering all reported monogenic

causes of female infertility and DSD. Subsequently, we developed a genetic diagnostic flowchart including 105 genes with at least moderate evidence for female infertility.

Wider implications

There is incremental evidence for a number of genes significantly involved in female fertility. We have comprehensively reviewed the existing research on the genetics of female infertility and DSD, enabling the development of diagnostic panels using validated genes. Further research is needed to establish the health-economic benefit of a fertility gene panel.

P36. Expanding the phenotype of copy number variations involving NR0B1 (DAX1)

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46,XY gonadal dysgenesis (GD) is a disorder of sex development due to incomplete gonadal differentiation into testes, resulting in female to ambiguous external genitalia. Duplications at the Xp21.2 locus involving the NR0B1 (DAX1) gene have previously been associated with 46,XY GD. More recently, a complex structural variant not directly involving NR0B1 has been reported in 46,XY GD illustrating that the mechanism of how CNVs at Xp21.2 may cause 46,XY gonadal dysgenesis is not yet fully understood. To date, these Xp21.2 duplications have only been reported in studies focused on the DSD patient population.

Here, we report on three families in which a duplication involving the NR0B1 gene was detected in the context of prenatal screening. We describe the first cases of duplications involving NR0B1 in phenotypically normal males. A 1,1 Mb gain was identified in an adult male with fertility problems. In another family, two healthy young boys with unambiguously male external genitalia carried a 712 kb duplication involving NR0B1.

Given the increasingly widespread use of genome-wide noninvasive prenatal screening, we can expect to detect similar CNVs at the Xp21.2 locus in the general population. The data reported here broaden the phenotype associated with CNVs involving NR0B1, and this may aid clinicians in counseling and decision making in the prenatal context.

P37. Prenatal diagnosis of a homozygous nonsense MPDZ variant in a foetus with severe congenital hydrocephalus

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A 29-year-old woman was referred for prenatal genetic counselling regarding suspicion of fetal septo-optical dysplasia. Prenatal ultrasound revealed a bilateral ventriculomegaly, absent cavum septum pellucidum and hypoplastic corpus callosum at 21 weeks of gestation. Next generation sequencing detected the presence of a homozygous, likely pathogenic nonsense variant in the MPDZ gene associated with congenital hydrocephalus. This abnormality is known to have an in utero onset. Clinical presentation is highly variable in severity, varying from stillbirth, severe neurodevelopmental problems and infancy death to milder symptoms in adult patients. Aside from hydrocephalus, other reported congenital anomalies are variable heart defects and ophthalmologic abnormalities. The present case, to our knowledge is the first prenatally diagnosed homozygous MPDZ pathogenic variant.

P38. The IPG experience in maternal cancer detection by noninvasive prenatal testing (NIPT) – retrospective study.

Sonia ROMBOUT, Axel MARICHAL, Jean-Martin BILLARD, Céline EVRARD, Colombine MEUNIER, Aude TESSIER & Julie DESIR

IPG

Since the introduction of noninvasive prenatal testing, incidental findings of relevant maternal abnormalities occurs frequently and among them, maternal presymptomatic cancers are also reported. The frequency of such observation is now estimated at 1/5000. Effectivelly, NIPT screening originally used to detect placenta-derived fetal cell-free DNA (cfDNA) in the maternal circulation, also detects the presence of tumor derived cell-free DNA. Malignancy in pregnant women potentially affects the copy number variation (CNV) profile in NIPT results. From our experience at IPG, this circulating tumor DNA is often characterized by multiple chromosomal rearrangements but, in some cases, only one chromosome may be involved. Such clinical relevant and actionnable (treatment and prevention) incidental findings are of course communicated and the pregnant woman should be adressed in oncology for a screening of malignancy.

P39. Exploring synthetic lethality caused by novobiocin in homologous recombination-deficient zebrafish: a novel preclinical in vivo model for POLθ inhibitor development?

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Double strand break (DSB) repair through homologous recombination (HR) is essential for maintaining the genomic stability of the cell. Inactivating the HR pathway (e.g. by a pathogenic BRCA2 variant) confers an increased risk for several cancers. PARP inhibitors (PARPi) specifically target HR-deficient tumours by inducing synthetic lethality. This effect was reproduced in brca2-/- zebrafish embryos, which led to the development of cost- and time-effective in vivo zebrafish assays that are capable of accurately quantifying PARPi efficacies. Moreover, differences in PARP trapping potencies of the inhibitors that are applied in the clinic were also reflected in the read-outs of our assays (PMID:33341473).

Screens for other synthetically lethal interactions are ongoing. DNA polymerase theta (POLθ) is important for the repair of DSB through alternative end-joining, and inhibition of POLθ causes cell death in HR-deficient cancers. Several pharmaceutical companies are developing POLθ inhibitors (POLθi) and one clinical trial is ongoing. Besides the relatively expensive compounds that are being developed, an inexpensive antibiotic, novobiocin, has also been identified as a specific POLθi that selectively kills HR-deficient cells in vitro and in patient-derived xenografts in mice (PMID:34179826). Moreover, It has previously been shown that POLθ-mutant zebrafish embryos cannot repair DSBs (PMID:27149851).

To streamline the development process for POLθi, there is a need for a cheap, accurate, and fast in vivo model that can establish POLθi efficacy. In this study, we are assessing if zebrafish could be used for this purpose. We are evaluating if novobiocin causes synthetic lethality in brca2-/- zebrafish embryos and if our previously developed read-outs for PARPi can also be applied to POLθi. We established that novobiocin does not cause significant toxicity in wild-type embryos and are currently testing a broad range of doses in brca2-/- embryos. After demonstrating synthetic lethality with novobiocin, commercially available specific POLθi will also be assessed.

P40. An optimised protocol for isolation of single cells from mouse aorta for single-cell RNA sequencing

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Single-cell RNA sequencing (scRNA-seq) has emerged as a powerful technique to provide valuable insights into disease mechanisms. Pathomechanistic interrogation of aortic pathologies, due to the involvement of a heterogeneous group of cell types, especially benefits from the in-depth information that scRNA-seq provides. A critical step in the scRNA-seq protocol is the preparation of a single-cell suspension that reflects the native tissue composition. Parameters to estimate the quality of a suspension are cell viability, cell yield, and absence of debris and clumps. We optimized a protocol to isolate high-quality single cells from mouse aortic root and ascending aorta tissue samples. Different enzyme mixes and concentrations, as well as different incubation times, are compared. Our results demonstrated that using a mix of 1.8 U/ml liberase, 1.8 U/ml elastase and 550 U/ml of DNase I during 1.25h is the optimal way to preserve live cells while allowing the full digestion of the tissue. After digestion, the suspension is centrifuged twice at 300g for 5 minutes since low speeds prevent cell damage. Adding 1.5% BSA is crucial to maintain viability and to minimise cell loss during washing steps. Finally, the suspension is filtered through a 35 µm strainer to remove clumps. Cell number and viability are measured with an automated cell counter using acridine orange/propidium iodide (AO/PI) staining. We obtained a single-cell suspension, with minimal cell debris and clumping, of over 25,000-40,000 cells per aorta segment and a viability greater than 90%. The protocol was also tested in the Fbn1 C1041G/+ mouse model (~Marfan syndrome) that develops aortic dilation early in life, yielding over 50,000-60,000 cells per aortic segment with a cell viability of more than 90%. Overall, this protocol delivers a good number of highly viable cells from murine aortic root and ascending aorta samples for scRNA-seq experiments.

P41. Developing a dual-reporter iPSC-line using Genetically Encoded Calcium and Voltage Indicators (GEVI/GECI)

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Genetically encoded voltage and calcium indicators (GEVI/GECI) are a novel tool that allow electrophysiological analysis of electrically active cells in a high-throughput and non-invasive manner. Cells are transfected with constructs containing a GEVI and/or a GECI and upon protein expression, a flux in fluorescence intensity can be recorded with a fluorescence microscope when the cell fires an action potential and/or when a calcium flux takes place inside the cell. Our goal is to select a GEVI and a GECI suitable for use in hiPSC-derived cardiomyocytes (hiPSC-CM) and develop a dual-reporter hiPSC-line containing both indicators that have been inserted into the genome using CRISPR/Cas9. This stable dual-reporter line can then be differentiated towards hiPSC-CM expressing both indicators without the need of extra transfection procedures and can be used to create disease models to study inherited cardiac arrhythmias.
We have tested two GECIs (GCaMP6f, NCaMP7) and five GEVIs (QuasAr2, paQuasAr3, Archon1, ArcLight-A242 and AcemNeon) on in-house differentiated hiPSC-CM and commercial CDI iCell2 cardiomyocytes. The constructs were brought into the cells via viral transduction and/or lipofection. We used the voltage dye FluoVolt to compare its signal to the GEVI signal. Imaging was performed between two to seven days after transfection using spinning disk confocal microscopy.
So far, we obtained good signal recordings with both GECIs in both cell models, while we did not record any signal with any of the tested GEVIs . We have selected NCaMP7 to use in our future dual-reporter hiPSC line as it showed a brighter signal compared to GCaMP6f. Further testing to find a GEVI will include optimizing the transfection protocol, electrically/chemically stimulating the cells as well as using commercially available cardiomyocyte lines for validation such as the murine HL-1 Cardiac Muscle Cell Line and rat ventricular iAM-1 cell line next to in-house differentiated hiPSC-CM.

P42. Optimizing monoclonal establishment of CRISPR/Cas9-edited non-small cell lung cancer lines

Marc Terrones[1], Geert Vandeweyer[2], Ken Op de Beeck[1] & Guy Van Camp[1]

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There are several approaches to obtain monoclonal cell mutant cell lines after gene edition using CRISPR/Cas9 technology. However, not all cell lines tolerate processes like single-cell culture. Thus, we optimized a low-density seeding assay to select mutant cells of interest without compromising cell proliferation and further expansion. By using patient-derived non-small cell lung cancer lines we propose a less agressive selection method that can be extrapolated to other cell line types.

P43. Identification of modifier genes underlying intra-familial phenotypic variability in zebrafish OI models

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Clinical variability in OI patients carrying an identical causal variant is frequently observed. This suggests that modifier genes contribute to the phenotypic severity through a network of interactions with the causative gene. Zebrafish is known to be a powerful model to study skeletal disorders. They are highly suitable to study intra-familial variability because of a high level of genomic variation and because high numbers of progeny with the same causal mutation can be obtained.
We studied two mutant zebrafish lines, the first line carrying a mutation in the col1a2 gene and the second line carrying a mutation in the fkbp10a gene. Deep skeletal phenotyping was done using a combination of X-ray imaging and Alizarin red staining. Exome sequencing of tail fin DNA was performed on a NovaSeq 6000 illumina sequencer, followed by SNP-based linkage analysis using Superlink Online SNP tool.
Deep phenotyping in a large number of col1a2 mutants (n=18) and fkbp10a mutants (n=27) obtained from a single set of parents from each model, revealed a wide phenotypic variability in the vertebral column with variable mineralization, fracture incidence, scoliosis, and other skeletal abnormalities. Exome sequencing of the most mildly and most severely affected mutants followed by SNP-based linkage analysis revealed a potentially linked region on chromosome 14 in the col1a2 model and on chromosome 13 in the fkbp10a model which segregates with the phenotypic severity. Haplotype analysis revealed that the genomic regions are ~3Mb for col1a2 model and ~15Mb for fkbp10a model in size and contain 61 and 268 protein coding genes respectively. We are currently validating the candidate regions to identify potential modifier(s).
We showed that zebrafish is a promising model for the analysis of modifier genes involved in skeletal diseases, and most likely also in other disorders. Modifier genes represent promising targets for intervening in disease progression.

P44. Zebrafish Facility Ghent – A new Ugent CORE Facility

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The Zebrafish Facility Ghent (ZFG) has been established more than 10 years ago at the Center for Medical Genetics (Ghent University Hospital) and is now integrated as an official Ugent CORE Facility. The zebrafish (Danio rerio) is an increasingly popular vertebrate model organism which offers several advantages over other model organisms for basic research, disease modeling and toxicology testing; The low husbandry cost, short reproductive cycle, external fertilization and development, production of large numbers of synchronous and rapidly developing embryos per mating and the optical transparency of zebrafish embryos make it an excellent tool for high-throughput screenings. The availability of a wide range of molecular techniques such as large-scale genome mutagenesis, transgenesis and overexpression/knockdown approaches, have also increased the power of zebrafish as a model organism. Furthermore, due the high genomic and molecular similarities between zebrafish and other vertebrates, many of the important discoveries in zebrafish development are applicable to humans. Finally, the zebrafish model can both refine and reduce animal experiments carried out in traditional rodent systems (3R principle), as zebrafish are not regarded as a laboratory animal until the age of 5 days according to EU directive 2010/63/EU. The ZFG provides services to both UGent and external users. These services mainly include zebrafish caretaking, breeding and genetic management, zebrafish disease modeling and toxicology/behavior testing, besides custom services for more extended projects.

P45. Proteomic analysis of the vertebral column in dominant OI zebrafish models reveals biomarkers for phenotypic outcome

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Osteogenesis imperfecta (OI) is mainly caused by dominant mutations in either the COL1A1 or COL1A2 genes, encoding type I collagen. Different mutations in these genes can lead to a different clinical outcome, while the molecular mechanisms underlying this phenotypic variability are largely unknown. The identification of a molecular signature ('biomarkers') for these different outcomes is therefore highly desirable. To identify potential biomarkers in dominant OI, we employed four OI zebrafish models, that show variability in phenotypic severity and carry either different glycine substitutions in type I collagen (col1a1adc124/+, col1a2mh15/+, col1a1amh13/+) or carry a double-heterozygous knockout mutation in type I collagen (col1a1asa1748+/-;col1a1bsa12931+/-). Protein was extracted from the vertebral column of each of these mutants (n=5) and matched controls (n=5), and subjected to mass spectrometry (MS)-based shotgun proteomics. Comparative analysis of differentially abundant proteins between mutants and their respective controls was done to reveal mutant- and OI-specific biomarkers. After optimization, we used Trizol to extract protein from the above-described OI zebrafish models and matched controls and performed LC-MS/MS analysis. Comparative analysis revealed respectively 13, 64, 6 and 11 differentially abundant proteins in the col1a1adc124/+, col1a2mh15/+, col1a1amh13/+ and col1a1asa1748+/-;col1a1bsa12931+/-mutants, including both mutant-specific and mutant-overlapping proteins. We are currently validating these findings in a selected set of proteins (Dkk1a, Bcan, Crtap) via western blotting and via RT-qPCR of the corresponding genes. Pending further validation, these proteins have potential as OI type-specific or general OI biomarkers.

P46. Artificial intelligence-assisted exome analysis for hearing loss

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Background
Hearing loss (HL) has a genetic origin in up to 60% of cases, depending on age-group. Establishing a genetic diagnosis is challenging given the large genetic and phenotypic heterogeneity. Different artificial intelligence (AI)-based genomic prioritization tools have been developed to aid in the filtering of genetic variants. Here we report on our 1-year experience with a commercial validated platform for AI-driven variant analysis called MOON (Diploid/Invitae).

Methods
Samples referred for HL were analyzed between August 2021 and December 2022 at the Center for Medical Genetics, Antwerp using an in-house standard diagnostic pipeline. This consists of an exome-based (TWIST Bioscience) gene panel for non-syndromic HL including 145 genes (WES-HL panel v3.0), complemented by an exome-wide AI-driven variant analysis based on HPO terms (MOON software, Diploid/Invitae). All detected variants were evaluated by a multidisciplinary expert team.

Results
We analyzed a total of 653 samples. In this cohort MOON identified 44 (possibly) disease causing variants in 31 genes, not identified by the dedicated gene panel. 21/44 variants were classified as (likely) pathogenic and 23/44 were of unknown clinical significance. Genes that were affected in >1 patient were LMX1A, PLS1, SLC12A2, CRYM, ESPN, FGFR3 and ATP2B2. These genes were not included in the exome based panel because: i) evidence for association with non-syndromic HL has only recently been published; ii) association with complex phenotype in which HL is usually not the major feature. Incidental findings were present in 8 cases.

Conclusion
AI-assisted exome analysis revealed a definite or probable genetic diagnosis not identified by the panel approach in 21 of 653 samples, thereby increasing the diagnostic yield of exome sequencing for HL by 3,2%. This approach seems especially valuable to detect variants in recently identified HL genes and in patients with mild or atypical presentations of syndromic HL. Pre-test counseling for incidental findings is essential.

P47. The added value of RNA-sequencing in exome variant interpretation

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UGent

Introduction:

Several studies have shown that transcriptomics (RNA-seq) nicely complements whole exome and whole genome sequencing (i.e., WES and WGS) in variant interpretation and leads to a 7-36% increase in diagnostic yield.

However, several hurdles remain to be taken before RNA-seq can be implemented in routine genetic diagnostics. First of all, the tissue under investigation is not always representative for the diseased tissue, and hence, spatiotemporal differences in expression and expressed isoforms might complicate the analysis. Furthermore, nonsense mediated decay (NMD) can mask aberrant events.

Here we present a minimally invasive ready-to-use protocol and analysis pipeline to perform RNA-seq analysis on short term cultured peripheral blood mononuclear cells (PBMCs).

Material and Methods:

Short cultured PBMCs are divided in two cultures, one of which is treated with cycloheximide to allow detection of NMD sensitive transcripts. Thereafter, RNA is extracted from both cultures followed by polyA-sequencing. After alignment of the RNA-seq data and gene count generation, the data are respectively processed with OUTRider and FRASER to detect aberrant expression or splicing events. Alternatively, a manual targeted analysis was performed using the integrative genomics viewer (IGV). If a suspicious splice variant was identified via whole exome sequencing (WES), targeted cDNA sequencing was performed in parallel.

Results & Discussion:

We have currently sequenced RNA from 50 individuals. In nine of them, a suspicious splice variant of uncertain significance was detected via WES. RNA-seq revealed aberrant splicing events in 7/9. As the aberrant splicing effects were often much more complex than anticipated, in 4/7 they were not picked up by targeted cDNA sequencing. In 7/9 we were able to reclassify the variant using RNA-seq data, thereby clearly highlighting that RNA-seq is crucial for correct interpretation but also for classification of splice site variants detected by WES.
Furthermore, our experiments with cycloheximide shed a more complex picture on NMD, as several aberrant transcripts seem to escape this mechanism.

In conclusion, we present an optimized RNA sequencing protocol and analysis workflow and show its added value for interpretation and classification of putative splice site variants.

P48. Comparison of accuracy of short and long read sequencing technologies

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Short read sequencing is commonly used to diagnose rare hereditary diseases via small panels and Whole Exome Sequencing (WES). Short read Whole Genome Sequencing (WGS) is also being implemented in diagnostics as it allows the detection of both Copy Number Variants (CNVs) and Single Nucleotide Variants (SNVs) and, as such can replace the standard of care dual approach of running arrayCGH (aCGH)/shallow WGS and WES for CNV and SNV detection respectively. Although long read sequencing technologies such as Oxford Nanopore and PacBio are increasingly used in research and some papers demonstrated their utility in rapid diagnosis of rare disorders, they are not yet implemented in routine diagnostics of rare diseases. Using the Genome In A Bottle (GIAB) cell line HG002 we assessed the performance of long read sequencing technologies for SNV and Structural Variant (SV) calling using several variant callers. Precision and recall of short and long read sequencing technologies have been compared on all type of variants to highlight the pros and cons of each technology and assess whether one long read technology is more suited for diagnostic use than the other.

P49. Use of Oxford Nanopore Long Read Sequencing to solve complex genetic cases

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Institut de Pathologie et de Génétique

Short read sequencing is widely used in routine diagnosis thanks to its accessibility and cost effectiveness. However, some cases remain unsolved. For some of those most challenging cases, IPG use Oxford Nanopore Technology (ONT) for long read DNA sequencing since 2019, to resolve cold cases or to obtain new relevant information about a pathogenic variant. Here, we report 4 cases solved using an ONT GridION device producing long reads.
Patient 1 presented biomarkers consistent with Niemann-Pick disease (autosomal recessive transmission). Short read exome sequencing revealed a pathogenic variant c.3176G>A p.(Arg1059Gln) in the NPC1 gene (NM_000271.4, GRCh38), inherited from the father. Long read sequencing with ONT allowed us to identify a gene inversion inherited from the patient's mother.
Patient 2 presented a severe phenotype of hypotonia, cerebral malformation, absence of language. Two variants of uncertain significance were identified in the ACACA gene by short reads exome sequencing. One variant was inherited from the father, the second arose de novo. Using a specific PCR construct, long read sequencing with ONT allowed us to verify that both variants were located in trans.
Patient 3 presented a clinical suspicion of RETT syndrome. Short reads sequencing data analysis suggested a heterozygous deletion of MECP2 exons 3 and 4 (NM_004992.3, GRCh38). X chromosome sequencing (using the adaptive sampling enrichment) showed that MECP2 gene was actually interrupted by a duplication of ZNF804A gene (a gene located on chromosome 2).
Patient 4 presented a deletion including the first exon of GABRG2 gene. Targeted long read sequencing allowed us to identify the breakpoints of the deletion and to design PCR primers for further parental testing and segregation analysis.
These cases illustrate the added value of long read sequencing in the field of genetic diagnosis, for the patients and their families.

P50. Long-read sequencing resolves (complex) cryptic structural variation in patients with syndromic intellectual disability

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AIMS: Intellectual disability (ID) affects 1-3% of the global population, yet current molecular techniques fail to identify the genetic defect in ~40% of patients. This diagnostic gap can be partly explained by missed structural variants (SVs). These are genomic rearrangements that due to their large size can cause abnormal phenotypes. Here, we investigated the ability of nanopore long-read sequencing (LRS) to identify cryptic (de novo) SVs in patients with unexplained (syndromic) ID.

METHODS: Using LRS, we sequenced five individuals with ID where a de novo SV was found through conventional methods, but exact breakpoints and thus a genetic diagnosis could not be established. Additionally, we sequenced 13 proband-parent trios with unexplained ID to identify (causal) de novo SVs.

RESULTS: For all five individuals, LRS resolves the interrogated SV and allows for a concrete genetic diagnosis. Remarkably, in three out of five cases the rearrangement is much more complex than anticipated through previous testing. We additionally detect twice the number of SVs compared to conventional methods, with an average of 28000 SVs per individual. Most of these variants consist of small non-coding insertions and deletions. Out of all 28000 SVs, we identify five de novo events in five out of 13 trios, with varying sizes (1.9 - 515 kb) and types (2x deletion, insertion, tandem duplication, complex rearrangement). Investigation of the causal relationship between these de novo variants and the phenotype is currently ongoing.

CONCLUSION: This study highlights the potential of LRS as a future diagnostic method for SV identification. LRS not only uncovers a significant number of SVs missed by current diagnostic techniques, but it also reveals exact breakpoints and previously hidden complex rearrangements. This allows for unexplained ID cases to receive a definitive genetic diagnosis through LRS, thus narrowing down the diagnostic gap.

P51. GWAS conditioned by SNP-specific shape effects identifies 11 loci underlying shape covariation of the cerebral cortex and cranial vault

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Covariation in brain and cranial vault shape inevitably arises from their structural relationship and proximity during development, where close coordination between both tissues is required for “normal” craniofacial development. Several studies have suggested cell-to-cell signaling as an important driver for their integrated co-development, with the FGF, BMP, Wnt, and Hedgehog signaling pathways playing essential roles in both brain and cranial vault development. Functional studies have supported this hypothesis but are limited to candidate genes and pathways. GWAS provides an unbiased framework and could potentially implicate novel genes and mechanisms underlying brain-vault covariation. A recent study scanned the mouse genome and identified a single locus associated with global axes of shape covariation between the brain and cranial vault. Here, we propose a novel approach that differs in that it extracts a SNP-specific latent phenotypic axis for one multivariate trait that is used for conditioning on another multivariate trait under coinvestigation. Specifically, we assessed the dependency of genome-wide associations for multivariate 3D cortical and cranial vault shape derived from MRIs of 4,148 participants of European descent from the ABCD study cohort. We demonstrate an improved discovery rate over previous methods and identify genes involved in signal transduction (BMP2, PTHLH, ABR, and TIAM2) and regulation of ossification and osteoblast differentiation (RUNX2, BMP2, PTHLH, and DLX5). Among the 11 identified loci, 4 could not be identified in separate GWASs on brain and cranial vault shape using the same data and were hence a product of our novel approach. Altogether, we demonstrate a new, powerful method for identifying the genetics underlying brain-vault covariation, thereby identifying genes that support the current hypothesis that close regulation of calvarial ossification is crucial for their integrated co-development and morphological covariation.

P52. An optimized workflow for CRISPR/Cas9-mediated generation of indels and large deletions in induced pluripotent stem cells and neural stem cells

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The innovative CRISPR/Cas9 technology has transformed our ability to manipulate the genome. As it is not always evident to receive relevant patient material, CRISPR/Cas9 technology provides a cheap and simple alternative to study how dysfunction can cause disease. Here, we present our workflow in human induced pluripotent stem cells (hiPSCs) and neural stem cells (hNSCs) to generate in vitro knock-out models based on indel generation. Moreover, we successfully optimized this workflow to generate kb-sized deletions, thereby making it possible to also study the impact of (non-coding) structural variation in relevant in vitro models.

We select the most promising single guide RNAs (sgRNAs) via in silico analysis. Subsequently, one RNP complex (indel generation) or two RNP complexes in equimolar ratio (larger deletions) are transfected into hiPSCs or hNSCs via nucleofection. After that, successful editing is assessed in the bulk of the cells via DNA isolation and amplification of the target region, followed by targeted next-generation-sequencing (NGS). For indel generation, primers are designed to generate an amplicon containing the theoretical cut site. For larger deletions, both primers outside and inside the deletion are designed. To obtain clonal cell lines, the transfected hiPSCs or hNSCs are single cell isolated through serial limiting dilutions with conditioned medium. Finally, monoclonality is confirmed by targeted NGS (indels) and CNV-seq (kb-sized deletions).

For indel generation, we obtained editing efficiencies ranging between 49-79% and observed that InDelphi correctly predicted the most frequent indels, 1 bp insertion and 1 bp deletion. Via nucleofection of two RNP complexes, we were able to generate clones harboring heterozygous or homozygous deletions ranging in size from 10 to 90 kb. During this process, we observed that it is crucial that both RNP complexes have similar editing efficiency. Via this workflow, it is possible to obtain monoclonal genome edited cells within a time frame of 6-8 weeks. After obtaining an engineerd cell line, these cells can be differentiated to the desired cell lineage and further functional testing can be performed.

P53. WiNGS - Federated approach for genomics data sharing and analysis

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WiNGS is a platform for next-generation sequencing data interpretation. It aims to provide a secure environment for researchers and clinicians to collaboratively analyze and interpret sensitive data. One of the key features of WiNGS is its secure federated data storage, which ensures that sensitive data is only accessible to authorized users and is kept confidential. In addition, the platform offers full API functionality, allowing researchers to easily integrate the platform into their own analysis tools or pipelines.

Alternatively, it offers a web-based user interface to analyze data, under a range of analysis approaches. These include single sample analysis, trio-based analysis under different inheritance models, and cohort-based analysis. Single sample analysis allows researchers to analyze a single individual's genetic data in order to identify potential genetic variants that may be associated with a particular disease or trait. Trio-based analysis, on the other hand, allows researchers to analyze the genetic data of a child and both of their parents in order to better understand the inheritance patterns of a particular trait or disease. Finally, cohort-based analysis allows researchers to aggregate data from multiple institutions in order to generate anonymous statistical insights. This approach can be particularly useful for large-scale studies involving multiple institutions.

Overall, WiNGS is a powerful and flexible platform that provides researchers and clinicians with a secure and federated environment for collaborative next-generation sequencing data interpretation. Its range of analysis approaches, combined with its secure data storage and full API functionality, make it an essential tool for researchers working in the field of genomics.

P54. Methylation-based deconvolution of cell-free DNA allows for non-invasive multi-cancer typing

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Plasma cell-free DNA (cfDNA) preserves the epigenetic characteristics of its cellular origin. In the presence of cancer, part of the cfDNA may originate from tumour cells and detection of cancer-specific signatures in the cfDNA pool holds the promise for non-invasive cancer detection and identification of the tumour location. Currently available deconvolution methods, exploiting methylation features to estimate tissue contribution to plasma cfDNA sample, are not focused on multi-cancer typing. We propose a novel reference-based deconvolution method, MetDecode, that allows scrutinizing cfDNA methylation data for the presence of potential cancer tissue contributors. We built a reference atlas of 7 blood cell types and 4 different tumour tissues (breast, colorectum, cervix and ovary) based on whole-genome methylation sequencing data. MetDecode uses tissues specific methylation markers to model cfDNA methylation profiles as convex linear combination of atlas entities, to estimate relative contribution of each entity in a cfDNA sample. One of the key innovations is the mitigation of atlas-specific biases, enabled by the non-linear correction of the atlas and alleviating discrepancies in data distribution between these cfDNA samples and the atlas. Using in-silico mixtures of tumour genomic DNA spiked into cfDNA from healthy individuals, MetDecode accurately estimated the tumour proportion and correctly assigned the tissue-of-origin in mixtures with as low as ~4% tumour fraction. The performance of the algorithm for multi-cancer typing was assessed using cfDNA samples from healthy controls (n=93) and cancer patients (n=16). For 13 out of 16 cancer samples the tissue-of-origin was correctly assigned (overall accuracy: 81.25%). Among the different cancer types, colorectal was performing the best (accuracy: 100%) followed by ovarian (accuracy: 85.71%). To conclude, we developed a novel nonlinear deconvolution algorithm outperforming existing models and demonstrate its value in non-invasive multi-cancer typing.

P55. Genome-wide DNA methylation sequencing methods for cancer biomarker discovery: their potential and limitations

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DNA methylation represents an important marker of gene expression in various human cancers and is extensively explored as a source of specific biomarkers for diagnosis, prognosis, prediction and follow-up. Numerous methods exist for profiling methylation, but many do not provide a comprehensive insight into the disease. Bisulfite sequencing remains the reference method for investigating methylation, despite its shortcomings. Although several methods have been proposed to circumvent associated limitations, there is no clear consensus on which one is most appropriate for genome-wide methylation profiling.

We conducted an extensive examination of four different DNA methylation analysis methods to find the strengths and weaknesses of each and make recommendations on which method is most suited for different methylation profiling applications. We assessed DNA methylation profiles from one healthy genome derived from blood and four human cancer genomes derived from fresh frozen tissue (n=2) and cell lines (n=2). Genomic DNA was subjected to short-read whole-genome bisulfite sequencing (WGBS), Illumina EPIC array, enzymatic methyl-sequencing (EMseq) and long-read third-generation sequencing by Oxford Nanopore Technologies (ONT). We compared the methods based on feasibility, coverage, accuracy, cost, and applicability for clinical diagnostics. We found overall high concordance between the methods, but differences in efficiency of read mapping, CpG calling, and coverage were observed. ONT detected a significant number of sites that are inaccessible to short-read assays, many of which might have biological significance. Moreover, longer fragments benefited from fewer overlapping reads, which increased mapping efficiency and mean coverage per CpG. We obtained high concordance of ONT sequencing with WGBS ($r = 0.936$), EMseq ($r = 0.930$) and EPIC array ($r = 0.918$).

Our findings provide a decision-making tool for future studies based on budget, DNA input, and required coverage. Overall, we provide a way to guide the selection of the most appropriate method for genome-wide DNA methylation profiling.

P56. Long read whole genome sequencing for the detection of structural and epigenetic variation in developmental disorders

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Technological improvements over the last decades have been instrumental for the identification of numerous chromosomal anomalies and monogenetic diseases, shedding light on the extreme genetic heterogeneity underlying developmental disorders. Despite the associated significant increase in diagnostic yield, about 60% of patients remain without molecular diagnosis. There is growing evidence that cryptic structural variations and methylome anomalies are an important cause of hitherto unsolved cases. Promising recent reports indicate that this diagnostic gap could be bridged by long-read sequencing platforms. The broad adoption of these methods has long been hindered by their high costs and error rate but should become reality as both, costs and error rates, keep dropping.
Further research is however needed to evaluate the clinical relevance of this new technology and enable its implementation in a diagnostic setting. To address these objectives, we perform trio whole genome nanopore sequencing in 50 patients with intellectual disability and/or multiple congenital anomalies without molecular diagnosis after short read whole exome or genome sequencing. We developed an analytical pipeline to assess coding and non-coding structural variants as well as methylome disturbances. As a proof-of-concept, we first evaluated the detection of structural variants, short tandem repeats, repeat associated methylation disturbances (FMR1, FSHD) as well as differential methylation of disease associated loci (11p15.5, 14q32 and 15q11q13) and episignatures in controls with a known disorder. The assessment of the added value of the technology in 50 unsolved patients as well as the construction of the essential population reference of normal structural variation is ongoing.
We will present our project, the results of the proof-of-concept phase as well as the first results, perspectives and challenges in the unsolved cohort. We envision a significant increase in the number of patients that will receive a molecular diagnosis, improving the understanding of molecular disturbances underlying diseases and patient care.

P57. Digital droplet PCR: an efficient, rapid, and simple methodology for CNV confirmation.

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Nowadays, next-generation sequencing (NGS) is routinely used to attempt to diagnose genetic diseases. It allows to detect a lot of variation including small copy number variations (CNV). Indeed, NGS base-by-base high coverage view of the coding regions allows detection of small or novel CNV that arrays or SWGS often miss. However, the presence of those small CNV must be confirmed by another method as artefacts are not uncommon with NGS. This confirmation could be challenging due to a lack of resolution or availability for most of other methods routinely used, like CGH-array, MLPA or SWGS.

We developed an efficient, rapid, and simple method based on digital droplet PCR (ddPCR) for small CNV confirmation. It allowed us to confirm small (as small as 120 pb) variations with small fold change (1.3-fold). We notably confirmed a potentially causal mosaic deletion with an allele frequency of 0.25%.

Digital droplet PCR is an easy way to quickly confirm CNV detected by NGS. It allows us to confidently reports some potentially causal small or novel CNV that would not have been highlighted by array, MLPA or SWGS analysis.

P58. Our Whole Exome Sequencing analysis at IPG: Outcomes of the first 1100 patients

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For patients with complex symptoms, physicians often have hard time carrying out genetic analyzes, especially in the absence of clear differential diagnosis. In such cases, a whole exome sequencing (WES) can allow rapid diagnosis of genetic diseases. WES was implemented routinely at the Institute of pathology and genetic (IPG) in late 2020. Since then, the number of WES analyzed has increased rapidly. We present here the outcomes of our first 1100 WES cases based on 2323 sequenced samples from a wide phenotypic spectrum.
WES analyses were performed in trios with the index case and the two parents in 50% of the families, 28 % involving index cases only. The last 22 % of the analyses included families with 2, 4 or 5 members. About 45% of the WES analyses were performed either for patients with intellectual disability/developmental delay (21%), neuromuscular disorders (14%) or multiple congenital anomalies (10%).
Among the 1100 families tested, we identified the underlying pathogenic or likely pathogenic variants in 221 cases (20 %). In further 368 families (33.5%) a variant of unknown significance, possibly explaining the clinical symptoms of the index patient was identified.
WES has a clear clinical benefit for the patients and their families and is an essential part of the personal medical care in 2023.

P59. Study of nonsense-mediated decay triggered by splicing variants: interest of purromycin use?

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IPG

The nonsense-mediated decay (NMD) is a mRNA surveillance pathway degrading defective mRNA harboring premature STOP codons (PTC), aiming to protect cells against the formation of truncated proteins. Variants resulting in PTC include nonsense, frameshift, and some splice-site variants. Consequences of splicing variants are based on predictions tools only and additional tests are required to determine the real impact on splicing. These tests, performed on patient's mRNA, may be compromised when the altered mRNA is degraded by the NMD during the translation. We thus specifically develop a method in which patient's cells are cultured in presence of puromycin, a strong translation inhibitor, to avoid NMD. We herein present two patients: (1) a 60-years old women affected with Charcot-Marie-Tooth disease and identified with the homozygous c.536-1G>T splice-site variant in the MME gene. Our method allowed us to observe the total degradation of the mRNA by NMD, probably leading to the complete absence of the neprilysin protein. Moreover, the use of puromycin to study the effect of the variant on mRNA level highlights the skipping of exon 7 of the gene, confirming the implication of this homozygous variant in patient's symptomatology; (2) a 2-years old girl presenting with Kabuki syndrome and carrying the heterozygous c.15921+1_15921+2del variant in the KMT2D gene. Splicing analysis in cultured cells revealed the skipping of exon 49 of the gene without degradation of the mRNA by the NMD, probably leading to the formation of a truncated and non-functional protein responsible for the patient's phenotype. Taken together, these data indicate that use of puromycin for splicing analysis is useful to identify molecular mechanism of splicing pathogenic variants even if NMD occurs.

P60. The Belgian Genetic Tests Database: a centralized and accessible tool for genetic testing allowing transparent access to comprehensive and quality information

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Sciensano

The Belgian strategy for rare diseases (RD) describes delimited actions and concrete measures to improve the management and quality of care associated with RD. One of the actions concerns quality management in the officially designated Belgian Centers for Human Genetics (CHG). It was recommended to develop and implement a database providing a comprehensive and uniform overview of genetic tests offered. This task was entrusted by NIHDI to Sciensano and controlled by a multistakeholder committee.

The main objective of this open access Belgian Genetic Tests Database (BGTD https://gentest.healthdata.be/) is to provide a tool to visualize, search, update and retrieve information on available genetic tests, their characteristics and to be able to evolve at the request of stakeholders. The BGTD also aims at reducing the burden of CHGs by facilitating the updates of genetic tests in Orphanet and by providing the necessary documents to various Belgian health authorities.

During the project we focused on the definition, implementation and testing of different tables, variables and relations between tables/variables, the search engine based on keywords. Also the access hierarchy, traceability and the modules/data subject to export/printing were defined. Agreement with Orphanet-Inserm made it possible to populate BGTD with genes associated with RD. Genetic test information was initially retrieved from resources including laboratory websites and government organizations.
To ensure validity of the data, tests are updated at least yearly by the CHGs and regularly by Sciensano (BELAC, external quality assessments (EQA), genes, diseases).
As of 2022, the database contains 895 genetic tests associated with at least a name, laboratory, gene, disease, sample type, method, documents necessary for prescription, reimbursement by NIHDI, turnaround time, and date of last update.
In conclusion, the BGTD complies with international, European and national directives and recommendations and is a valuable tool for anyone affected by or working with RD.

P61. Challenges with a MAGEL2 inframe deletion variant: two clinically distinct families and sequencing issues

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The aim of this study is to evaluate the clinical spectrum for a class 3 inframe deletion MAGEL2 variant (NM_019066.5): c.639_668del & p.(His221_Ala230del) causing two very district clinical features in two families. Furthermore, we faced and tackled some sequencing challenges to pick up and confirm this inframe variant. Lastly its pathogenicity will be discussed.

The first family came to presentation due to a fetus with hydrops fetalis, pulmonary hypoplasia, subcutaneous ascites, fetal akinesia and arthrogryposis. The second family presents with a monozygotic twin showing a Prader-Willi like phenotype with mild intellectual disability, autism spectrum disorder and obesity. Whole exome data processed with mapper BWA and variant caller GATK4 revealed a heterozygous MAGEL2 variant in both families. When analyzing the mapped data with IGV, the variants were only shown in about 1% of the reads, looking more like a sequencing error. The presence of this variant in the exome data was not shown with SNAP, GATK3 or VARDICT mapping tools. Therefore, Sanger sequencing was performed to confirm this variant in both families. This was challenging since MAGEL2 has some homologous regions and tools for variant interpretation were not always able to call this variant properly. Since we observed an inframe deletions of 30 nucleotides we performed a fragment analysis and were able to visualize two fragments for the individuals carrying the variant and only one fragment for the other family members.

The inframe deletion variant is present in both families and segregation analysis does not reject the pathogenicity of this variant nor confirms it. Functional studies might help to further elucidate the pathogenicity of this variant, but the clinical symptoms and segregation results fit within the spectrum of MAGEL2-disorders. Our study underlines that interesting MAGEL2 variants can be missed when certain mappers and variant annotation tools are used to process the sequencing data.