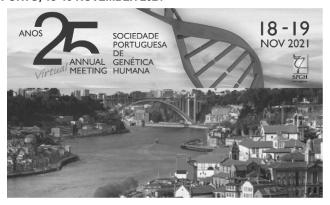# Medicine

Proceedings of the 25th Annual Meeting of the Portuguese Society of Human Genetics (SPGH – *Sociedade Portuguesa de Genética Humana*)

PORTO, 18-19 NOVEMBER 2021

Letter from the SPGH President

The 25th Annual Meeting of the Portuguese Society of Human Genetics (SPGH – Sociedade Portuguesa de Genética Humana) took place on November 18th and 19th, 2021 in virtual format, for the second consecutive year. It represents the largest annual event in the field of Medical and Human Genetics in Portugal, bringing together the national experts in the field, as well as some invited international scientists.

This meeting was also an occasion to celebrate the 25th anniversary of the Society. To this purpose, this year's program included a commemorative session where the beginning of Medical Genetics in the country and the creation of the Society were revisited.

The scientific sessions addressed topics such as the advances in knowledge of mechanisms and treatment of genetic diseases, the importance of quality in the provision of genetic care and the challenges that the future brings. We registered the submission of nearly one hundred scientific abstracts for oral or poster communication, hereby published.

We would like to thank all the invited speakers and session moderators, that ensured the scientific quality of the sessions.

Jorge Pinto Basto
SPGH President

ORAL PRESENTATIONS

Basic Research

OP1   MUTANT ATAXIN-2 PATHOLOGICAL FEATURES IS ALTERED BY THE AGING PROCESS

Inês Torquato Afonso[1], Patrícia Lima[2], Carlos A. Matos[1], Clévio Nóbrega[1]

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Aging is a natural process which can be defined as a time-dependent functional decline of an organism. This process is characterized by different molecular hallmarks, including oxidative stress, epigenetic alterations, loss of proteostasis, dysregulated nutrient-sensing (1). Some genetic disorders like Spinocerebellar Ataxia type 2 (SCA2) occur later in life and are associated with neurodegeneration of specific brain regions (2). SCA2 is an autosomal dominant disorder where the product of the mutant gene ATAXIN-2, originates a mutant protein with propensity to aggregate which seems to be toxic for neurons. This project aims to investigate the impact of aging upon in the neuropathological features related with mutant ataxin-2 expression.

Briefly, 3 and 18-months-old C57BL/6 mice were injected in the right hemisphere of the striatum with lentiviral vectors encoding for the human mutant ATAXIN-2 gene, while the contralateral hemisphere was injected with wild-type ATAXIN-2, as control. Twelve weeks post-injection the animals were sacrificed, and brain tissue was analyzed.

The 18-month-old animal group injected with mutant ATAXIN-2 had significantly increased number of pathological aggregates, as well as higher volume of neuronal loss in comparison with the 3 months old animals. Other aging hallmarks, such as inflammation, epigenetic, mitochondrial, and apoptotic markers were evaluated in this project.

The results indicate that mutant ATAXIN-2 expression has more prominent effect in old animals, supporting an impact of aging in the neuropathological abnormalities caused by mutant ATAXIN-2 expression.

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OP2 THE CDH1 LOCUS REGULATORY ARCHITECTURE: CDH1 NONCODING ELEMENTS CONTROL E-CADHERIN CANONICAL FUNCTIONS

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Introduction: E-cadherin is a cell-cell adhesion molecule involved in homeostasis, mobility, polarity, proliferation and differentiation. Loss of expression of E-cadherin is classically associated with epithelial tumour progression and is one of the epithelial to mesenchymal transition (EMT) hallmarks. Herein, we wish to disclose the contribution of the noncoding portion of CDH1 to the canonical functions attributed to this molecule.

Materials and Methods: We performed a bioinformatics analysis based on open chromatin and chromatin-interaction profiles and prioritized two CDH1 intronic Cis-Regulatory Elements (iCREs) for CRISPR-Cas9 editing in two gastric cancer cell lines. RNA-seq, ATAC-seq, and 4C-seq with a viewpoint in the CDH1 promoter, were performed in parental cell lines and iCREs CRISPR-Cas9 edited clones.

Results: 4C-seq revealed CDH1 promoter interactions with the two selected iCREs. RNA-seq and pathway analysis showed that deletion and/or inversion of CDH1 iCREs impaired most E-cadherin canonical functions. Edited clones with E-cadherin loss of expression presented genome-wide closed chromatin at CpG islands, promoters, TSS and introns, as opposed to clones retaining normal E-cadherin expression. Chromatin accessibility was directly correlated with differential RNA expression profiles. Specifically, pathways associated with E-cadherin impairment, such as adhesion, migration, cadherin binding and EMT were deregulated in both the transcriptome and chromatin accessibility. This effect was even stronger if both iCREs were simultaneously disrupted.

Conclusion: We disclosed a chromatin-regulatory network involving two CDH1 intronic regulatory elements that interact with the CDH1 promotor, profoundly affect the genome-wide chromatin accessibility and expression of adhesion, migration and EMT-associated molecules, and globally control fundamental functions attributed to E-cadherin.

OP3 TRANSCRIPTOMIC CHARACTERIZATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Introduction: Cardiomyocytes derived from human iPSCs (iPSC-CMs) are rapidly becoming a promising model to study the pathogenesis of genetic heart diseases and to develop new therapies. Nonetheless, in order to use iPSC-CMs as a disease model, it is important to characterize the extent to which they are able to recapitulate the biological processes of a real heart.

Methodology: We performed a transcriptomic characterization of iPSC-CMs derived from healthy patients using single-cell RNA-seq and bulk RNA-seq data. The results were compared with publicly available datasets of human hearts, both adult and fetal.

Results: Based on single-cell sequencing data, we found the majority of cells in iPSC-CM cultures correspond to atrial- and ventricular-like cardiomyocytes. Genes that are significantly upregulated in hearts over iPSC-CMs are enriched for angiogenesis and immunity-related pathways, revealing expected biological differences between in vitro cell cultures enriched for cardiomyocytes, and a complex tissue containing cells other than cardiomyocytes. As it concerns splicing, we found an enrichment in muscle and cardiac-related pathways, similarly to what is observed between fetal and adult hearts, and in line with the known role of splicing in heart development. Specifically among sarcomeric and ion

channel genes, few are differentially expressed or spliced between iPSC-CMs and hearts, with the iPSC-CMs mostly showing fetal-like patterns for both gene expression and splicing. Moreover, many of the alternative splicing events found in these genes were also observed when comparing fetal and adult hearts. While some have been described as cardiac maturation related isoform switches, many were previously undescribed.

Discussion: Our results show that the transcriptome of human iPSC-CMs recapitulates the gene expression and splicing patterns observed in the human fetal heart. We further identified many novel alternative splicing events between fetal and adult hearts, underscoring the role of splicing regulation in heart development.

OP4 PERMISSION NOT GIVEN BY THE AUTHORS

OP5 WHOLE GENOME SEQUENCING ANALYSIS: EXPLORING GERMLINE CNV LANDSCAPES

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Introduction: Genomes are naturally prone to aberrations, such as single nucleotide variants (SNVs) or structural variants (SV), which need continuous correction. Those that remain uncorrected, likely explain part of the missing heritability that exome analysis could not reveal, particularly in non-coding regions of disease-causing genes. Genome-wide analysis is therefore pivotal to understand genome variation and Whole Genome Sequencing (WGS) is growing as the preferred method for that purpose. Among others, lack of user-friendly WGS data analysis pipelines and interpretation tools are the major barrier to routine use of these techniques. Herein, we developed a WGS analysis pipeline to explore causal mechanisms in hereditary diseases.

Methodology: Germline WGS data from 22 samples were submitted to conventional quality control, alignment and post-processing. Multiple variant callers (DELLY, Lumpy and GRIDSS) were used to call CNVs. An integrative pipeline was developed for the overlap analysis of multiple callers to improve the pick-up rate of true positive CNVs.

Results: The percentage of mapped reads in all WGS samples was on average above 97%, with on average 2% of marked duplicates. DELLY retrieved 23306 and Lumpy retrieved 13069 variants, while GRIDSS delivered approximately 959648 breakpoints/breakends. The overlap analysis prioritized 19717 multiple called variants by at least two callers that we classified as high confidence. An intronic deletioncalled by 3 callers and two inversions were called by 2 callers (DELLY and GRIDSS) and affect a cis-regulatory element of a gene that matches the patient's phenotype. These have already been validated in original patient's material, and all GRIDDS-predicted breakpoints have been confirmed. An additional CNV, also matching the patient's phenotype, is currently in the pipeline for validation.

Discussion: Herein, we developed a WGS analysis pipeline that proved feasible in prioritizing true-positive CNVs with potential to be disease-causing. This work allowed the implementation of a WGS workflow for WGS germline analysis that is fast and accurate in producing user-friendly readouts.

Clinical Research

OP1 GERMLINE COPY NUMBER VARIANTS: AN UNDERREPORTED GENETIC DIAGNOSIS IN GASTROINTESTINAL TUMOUR RISK SYNDROME SUSPECTED INDIVIDUALS

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Germline pathogenic variants, including rare copy number variants (CNVs), in cancer predisposing genes (CPG) cause genetic tumour risk syndromes (TRS). TRS-causative variants can be clinically actionable and lead to intensive surveillance and/or risk reducing surgery that improve morbidity and mortality. Regrettably, causative and actionable variants cannot be found for all TRS-suspected individuals. While for SNV-calling specificity/sensitivity is almost 100%, CNV detection in exome-data remains challenging. We hypothesized that pathogenic CNVs in CPG may solve some of the yet unexplained, but clinically suspected gastrointestinal TRS-cases. The ERN-GENTURIS/SOLVE-RD project, re-analyzed exomes from 293 unsolved TRS-cases: adenomatous polyposis (AP; n=105), hyperplastic polyposis (HP; n=98), hereditary gastric cancer (HGC; n=83) and hereditary colorectal cancer (hCRC; n=7). CNVs were called with four different variant callers (ClinCNV, ExomeDepth, Conifer, VarGenius). 341 CNVs filtered from 229 CPGs were prioritized for their involvement in GI tumours, quality and calling by >1 caller. High-quality and/or 'multiple-called' CNVs were evaluated using IGV and focused paired-end mapping/split-read analysis. Eight CNVs (6-del; 2-dup), 3 'multiple-called', were found in 11/293 TRS-cases, sometimes in cases with an atypical phenotype. A CDH1 deletion, validated by MLPA, is found in 4 HGC-relatives. Supported by split-reads/paired-end mapping, it was considered an actionable diagnosis (4.8% among HGC cases). A deletion affecting PALB2 in 1/83 (1.2%) HGC cases was validated by qPCR. In an AP case, the last fraction of the APC and the beginning of the SRP19 gene was found deleted and supported by split-reads/paired-end mapping. Afterwards, validated by MLPA. Using a different approach, looking for 'multiple called' CNVs in CPG not directly associated with GI-TRS, a big deletion in SLX4, previously associated with sporadic CRC, was also validated by qPCR, solving 1/98 (1%) HP cases. Altogether, this approach delivered a potential diagnosis in at least 2.3% of unsolved GI TRS-cases.

OP2 HEAD AND NECK SQUAMOUS CELL CARCINOMA SIGNATURES: AN INTEGRATIVE MULTI-OMICS APPROACH

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Introduction: Head and neck squamous cell carcinoma (HNSCC) develops in the mucosal epithelium of the upper aerodigestive tract by the accumulation of copy number aberrations and methylation changes that lead to altered genetic expression. The objectives of this work were to analyse these alterations in an integrative manner allowing for the complete study of the genetic background of HNSCC in order to better understand its progression and to develop a novel methodological approach in integrative studies.
Methodology: The study's cohort consists of 410 HNSCC patients, from The Cancer Genome Atlas. Copy number alteration, methylation and RNA-Seq data from tumour tissue was retrieved. After initial pre-processing of the data, multi-variable techniques were applied in order to reduce data dimensionality. Based on multi-omics clustering profiles, two groups with different survival curves were established, using the Kaplan-Meier method. A multi-omics signature was established by using an importance plot algorithm and LASSO regression, and was evaluated by supervised classification methods.

Results: Two different clusters were established in the cohort: one including 58% of patients and another containing the remainder 42%. Survival analysis performed in these two groups showed a median difference in survival of 836 days (2 years and 4 months). Having associated the survival of the patients with the clusters they belong to, a nine gene multi-omics signature capable of distinguishing the two groups, was determined. This signature, that included copy number alterations in two genes, the methylation profiles of three genes and the expression of four genes, shows good predictability with an accuracy of 96% and 95% determined by two different methodologies.
Discussion: This is an innovative study that shows that signatures composed of different omics can be auxiliaries in the clinical practice, as demonstrated here with the prediction of survival in HNSCC patients. Integrating multi-omics profiles allows for a more comprehensive study of cancer, creating the prospect to positively influence the clinical outcome of these patients.

OP3 INCIDENTAL CARRIER DETECTION OF 639 VARIANTS IN PATIENTS TESTED FOR DIAGNOSTIC PURPOSES: ADDED VALUE FOR GENETIC COUNSELLING AND A GLIMPSE ABOUT RECESSIVE DISEASES IN PORTUGAL

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Introduction: Implementation of whole-exome sequencing (WES) in routine diagnosis allowed great advances in the study of human genomic variation. With the application of broad genomic approaches, there is the possibility of identifying pathogenic (PAT) or likely-pathogenic (L-PAT) variants, irrelevant for the condition for which the patient is being testing, but still with medical and/or genetic counselling value.
Aim: We evaluated the number and nature of such variants at our center (Jan 2019 – Aug 2021).
Methods: Genetic data from 1,567 consecutive diagnostic tests, using WES-based multigene panels, were reviewed. Carrier status was reported only in tests requested by a medical geneticist and when specific informed consent had been obtained. Only PAT/L-PAT variants in heterozygosity, in genes known to be associated with a recessive disorder and fulfilling the following criteria are reported: (a) disease without high clinical variability/expressivity; (b) onset in infancy and resulting in severe disease, (c) disease affects other than just vision or hearing.
Results: Incidental carrier detection occurred in ~20% (n=329) of individuals (mean: 1.94 variants/patient; ranging: 1 to 6), in a total of 639 reported variants (536 distinct) in 402 genes. The most frequent variants were in CFTR (7 distinct variants, 16 cases), ATP7B (9/9), and PAH (10/10); associated to cystic fibrosis, Wilson disease or phenylketonuria, respectively. Overall, the p.(Phe508del) variant in CFTR was the most frequent (9 cases). Several variants had already been described specifically in Portuguese patients: NM_052859.4(RFT1):c.208T>C, associated with a congenital disorder of glycosylation (type In); NM_004562.3(PRKN):c.155del, in early-onset Parkinson; NM_000426.4 (LAMA2):c.2461A>C, in muscular dystrophy; and NM_024570.4(RNASEH2B):c.529G>A, in Aicardi-Goutieres syndrome.
Discussion: As expected, the most frequent AR diseases were represented in our cohort. These data should only reflect the tip of the iceberg concerning burden of recessive diseases in our population. This dataset will be further expanded resorting to the variants derived from the ~7200 exomes processed so far in our laboratory.

OP4 THE FIRST CDH1 FOUNDER VARIANT IN THE PORTUGUESE POPULATION: A MISSENSE WITH SEVERE IMPACT IN MRNA SPLICING

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Introduction: Hereditary diffuse gastric cancer (HDGC) caused by CDH1 germline pathogenic (P) or likely pathogenic (LP) variants predisposes to early onset diffuse gastric (DGC) and lobular breast cancer (LBC). In Northern Portugal, an unexpectedly high number of early-onset DGC and LBC in apparently unrelated families carrying the same CDH1 c.1901C>T variant suggested this as a CDH1-founder variant. We aimed to demonstrate that c.1901C>T (formerly known as p.A634V) is a bona fide truncating variant inducing cryptic splicing, to calculate the timing of a potential founder effect, and to characterize tumour spectrum and age of onset in carrying families.

Methods: The impact in splicing was proven by using carriers' RNA for PCR-cloning sequencing and allelic expression imbalance analysis with SNaPshot. Carriers and non-carriers from four distinct families were haplotyped for 12 polymorphic markers, and the decay of haplotype sharing (DHS) method was used to estimate the time to the most common ancestor of c.1901C>T. We collected and analyzed the clinical information from 58 carriers to explore stomach and breast-associated clinical presentations.

Results: We validated the cryptic splice site within CDH1-exon 12, which was preferred over the canonical one in 100% of sequenced clones. Cryptic splicing induced an out-of-frame 37bp deletion in exon 12, premature truncation (p.Ala634ProfsTer7), and consequently RNA mediated decay. The haplotypes carrying the c.1901C>T variant were found to share a common ancestral estimated at 490 years (95% Confidence Interval 445–10,900). Among 58 carriers (27 males (M)–31 females (F); average age 13–83 years), DGC occurred in 11 (18.9%; 4M–7F; average age 33 ± 12) and LBC in 6 females (19.4%; average age 50 ± 8).

Conclusion: We demonstrated that the c.1901C>T variant is a loss-of-function splice-site variant that underlies the first CDH1-founder effect in Portugal. Knowledge on this founder effect will drive genetic testing of this specific variant in HDGC families in this geographical region and allow intrafamilial penetrance analysis and better estimation of variant-associated tumour risks, disease age of onset, and spectrum.

OP5 DISCLOSURE OF GENETIC INFORMATION TO PATIENT'S RELATIVES: HEALTHCARE PROFESSIONALS' PERSPECTIVE ON PERCEIVED RESPONSIBILITIES AND CONFIDENTIALITY OF GENETIC INFORMATION

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Results from genetic and genomic testing are relevant for patients, but also their relatives. The degree of responsibility and proactivity of genetic healthcare services to ensure relatives' awareness of their risks has long been debated, but research exploring healthcare professionals' (HPs) views on this topic was inexistent in Portugal.

We conducted 10 focus groups with 34 HPs, working in 7 medical genetic services. We aimed at exploring their views and reasoning about disclosure of genetic information to patients' relatives, perceived responsibilities to patients and families, confidentiality of genetic information, and how those considerations related to their practice. Data were analysed thematically.

Discussion centred mainly on conditions amenable to medical intervention. The majority of participants perceived to be their responsibility to provide objective genetic risk information to their patients and its implications for relatives, patients having a moral responsibility to share relevant information with them. Notably, primacy of medical-patient relationship and the national law on genetic information were often mentioned to discard direct responsibility to patients' relatives. When identifying barriers to family communication, however, participants reported using diverse strategies to encourage disclosure. Although recognising the shared dimension of genetic information, in practice, participants had a strict individual approach to confidentiality and

considered that applying a relational approach – e.g. treating personal and familial information differently – would be problematic. Contacting at-risk relatives directly was seen as the ideal scenario, but difficult to implement due to insufficient multidisciplinary HPs in genetic services. Some demanded for broader discussion and clear national guidelines. Results provided a rich empirical basis for further debate on the roles and responsibilities of HPs in supporting intrafamilial communication of genetic risks and how to appropriately cascade relevant information to at-risk relatives.

Clinical Case Reports

OP1 TRICHOTHIODYSTROPHY, A CASE-REPORT HIGHLIGHTING THE IMPORTANCE OF CONNECTING GENOTYPE AND PHENOTYPE DATA TO REACH A DIAGNOSIS

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Introduction: Trichothiodystrophy (TTD) comprises a group of ultra-rare autosomal recessive conditions, characterized by stiff and brittle sulfur-deficient hair and systemic abnormalities, and a history of maternal pregnancy complications. TTD is caused by biallelic deleterious variants in specific DNA repair/transcription genes. Tiger tail banding (TTB) hair under polarizing microscopy (PM) is a pathognomonic pattern of TTD.

We report the diagnostic journey of a 5-yo female patient with TTD.

Clinical Case: At two years of age, our patient presented to the emergency room with enteropathy where her low height/weight and short brittle hair were noticed. Parents were healthy and non-consanguineous and had had a son deceased at 16mo with an unknown polymalformative syndrome. She had immunodeficiency and anemia and a previous history of severe allergies. A Mendeliome gene panel was done and four VUS were found, including two novel variants on ERCC2(NM000400.3): c.1742T>C (p.L581P) and c.949+5G>A. A strong clinical suspicion of Netherton syndrome due to association of brittle hair, enteropathy and allergies prompted a referral to Medical Genetics for further SPINK5 studies. The Medical Geneticist's appreciation of the patient's phenotype and genotype shifted the focus to TTD. The ERCC2 variants were compound heterozygous. Dermatology referral to assess hair morphology under PM confirmed the presence of TTB. UV light sensitivity and DEB-induced chromosome breakage studies showed abnormal results. Considering the importance of a molecular diagnosis for reproductive options, a minigene assay was performed and confirmed the variant's impact on splicing, allowing for reclassification of the intronic variant as likely pathogenic, and the trans missense variant as likely pathogenic. Thus, a TTD diagnosis with molecular confirmation was achieved in our patient.

Discussion: This case-report describes a 3-year diagnostic odyssey, highlighting the crucial need to integrate genotype and phenotype data in the quest for the correct diagnosis. It also underlines the importance of a skilled multidisciplinary team to confirm a diagnosis and to provide specific genetic counselling.

OP2 HEART DEFECTS, ORAL CLEFT, AND POLYDACTYLY CAUSED BY BIALLELIC VARIANTS IN WDPCP GENE INVOLVED IN CILIOGENESIS

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Introduction: Ciliopathies are a group of disorders with overlapping phenotypes, caused by dysfunctional cilia. WDPCP is a planar cell polarity protein, which plays an important role in recruiting molecules essential for ciliogenesis. Wdpcp mutant mouse exhibited developmental defects including anophtalmia, polydactyly, kidney cysts, heart defects, and facial clefts. In humans, only four patients with biallelic pathogenic variants in WDPCP have been reported, two of them presenting with congenital heart defects, hamartomas of tongue, and polysyndactyly (CHDTHP), and the other two with Bardet-Biedl Syndrome 15.

Case Report: We describe a 2-year-old girl, the first child born to non-consanguineous parents with no relevant family history. Prenatal ultrasound revealed multiple anomalies including heart defects, lip and palate clefts and polydactyly. Chromosomal microarray analysis and molecular study of Ellis-van Creveld Syndrome were negative. The parents proceeded with the pregnancy and delivery occurred at term. Physical examination of the newborn showed left cleft lip and palate, postaxial polydactyly of the left hand and hallux duplication bilaterally. Echocardiography revealed a large atrial septal defect (almost a single atrium) and left atrioventricular regurgitation. Transfontanellar and abdominal ultrasounds were unremarkable. Currently, her psychomotor development is slightly delayed. In trio whole-exome sequencing analysis identified two novel compound heterozygous variants in WDPCP: c.1486T>G p.(Cys496Gly) and c.852_860delinsG p.(Asp285Alafs*4).

Discussion: To our knowledge, only two patients with WDPCP related "CHDTHP" have been reported so far. Interestingly, our patient is the first one presenting oral clefts, resembling the phenotype of the Wdpcp knockout mouse. This study further expands the molecular and phenotypic spectrum of this rare and still poorly known disorder, contributing to a deeper understanding of the function of WDPCP in ciliogenesis. To the patient´s family, molecular diagnosis allowed proper genetic counselling and informed reproductive choices.

OP3 MANDIBULOFACIAL DYSOSTOSIS TYPE GUION-ALMEIDA: NATIONAL CASE SERIES WITH CLINICAL AND MOLECULAR CHARACTERIZATION

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Introduction: Mandibulofacial dysostosis type Guion-Almeida (MFDGA, MIM#610536) is an autosomal dominant condition caused by haploinsufficiency of EFTUD2 gene, which encodes a component of the major spliceosome that removes introns from pre-mRNA transcripts. Clinically, MFDGA presents with malar and mandibular hypoplasia as the core phenotype, associated with microcephaly, developmental delay/intellectual disability (DD/ID), and structural congenital anomalies. Its highly variable manifestations make MFDGA an underdiagnosed disorder, and frequently misdiagnosed with CHARGE syndrome, oculoauriculovertebral spectrum, and other mandibulofacial dysostosis.

Aim: Here, we present a national case series of patients diagnosed with MFDGA.

Methods: Retrospective review of clinical and molecular data from MFDGA patients diagnosed in Portuguese Clinical Genetics Departments.

Results: We identified 7 patients (5 males, 2 females) from 7 families. All patients had mandibular hypoplasia, DD/ID and microcephaly. The most frequent facial dysmorphisms were micro/retrognathia (6/7), ear anomalies (5/7), prominent nose (3/7), and facial asymmetry (3/7). The most common structural anomalies were airway abnormalities [esophageal atresia (4/7), choanal atresia/stenosis (2/7), and velopharyngeal insufficiency (1/7)], followed by heart defects (3/7), and genitalia anomalies (1/6). Additional relevant phenotypic features were growth delay/short stature (5/7), feeding difficulties (3/7), and vision problems (3/7). Pregnancy complications were reported in 3 cases. 4 out of 7 patients required invasive interventions. Regarding the diagnostic approach, other specific genetic diagnosis, namely CHARGE and Dubowitz syndromes, were first considered in 2 cases, while MFDGA was the first

diagnostic hypothesis in 1 case. A total of 7 different EFTUD2 sequence variants were identified: 4 frameshift [pathogenic (P)/likely pathogenic (LP)]; 2 affecting splicing [2 (P/LP)]; and 1 stop gain (LP).

Discussion: Our data is consistent with the literature. Also, this study adds information to the clinical level, expanding MFDGA phenotypic spectrum, and molecular profile, 6 novel EFTUD2 variants are reported.

OP4 THREE PATIENTS WITH PHIP-RELATED SYNDROME - FURTHER PHENOTYPIC DELINEATION

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Introduction: Chung-Jansen syndrome/PHIP-related syndrome is a recently described developmental syndrome characterized by developmental delay (DD)/intellectual disability (ID), behavioral problems, obesity, and facial dysmorphisms. Heterozygous pathogenic variants in PHIP gene are thought to be deleterious through haploinsufficiency.

Methodology: Retrospective review of medical records and clinical reevaluation of patients with PHIP variants.

Results: We report 3 index cases of Chung-Jansen syndrome, two aged 16 and the other 10. DD was present in 2/3 individuals, and all patients had mild ID, and behavioral problems. Obesity was present in 1/3 patients, musculoskeletal abnormalities in 2/3, cryptorchidism in 2/3, and sleep disorders in 2/3. Thickened nasal alae (2/3), anteverted nares (2/3), large ears with thickened helix (2/3), tapered fingers (2/3), clinodactyly of the 5th finger (3/3), and 2-3 toe syndactyly (2/3) were the most frequently reported dysmorphisms. Molecular characterization was performed by whole exome sequencing (WES) and 3 likely-pathogenic variants (nonsense, frameshift, and splice site) were identified. Subsequent parental studies confirmed the variants were de novo in patients 1 and 3. Patient 2 inherited his PHIP variant from a clinically affected mother with learning difficulties, depression, obesity, and dysmorphisms. He has an older sister with an overlapping phenotype who is awaiting clinical reevaluation and targeted analysis for the familial variant in PHIP gene.

Discussion: Our cohort highlights the typical mild presentation of DD/ID in Chung-Jansen syndrome. The low rate of obesity supports the proposed higher incidence of this feature in post-pubertal individuals. Comparing with previous studies, sleep disorders were more prevalent, but autism spectrum disorder was not reported. There was no obvious facial gestalt. Though only 2 familial cases have been reported so far, we believe that milder phenotypes can be more prevalent among affected families than we first expected. Chung-Jansen syndrome highlights the role of WES in diagnosing neurodevelopmental syndromes with no unequivocally distinguishable phenotypic traits

OP5 EXPANDING THE CLINICAL SPECTRUM OF COMBINED OXIDATIVE PHOSPHORYLATION DEFICIENCY ASSOCIATED WITH MRPS34 GENE: A MILD CASE WITH SLENDER HABITUS

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Introduction: Combined oxidative phosphorylation deficiency (COXPD) is a group of multisystem disorders with variable manifestations resulting from a defect in the mitochondrial oxidative phosphorylation system due to variants in about 50 genes. In 2017, the MRPS34 gene was associated with COXPD (type 32). Six patients with variants in this gene, all with a severe Leigh syndrome phenotype, have been reported so far.

Case report: We describe a 16-year-old girl referred to Genetics at the age of 5 years due to developmental delay (DD). Unremarkable family history. Pregnancy complicated by oligohydramnios. Vaginal delivery at 36 weeks gestation. Birth somatometry appropriate for gestational age. No neonatal complications.

Growth evolved with weight and length below the 3rd centile until 6 months and then on the 5th-50th centiles. She had mild to moderate DD/intellectual disability (ID), affecting more markedly language. At 16 years, she read slowly and had articulation problems; comprehension and memory were limited. Other medical problems were

hypogonadotropic hypogonadism, slender habitus with joint laxity and kyphoscoliosis, misaligned teeth and unspecific dysmorphic features.

Head MRI at 2 years showed bilateral olivary nucleus degeneration; however, a subsequent MRI scan performed 3 years later was normal. Metabolic investigation was normal except for mildly increased lactate and pyruvate with a normal ratio. Chromosomal microarray analysis was normal. Finally, exome sequencing identified a previously described homozygous pathogenic variant in MRPS34, c.322-10G>A (p.?), establishing the diagnosis of COXPD type 32.

Conclusions: We present a patient with syndromic moderate DD/ID due to a homozygous pathogenic variant in MRPS34. This is the 7th patient reported to date with COXPD type 32. All other patients, including 4 carrying the same variant, died in the first months of life or survived with severe DD/ID, and presented neuroimaging findings suggestive of Leight syndrome.

This case is significantly milder comparing to previous patients. In addition, this girl had slender habitus with joint laxity and kyphoscoliosis, amplifying the clinical spectrum of COXPD type 32.

POSTER PRESENTATION

Basic Research

P1 THE 2020S TOOTH FAIRY: FROM LOOSE TOOTH TO NEURONAL CELL CULTURES, AN INNOVATIVE METHOD TO MODEL NEUROLOGIC LYSOSOMAL STORAGE DISEASES IN VITRO

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Lysosomal storage disorders (LSD) are rare genetic diseases caused mutations in genes that encode lysosomal enzymes, membrane proteins or transporters. This leads to an accumulation of undegraded substrates, which ultimately causes a broad range of symptoms affecting multiple organs/systems, including the central nervous system. Yet, most therapies for LSD are limited to treating non-neurological signs. Thus, there is an urgent need for the development of new ones that tackle the neuronal pathogenesis.

Taking this into account, some of our recent studies focus on the design of RNA-based therapies to correct/ameliorate the LSD cellular phenotype. While exploratory, these studies led us to reflect on the major obstacles that may hinder their clinical translation, triggering some of our current interests: the need for adequate delivery vectors and suitable disease models.

Here we address this second concern, showing how we are developing and characterizing innovative patient-derived cell models for early onset neurodegenerative LSD.

Briefly, we are collecting deciduous "baby" teeth from children with neurological forms of LSD and breaking them open to access the pulp tissues, which contain dental pulp stem cells (DPSC). The tissues are then digested with appropriate enzymes and primary DPSC lines established. DPSC hold potential to give rise to a variety of cells including neurons. So far, we have successfully implemented the protocol for the establishment of this sort of cultures and are currently working on the differentiation steps, which will allow the formation of mixed neuronal and glial cultures. Plus, we have successfully cryopreserved DPSC from

several controls and from one LSD patient. Those cells may be differentiated with this method but there are other potential applications: p.e, their differentiation into chondrocytes, one of the major components of cartilage and primary site of accumulation in several LSD.

This is a total innovation in the field and we believe it holds potential to set a new trend for investigating LSD as it relies on a non-invasive, cost effective approach that can be set in virtually any lab with standard cell culture conditions.

P2 UPREGULATION OF TRNASER DRIVES GENOME ADAPTATION OF NSCLC TUMORS

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tRNAs are a driving force of genome evolution in Yeast and Bacteria. Their deregulation is frequently observed in tumors with Serine tRNAs being often overexpressed. This has important functional consequences, such as increased metabolism and tumor growth. Therefore, we hypothesized that tRNA deregulation may contribute to the increased genome instability observed in tumors.

To study the effect of tRNA deregulation in tumors, we overexpressed tRNA-Ser-AGA-2-1 in a NSCLC cell line, H460. This cell line and a Mock (control) were xenografted in nude mice and collected at 3 timepoints: T1-Naïve; T2-Treated once with cisplatin/vehicle and; T3) treated twice with cisplatin/vehicle. DNA and RNA were extracted for WES and RNAseq.

The tumor mutation burden remained unchanged along time, however, tRNASerOE tumors in T3 treated with either cisplatin or vehicle showed a higher mutation burden. tRNASerOE are rich in MSI and DNA damage response Stratton signatures. Moreover, although in T1 tumors have a similar number of variants, in T2&3, tRNASerOE tumors display two times more variants than Mock tumors regardless of treatment. These variants are mainly synonymous and missence variants. Interestingly, tRNASerOE exclusive variants favor proliferation and therapy resistance, which is in line with the phenotypes observed and the RNAseq data.

In conclusion, tRNASerOE increases the tumor mutation burden and the variants detected favor tumor growth, proving that deregulation of a single tRNA is enough to fixate important and adaptive mutations in the genome of cancer cells, highlighting a new and unexplored mechanim.

P3 INTERNAL RIBOSOME ENTRY SITE (IRES)-MEDIATED TRANSLATION AS A PUTATIVE CANDIDATE MECHANISM TO MRNA-BASED THERAPIES

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Untranslated regions in the messenger RNA (mRNA) are susceptible to the interaction of regulatory elements including proteins or non-coding RNA molecules, being an important hotspot to the study of gene expression regulation. Internal Ribosome Entry Sites (IRES) are secondary structures usually located on the 5'UTR of an mRNA molecule that can recruit the ribosome during initiation of protein synthesis without the involvement of the cap structure. This mechanism tends to appear when the cell is under stress conditions, which might include the presence of oncogenes, growth factors or proteins involved in programmed cell death.

This work focuses on the human AGO1, an important key player for RNA-mediated gene silencing, and whether its 5'UTR is capable of driving cap-independent translation initiation.

To achieve this goal, a construct containing the 5'UTR of human AGO1 was cloned, taking advantage of a bicistronic vector containing two reporter genes, Renilla luciferase (RLuc) and firefly luciferase (FLuc), the last one cloned downstream from the 5'UTR. We performed luminometry assays to assess the relative translation efficiency of FLuc, which is under the control of AGO1 5'UTR.

The results showed that human AGO1 5'UTR mediates a cap-independent eIF4G-dependent mechanism of translation initiation enhanced by a free 5' end. Also, we saw that this alternative mechanism is maintained, and even enhanced, under stress conditions, such as the knock-down of

eukaryotic initiation factor 4E, the protein that directly binds to the cap structure. We are currently investigating what is the minimal sequence required for IRES-mediated translation.

Combining these results with the emerging RNA-based therapies will be helpful to develop novel strategies to prevent and treat disorders, such as cancer, involving dysregulation of AGO1 translation.

P4 A PRO-INFLAMMATORY MICROENVIRONMENT TRIGGERS OVEREXPRESSION OF TUMOR-RELATED RAC1B IN POLARIZED COLORECTAL CANCER CELLS

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An inflammatory microenvironment is a tumor-promoting condition that provides survival signals to which cancer cells respond with changes in their gene expression. One key regulatory mechanism is alternative splicing. For example, RAC1B is a RAC1 alternative splicing variant that we previously identified in a subset of BRAF-mutated colorectal tumors and that was found increased in colon mucosa under inflammatory conditions, such as samples from inflammatory bowel disease patients or following experimentally-induced acute colitis in a mouse model.

Based on these findings, the main goal of this work was to determine which pro-inflammatory signals from stromal cells lead to an increase in RAC1B expression levels in colorectal cancer (CRC) cells.

For this, we used a physiologically relevant epithelium-like monolayer of fully polarized Caco-2 CRC cells grown on porous membrane inserts, and then co-cultured underneath with stromal cells, including fibroblasts, monocytes and macrophages. RAC1B expression was analyzed in Caco-2 cells by RT-qPCR, Western blot and confocal fluorescence microscopy. Co-culture experiments revealed that the combined presence of cancer-associated fibroblasts and/or M1 macrophages induced an increase in RAC1B levels in Caco-2 cells, accompanied by a loss of epithelial organization. Moreover, using a human inflammation antibody array, we were able to identify from the conditioned co-culture media that cytokine IL-6 was associated with increased RAC1B expression. Remarkably, the incubation of polarized Caco-2 cells with purified IL-6 was sufficient per se to trigger an increase in RAC1B expression in a dose-dependent manner, and the presence of anti-IL-6 antibodies during the co-culture prevented the increase.

Overall, our data indicate that pro-inflammatory signals from the microenvironment can modulate RAC1B expression in colon epithelial cells. Since RAC1B was shown to sustain tumor cell survival and promote escape form oncogene-induced senescence, the data further strengthen the causal connection between inflammatory conditions and the development of colorectal cancer.

P5 FABRICATION OF A HUMAN STOMACH-ON-A-CHIP DEVICE DISPLAYING ORGAN-LIKE FEATURES

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Introduction: The stomach has a central role in human physiology. Complications leading towards stomach disease pose serious consequences and treatment is generally associated with high morbidity and mortality. The lack of biomimetic models capable of reproducing the complex architecture and dynamic environment of the gastric mucosa, still hampers the development of new diagnostic and therapeutic tools. Recent advances in microengineering, made possible the fabrication of bioinspired devices capable of replicating the physiology of an organ, inside a microfluidics chip. Here we describe a bioinspired stomach-on-a-chip (SoC) device supporting peristalsis-like motion and reconstituting organ-level architecture and function.

Methodology: A fabrication method based on xurography was used to fabricate a multilayered organ-on-a-chip device. The 9 layered device was engineered by intercalating layers of fluidic channels made of a flexible silicone, separated by perforated PET membranes. The latter allow physical and chemical communication between every layer of the device. Bellow the fluidic assembly, a flexible PDMS microactuator can be added to impart mechanical stretching.

The resulting SoC represents the inner layers of the gastric mucosa, namely the epithelial barrier, the basement membrane and the lamina propria. The SoC was characterized regarding its response to the dynamic conditions imparted by the chip. Resulting phenotype and in vivo gastric function were evaluated.

Results: The dynamic environment imparted by the SoC, elicited epithelial polarization and differentiation. Interestingly, these phenotypic traits are characteristic of the normal gastric mucosa but are commonly lost in 2D gastric cell culture. Furthermore, the SoC recapitulated some functional features of the native organ, namely, enhanced epithelial barrier function and increased pepsin activity.

Conclusion: The engineered SoC demonstrated the importance of considering the effect of mechanotransduction, in order to recapitulate key in vivo features of the native organ. The proposed SoC represents an interesting tool in a personalized medicine context, as a biorelevant tool to study gastric disease.

P6 LEARNING FROM MRNA: THE RELEVANCE OF THE TUMOUR SUPPRESSOR PROTEIN UPF1'S INTERNAL RIBOSOME ENTRY SITE-MEDIATED TRANSLATION IN TUMORIGENESIS

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Crucial in several cellular processes, such as nonsense-mediated mRNA decay, cell cycle progression, and telomere maintenance and homeostasis, Up-frameshift 1 (UPF1) has also been considered a tumour suppressor protein in hepatocellular carcinoma and gastric cancer, as it is underexpressed in the latter and negatively correlated to MALAT1 (long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1) expression. UPF1 overexpression inhibits some cancer-specific features, like proliferation, cell cycle progression, cell migration and invasion, and enhances apoptosis, turning the UPF1/MALAT1 pathway a potential therapeutic target for gastric cancer.

Here, we investigate the importance of UPF1 internal ribosome entry site (IRES)-dependent translation in tumorigenesis, specifically in colorectal cancer. Thus, we used a bicistronic system with two reporter genes, in which we cloned UPF1 5' untranslated region (UTR) upstream the second cistron, whose translation will only occur internally. We transcribed the bicistronic mRNA in vitro and transfected HeLa (cervical cancer), NCM460 (normal intestinal-derived colonocytes) and HCT116 (pre-metastatic colorectal carcinoma) cells with such mRNA, along with the positive and negative controls. The results show a significant increase in IRES-mediated translation levels compared to those of the negative control, both in normal conditions and under endoplasmic reticulum stress. Also, internal initiation occurs in the absence of the cap structure. Deletional and mutational analysis of UPF1 5'UTR showed that nucleotides 1–100 (stem-loop (SL) I) and 151–275 (SL III) — out of 275 nucleotides — are the minimal required sequences for the IRES to work properly. Also, we used RNA antisense oligonucleotides (ASOs) targeting UPF1 IRES SL I and III and observed a reduced UPF1 expression. Cellular viability increases in HCT116 cells and decreases in NCM460 cells treated with ASOs targeting SL III and SL I, respectively, whereas apoptosis increases in NCM460 cells and decreases in HCT116 cells treated with ASOs targeting SL I. This may be the dawn of a new RNA-based therapeutic approach regulating colorectal cancer development.

P7 PERMISSION NOT GIVEN BY THE AUTHORS

P8 ASSESSING THE POTENTIAL OF RNA-BASED THERAPEUTICS FOR A GROUP OF LYSOSOMAL STORAGE DISEASES WITH NEUROLOGICAL INVOLVEMENT

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During the first two decades of the 21st century, remarkable progresses have been achieved in the field of RNA-based therapeutics. From antisense RNA to RNA modification, the therapeutic potential of RNA-based technologies has nothing but increased. In our lab, we have been addressing the potential of different RNA-based drugs to either correct or ameliorate the sub-cellular phenotype of a number of severe, life-threatening diseases: the so-called Lysosomal Storage Disorders (LSDs). Among them, we are focusing our efforts on those which present with a predominant neurological phenotype, since there are virtually no approved treatments for any of them.

Briefly, two major research lines are being pursued: the first relies on the design of mutation-specific approaches to correct abnormal splicing processes in LSD-related genes, whenever they underlie pathology. The second depends upon selective downregulation of genes involved in the biosynthethic cascades that give origin to the substrates that accumulate in each pathology.

Here we present an overview on our results with both approaches on Sanfilippo syndrome, a sub-group of severe neurodegenerative LSDs.

For the mutation-specific, splicing correction approach, we are using U1snRNA vectors to restore the splicing defect caused by the HGSNAT mutation c.234 + 1G>A, that leads to Sanfilippo C disease. We started by demonstrating in vitro that a modified U1snRNA vector designed to improve the definition of HGSNAT exon 2 could partially restore its normal splicing process (1). Now, we are evaluating its therapeutic potential in vivo, in mice expressing the human splicing defect.

For the substrate reduction approach, we are using siRNAs. By acting over a specific biosynthethic cascade, siRNAs promote an overall decrease of the accumulating substrate. So far, we have already tested this approach in patients' fibroblasts and observed a high inhibition of the target mRNAs and a decrease in storage.

Overall, there are substantial differences between these two approaches but they also face common challenges and show equally promising results.

1. Matos et al., 2014 (DOI: 10.1186/s13023-014-0180-y)

P9   ANTISENSE OLIGONUCLEOTIDE EXON-SKIPPING AS A THERAPEUTIC APPROACH FOR MUCOLIPIDOSIS TYPE II ALPHA/BETA: IN VITRO AND IN VIVO STUDIES

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Mucolipidosis type II alpha/beta (ML II) is one of the most severe Lysosomal Storage Disorders and is caused by the deficiency of the enzyme GlcNAc-1-phosphotransferase. This enzyme is responsible for the addition of the mannose 6-phosphate marker to lysosomal enzymes, which allow their targeting to lysosomes. Of the several mutations that occur in ML II, the deletion of 2 nucleotides from GNPTAB exon19 (c.3503_3504del) is the most frequent, making it a good target for a

specific mutation therapy as there is no therapy for this disease. In this study, we explored the possibility of an innovative therapeutic strategy based on the use of antisense oligonucleotides (AOs) for ML II. In a previous in vitro study in ML II patient fibroblasts, AOs were used to promote the exon 19 skipping from the GNPTAB pre-mRNA, resulting successfully in the production of an in-frame mRNA1. Currently, our objective is to evaluate the therapeutic potential of this approach, both in vitro in C57BL/6 fibroblasts and in vivo in C57BL/6 mice. For this, 18 animals were used, divided into 6 groups: groups 1 and 4 were injected with saline solution, groups 2 and 5 with AO at 25 mg/kg and groups 3 and 6 with AO at 50 mg/kg. All animals were injected by intraperitoneal route and were sacrificed after 4 or 7 days post-treatment. At the end of the experiment, the organs were collected and frozen at -80ºC, for later RNA extraction, cDNA synthesis and RT-PCR. After results analysis, the exon 19 skipping was not observed using any of the tested doses or incubation periods. So, we can theorize that the doses administered were not sufficient to achieve a response or the AO might have had a high clearance rate. As for the in vitro experience, the C57BL/6 fibroblasts were seeded in 6-well plates and subsequently transfected with concentrations of AO ranging from 10nM to 600nM. After 24/48h of incubation, cells were collected and cDNA analysis revealed a full length transcript but also another one of lower molecular weight compatible with exon-skipping. These are preliminary data, so in the near future more experiments will be done.

Matos L, et al. Hum Gene Ther, 2020, 31(13-14):775-783.

P10   A CHRONOLOGY OF MTDNA AFRICAN LINEAGES OUTSIDE AFRICA REVEAL THE INFLUENCE OF CLIMATE CHANGE AND SLAVERY IN MIGRATIONS DURING THE HOLOCENE

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Africa is likely the birthplace of the Modern Human about 200,000 years ago. This is visible across human diversity including on the female-inherited mitochondrial DNA (mtDNA). The most ancient clades of the mtDNA tree exist in Africa, haplogroups L0 to L6, while all diversity outside Africa emerges from a single of these clades, the Eastern African L3, that left the continent about 70-60 thousands years ago. However that was not the only time when migrations out of Africa occurred. Here we investigated migration of lineages from African haplogroups using a phylogeographic approach. We built a mtDNA tree of haplogroups L0-L6 containing nearly 7000 sequences and we applied a founder analysis, aiming to detect periods of migration to either Europe, Asia or the Americas. We obtained two major periods of migration. One occurred in the early-middle Holocene, from Northern, Western, Central and Eastern Africa into Europe and Southwest Asia. This corresponded to a period where climate changes allowed crossing of the Sahel Belt, into Northern Africa and ultimately, Europe. The second migration showed a completely different nature reaching not only Europe but also the Americas, likely corresponding to the forced transport of African individuals as slaves. Here, the signal in Europe is greater in the Iberian Peninsula and it is obviously the only signal in the Americas. The population source of these groups is also distinct from the earlier one. There is a high percentage of West African lineages (73.4% in North America and about 50% in the Iberian Peninsula), which matches the location of the major ports of transportation of Slaves in the African coast. The migration of Southern African lineages, that were not detected in early migrations, represented the source of a substantial number of lineages in Iberia, and mostly, in South America, reflecting the fact that Portugal was the only country with slave ports located in the Southern part of the continent, namely in Angola.

P11   DECIPHERING COPD AS A RISK GROUP FOR COVID-19: CAN WE BLAME GENETICS?

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People with chronic obstructive pulmonary disease (COPD) constitute one of COVID-19 risk groups. Variability in predisposition and clinical response to COVID-19 exist but our understanding of these factors in the COPD population is limited. This study explored the genetic background as a possible answer to COVID-19 infection response heterogeneity, either for the poor prognosis in people with COPD or across worldwide populations. Our cohort comprises 255 people with COPD (66±9 years; 72% male; FEV1 53.01±20.31% predicted) and 243 controls (67±10 years; 80% male; FEV1 100.46±19.19% predicted) clinically characterized and genotyped using saliva samples. COVID-19 associated SNPs (susceptibility: rs286914 and rs12329760; and severity: rs657152 and rs11385942) were assessed in our cohort and in the major world populations. Allelic frequencies were used to calculate the probability of having multiple risk alleles. Polygenic risk analysis was also conducted, in our cohort, for the two mentioned phenotypes (susceptibility and severity).

No differences in genetic risk for COVID-19 susceptibility or severity were found between people with COPD and the control group (all p-values>0.01), either considering risk alleles individually, allelic combinations or polygenic risk scores. All populations, even those sharing European ancestry (Portuguese, Spanish and Italian), showed significant differences from the European (all p-values<0.0001).

Our results indicated a low genetic contribution for COVID-19 infection predisposition or worse outcomes in people with COPD. We quantified also the high genetic heterogeneity across major world populations for the same alleles, even within European subpopulations.

P12 GENETIC PROFILING OF GLIOBLASTOMA MULTIFORME AS A POTENTIAL SURVIVAL BIOMARKER: A PRELIMINARY IN SILICO ANALYSIS USING TCGA DATA

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Introduction: Glioblastoma Multiforme (GBM) is the most aggressive and common form of astrocytic tumour. It accounts for over 60% of all adult brain cancers and remains incurable, with a mean survival time of 15 months after diagnosis. The objective of this work is to assess whether there is a causal relation between patient survival time and the tumoural genetic alteration profile utilizing data from The Cancer Genome Atlas (TCGA) database.

Methodology: Patient clinical and genetic data was retrieved from the TCGA database. Survival time was used to separate the 573 selected subjects into two groups using median survival as threshold, following application of the Kaplan-Meier estimator.

Genetic data was then cross referenced with the two previously defined groups using an R algorithm, which permitted the sorting of the gene alterations by frequency in both.

CNVs that were present in at least 30% of patients in each group were selected and compared between the categories. Those with a difference in alteration frequency over 27,9% were selected for further analysis. Fisher´s exact test was then performed to identify the genes that serve as discriminators between the groups.

Results: After statistical analysis, three genes, BMI1, COMMD3_BMI1, MYO3A were selected (p-value < 0,001 in Fisher's test).

An Odds Ratio = 2,41 (95% CI = [1,45;4,01]) was calculated for each gene individually and was identical for the three, as the observed

alteration profiles were very similar. This corresponds to a 2,4 times greater likelihood that, if the gene is deleted, the patient belongs to the group with lower survival (<=430 days); and, if the gene is amplified, they belong to the longest surviving group (>430 days).

Patients with CNV deletions in these three genes survive for significant less time and those with amplifications survive for noticeably longer.

Discussion: The results confirm the observed differences between groups, with a significant association between alterations in these three genes and GBM patient survival time. This approach affirms the potential of using GBM genetic heterogeneity as a possible biomarker for patient survival, positively impacting clinical management.

P13 GENE EXPRESSION DYNAMICS OF AGING

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Introduction: Gene expression alterations occurring with aging have been described for a multitude of species, organs, and cell types. However, most of the underlying studies rely on static comparisons of mean gene expression levels between age groups, not accounting for the dynamics of gene expression throughout the lifespan. These studies also tend to disregard the pairwise relationships between gene expression profiles, which may underlie commonly altered pathways and regulatory mechanisms with age.

Methodology: To overcome these limitations, we have re-analyzed a publicly available RNA-Seq dataset (GSE132040), combining segmented regression with weighted gene correlation network analysis (WGCNA) to identify high-confidence signatures of aging in the brain, heart, liver, skeletal muscle, and pancreas of C57BL/6 mice.

Results and Discussion: Functional enrichment analysis of the overlap of genes identified in both approaches showed that immune- and inflammation-related responses are prominently altered in the brain and the liver, while in the heart and the muscle, aging affects amino and fatty acid metabolism, and tissue regeneration, respectively, which reflects an age-related global loss of tissue function. We also explored sexual dimorphism in the aging mouse transcriptome and found the liver and the muscle to have the most pronounced gender differences in gene expression throughout the lifespan, particularly in proteostasis-related pathways, which we highlight as important determinants of health and lifespan.

Acknowledgments

P14 CONTRIBUTION OF HFE AND HPSE GENES AND METHAEMOGLOBIN REDUCTASE ACTIVITY TO HEART FAILURE

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Introduction: Heart failure can be defined as a syndrome caused by a structural anomaly and/or by a committed cardiac function, which leads to an inadequate cardiac output unable to meet the metabolic necessities of the organism. We aim to understand if HFE and HPSE genes as well as methaemoglobin reductase activity, may influence the development of heart failure.

Methodology: It was performed a case-control study, in which 252 DNA samples from Portuguese individuals were analysed, 143 derived from subjects with heart failure, and 109 from healthy controls. For HPSE genotyping (rs4693608), we performed endpoint PCR analysis. A multiplex ARMS (Amplification-Refractory Mutation System) assay was used for the simultaneous detection of two HFE polymorphisms (C282Y and H63D). Reductase methaemoglobin activity was determined by spectrophotometric methods. All statistical tests were performed with IBM® SPSS® Statistics 26.0 software. Statistical significance was defined as a p-value < 0.05.

Results: Regarding the H63D polymorphism, results show the CG genotype as a risk factor [OR (95% CI) = 2.889 (1.041-8.018); p=0.042]. In what concerns HPSE gene, the GG genotype was found to have a protective effect [OR (95% CI) = 0.435 (0.193-0.982); p=0.045] while the presence of the A allele is a risk factor [OR (95% CI) = 2.297 (1.018-5.179); p=0.045]. Considering methaemoglobin reductase, its activity was lower in patients than in healthy controls (p=0.019).

Discussion: Intravenous iron supplementation is sometimes considered in heart failure treatment, emphasizing the results presented in the present study. Considering the high prevalence of heart failure in Portugal (400.000 individuals, according to Sociedade Portuguesa de Cardiologia), it is important to identify iron-related markers, since it may allow an earlier and more expert approach, which may provide better prevention and therapeutic strategies for this pathology.

P15 EXPRESSION ANALYSIS OF EPIGENETIC REGULATORS IN HUMAN PLACENTAS FROM IDIOPATHIC PREGNANCY LOSSES

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Pregnancy loss (PL) is a common event that has a strong clinical and social impact. About 10-15% of clinically recognized pregnancies terminate spontaneously. Despite the heterogeneity in the etiology, about 50% of recurrent PL remain idiopathic.1 The enzymes that catalyse DNA methylation (DNA Methyltransferases) and hydroxymethylation (TET enzymes) are crucial in the differentiation and regulation of trophoblast cell lineages.2 Therefore, dysregulation of these epigenetic regulators might underlie idiopathic PL.

A total of 61 human placental samples from PL were studied. Sixteen of these samples were obtained from first trimester PL (5-12 gestation weeks (GW); 3 controls vs 13 cases); 27 placentas were obtained from second trimester PL (13-24 GW; 14 controls vs 13 cases) and 18 placentas from PL of third trimester (25-40 GW; 8 controls vs 10 cases). Cases were selected from idiopathic PL and controls were selected from PL due to infections or voluntary termination of pregnancy or maternal anatomical conditions. Transcript levels of DNMT1, DNMT3A, DNMT3B, TET1, TET2 and TET3 were analyzed by RT-qPCR. Additionally, transcriptomic analysis of 8 placental samples (4 cases and 4 controls) of each trimester is currently being performed.

In the first trimester, we observed a significant upregulation of DNMT3B and an upregulation of DNMT1 in idiopathic PL. In the second trimester, an increase of TET2 and TET3 expression was observed. Regarding the third trimester, no significant differences were observed between the groups.

We performed expression analysis of six epigenetic regulators in placentas from idiopathic PL throughout the three trimesters of gestation. We identified that DNMTs and TETs are expressed in the three trimesters of gestation. We also observed an upregulation of DNMT3B, TET2 and TET3 in cases; in the future, it will be important to analyse protein levels to validate the observed changes.

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P16 MUTATIONS IN NEUROTRANSMITTER AND SYNAPTIC GENES AND BIOLOGICAL NETWORKS AFFECTED IN AUTISM SPECTRUM DISORDER

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Introduction: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by communication deficits and repetitive behavioral patterns, and an important genetic component. There is strong evidence indicating that neurotransmission and synaptic processes are altered in ASD. Our objective was to identify ultra-rare variants in neurotransmission and synaptic (NS) genes that play a role in ASD, linking mutations to the biological pathways affected.

Methods: We defined 1216 NS candidate genes by filtering for 'neurotransmitter' and 'synapse' in Gene Ontology, Reactome and KEGG; we overlapped the gene list obtained with the databases SynaptomeDB and SynSysNet, and with a gene list obtained from a literature review. We searched for ultra-rare loss of function Single Nucleotide Variants (MAF<0,1%) in these genes in the Whole Exome Sequencing dataset from the Autism Sequencing Consortium (N=3938 cases) and in gnomAD (N=60146 controls; MAF<0,1%). We constructed a network of protein-protein interactions using the genes affected, and communities were identified applying the Leiden algorithm.

Results: We identified 357 variants in 208 genes, defining 7 network communities: Cytochrome P450 metabolism; Ion channel activity; Chemical synapse transmission; Neuronal development; G protein-coupled receptors; Energy metabolism and Neurotransmitter release cycle. The neuronal development community (27,6% of cases; 54 genes), the Cytochrome P450 metabolism community (18,8% of cases; 33 genes) and the G protein-coupled receptors community (16,1% of cases; 32 genes), are the biological communities with the highest number of ASD cases affected.

Discussion: The biological processes affected are in pathways more specific from the synapse as the neuronal development or the neurotransmitter release cycle, in combination with general cellular processes associated with Cytochrome P450 metabolism or ion channel activity. The majority of the cases have mutations in genes involved in neuronal development, supporting the relevance of this pathway in ASD. Our study reinforces the synaptic and neurotransmitter pathway hypothesis as one of the disease-relevant genetic drivers for ASD.

P17 THE HUMAN TRANSCRIPTOMIC LANDSCAPE OF OOCYTES AND CUMULUS CELLS DURING OOCYTE MATURATION

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Reproductive success is controlled by several complex and coordinated processes that occur during oogenesis. The developing oocyte undergoes transcriptional and epigenetic alterations during oocyte maturation that

will culminate in a mature metaphase II oocyte. Here we addressed these mechanisms by analysing human oocytes in different stages - germinal vesicle (GV), metaphase I (MI) and metaphase II (MII) stages - and cumulus cells (CC). Samples were obtained from surplus materials of oocyte donations, after donor's informed consent and FMUP/HSJ Ethics Committee approval. We analysed epigenetic regulators (TET and DNMT) expression in GV, MI and MII oocytes and CC by RT-qPCR. We also performed immunostaining for TET2 and TET3 proteins in GV oocytes. A preliminary transcriptomic analysis of GV and MII oocytes and cumulus cells was also performed by Smart-Seq in Genomics Unit at IGC, Lisbon.

Our RT-qPCR data showed the presence of TETs and DNMTs transcripts in oocytes from the three maturation stages, with TET3 and DNMT1 expression being most abundant and TET1 almost undetectable. We observed that transcript levels were stable during oocyte maturation, from GV to MII oocyte; additionally, immunostaining showed that TET2 and TET3 are present in the cytoplasm of GV oocytes. Comparison between germinal and somatic cells revealed a significant increase in transcript levels of DNMT1 and TET3 in MII oocytes comparing to the respective cumulus cells.

Preliminary analysis of the transcriptomic data allowed to identify highly expressed genes in GV and MII oocyte stages, with gene ontology analysis revealing association with translation process and the cell cycle, respectively.

We here provide novel insights into the epigenetic and transcriptional regulation of human oogenesis. A better understanding of oocyte maturation and the interaction with surrounding cumulus cells, including their transcriptomic signature and epigenetic features, will potentially contribute to improve fertility treatments.

P18 AUTISM SPECTRUM DISORDER: CONTRIBUTION OF GENETIC VARIANTS INVOLVED IN THE NONSENSE-MEDIATED MRNA DECAY

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Introduction: Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by impaired social/communication skills and stereotyped/repetitive behaviors. Genetic factors account for 50-80% of the familial risk of ASD, but genetic determinants are not fully understood and a role for regulatory processes is plausible. In this study, we explored the contribution to ASD etiology of genes involved in an important post-transcriptional regulatory mechanism implicated in neurodevelopment, the Nonsense-Mediated Decay (NMD).

Methods: We first compiled a group of 46 genes encoding NMD factors and regulators. In these genes we searched for Single Nucleotide Variants (SNVs) and Copy Number Variants (CNVs) in two samples of ASD patients (N=1828 and N=3570, respectively). We observed the frequency of these variants in 60146 controls from gnomAD v2.1.1 (for SNVs) and in 10355 controls from the Database of Genomic Variant (for CNVs). In genes with rare variants (MAF<1% in controls) predicted to be pathogenic in silico, we further investigated whether these variants affect protein domains required for NMD.

Results: We identified 270 predicted pathogenic SNVs within 38 genes in 524 ASD patients (28.7% of the total ASD cases) and 38 CNVs located in 18 genes in 38 ASD patients (1% of the ASD cases). Five of these genes, RBM8A, UPF2, FMR1, SMG6 and EIF4G1, were previously associated with ASD. We found that 136 variants (122 SNVs and 11 CNVs), in 23 genes, were located within known protein domainsrequired for NMD. These variants, identified in 258 ASD patients, may affect proper NMD function and consequently contribute to changes in the expression of NMD targets.

Discussion: In this study we identified genetic variants that may affect NMD function in ASD patients. Since most NMD targets encode proteins expressed in the brain, we hypothesize that NMD impairment can constitute a risk factor to ASD pathophysiology. Further studies are needed to better understand the impact of these genetic variants on NMD function and their relevance for ASD.A full understanding of these regulatory mechanisms may constitute an opportunity for the development of therapeutic interventions.

P19 REPORTING OF SECONDARY FINDINGS IN CLINICAL GENOMIC SEQUENCING: NATIONAL GUIDELINES ARE REQUIRED

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Introduction: The rapid and growing integration of exome and genome sequencing into clinical genetic diagnosis raises awareness regarding the identification of variants of potential clinical value unrelated to the primary reason for testing (secondary findings, SF). SF pose major challenges, as multiple issues (medical, legal, ethical, economic) and different contexts (e.g. paediatric and prenatal diagnosis, patient and family management, research in rare diseases) must be considered, highlighting the importance to promote standardized reporting of SF. We aim to bring to the consideration of the Portuguese Society of Human Genetics (SPGH) the urgent need for issuing national guidelines for reporting SF from clinical sequencing.

Methodology: Consultation and review of guidelines for reporting SF in clinical exome and genome sequencing from different organizations, focusing on the ones issued by the American College of Medical Genetics and Genomics (ACMG)1, the European Society of Human Genetics (ESHG)2 and the French Society of Predictive and Personalized Medicine (SFMPP)3.

Results: The ACMG recently published SF v3.0 list4 includes 73 clinically actionable genes mainly related to cancer and cardiovascular phenotypes, for which causal SF should be reported unless patients opted out. The ESHG recommends a more cautious approach, stating that genomic analysis should be as targeted as possible for the time being because a broader analysis raises complex issues in clinical practice. The SFMPP restricts its guidelines for reporting pathogenic SF to a list of 36 actionable cancer genes, requiring a double consent from the patient.

Discussion: Consideringthe diversity of approaches and the complexity involved in reporting SF, we propose that the SPGH should promote the creation of a multidisciplinary workgroup involving all the stakeholders to put forth official national guidelines for reporting SF in clinical sequencing.

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P20 EPIGENETIC ANALYSIS OF THE CGRP PATHWAY GENES INVOLVED IN MIGRAINE

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Introduction: Migraine is a complex debilitating neurovascular disorder that affects ~1 billion people worldwide, mainly females. It is characterized by attacks of moderate to severe headache pain and associated symptoms.

Calcitonin Gene Related Peptide (CGRP) is often implicated in migraine and is widely expressed in the trigeminal region. Its receptor consists of two proteins: calcitonin receptor-like receptor (CLR) and receptor activity modifying protein (RAMP1).

Epigenetic processes, such as DNA methylation, have been shown to play a contributing role in many diseases. DNA methylation occurs mostly at cytosine residues in CpG dinucleotides in the gene promoter and controls gene expression by recruiting involved proteins or impeding the binding of transcription factors to DNA.

Methodology: So far, we have investigated the methylation state of the RAMP1 promoter in 39 women blood DNA samples: 11 diagnosed with migraine and 28 controls.

We treated DNA with sodium bisulfite, converting unmethylated cytosine to uracil while methylated cytosines remain intact. Next, PCR was performed to amplify the RAMP1 promoter followed by Sanger Sequencing analysis.

Results: We identified the methylation of 5 CpG islands. We found that 64% of migraneurs have at least one CpG site methylated, showing generally higher levels of promoter methylation than controls.

Controls tend to have the -334 CpG unit hypermethylated when compared to migraneurs.

Discussion: Only few studies, relying on small sample sizes, analyzed the methylation of the RAMP1 promoter in migraine's context. To strengthen our findings, we will broaden this study to 100 samples. We will also explore methylation levels of other genes involved in the CGRP pathway. Ultimately, we hope to find epigenetic biomarkers to predict female migraine risk in an accessible body fluid.

P21   ANALYSIS OF EPIGENETIC REGULATORS IN SPERMATOZOA FROM INFERTILE PATIENTS

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Infertility affects around 15% of couples worldwide, with male infertility accounting for 20-30 % of the cases [1]. Male infertility has been associated with epigenetic defects, namely in DNA methylation, which is regulated by DNA methyltransferases (DNMT). DNA demethylation occurs during epigenetic reprogramming and involves intermediates, such as 5-hydroxymethylcytosine (5hmC), a product of oxidation of 5-methylcytosine (5mC) by TET enzymes. 5hmC has been linked with active gene transcription in spermatogenesis [2]. The aim of this study was to assess the expression levels of epigenetic regulators in spermatozoa from infertile patients, with oligozoospermia (OZ; decreased sperm counts), comparing to normozoospermic (NZ) controls. Quantitative Real-Time PCR was performed in samples from 13 OZ and 12 NZ men to evaluate transcript levels of 6 epigenetics regulators (DNMT1, DNMT3A, DNMT3B, TET1, TET2 and TET3). Additionally, protein expression was analysed by immunostaining together with analysis of 5mC and 5hmC DNA modifications. DNMT1 and DNMT3A transcript levels were significantly lower in OZ samples, in accordance with a decrease in positive stained cells. DNMT3B and TET3 also showed a smaller decrease in mRNA levels, with a concomitant decrease in DNMT3B-positive cells. No changes were observed in TET1 and TET2 mRNA and protein expression as well as in the number of 5hmC-positive cells. However, 5mC-positive cells were increased in OZ samples. Although preliminary, these results support the association of epigenetic deregulation with male infertility and warrant further investigation to assess their role in the spermatogenic process and implications for successful fertility treatments.

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P22   CAN CELL-TYPE SPECIFIC VARIABILITY BE INVOLVED IN A RARE VARIANT OF UNVERRICHT-LUNDBORG? INVESTIGATION WITH IPSC GENERATED MODELS

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Homozygosity for a private synonymous mutation in the cystatin-B gene (CSTB, MIM:601145; c.66G>A; p.Q22Q) was detected in a Portuguese patient with a rare, atypical form of Unverricht-Lundborg disease (ULD, MIM #254800). This apparently silent mutation leads to mis-splicing of CSTB pre-mRNA where a normal and an abnormal transcript were detected. The abnormal transcript, with a 354 bp inclusion from intron 1, due to the activation of a cryptic 5′splicing site, is expected to result in the production of an altered peptide with a premature truncation. In the patient's fibroblasts, cystatin B protein has nuclear location and presents a shift from lysosomal distribution. The abnormal transcript might be promoting aggregate formation leading to a toxic gain of function and being involved in the mis-localization of the protein. The use of derived cells from induced Pluripotent Stem Cells (iPSC) can be used as tools for modelling biological processes, particularly in the case of cell types that are difficult to obtain, such as in neurologic diseases like ULD.

Given our experience with ULD [1, 2], the effective iPSC skills [3, 4] and bearing in mind that the splicing process may vary among different tissues [5] we propose a new approach to: A) generate iPSCs from the patient's fibroblasts using the currently established method; B) differentiate those iPSCs into neuronal cells; and C) analyse the transcripts and the protein localization in different types of cells.

Using iPSCs as a source of different cell types, we intend to clarify if the observed abnormal RNA splicing is cell-type specific, and to characterise the subsequent protein mislocalization.

In conclusion, we hope to be able to contribute to the understanding of cell-type specific implications in the pathogenesis of ULD.

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P23   PRELIMINARY CHARACTERIZATION OF LYSOSOMAL-RELATED GENES IN TWO TAY SACHS VARIANT B1 FIBROBLAST CELL LINES

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Tay Sachs disease (TSD) variant B1 is a neurodegenerative lysosomal storage disease (LSD) which, although rare, is the most frequent form of TSD in Northwestern Iberia. The mutation p.R178H (c.533G>A) associated with the TSD variant B1 leads to a mutant HexA protein with altered kinetics and reduced residual activity.

The ability to reprogram somatic cells back to a pluripotent state created new opportunities for generating models of disease-relevant cells by inducing patient cells into induced pluripotent stem cells (iPSCs). Availability of disease-relevant cell types derived from TSD variant B1 patient iPSCs will provide a model for studying the pathogenic mechanisms and testing therapeutic agents.

This work focus on the characterization of lysosomal-related genes of two TSD variant B1 patient skin fibroblast cell lines with the mutation p.R178H (obtained from an international cell biobank) prior to being reprogrammed to the pluripotent state.

We extracted and purified the genomic DNA from TSD variant B1 patient fibroblast cell lines. For library preparation and sequencing, we used an in-house customized Next Generation Sequencing (NGS) panel of around 150 genes related or not related to lysosome function.

Analyzing the NGS data, we identified some point variants (missense and small indels) in exons and their respective intronic flanking regions in the genes analyzed that were found in the respective fibroblast cell line and may represent variants of interest in these particular cases. These findings will be further explored.

When generating iPSCs in order to obtain disease-specific cell models it is important to have characterization checkpoints of the different cell lines throughout all the manipulation stages. Therefore, in the future these NGS data will be compared with data obtained by NGS analysis of TSD variant B1 patient iPSCs and of neuronal cell lines differentiated from these TSD variant B1 patient iPSCs.

This work was financed by FCT (PTDC/BIM-MEC/4762/2014) and INSA-DGH.

P24 CHARACTERIZATION OF AN ONCOGENIC ISOFORM OF TP53: Δ160P53

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The transcription factor p53 is a key cell regulator, having roles in varied cellular processes. Widely known as a tumour suppressor protein, p53 is responsible for signalling the adequate response to DNA damage, oncogenic signalling, or other stress stimuli. The target genes of this protein are involved in cell cycle arrest, senescence, apoptosis, and DNA damage response, among other pathways. Besides the full-length p53 (FLp53), to which these functions are attributed, the TP53 gene encodes for eleven other protein isoforms that result from alternative splicing, internal initiation of translation, and transcription from an internal promoter. In striking contrast to FLp53, the N-terminally truncated Δ160p53 exhibits its pro-oncogenic traits1, although it only differs from FLp53 by the lack of its first 159 amino acids2. Δ160p53 promotes cell survival, proliferation, invasion, and adhesion, and it is overexpressed in cancer cells harbouring hotspot p53 mutants. The TP53 gene is frequently mutated in cancer, and hotspot mutants result from single missense mutations that convert p53 into a driver of tumorigenesis. As Δ160p53 presents many of the oncogenic roles attributed to p53 cancer mutants, it is plausible that this isoform could be responsible for the paradoxical mutant p53 functions. However, detailed knowledge on the mode of action of Δ160p53 is still lacking. We have performed additional tests to further characterize the oncogenic traits of this isoform. To evaluate the ability of Δ160p53 to promote anchorage-independent cell growth, we have used the soft agar colony formation assay. Preliminary results show a tendency of Δ160p53 to promote growth when compared to the control and other isoforms. Our new data complements our previous knowledge on Δ160p53 and reinforces the importance of studying this isoform for therapeutic targeting.

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P25 SKEWED X-CHROMOSOME INACTIVATION IN RECURRENT PREGNANCY LOSS

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Introduction: A Recurrent Pregnancy Loss (RPL) is a disorder characterized by the loss of two or more pregnancies before 20-24 weeks of gestation, according to the European Society of Human Reproduction and Embryology (ESHRE).1,2 RPL, which affects about 2-5% of pregnant women, can be caused by genetic and epigenetic events.1,2

The X-chromosome inactivation (XCI) is an event occurring in female mammals to inactivate part or all of one of the X chromosomes during the embryonic development.2,3 Typically, it is a random phenomenon occurring to compensate the difference in the X-linked gene dosage between females and males.3 However, when the inactivation occurs preferentially in one X chromosome it is called skewed XCI and this condition has been already reported in women with RPL.3 The goal of this study was to evaluate the X-inactivation pattern in women with RPL.

Methodology: This study included 8 DNAs extracted from peripheral blood of women with RPL whose spontaneous abortions samples were received in the Genetic Unit, Department of Pathology of the Faculty of Medicine of Porto for cytogenetic study (Karyotype or array CGH). X-inactivation analysis using HUMARA method (Restriction Enzyme Digestion/Polymerase Chain Reaction/Fragment Analysis) was performed.

Results: 3 of 8 DNA samples showed a skewed X-chromosome inactivation pattern. From these, one was classified as a non-random X-inactivation (>65%) with 78.31% of inactivation and the other two as extremely non-random X-inactivation (≥85%) with 89.96% and 97.32% of inactivation.

Discussion: This study suggested that a non-random X-inactivation pattern might be more frequent in women with RPL, as previously describe in the literature. However, in the future we will need to include more RPL cases and a control group (samples including women without RPL) to confirm the results.

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P26 MTHFR GENE AND IT'S ASSOCIATION TO FIBROMYOMAS DEVELOPMENT

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Uterine fibromyomas, also known as leiomyomas or fibroids, are benign smooth muscle tumors of the uterus, the most frequent benign tumors at the reproductive age. MTHFR gene is involved in the modulation of several events such as angiogenesis, apoptosis, cell cycle, DNA methylation, that are crucial factors for tumor's biology. In this study, it was intended to elucidate the potential role of this gene in fibromyomas development by studying the influence of its promoter methylation status and the C667T (rs1801133) variant in the disease. Results show lower promoter methylation in fibromyoma and an association of the CC genotype with the disease. Both results point to the importance of a higher activity of the enzyme for fibromyoma development. Higher MTHFR activity is associated with lower homocysteine levels, a precursor of glutathione, and an important molecule for oxidative stress response. Reactive oxygen species are well known tumorigenic molecules since they increase cell proliferation and survival.

Clinical Research

P27 MONOALLELIC EXPRESSION OF BREAST CANCER DRIVER GENES REVEALS FUNCTIONAL PASSENGER MUTATIONS

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Introduction: The traditional model of cancer development posits that a small number of mutations drive tumor progression, while thousands of other co-occurring mutations are thought to be passengers with little impact. However, this dichotomous classification generally does not take into account the expression level of these mutations, leading to the potential misclassification of non-expressed mutations as cancer drivers.

Methodology: Using TCGA breast cancer data, we integrated whole-exome sequencing with allele-specific gene expression of heterozygous coding mutations, gathering a dataset with 5,151 events from a cohort of 628 patients. Next, we searched for monoallelic expression of the wild-type allele, followed by the characterization of the mutational landscape and gene functions.

Results: We found that non-expressed mutations are common in breast cancer (6.4% of all heterozygous mutations), even when known imprinted genes are removed. Missense and synonymous mutations are the most commonly found consequences. They are present in driver genes and oncogenes, in breast cancer stem cell signature genes, in other non-cancer related genes, and tumor suppressors, where their role (as functional passenger or driver) depends on mutation consequence. Functionally, these mutations fall on genes enriched for transmembrane receptors, transcriptional regulation, cytoskeleton organization, adhesion, and metabolic regulation.

Discussion: The frequency of transcriptionally silenced mutations in breast cancer suggests that the expression of these alleles is either genetic or epigenetically cis-regulated, most commonly via mutations in upstream cis-regulatory elements of the mutated allele, or by imprinting or other epigenetic silencing. Further studies will be conducted to map these mutations to their respective regulatory mechanisms. Until then, this new class of mutations highlights the clinical relevance of using expression data to improve therapeutic choice by avoiding the usage of drugs targeting non-expressed mutations.

P28 FAMILIAL HYPERCHOLESTEROLEMIA - MONOGENIC, POLYGENIC OR BOTH?

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Introduction: Familial Hypercholesterolemia (FH) is a monogenic, common autosomal disorder of lipid metabolism. Genetic diagnosis includes the study of 3 genes: LDLR, APOB, PCSK9. About 50-60% of clinical FH patients present a negative result for these genes and their phenotype can be explained by a polygenic contribution or be due to other monogenic causes. The present work aims to determine the genetic cause (monogenic or polygenic) of hypercholesterolemia in clinical FH patients.

Methodology: A NGS panel was implemented including 8 genes (LDLR, APOB, PCSK9, LDLRAP1, APOE, LIPA, ABCG5, ABCG8) and 6-SNPs determining the genetic risk score (GRS). High polygenic risk score (PRS) was considered for GRS≥0.76. Variants were classified according to American College of Medical Genetics and Genomics (ACMG) guidelines or gene specific specifications.

Results: Using this screening approach 161 index cases (IC) with clinical FH diagnosis were analysed and 59 IC (30 children + 29 adults) were identified with a pathogenic or likely pathogenic (P/LP) variant (ACMG classification): 58 IC have heterozygous FH (56 with an LDLR variant and 2 with a PCSK9 variant) and 1 IC has homozygous FH (2 LDLR variants). Putative pathogenic variants were identified in heterozygosity in genes associated with recessive dyslipidemia (1 in APOE, 2 in ABCG5 and 2 in ABCG8). No other P/LP variants were identified in the remaining IC; however, several variants of unknown significance (VUS) were found in APOB (n=24). Among the 97 FH negative IC, 48 have a GRS≥0.76 and 15 have a GRS<0.51. In the FH positive IC 14 also have a GRS≥0.76.

Discussion: A causative FH variant was identified in 37% of the clinical FH patients. Additionally, 5 IC (3%) have putative pathogenic variants in other genes that can contribute to their phenotype. Among the FH negative IC 50% have a high PRS and 16% have a low PRS, highlighting that they can have an unidentified monogenic cause. The identification of the genetic status (monogenic or polygenic) of an individual with FH phenotype may have implications in their risk stratification, cascade screening of relatives, disease management and therapeutic measures.

P29 GENOTYPE, PHENOTYPE AND CLINICAL FOLLOW-UP OF A MULTICENTRIC COHORT OF PATIENTS WITH PTEN HAMARTOMA TUMOR SYNDROME

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Introduction: PTEN hamartoma tumor syndrome (PHTS) is caused by PTEN haploinsufficiency. Phenotype includes macrocephaly, neurodevelopmental disorders (NDD), cancer susceptibility, and skin alterations. Given PHTS´s broad-spectrum, patients might be followed in different medical settings. To improve diagnosis and follow-up, we aim to characterize a cohort of Portuguese PHTS patients.

Methods: PHTS patients' data from three Portuguese Medical Genetics Departments were reviewed. Cleveland Clinic PHTS risk score and NCCN diagnostic fit were computed.

Results: Twenty-four patients from 17 families were included in our study, including nine adults. All patients had a pathogenic/likely pathogenic variant in PTEN, with 11 families presenting a frameshift type. Age of clinical and molecular diagnosis showed a bimodal distribution with a median age of 8 years. Common phenotypic features were macrocephaly, present in all patients, benign thyroid disease (n=10), and lipomas (n=6). Pathognomic PTHS features were not frequent, such as Lhermitte-Duclos disease (n=1), trichilemmomas (n=2), and papilomas (n=2). Nine patients present NDD. Two adult patients presented early-onset cancer: one male with papillary thyroid cancer and one female with papillary thyroid and breast cancer. Adult patients were integrated into screening for PTHS-associated cancers, but the physician responsible for this screening varies, with 11 patients being followed by a non-oncologist hospital specialist, five by Oncology, three by their family doctor and other hospital specialists, and one patient only by a family doctor. PHTS risk scores were highly variable in our cohort with confirmed cases presenting between 1% and 99% of risk. In patients with low-risk scores, positive family history elicited genetic investigation. Three adult patients with confirmed disease did not fit clinical NCCN criteria for PHTS.

Conclusions: PHTS diagnosis remains a challenge due to its clinical broad-spectrum, with few patients presenting pathognomonic features. PHTS diagnosis should be considered in first-degree family members, even if clinical suspicion is low. Further studies are needed on follow-up optimization.

P30 TO REPORT OR NOT TO REPORT? – 15Q11.2 (BP1-BP2) RECURRENT MICRODELETION

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Introduction: 15q11.2 microdeletion (BP1-BP2), including NIPA1, NIPA2, CYIP1 and TUBGCP5 genes, is assigned as a susceptibility locus for neurodevelopmental disorders (NDDs) with low penetrance and variable unspecific clinical expression. Recent studies indicate a mild subclinical effect on neurocognitive function. The interpretation of this recurrent copy number variation (CNV) can be particularly challenging, leading to high report variability. The aim of this work was to gain insight into laboratory reporting policies.

Methods: We conducted a "15q11.2 microdeletion (BP1-BP2)" survey concerning clinical classification and reporting strategies in the postnatal and prenatal settings. Participants included 7 national and 4 international genetic laboratories.

Results: 8 out of 11 laboratories (73%) report the 15q11.2 CNV in the postnatal setting. Out of the remaining 3 laboratories, 1 reports it only if NDDs are present, 1 doesn't report it and 1 didn't respond. Regarding clinical classification, 3 classify the variant as pathogenic (P), 3 as likely pathogenic (LP), 2 as uncertain (VUS), 1 classifies it differently (LP or VUS) depending on clinical presentation and 2 didn't respond. In the prenatal setting, 7 out of 11 laboratories (64%) don't report the variant, 2 (18%) report it and 2 (18%) may report it or not based on clinical and / or informed consent criteria. Clinical classification varies among laboratories: 2 classify it as P, 1 as LP, 4 as VUS and 4 didn't respond.

Discussion: The majority of laboratories report the 15q11.2 deletion in postnatal as P or LP, while in prenatal it is mainly classified as VUS and not reported. Our data highlights a high variability and elaborate conditional reporting criteria for this CNV. This heterogeneity reinforces the undeniable need for stronger collaborations between national genetic laboratories. Establishing consensual guidelines for CNV classification and reporting in the frame of a unified strategy to address these ill-defined variants is essential to improve quality and minimize distress among patients and clinicians.

P31 KBG SYNDROME IN THE PORTUGUESE POPULATION: CLINICAL AND MOLECULAR CHARACTERIZATION OF 41 PATIENTS

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Introduction: KBG syndrome (MIM#148050) is an autosomal dominant syndromic developmental delay/intellectual disability (DD/ID) disorder caused byhaploinsufficiency variants in ANKRD11 gene, characterized by a typical facial gestalt, macrodontia and short stature. It is presumed to be a frequent and underdiagnosed aetiology of syndromic DD/ID. We present the clinical and molecular characterization of 41 Portuguese patients with KBG.

Methodology: Retrospective review of clinical and molecular data from KBG patients diagnosed in Portuguese medical genetics centres. Informed consent was obtained, and the study was approved by the Ethics Committee of the Lisbon Academic Medical Centre.

Results: We collected data from 41 patients (21 females, 20 males) from 34 families. All patients had DD/ID and learning difficulties. The typical gestalt was recognized in 76%, and in 11% Cornelia de Lange syndrome was first considered. Other common findings were 5th finger clino/brachydactyly (86%), macrodontia (79%), attention deficit hyperactivity disorder (ADHD) (63%), hearing loss (56%) and recurrent otitis media (43%). Growth delay/short stature (<3rd centile) was observed in 38%. Additional relevant phenotypic features were cryptorchidism (44%), auricular septal defects (23%) and epilepsy (16%). A total of 24 different ANKRD11 sequence variants were identified: 19 frameshift [pathogenic (P)/likely pathogenic (LP)]; 4 missense [2 (P/LP), 2 uncertain (VUS)]; and 1 affecting splicing (P). Four unrelated patients had large deletions involving ANKRD11 gene, including one case without the typical KBG phenotype. Variants were found in all but two unrelated patients, who both had the typical gestalt.

Discussion: This data is consistent with previous literature. KBG syndrome has a distinctive gestalt which is easier to recognize later in childhood when macrodontia becomes evident. DD/ID is usually mild; behaviour disorders, particularly ADHD, are common and should be addressed for better developmental outcomes. Target gene sequencing should confirm most clinical diagnoses, since the great majority is due to frameshift variants in ANKRD11.

P32 CIRCULATING CELL-FREE DNA LEVELS AND MOLECULAR PROFILING OF GLIOMA PATIENTS

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Introduction: Glioma is the most common form of central nervous system neoplasm that originates from glial cells, and account for over 70% of malignant brain tumors. This neoplasm remains among the most difficult cancers to treat and, therefore, it is essential to better understand the molecular mechanisms of this disease to optimize therapeutic approaches. This study aimed to perform a (epi)genomic characterization of tumor tissue samples from glioma patients and to evaluate plasma cell-free DNA (cfDNA) levels before and after the treatment.

Methods: A total of 20 samples of plasma were collected from 7 patients, before and at several timepoints after initiation of treatment.After cfDNA isolation, concentrations were determined, compared between patients and controls, and monitored throughout the patients' clinical course. Tumoral tissue samples were analyzed by array Comparative Genomic Hybridization (aCGH) and Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA).

Results: The quantitative analysis of cfDNA revealed that patients with glioma had higher plasma levels of cfDNA (0,571±0,574) than controls (0,303±0,092) and most of the patients revealed an increase in cfDNA levels after starting treatment, before decreasing. aCGH analysis revealed alterations in several chromosomes, mainly on chromosomes 10, 14, 17, and 19, where are mapped several key genes for carcinogenesis. MS-MLPA analysis revealed that the genes that presented most frequently gains were CDK6, CFTR, CD44, GATA5, STK11 and those that presented most frequently losses were CDKN2A, PAX5, PTCH1, CREM, KLLN, PTEN, PYCARD. The genes that were shown to be frequently methylated were ESR1, WT1, GATA5.

Discussion: More studies with larger cohort and longer follow up are needed; however, our results showed that it is possible to isolate cfDNA from plasma of glioma patients and that an integrative analysis of liquid biopsies and tumor (epi)genomic characterization seems to be crucial to better understand this neoplasm. The correlation of molecular and clinical-pathological data is vital to help in the prognosis and monitoring of glioma patients.

P33 PARKINSON DISEASE: EXPERIENCE OF TWO DECADES IN THE ANALYSIS OF PRKN GENE

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Introduction: Parkinson disease (PD) is a progressive severe neurodegenerative disorder, characterized by rest tremor, rigidity of muscles, bradykinesia, postural instability, and dementia. Although predominantly sporadic, there are PD families/patients with autosomal recessive (AR) or dominant inheritance. The most frequent autosomal dominant PD is caused by disease-causing variants in the leucine-rich repeat kinase 2 (LRRK2) gene. Biallelic variants in the gene for parkin RBR

E3 ubiquitin protein ligase (PRKN) is an important cause of AR PD, occurring in up to 50% of all early-onset cases.

Methodology: We describe the PKRN variants profile in a cohort of 524 patients with PD, tested between 2000-2020. PRKN analysis was performed by Sanger sequencing and/or MLPA, or a NGS multigene panel, according to the clinical requests.

Results: A molecular diagnosis of PRKN-related PD was established in 63 patients, with 3 additional cases associated to variants of unknown clinical significance (VUS). A single pathogenic variant in heterozygosity was also identified in 16 patients. Altogether, 29 PRKN variants have been identified: 7 missense, 3 affecting splice-sites, 2 small frameshift deletions and 1 insertion-deletion, plus 16 copy number variants (14 deletions and 2 duplications).

Overall, 25 variants were classified as pathogenic or likely-pathogenic, whereas 4 are VUS.

Discussion: The obtained diagnostic yield (12%) is quite relevant considering the high clinical and genetic heterogeneity of PD. The c.155del variant is the most frequently found variant in our cohort (65% of cases). Surprisingly, large PRKN rearrangements were also identified in a significant proportion (54%) of patients.

A definitive diagnosis of PD allows proper patient management and more precise genetic counselling of patients and families. As several gene-targeted therapies for PD have now reached the clinical trial stage (although not yet the case for PRKN-related entity), the clinical utility of genetic testing for PD has now expanded considerably.

P34 CLINICAL AND GENETIC ASPECTS OF BERNARD-SOULIER SYNDROME IN A COHORT OF EIGHT FAMILIES

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Introduction: Bernard-Soulier Syndrome (BSS) is caused by variants in genes that encode for a major platelet (PLT) membrane glycoprotein (GP) complex, GPIb/IX/V, which is the receptor for von Willebrand factor (VWF) and plays an important role in blood clotting. The complex consists of 4 subunits (GPIbα, GPIbβ, GPIX and GPV), encoded by different genes (GP1BA; GP1BB; GP9 and GP5). To date, only variants in GPIBA, GPIBB, and GP9 genes were found. Besides the classical autosomal recessive inheritance, there are reports of monoallelic BSS cases with a mild phenotype and less pronounced PLT defects.

Objective: To report the clinical and laboratory data from 8 families (19 patients) diagnosed with BSS, from our cohort of patients with inherited macrothrombocytopenia.

Material and Methods: Data collected included family history, bleeding score (BS) with BAT-ISTH, and results from laboratory tests such as PLT count, mean platelet volume (MPV), PLT function assay (PFA100), PLT agglutination with ristocetin by lumi-aggregometry, and GP quantification by flow cytometry – GPIbα (CD42b) and GPIX (CD42a). GP1BA, GP1BB and GP9 genes were screened by Sanger sequencing.

Results: From the 8 BSS families, 4 were biallelic (6 patients, all females) and 4 were monoallelic (13 patients, 7 females). Differences between biallelic and monoallelic BSS were observed in the median BS (8 in biallelic and 2 in monoallelic), as well as in median PLT counts (45x103 and 131x103, respectively), median MPV (21.9fL and 13.8fL, respectively), and median GP levels (8% for GPIb and 9% for GPIX in biallelic; 65% for GPIb and 73% for GPIX in monoallelic). Concerning the genetic defects in biallelic BSS, 2 families share the same (already described) variant in GP9 and the other 2 families had different variants in GP1BA, one of which was new. All the variants found in monoallelic BSS families were new, three in GP1BA, and one in GP1BB.

Conclusions: Using an adequate diagnostic workflow we identified 8 BSS families. Seven genetic variants were found, amongst them 5 were new. Future studies are necessary to better address the pathogenicity of the new variants.

P35 AR GENE EXON 1 CAG REPEAT LENGTH CHARACTERIZATION IN A COHORT OF INFERTILE FEMALES

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Introduction: Methylation status of the androgen receptor (AR) gene CAG tract located in exon 1, is commonly used to assess X-chromosome inactivation pattern. AR is expressed in the ovaries and normal longer CAG repeat lengths (above 24) have been associated with premature ovarian failure, poor pregnancy outcomes, and recurrent spontaneous abortions. Studies in mice indicate that AR interacts with genes required for normal folliculogenesis, but the mechanism underlying the role in female reproduction remains elusive. Aiming to correlate the AR gene with ovarian reserve markers, the number of CAG repeats was determined among females followed at our hospital center.

Methodology: CAG repeats were assessed by PCR methodology in 120 samples: 98 infertile females undergoing ICSI (mean age 34.8 ± 3.6) and 22 oocyte donors (mean age 27.1 ± 3.5). Ovarian reserve markers, namely anti-Müllerian hormone (AMH) levels and antral follicle count (AFC), were anonymously recorded.

Results: CAG number ranged from 15 to 30, mean ± SD 22 ± 2.6 (infertile females) and 14 to 27, mean ± SD 21.7 ± 2.6 (oocyte donors). Longer CAG repeats are observed in 37% of the infertile females and in only 27% of oocyte donor samples. Interestingly, 41% of the infertile females with longer CAG repeats showed levels of AMH, ACFs, or both, below reference values, whereas these markers were within normal reference values in the oocyte donor group.

Discussion: Our results support previous studies that associate longer CAG repeats with a poor ovarian reserve. Given that in males long CAG repeats are associated with hypoandrogenicity, we speculate that in females the same mechanism is responsible for a reduced androgen receptor activity, reflected in the low levels of AMH and AFCs. Despite the lack of statistical power due to the small sample size, our results seem to support the involvement of the AR gene in the normal follicular development with impact on female (in)fertility.

P36 PRENATAL DIAGNOSIS OF SKELETAL DYSPLASIAS: STATISTICS OF THE LAST 6 YEARS OF CDPN-MAC

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Introduction: Skeletal dysplasias (SD) constitute a numerous and heterogeneous group of conditions resulting from abnormalities in bone shape, size, growth and/or density. They are rare anomalies (1.6-2.4/10,000 births) and 50% are lethal. Prenatal diagnosis is mainly based on ultrasound suspicion. The absence of a family history in most cases (de novo mutations), the absence of an identifiable extrinsic cause, the phenotypic variability and the overlapping of features make specific diagnosis very difficult. Clinical evaluation by Medical Genetics and the advances in next generation sequencing (NGS) techniques have contributed to increase the diagnostic capacity.

Objectives: Analyze the results of genetic studies requested in suspected cases of SD.

Methods: Retrospective study of suspected cases of SD in which were asked molecular studies by the CDPN-MAC between March 2015 and June 2021. 29 cases were reviewed and the variables collected were: personal/family history, gestational age (GA) of suspicion, ultrasound changes, molecular study results, pregnancy outcome and postnatal evaluation.

Results: Of the 29 cases analyzed: 14 had a confirmed molecular diagnosis (5 cases of Osteogenesis imperfecta, 3 of Thanatophoric dysplasia, 1 of Diastrophic dysplasia, 1 of Achondrogenesis, 1 of Achondroplasia, 1 of Apert´s Syndrome, 1 of Bohring Opitz Syndrome, 1 Chondrodysplasia punctata), 3 had clinically relevant variants waiting for validation (family study on course), 2 have ongoing studies, 10 have a molecular study without relevant changes. Mean GA of ultrasound suspicion

was 21 weeks. Outcome of pregnancy in cases of confirmed diagnosis: 10 medical interruptions of pregnancy and 4 live births (Osteogenesis imperfecta, Diastrophic dysplasia, Achondroplasia, Chondrodysplasia punctata).

Conclusions: We highlight the need for a multidisciplinary approach to these pathologies, as well as the importance of NGS sequencing to establish a diagnosis. A correct genetic counseling is extremely important for couples to be able to make informed decisions in the current and future pregnancies.

P37 WIDENING THE SPECTRUM OF TMPRSS6 GENE PATHOGENIC VARIANTS RELATED WITH HEREDITARY IRON DEFICIENCY

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Iron-Refractory Iron-Deficiency Anemia (IRIDA) is a rare autosomal recessive hypochromic microcytic anemia derived from loss-of-function mutations in the TMPRSS6 gene, which encodes Matriptase-2, a negative regulator of hepcidin expression. IRIDA patients have high hepcidin levels that prevent iron absorption and recycling. Very few studies concerning this pathology have been carried out in the Portuguese population and its molecular basis is still largely unknown.

In this study, we aimed to identify genetic variants in TMPRSS6 in a sample of the Portuguese population with a hematological phenotype suggestive of iron deficiency. In addition, we intended to evaluate the performance of NGS for genetic screening of this large gene.

We studied 100 adults with anemia and/or microcytosis and/or hypochromia collected by the Portuguese National Health Examination Survey (INSEF). Other possible genetic causes for these abnormal phenotypes, namely α- and β-thalassemia, were discarded after HBA1, HBA2 and HBB genetic screening. The TMPRSS6 gene (18 coding regions, exon/intron boundaries and regulatory regions) was amplified in 3 long-PCR fragments that were screened by NGS using Nextera XT libraries in a MiSeq platform. The genetic variants found were validated by Sanger sequencing (transcript ENST00000676104.1).

Several known variants were identified along with two unreported mutations, c.1585T>C (p.Cys529Arg) and c.1580T>G (p.Phe-527Cys). These novel mutations were classified as pathogenic by in silico analyses through Polyphen2, SIFT, and Missense3D. Moreover, Phyre2 software was used to produce a 3D structure of the mutated proteins, based on alignments with known protein structures, as there is no 3D model for Matriptase-2 on online databases. The two novel mutations were found in heterozygosity, explaining the mild abnormal hematological phenotypes and serum iron biomarkers presented by both patients. Functional studies should be performed to validate these findings.

Our results widened the spectrum of TMPRSS6 pathogenic variants underlying hereditary iron deficiency-related pathologies. In addition, NGS revealed to be an appropriate tool for TMPRSS6 genetic screening.

P38 BRCA1 AND BRCA2 VARIANTS IDENTIFIED IN PATIENTS WITH A PERSONAL/FAMILIAL HISTORY OF HEREDITARY BREAST/OVARIAN CANCERS AND OTHER HEREDITARY CANCER SYNDROMES: CHALLENGES RELATED WITH VARIANTS OF UNCERTAIN SIGNIFICANCE

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Introduction: Screening for BRCA1 and BRCA2 variants (Vs) in patients with Hereditary Breast/Ovarian Cancer (HBOC) or other Hereditary Cancer Syndromes (HCS) is performed using next-generation sequencing (NGS), allowing detection of a high number and types of Vs. The growing use of PARP inhibitors (PARPi) in the treatment of patients with homologous recombination-deficient tumors contributes to an increasing number of patients being screened for BRCA Vs even when family history of HBOC/HCS is absent. These approaches result in a growing number of identified Vs that need to be classified. The goals of this study, apart from identifying pathogenic and likely pathogenic Vs, were to identify uncertain significance Vs (VUS) and bring to discussion their uncertainties and impact on patients and family members.

Methodology: BRCA1 and BRCA2 were analyzed in 207 patients mainly with HBOC/HCS, using TruSight® Cancer and MiSeq. Annotation was performed with Variant Interpreter, VEP, HSF, IGV, Alamut and Varsome. Vs were divided in 3 groups (G) according to allele frequency (AF) in population databases (G1: AF>5%, G2:1%≤AF≤5% and G3: AF<1%) and classified according to ACMG-AMP guidelines.

Results: In BRCA1 and BRCA2, 45 and 96 Vs were detected, respectively. While in BRCA1 G3, we detected 6 pathogenic (P) Vs and 9 VUS, in BRCA2 G3, we found 9 P Vs, 2 likely pathogenic (LP), and 15 VUS. We highlight that in G3, VUS were more frequent than P and LP Vs.

Discussion: Among G3, 28% of BRCA1 and 25% of BRCA2 Vs were VUS. VUS give rise to difficulties related to management of patients and families. Functional studies of missense or putatively affecting splicing VUS are of major importance to assess their biopathologic impact, as some of them may be hypomorphic and reclassified as P/LP. Accordingly, some VUS may have impact in therapeutic decisions (e.g. PARPi) as well as in patient's cancer-risk management protocols, including appropriate genetic counselling and VUS screening in selected family members. We predict that new challenges related to VUS will emerge.

P39 A GENETIC COUNSELLING NARRATIVE INTERVENTION GROUP AS A TOOL TO SUPPORT PATIENTS WITH HEREDITARY ATAXIAS IN PORTUGAL

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Introduction: Living with a late-onset neurological condition can pose difficulties regarding psychological adjustment along the life cycle. Genetic counselling interventions aimed to foster psychosocial support in affected or at-risk individuals for conditions such as hereditary ataxias (e.g. spinocerebellar ataxias) are very scarce. No interventions of this kind have been reported in Portugal so far, and there are only very limited examples of support interventions elsewhere. Therefore, we aimed to respond to that need by designing and implementing a service improvement online intervention for patients with hereditary ataxias.

Methodology: We adapted a group intervention of a structured narrative exercise to be delivered remotely in Portugal. In parallel, we set a study to explore, respectively, i) the experiences of people participating in the intervention, and ii) to assess the impact of the intervention in their psychological wellbeing. Data collection involved observations and a post-intervention focus group.

Results: The Portuguese Hereditary Ataxias Association advertised the narrative group intervention in social media and 13 individuals stated their interest in participating. Of these, 9 people took part in a preliminary session aimed to present the goals and nature of the intervention and allow participants and facilitators to introduce to each other. 6 of those participants attended the narrative session (2,5 hours). Participants' mean age was 48 (39-53), 3 men, with different types of

Ataxias (mostly Machado-Joseph Disease). A focus group took place 2 weeks after the narrative session aiming to collect the participants' views and experiences with the group intervention. Participants largely accepted the intervention format and reported high levels of satisfaction with the narrative session, as it allows them to know different perspectives and to gain new insights on how to cope with their genetic conditions.

Discussion: This approach has potential to be extended and adapted to other types of genetic conditions and populations and may be used as a follow-up support tool in genetic counselling protocols.

P40 SCREENING FOR THE FMR1 PREMUTATION IN INFERTILE FEMALES

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Introduction: FMR1 gene 5'UTR CGG tract expansions above 200 repeats cause fragile X syndrome (FXS), while premutations (PM, 55 to 200 CGGs) are associated with tremor/ataxia syndrome and premature ovarian failure (POI). PM frequency varies across different populations being estimated as 1/130–300 females. Convincing evidence now links mRNA gain-of-function toxicity in PM carriers with impaired ovarian function and fertility. The aim of this work was to identify and characterize FMR1 PM frequency in infertile females undergoing assisted medical procreation and oocyte donor candidates attending our centre for fertility treatment and gamete bank donation, respectively.

Methodology: 95 infertile females and 300 oocyte donor candidates were included in this study. TP-PCR methodology was used to size CGG repeats in both groups of women and AGG interruption patterns in the infertile group only.

Results: No CGG expansions were detected in oocyte donor candidates, whereas expansions within the PM range were identified in two females of the infertile cohort, corresponding to a 1/48 carrier frequency, with the following genotypes: [CGG12AGGCGG10];[CGG10AGGCGG48] and [CGG10AGGCGG9AGGCGG9];[CGG9AGGCGG9AGGCGG36]. The two patients with PM had an exacerbated response to controlled ovarian stimulation with need to postpone embryo transfer due to risk of ovarian hyperstimulation syndrome, in contrast to the poor response and risk of POI that was expected.

Discussion: Owing to the reduced sample size and recruitment bias in the infertile cohort, the carrier frequency identified should not be transposed to the general population. Nevertheless, the number of premutations identified underscores the utility of FXS testing in infertile females, besides the established oocyte donors' candidates screening. Screening of family members could enable the identification of CGG expansion carriers allowing informed decisions concerning reproductive plans.

P41 GENETIC MODULATORS OF HEMOLYTIC ANEMIA IN ANGOLAN CHILDREN WITH SICKLE CELL ANEMIA

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Sickle Cell Anemia (SCA) is a recessive genetic disease caused by the c.20A>T variant in HBB gene. It is characterised by sickled erythrocytes, chronic hemolytic anemia and vaso-occlusive events. However, these manifestations are heterogeneous due to environmental and genetic modifying factors. The aim of this study was to investigate genetic modifiers of hemolytic anemia in pediatric SCA patients living in Africa, where the disease is a severe public health problem.

The study was conducted on 200 Angolan SCA 3-12 year-old children. Thirteen polymorphic regions in genes previously associated with vascular cell adhesion (VCAM1 and CD36), vascular tonus (NOS3) or erythrocyte hemoglobinisation (HBA), were genotyped using PCR, RFLP, Gap-PCR and Sanger sequencing. Hematological and biochemical phenotypes were obtained at steady state and clinical adverse events were collected from patients' medical records.

Results revealed a high level (67,5%) of α-thalassemia co-inheritance (del. 3.7kb in HBA), which improve patients' health by delaying the onset of the disease, decreasing anemia and the number of blood transfusions. Two SNPs in CD36 (rs1984112 and rs1413661) showed impact on anemia severity. Particularly, genotypes containing the rs1413661_allele C revealed to be risk factors for severe anemia, as they were associated with lower hemoglobin levels, increased number of hospitalizations and transfusions. This is the first report associating this SNP with SCA pathology. Moreover, the rs1041163_allele C in VCAM1 was associated with lower LDH levels, inversely the rs2070744_allele C in NOS3 was associated with higher LDH levels and a higher number of hospitalizations, being a possible risk factor for increased hemolytic rate.

This study contributed to the understanding of SCA complex pathophysiology. It confirmed the positive role of α-thal., both in SCA related anemia and in its clinical manifestations. In addition, it reinforced the importance of vascular cell adhesion in hemolytic anemia variability. In this context, we propose the SNP rs1413661 in CD36 as an important novel genetic modulator of SCA in Africa.

P42 GENETIC VARIATION SPECTRUM OF ATP7B IN A COHORT OF 113 PATIENTS WITH WILSON DISEASE

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Wilson disease (WD) is an autosomal recessive disorder of the copper metabolism, caused by diallelic pathogenic variants in the copper-transporting gene, ATP7B. WD usually presents with hepatic, neurologic, and/or psychiatric disturbances. Molecular genetic testing is critical for a timely-adequate diagnosis and treatment, to prevent lifelong disabilities. This work aimed at expanding the mutational spectrum of disease-related variants in ATP7B, in a large cohort of WD patients.

Since 2004, a total of 301 patients were genotyped at CGPP, for confirmation or exclusion of WD. In the vast majority of patients, ATP7B gene was analysed by Sanger sequencing and, in patients heterozygous for one disease-causing variant (n=20), MLPA was also performed. Variants were classified according to the ACMG guidelines.

WD was genetically confirmed in 113 patients (99 families): 32 are homozygotes and 81 compound heterozygotes for pathogenic or likely-pathogenic variants. A total of 34 distinct variants (including 4 novel) and 64 different genotypes were determined. The three most common disease-causing variants were found in 75.2% of the cases, among whom 18 were homozygotes; NM_000053.3:c.3402del was the most frequent, being present in homozygosity in 6 and in heterozygosity in 19 patients.

This data expands the mutational spectrum of WD causing variants and contributes to the continuously demanding effort of interpreting variants causing WD. Interestingly, the c.3402del variant has also been reported as the most frequent in WD cohorts from Venezuela and Brazil. This contrasts with other European or Asian cohorts, where

p.His1069Gln or p.Arg778Leu, respectively, seem to be the most prevalent WD-causing alleles. Furthermore, with the emerging development of preconceptional carrier screening approaches, this data can also help design and interpretate such tests.

P43 CLINVAR LDLR VARIANT CLASSIFICATION VS FH VCEP LDLR SPECIFICATIONS

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Introduction: The American College of Medical Genetics and Genomics published the standards for interpretation of sequence variants and recently the ClinGen Familial Hypercholesterolemia (FH) variant curation expert panel (VCEP) thoroughly adapted them to the FH and LDLR context.
FH is a highly prevalent (1:250) genetic dyslipidaemia characterized by lifelong exposure to elevated low-density lipoprotein cholesterol levels and consequently high cardiovascular disease risk.
ClinVar is a public archive of genomic variation relating to human health and their clinical significance interpretations, derived from laboratory submission reports.
Methods: We classified 300 LDLR variants following FH VCEP specifications and compared them with interpretations in ClinVar. For 70 variants case level data was shared from FH VCEP associated laboratories, for the remaining variants only internal and publicly published data was considered. ClinVar classifications were extracted on 1/10/2021.
Results: The 300 LDLR variants spanned the whole gene and were 67% missense, 12% null (nonsense and frameshift), 10% splicing and 2% each in frame, synonymous, 5'UTR and large deletions.
There was concordance in classification in both methods for 158/300 variants. Of the 100 ClinVar conflicting variants, after classification with FH VCEP specification 40 became pathogenic/likely pathogenic (P/LP), 18 benign/likely benign (B/LB), 41 variants of uncertain significance (VUS) and 1 remained conflicting. Of the 14 ClinVar VUS, 2 were resolved by the new FH VCEP specification, 1 to B/LB and 1 to P/LP. Overall, 60 of the 114 unclassified variants in ClinVar (conflicting and VUS) received a definite classification (P/LP or B/LB) with the FH VCEP specification.
Discussion: With the widespread of affordable genetic diagnosis, the number of potential disease-causing variants has increased, and with it the importance of correctly determine variant pathogenicity.
Our results show that the FH VCEP LDLR-specific guidelines helped improve variant classification. This is imperative to achieve a consensus, accurate and standardized method for clinical interpretation of variants identified in FH patients worldwide.

P44 RARE DYSLIPIDAEMIAS ASSOCIATED WITH HDL CHOLESTEROL VALUES

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Introduction: Dyslipidaemia is a disorder of lipid metabolism, characterized by either an increase or decrease in lipid particles, usually associated with triglycerides (TGs), LDL cholesterol (LDL-C) or HDL cholesterol (HDL-C). Most hyperlipidaemia and HDL deficiency confer an increased cardiovascular risk while hypolipidaemia, such as abeta or hipobetalipoproteinemia, may present different manifestations ranging from poor weight progression to neurological manifestations. The aim of this study is to presents 7 index cases with rare dyslipidaemia associated with HDL cholesterol values referred to our lab.
Methods: Lipid profile was determined for each individual in an automated equipment Integra Cobas (Roche). Molecular analysis was performed by NGS with a target panel of 57 genes involved in lipid metabolism (Sure select QXT, Agilent) and samples were run in a NextSEQ Sequencer (Illumina). Only genes associated to rare forms of high and low HDL were analysed for this work, namely: ABCA1, APOA1, LCAT, CETP and SCARB1. All rare variants (MAF<1%) found in these genes were confirmed by Sanger sequencing.
Results and discussion: This study includes 7 index cases (IC), with the following clinical diagnoses: Fish Eye Disease (1), Hypoalphalipoproteinemia (1) and Hyperalphalipoproteinemia (HALP) (5). We have identified the cause of low HDL values in the 2 patients under study. One IC has a variant in ABCA1 in homozygosity causing Tangier disease and 1 IC has a compound heterozygosity in LCAT causing Fish Eye Disease. In the subjects with a clinical diagnosis of HALP only 1 heterozygous variant in CETP was found in 1 IC, in the remaining 4 IC we did not find any variants associated with HALP. In these cases the cause of high HDL values seems to be multifactorial and not due to a monogenic condition.
Conclusions: NGS proved to be a fundamental key for genetic testing of rare lipid disorders, allowing us to find the genetic cause of disease in 2 patients with low HDL. Patients with these rare conditions should be identified as early as possible in order to minimize or prevent the adverse effects of these conditions.

P45 MOLECULAR STUDY OF MODY: PRELIMINARY RESULTS FROM A SIX YEARS PROJECT

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Introduction: It is estimated that between 1 and 5% of diabetes cases result from point mutations in a single gene, originating monogenic diabetes. The most common from these are Maturity-Onset Diabetes of the Young (MODY) with 14 genes associated with it but most resulting from variants in GCK, HNF1A, HNF1B and HNF4A. These patients have distinct phenotypic and metabolic characteristics, depending on affected gene and variant found with each subtype requiring different monitoring and therapeutic strategies. These patients are also wrongly diagnosed with type 1 or type 2 diabetes and therefore an accurate genetic diagnosis is essential for adequate clinical decision. Here are presented preliminary results of this study which aims to provide clinicians with accurate diagnostic.
Methodology: Blood was collected and DNA extracted from a total of 118 individuals, 76 index patients and 42 family members. Promoter region, coding sequences and adjacent intronic regions of GCK, HNF1A, HNF1B and HNF4A were amplified and sequenced employing a cascade screening strategy. Large rearrangements studies were conducted with MLPA. In silico analysis was performed and variants classified according to ACMG recommendations.
Results: It was identified a pathogenic or probably pathogenic variant in 36% of index patients (48% in GCK, 32% in HNF1A, 16% in HNF1B and 4% in HNF4A). Additionally, 17 relatives were also diagnosed with MODY. For a total of 34% of individuals involved in this study it was possible give a definitive diagnostic of MODY.
Discussion: A probably pathogenic or pathogenic variant was detected in 36% of studied index patients. Still, in a relevant number of cases was not possible to identify a genetic etiology. Possibly this may be due to the fact that only 4 of the MODY genes were analyzed and we may have patients with variants in those other genes. However, given the high number of negative results it is unlikely that all these could have alterations in the rarer genes, making it highly probable that these are true type 1 and type 2 diabetics. In conclusion, for 44 individuals a MODY etiology was discovered and for them it is possible to adapt the clinical approach.

P46 MOLECULAR GENETIC SCREENING IN GAMETE DONORS: CARRIER PREVALENCE IN A MEDICALLY ASSISTED REPRODUCTION CENTRE

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Introduction: Genetic screening in donors aims to provide prospective parents with meaningful reproductive information, and the dominant view is that it should be focused on frequent, recessive, severe, congenital disorders of childhood. Presently in Portugal the established minimum set of molecular genetic screening tests performed in potential gamete donors comprises cystic fibrosis (CF), spinal muscular atrophy (SMA), and in women also fragile X syndrome (FXS). Other genetic disorders studied include chromosomal anomalies, thalassemia and hemoglobinopathies. The main goal of this study was to determine the prevalence of CF, SMA and FXS carriers amongst potential donors attending the collection centre in our hospital from February 2018 until September 2021.

Methodology: Results from CF, SMA and FXS screening in 144 gamete donor candidates were analysed retrospectively and anonymously. For CF, screening included 62 clinically relevant CFTR variants considered the most frequent in Europe and Iberia. Regarding SMA, the number of SMN1 copies (exons 7 and 8) was determined, as was the presence of the pathogenic variant c.770_780dup. Additionally, for FXS the [CGG] n repeat tract in the FMR1 gene was sized in female donor candidates. The prevalence of carriers for these three diseases was calculated.

Results: The majority of potential donors were women (68%). Regarding CF 3,5% of candidates (1/29) were carriers of pathogenic, CF-causing variants, namely: c.1521_1523delCTT, c.1519_1521delATC, c.350G>A, and c.3454G>C. A single SMN1 copy was found in 3,0% of candidates. In contrast, no female carriers of FXS were detected.

Discussion: This study showed that carrier status of gamete donors reflected the prevalence of CF found in the general population in Europe. A higher prevalence than previously determined in the general Portuguese population was found for SMA carriers (1/34 vs. 1/52) which may be explained, in part, by the various nationalities and ethnicities among donor candidates at our centre. The total number of women studied may have been too small to detect FXS carriers, as the premutation frequency reportedly varies between 1/151 and 1/300.

P47 BREAKING PERCEPTIONS: HOW TO EFFECTIVELY SUPPORT HEALTH PROFESSIONALS TO PARTICIPATE IN RESEARCH PROJECTS

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The community of health professionals (HP) performing research in Portugal and Europe, has been growing over the last few years. The engagement and active participation of HP in research projects led by basic scientists brings a clinical and practical perspective to basic research.

Clinical genetics is the area in which medical geneticists are responsible for diagnosing and advising on genetic diseases. They are responsible for determining the risk arising from family history, selecting cases in which further genetic investigations are needed and determining a surveillance plan and prevention measures appropriate to the risk level of disease.

These HP are in a privileged context to participate and lead research projects, due to their proximity to patients and to the opportunity to tackle clinically driven research questions. Moreover, clinical geneticists involved in research is an increasing necessity to facilitate the integration of research results in their healthcare practice and, ultimately, improve individual and community health outcomes.

Understanding how clinical geneticists perceive organizational support for research and how this influences their research engagement will shed light into this subject.

Our aim is to understand what the main enablers are and barriers for clinical geneticists successfully engage in research.

A systematic literature review allowed us to identify more than 30 enablers/barriers for the involvement of these HP in research, the most prominent of which are: time, recognition and awards, organizational support, accessible funding, consideration of research in career appraisal, training, and organizational culture.

The practical relevance of the identified variables will be assessed through a questionnaire survey, addressed to health professionals and hospital administrators in public health organizations from north to south of Portugal.

The main contribution of this project is to provide a better understanding of the impact that organizational support and management have in health professionals´ research engagement, and how to effectively support them within their clinical context to participate and/or lead research projects.

P48 ESTABLISHING AN APPROACH TO REDUCE SANGER CONFIRMATION IN NGS-BASED STUDIES

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Introduction: Clinical diagnostic testing has been revolutionized by Next-Generation Sequencing (NGS). This powerful technology has been replacing Sanger sequencing, the gold standard methodology for clinical molecular diagnosis. Currently, and given the complexity of NGS, no specific guidelines are addressing NGS quality parameters or providing concrete guidance for confirmatory analysis of NGS results. Sanger sequencing is used as a broad methodology to validate each reported variant, however, these confirmations on all NGS-detected variants not only increase the test cost, but also delay the turnaround time. Some studies propose that independent confirmations of NGS findings are unnecessarily redundant and should not be required for high-quality variants. The aim of this work is to establish internal quality thresholds, above which Sanger confirmation of NGS variants will not be necessary.

Methodology: A retrospective analysis of 500 Single Nucleotide Variants (SNVs), detected by NGS and Sanger sequenced, was performed to assess the need for further confirmation.

Results and Discussion: Sanger sequencing confirmed 99.6% of NGS variants. Of the 500 total SNVs analyzed, 483 variants had a Read Depth (RD) ≥ 20, which represents 96.60% of the variants. From these 483 variants, and taking into consideration the zygosity, quality criteria according to Call Quality (CQ) and Allelic Frequency (AF) parameters were established. Only 59 SNVs (11.8%) did not meet the filtering criteria and should, therefore, be Sanger-sequenced. In conclusion, performing Sanger sequencing of SNVs that conforms to appropriate high-quality thresholds could be noninformative and redundant. For SNVs, we will be able to reduce our Sanger confirmation workload by approximately 89%. Although Sanger confirmation of low-quality SNVs and all other variants remains necessary, this new approach will lead to a significant decrease in costs and labor.

Clinical Case Reports

P49 FAMILIAL 1P36.31P36.23 DELETION ENCOMPASSING CAMTA1 ASSOCIATED WITH VARIABLE INTELLECTUAL DISABILITY AND ATAXIA PHENOTYPE

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Introduction: Intragenic CAMTA1 rearrangements have been associated with a variety of neurobehavioral phenotypes, including developmental delay (DD)/intellectual disability (ID) and nonprogressive cerebellar ataxia (OMIM#614756). From the few families reported so far in the literature, the phenotypic spectrum appears to be very variable, with cognition ranging from normal to moderate ID, and cerebellar signs from unsteady gait to overt ataxia.

Methodology: The proband is a 9-year-old girl whowas referred to our clinic due to autism spectrum disorder (ASD) and learning difficulties. She had a history of impairment in gross and fine motor skills with unsteadiness while running. Brain MRI was normal. Her maternal half-sister had mild ID, ASD and attention deficit hyperactivity disorder (ADHD). She had a diagnosis of intrauterine CMV infection causing postlingual unilateral deafness. She also had marked impairment of gross and fine motor skills. Her brain MRI showed possibly CMV-related abnormalities, but no cerebellar involvement. Their mother had no ID or ataxia, but reported mild global DD, learning difficulties, and ADHD in childhood, plus a history of frequent falls.

Results: ArrayCGH (180K, CGX-HD, PerkinElmer) identified an interstitial 1.44 Mbp deletion in 1p36.31p36.23 (6967159_8402554; hg19) encompassing exons 4 to 23 of CAMTA1 gene. Subsequent FISH studies with a probe specific for region 1p36.23 confirmed the deletion to be maternally inherited and present also in the proband's maternal half-sister.

Discussion: This family illustrates the variable expression of the phenotype in individuals with intragenic rearrangements in CAMTA1 gene, namely regarding cognition and motor impairment. Although, to our knowledge, the deletion found in this family is larger than those previously reported in the literature (encompassing several genes downstream of CAMTA1), the phenotype fits well with the clinical manifestations described in other families, further contributing to establish CAMTA1 as a gene associated with a neurodevelopmental phenotype with variable cerebellar involvement.

P50   AP1B1-RELATED AUTOSSOMAL RECESSIVE KERATITIS-ICHTHYOSIS-DEAFNESS SYNDROME (KIDAR)

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Introduction: Autosomal recessive keratitis-ichthyosis-deafness syndrome (KIDAR MIM#242150) is a disorder caused by homozygous or compound heterozygous pathogenic loss-f-function variants in the AP1B1 gene. Only eight patients have been reported in the literature and additional descriptions are essential to further delineate the phenotype of KIDAR.

Methodology: Report of an additional case of KIDAR and comparison with other published cases with molecular characterization.

Results: We present a 14-year-old male with short stature, thin build, frontal bossing, small teeth and prominent abdomen. The skin examination was remarkable for an ichthyosis with conspicuous palmoplantar keratoderma, sparse and brittle hair with alopecia on the vertex and slight bilateral ectropion. He has no family history of the disease and has a personal history of fetal ascites, neonatal pancreatic insufficiency with consequent failure to thrive, feeding difficulties, recurrent infections and sepsis. He had congenital profound bilateral sensorineural deafness, photosensitivity and photophobia. He also had persistent mild anemia, neutropenia, thrombocytopenia, and low serum copper, ceruloplasmin and growth hormone. Brain MRI showed cerebral atrophy and thin corpus callosum and mild global developmental delay was noted. Genetic testing revealed a homozygous deletion in the AP1B1 gene, similar to two of the reported cases.

Discussion: We concluded that the phenotypes of all individuals are highly concordant and major features are enteropathy with feeding difficulties, failure to thrive, ichthyosis, palmoplantar keratoderma, sensorineural deafness and sparse and brittle hair. We report other features present in more than one patient that could be part of the phenotypic spectrum and, in case of suspicion, we suggest copy number variation analysis to be performed alongside AP1B1 sequencing.

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P51   PHENOTYPIC VARIABILITY IN GLYCOGEN STORAGE DISEASE TYPE IXA - FOUR CLINICAL CASES

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Introduction: Glycogen Storage Disease type IX (GSD IX) is caused by phosphorylase kinase (PhK) deficiency, impairing glycogen hydrolysis into glucose. One of the genes encoding the PhK molecule is PHKA2, with pathogenic variants being associated with GSD IXa, the most frequent GSD IX and the only X-linked form.

Case Reports: We present a case of a three-years-old boy, observed following 9 months of suspected symptomatic morning hypoglycemia episodes, subsiding after eating. An overnight 12h fasting investigation had been inconclusive. Similar symptoms were noticed in his ten-months-old brother, both with unremarkable physical exams. Home monitoring capillary blood glucose and beta-hydroxybutirate evidenced ketotic hypoglycemia. Molecular genetic testing identified the c.721A>G p.(Ile-241Val) variant in PHKA2 gene, in hemizygosity in both with confirmed maternal inheritance.

We report a second case of a two-years-old boy presenting at 12 months with persistent asymptomatic elevated aminotransferases. Clinically, he presented with abdominal distention, visible collateral circulation and hepatomegaly, along with speech delay. Dyslipidemia, elevated transaminases and abnormal urine organic acid profile were found. His dizygotic twin brother presented a similar clinical picture. Whole exome sequencing identified in both boys and their mother, respectively in hemizygosity and heterozygosity, the likely pathogenic c.1210C>T p.(Gln404*) variant in PHKA2 gene. Home monitoring disclosed occasional subnormal glycaemia and elevated ketones, according to the diagnosis of GSDIXa. Fasting avoidance, adequate protein intake and nocturnal maltodextrine supplement were implemented, with regression of liver dysfunction stigmata and normalization of aminotransferases and lipidemia in all patients.

Conclusion: GSDIXa is a condition with great variability both in clinical presentation and severity. It should be considered in all cases of ketotic hypoglycemia and/or hypertransaminasemia with dyslipidemia, particularly in male patients. Genetic studies are essential for diagnostic molecular confirmation and adequate therapeutic measures as well as for appropriate family counselling.

P52   PERMISSION NOT GIVEN BY THE AUTHORS

P53   EXPANDING THE MUTATIONAL SPECTRUM OF KMT2B GENE: SIX NOVEL VARIANTS AND TWO CASES WITH ATYPICAL PRESENTATIONS

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The gene lysine-specific methyltransferase 2B gene (KMT2B) encodes for a member of the SET/MLL protein family, involved in the generation of epigenetic marks, pivotal for gene activation and essential for normal development.KMT2B was initially associated with early-onset dystonia, but recently emerged as a genetic factor for more complex and syndromic presentations including neurological symptoms/signs.

We present clinical and genetic data from seven patients with KMT2B (pathogenic or likely pathogenic) variants. NGS multigene panels (based

on whole-exome sequencing) were used in six patients, whereas one was directly tested for variants in KMT2B by Sanger sequencing, according to the clinical request. Whenever possible, variant segregation analysis was performed in the patients' parents.

Two patients showed an atypical phenotype. One presented with ataxia, subtle dystonia and polyneuropathy, and harboured a novel splice-site variant (c.3334+1G>A) which abolished the splice-site, as confirmed through analysis of mRNA extracted from fibroblasts. The second, had a syndromic presentation including dysmorphic features and intellectual disability - but no dystonia –, and carried a novel frameshift variant (c.5565_5567delinsT, p.Ala1856Profs*115). In both patients, these KMT2B loss-of-function variants arose de novo and were classified as likely-pathogenic.

Among the five remaining patients, two had isolated dystonia and carried different unreported variants: a missense (p.Arg1762His) and a splice-site affecting variant (c.5198-4_5206del); both were classified as variants of unknown clinical significance (VUS); the latter occurred de novo. Three other patients had complex dystonia; one carried a frameshift (c.3800_3807del, p.Glu1267Alafs*35) and two had missense variants (p.Arg145Gln and p.Arg1777Pro); two were classified as pathogenic or likely-pathogenic, whereas the p.Arg145Gln variant was a VUS.

In summary, we expand the mutational spectrum of disease-causing variants in KMT2B with a total of six previously unreported variants. Release of these data will contribute to explore further their association with atypical and syndromic phenotypes related to this gene.

P54 A NOVEL HOMOZYGOUS VARIANT IN CDK10 – AL KAISSI SYNDROME AS AN EMERGING DISORDER

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Introduction: CDK10 is a poorly known cyclin M-dependent kinase, acting in the control of actin network architecture and ciliogenesis. Biallelic loss-of-function variants in CDK10 gene cause Al Kaissi syndrome (AKS) (OMIM#617694), a recently identified neurodevelopmental disorder with only 10 patients reported in the literature. AKS is characterized by growth retardation, global developmental delay/intellectual disability, facial dysmorphisms, and spine malformations.

Methodology: We describe a 2-year-old male, first child of non-consanguineous Nepali parents, with no relevant family history. Pregnancy was complicated by acute foetal distress (two umbilical cord loops around trunk and upper limb), leading to an emergency C-section at term. He was small for gestational age and evolved with hypotonia, feeding difficulties and failure to thrive, requiring a gastrostomy and multiple hospital admissions. Psychomotor development was moderately delayed. On physical examination, dysmorphic features (bilateral epicanthal folds, bulbous nose, thin upper lip vermilion, low-set posteriorly rotated ears), sacral dimple and axial hypotonia were noted. Previous investigation included biochemical studies, cranial, cardiac and abdominal ultrasound, and brain MRI, all with normal results. Spinal MRI showed an intradural cyst at L5-S1 level. Pathogenic copy number variations (CNVs) and Prader-Willi syndrome (PWS) were excluded by array-CGH and MS-MLPA, respectively.

Results: Whole exome sequencing with CNV analysis and mitochondrial genome sequencing detected a novel homozygous variant of uncertain significance in CDK10 gene: c.87+5G>A. Parental studies are still ongoing.

Discussion: The reported variant is predicted to disrupt the highly conserved donor splice site, was not found in controls, and is highly specific for the phenotype, which is suggestive of causality. Functional studies will be performed. Identification of a genetic aetiology is crucial for clinical management and genetic counselling. To our knowledge, this is the first patient with AKS described in Portugal, contributing to a better characterization of the phenotypic spectrum.

P55 A CASE OF TRABOULSI SYNDROME DUE TO A NOVEL SPLICE SITE VARIANT: CASE REPORT AND REVIEW OF THE LITERATURE

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Introduction: Traboulsi syndrome (TS) or Facial Dysmorphism, Lens dislocation, Anterior-segment abnormalities, and spontaneous filtering Blebs syndrome (# 601552) is a syndromic form of ectopia lens. It is characterized by ocular features (spontaneous lens (sub)luxation, subconjunctival bleb formation, shallow anterior chamber, iridocorneal adhesions, glaucoma, corneal edema, scleral thinning, and decreased visual acuity), craniofacial dysmorphisms, skeletal signs, hypermobile joints, and cardiac involvement. TS results from biallelic pathogenic variants in the ASPH gene, which encodes an enzyme that hydroxylates the aspartic acid and asparagine in epidermal growth factor-like domains of several proteins. So far, 19 cases were reported in the literature.

Case report: We report a 25-year-old male with a severe ophthalmopathy. Third child of apparent non-consanguineous healthy parents. He has a brother with similar phenotypic features. First evaluated with 8 years at an ophthalmology appointment with high myopia that progressed through the years to bilateral ectopia and subluxation of crystalline lenses and amblyopia of the left eye. At our observation he showed facial dysmorphisms, elongated face, and prominent nose with a convex nasal bridge, malar hypoplasia, malocclusion, and retrognathia. Due to persistent lumbar arthralgias, an MRI was performed but no structural changes were identified. Echocardiogram revealed a mitral valve prolapse. Exome sequencing was performed and identified a homozygous splice site variant in ASPH: c.1765-1G>A, classified as pathogenic, that explains our patient phenotype and supported the diagnosis of TS. Segregation testing is ongoing.

Conclusion: TS shows many overlapping features with other connective tissues disorders, such as Marfan syndrome, and should be considered as a differential diagnosis. Also, this case contributes to the expansion of TS genotype with a novel splice site pathogenic variant in the ASPH gene. From a practical standpoint, it provided an appropriated genetic counselling and a better surveillance to this patient and family.

P56 MATERNAL DELETION ENCOMPASSING EXONS 4 TO 7 OF THE UBE3A GENE IN A FAMILY WITH ANGELMAN SYNDROME CONFIRMED BY MLPA

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Introduction: Angelman syndrome (AS) patients are characterized by severe intellectual disability including absence of speech, dysmorphic craniofacial features, ataxia and distinctive behavioural phenotype. AS is caused by lack of expression in neurons of UBE3A gene, located in the imprinted region 15q11.2-q13. Functional loss of UBE3A can be due to 15q11.2-q13 deletions, paternal uniparental dissomy or mutations in the maternal inherited UBE3A allele. Currently, genetic diagnosis includes array Comparative Genomic Hybridization (aCGH), 15q11.2 methylation analysis and/or UBE3A sequence analysis which identify anomalies in approximately 90% of individuals. We report a family of two healthy unrelated parents with three siblings, one healthy girl and two boys with epilepsy and severe intellectual disability. The oldest affected brother was previously referred for aCGH and Methylation Specific-Multiplex Ligation-Dependent Probe Amplification (MS-MLPA) for 15q11.2-q13 region, both tests with normal result. After the birth of the youngest affected brother, a clinical revaluation of the affected brothers led to AS suspicion.

Methodology: UBE3A gene sequencing followed by MLPA confirmation using UBE3A probemix panel.

Results: The UBE3A gene sequencing revealed a 6kb intragenic deletion, encompassing exons 4 to 7 in the youngest brother. The genetic analysis in the oldest brother and in the parents, using MLPA P336-B1, confirmed the presence of the same deletion in the eldest brother and in the mother who is unaffected due to methylation of the allele with the deletion.

Discussion: UBE3A intragenic deletions are very rare but may represent an important fraction of AS patients without a genetic diagnosis. These small abnormalities might be missed by sequencing or by aCGH that is still the first diagnostic tool in neurodevelopmental disorders. In patients with AS suspicion the UBE3A intragenic exonic deletions as well as rare types of structural rearrangements that might disrupt this locus, should be tested by MLPA or by qPCR. Regarding that point mutations in UBE3A gene can be inherited from healthy mothers they may represent an underestimated cause of patients with AS.

P57   TAB2 HAPLOINSUFFICIENCY - FROM ISOLATED HEART DISEASE TO A NEWLY RECOGNIZED SYSTEMIC PATTERN

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Introduction: TAB2 (TGFβ-activated kinase 1 binding protein 2) is expressed in the endocardial cushion and plays an important role in cardiac outflow tract and valvular formation during embryonic development. TAB2 haploinsufficiency syndrome is a newly recognized disorder associated with cardiomyopathy and congenital heart defects (CHD), namely variable polyvalvular disease and cardiac outflow tract structural abnormalities. Other features include intrauterine and/or postnatal growth restriction, short stature, hypotonia, connective tissue abnormalities, facial dysmorphism and intellectual disability. We are aware of at least 72 patients reported in the literature, of whom 36 bearing microdeletions in 6q25.1 involving TAB2 gene.
Methodology: We describe a 13-year-old boy, who is the second child of non-consanguineous parents. His father and brother have a trabecular cardiac left ventricle. Uneventful pregnancy and delivery at full-term, needing ventilatory support a few hours after birth due to pneumomediastinum. Throughout the years, he presented axial hypotonia, failure to thrive, umbilical hernia, gastroesophageal reflux, recurrent respiratory infections, hyperlaxity, lower limb dysmetria, relative macrocephaly and dysmorphisms. Psychomotor development was normal and he has normal cognition. Echocardiographic assessment revealed mild mitral valve regurgitation with left ventricular (LV) hypertrabeculation progression.
Results: Solo whole exome sequencing (including NGS-CNV analysis) was performed revealing the presence of a heterozygous 566 kb pathogenic deletion of chromosomal region 6q25.1 encompassing TAB2 gene. The latter was confirmed by arrayCGH. Paternal array-CGH analysis was negative.
Discussion: Besides the cardiac phenotype, the clinical picture may include non-cardiac findings, some overlapping with Noonan syndrome. There are no significant phenotypic differences between point mutations and microdeletions, supporting TAB2 haploinsufficiency as the primary mechanism. Patients are predisposed to develop a form of cardiomyopathy with reduced systolic function and should be screened longitudinally for systolic heart failure, even in the absence of CHD.

P58   CNOT2 IS PHENOCRITICAL FOR 12Q15 MICRODELETION SYNDROME

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Introduction: Microdeletions of 12q15 region are rare. The clinical phenotype includes neurodevelopmental problems with nasal speech, facial dysmorphism and variable skeletal anomalies (OMIM#618608). Recently, haploinsufficiency of CNOT2 was associated with an autosomal dominant disorder and suggested to be paramount for the phenotype of 12q15 microdeletion. We report the third case of CNOT2 deletion, aiming to contribute to the validation of this gene as phenocritical for this syndrome.
Methods: The proband is an 8yo girl, referred to Genetics for language delay, mild intellectual disability and learning difficulties. On examination, she was obese and had minor dysmorphic features, including low-set posteriorly rotated ears, fleshy earlobes, thin straight eyebrows, hypertelorism, upslanting palpebral fissures, wide nasal base, depressed nasal bridge, smooth philtrum, bilateral brachydactyly (hands/feet), tapering fingers, unilateral transverse palmar crease and broad halluces. She also had bilateral deafness.
DNA samples were analyzed by aCGH (180K, CGX-HD).

Results: aCGH revealed a 92.35kb deletion in 12q15 (70572114_70664463; hg19), encompassing the 5'UTR and first exons of all isoforms of CNOT2 gene. Parental studies showed this deletion occurred de novo.
Discussion: CNOT2 protein is a component of the CCR4-NOT complex, involved in the regulation of gene expression. Interestingly, CNOT1 and CNOT3, encoding other subunits of this complex, have also been associated with novel developmental disorders.
Previous studies indicate that CNOT2 is the single commonly deleted gene in the minimal overlapping region in patients with large 12q15 deletions. Recently, partial heterozygous deletions of CNOT2 were described in two patients with clinical features reminiscent of 12q15 microdeletion. Moreover, a truncating variant in CNOT2 was found in a patient with characteristic facial features and developmental delay, further suggesting that haploinsufficiency of this gene is causative of the phenotype.
We found a deletion encompassing CNOT2 in a girl whose phenotype resembles 12q15 microdeletion. Our case contributes to further validate CNOT2 as phenocritical for this rare syndrome.

P59   PERMISSION NOT GIVEN BY THE AUTHORS

P60   ABCB4 PHENOTYPIC VARIABILITY: A FAMILY COHORT

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Introduction: Variants in ABCB4 gene are associated with intrahepatic cholestasis of pregnancy-3 (ICP3) and gallbladder disease-1 (GBD1) in an autosomal dominant (AD) or autosomal recessive (AR) inheritance, and with progressive familial intrahepatic cholestasis-3 (PFIC3) in AR manner. These diseases prompt cholestasis and liver disease, with ICP3 resolving postpartum. Most patients develop cholelithiasis and symptoms often recur post-surgery.
Case report: A 42-year-old woman was referred to our genetics unit with intrahepatic cholestasis and inflammation on liver biopsy. With a history of cholecystectomy at age 27, she had never been pregnant and was currently undergoing fertility treatments. Further investigation identified a 49-year-old sister with hepatic fibrosis of unknown origin and severe liver enzymes increase in 2 pregnancies, resolving post-miscarriage. Their mother had also been cholecystectomized and presented chronic gamma-GT and alkaline phosphatase elevation, with recurrent cholangitis. The father had the gallbladder removed at age 52 and had some episodic bilirubin increases.
Results: A NGS panel for intrahepatic cholestasis revealed the index as a compound heterozygote for a known pathogenic variant in ABCB4 (c.959C>T) and a rare intronic variant (c.2784-12T>C) (NM_018849.2). Parents' segregation confirmed the biallelism of the two ABCB4 variants in the daughter. A single variant in UGT1A1 (c.-41_-40dup) was also identified, inherited from the newly discovered homozygous father, linked to Gilbert syndrome (AR). The older sister had also inherited both ABCB4 variants and, as expected, was a carrier for the father's UGT1A1 mutation.
Discussion: In a direct family of 5 people, 4 had important liver and gallbladder phenotypes, all diverse from each other. The daughters, compound heterozygous for ABCB4, were more severely affected, with neither being able to bare children so far. The reason for these problems had never been uncovered and was unsettling the family. Therapy with oral UDCA is indicated and both sisters should be counseled for the risks and thoroughly accompanied if another pregnancy happens. Further family studies are highly indicated.

P61   THE IMPRINTED ODDS IN A FAMILY WITH MAGEL2 PATHOGENIC VARIANT

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Introduction: Schaaf-Yang syndrome (SYS) (OMIM 615547) is an imprinted hereditary disease caused by pathogenic variants at MAGEL2 gene.

SYS is characterized by neonatal hypotonia, feeding difficulties, global developmental delay, joint contractures and respiratory distress. A minority of cases can be particularly hazardous.

Case Report: Healthy nonconsanguineous couple, with a healthy 9-year-old girl and previous termination of pregnancy at 18th week of gestation (wog) due to fetal akinesia, cardiac and renal defects, bilateral overlapping digits, rocker-bottom feet and cystic hygroma in a male fetus. Was referred to our outpatient clinic during third pregnancy at 28th wog, due to fetal akinesia, bilateral hydronephrosis, megacystis, polyhydramnios, retrognathia, overlapping left-hand digits, rocker-bottom feet and arthrogryposis in a female fetus. At 29 wog, a stillbirth was born. Fetal whole exome sequencing analysis detected a heterozygous pathogenic variant c.1996del p. (Gln666Serfs*36) at MAGEL2, segregation studies concluded inheritance from unaffected father, establishing SYS diagnosis. Molecular study of previous fetus was requested, and the same variant was present.

Discussion: MAGEL2 is located in the Prader-Willi critical region 15q11-13. Pathogenic variants in the maternally imprinted/paternally expressed MAGEL2 gene present variable phenotypes, from mild contractures to fetal death.

The imprinted nature of this condition can phenotypically skip generations if the mutation resides on the maternal chromosome. Male carriers of a MAGEL2 pathogenic variant have a 50% chance of having an affected child.

Literature review reports 6 cases with c.1996delC pathogenic variant at MAGEL2. All cases resulted in fetal or perinatal death, due to severe arthrogryposis and fetal akinesia. These findings were consistent with our case report, and highlight the severity of this variant, bringing to attention the importance of parental testing, determination of the allelic location of the variant and the challenges for genetic counseling when imprinting disorders are present.

P62   A RARE CASE OF ACUTE MYELOID LEUKEMIA WITH DER(22)T(1;22)(Q21;P10): A CASE REPORT

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Introduction: Acute myeloid leukemia (AML) is the most common acute leukemia in adults, with an annual rate of 4.3 new cases per 100.00. Characterized by immature myeloid cell proliferation and bone marrow failure, which can be subdivided into 9–11 pathogenetically different subtypes. Cytogenetic analysis plays an important role in the study of this pathology since the detection of specific chromosomal alterations are associated to subtypes and has prognostic and therapeutic implications. The most common cytogenetics abnormalities associated with AML are t(8;21)(q22;q22), inv/t(16)(p12q22) and t(15;17)(q22;q12).

Clinical Report: The authors present a case of a 68-year-old man with anemia, leukopenia with monocytosis and adenomegaly. The patient was admitted with suspect of AML. In peripheral blood sample, classical and molecular cytogenetics analysis were required to confirm the diagnosis.

Cytogenetic analysis revealed an unbalance translocation between the long arm of chromosome 1 (q21) and the short arm of chromosome 22 (p11), in 18 metaphases, which result in trisomy of long arm (q) of chromosome 1. Fluorescent in situ hybridization (FISH) analysis for t(15;17) and inv(16) had a normal pattern.

Discussion and Conclusion: The present case had an unbalanced translocation der(22)t(1;22)(q21;p11) resulting in trisomy of 1q. This is a rare event and usually detected as a secondary chromosomal abnormality in a complex karyotype. Recent studies have shown that the gain of 1q is a poor prognostic biomarker in acute leukemia associated to worse e survival and high risk.

The patient died two weeks after diagnosis with rapidly progressive disease, confirming the previous reports that trisomy 1q is associated with a poor prognosis.

To best our acknowledgment this is the first case reported with trisomy 1q involving the unbalanced translocation der(22)t(1;22)(q21;p10) in AML patients.

The present case highlights the importance of conventional cytogenetics, as it contributes to the diagnosis, prognostic and evaluation treatment of AML.

P63   COEXISTING JAK2 AND CALR MUTATIONS IN ESSENTIAL THROMBOCYTHEMIA: A CASE REPORT

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Introduction: Essential Thrombocythemia (ET) is a classical Philadelphia-negative myeloproliferative neoplasm (MPN), primarily affecting the megakaryocytic lineage. Attempts in understanding the molecular pathogenesis of MPNs have successfully identified somatic driver mutations in the Janus Kinase (JAK2), Calreticulin (CALR), and Myeloproliferative Leukemia (MPL) virus oncogene genes. These mutations initially thought to be mutually exclusive, have been recently reported to coexist in a low number of cases.

Case Report: Here we report an 81-year-old male patient with thrombocythemia (over 800,000 platelets/µL) harboring JAK2 and CALR mutations. This is, to the best of our knowledge, literature-wise, the fourth reported ET patient with co-occurrent JAK2 V617F and CALR type 2 (K385fs*47) mutation, and the first documented case of a double mutant for such variants in Portugal.

Discussion: The high frequency of JAK2 mutations in MPNs resulted in guidelines advocating an initial screening for variants in this gene alone. Only in absence of JAK2 mutations should CALR and MPL then be tested. This algorithm, practiced in most laboratories, is clearly to blame for the underreported cases of co-existing driver mutations in ET. Moreover, because there is no documented increase in severity associated with the coexistence of JAK2 and CALR mutations, only age, elevated platelet count, and presence of the JACK2 variant propelled our patient to the high-risk tier for thrombotic events.

The implicit inference from this clinical case supports the theory that JAK2 mutations have a greater impact on disease phenotype (rather than do CALR variants). However, the known heterogeneity of MPNs, whose hallmark is the constitutive activation of the JAK-STAT pathway, shifts our concern into understanding the interaction mode of these two driver mutations, their order of occurrence, and the possible effect of allele burden on disease phenotype. New invaluable insights into MPN-related molecular mechanisms can be attained from the careful clinical characterization of patients with coexisting driver mutations such as ours. Hence the ever so importance of comprehensive testing in MPNs.

P64   HOMOZYGOUS DELETION OF THE PRODH GENE RELATED TO TYPE I HYPERPROLINEMIA

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Introduction: Hyperprolinemia is a rare metabolic disorder that results from defects in the proline dehydrogenase enzyme. Both types of hyperprolinemia are characterized by neurologic manifestations, such as intellectual disability and seizures. However, the major difference between type I and type II is that the first one results from the defects on the enzyme responsible for the conversion of proline to 1-pyrroline-5-carboxylate (PC5) and type II results from defects on the PC5. This genetically heterogeneous disorder is caused by mutations on the PRODH and ALDH4A1 genes, located on chromosomes 22q11 and 1p36 respectively. The PRODH gene is located within the critical region of the DiGeorge syndrome and chromosomal deletions near this region can lead to a neurological phenotype and cardiac manifestations.

Methodology: A woman at 20th weeks of pregnancy was referred for prenatal diagnosis due to transposition of the great vessels, whose analysis by 180K oligoarray-CGH (aCGH) was requested.

Results: The aCGH showed a 115kb homozygous deletion on the long arm of the 22q11.21 chromosome that includes 6 genes, with 3 genes reported in OMIM database. After performing the aCGH using samples collected from the parents, we conclude that the homozygous deletion was inherited from both heterozygous parents. They decided to continue with the pregnancy.

Discussion: This likely pathogenic deletion located at 22q11.21 involved the PRODH gene, which is associated with type I hyperprolinemia and epilepsy. The breakpoints also disrupted the DGCR6, DGCR5 and DGR10 genes. Deletions involving the PRODH and DGRC6 genes were detected in individuals with conotruncal heart defects (Gao et al. 2015). Jacquet et al. 2003 described a case of an individual with a similar homozygous deletion affecting the PRODH gene presenting type I hyperprolinemia, epilepsy and severe psychomotor delay.

Homozygous deletions involving these genes can have a severe impact on the fetus phenotype. Therefore, these results and their implications should be discussed and evaluated at a genetic counselling consultation.

P65 RELEVANT MARKERS FOR MOLECULAR DIAGNOSIS OF 46,XX SRY-POSITIVE AZOOSPERMIC MEN

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Introduction: Molecular screening of Y-chromosome AZF microdeletions, along with chromosome analysis, is the first step of the genetic diagnosis routinely performed in infertile men with severe oligozoospermia or non-obstructive azoospermia. These deletions can cover one or more of the three AZF regions, each one with different testicular histological phenotypes ranging from Sertoli cell only (SCO) syndrome (AZFa) to gamete maturation arrest (AZFb) and hypospermatogenesis (AZFc).

Complete AZF deletions (AZFabc) are very rare and most likely related to abnormal karyotypes such as 46,XX, 46,X,del(Y)(q11.1) or 46,X,iso(Y)(p10), causing azoospermia with SCO, therefore it is not appropriate to propose testicular sperm extraction due to the impossibility to retrieve testicular spermatozoa for intracytoplasmic sperm injection.

Methodology: Since 2000, we analysed 348 samples of azoospermic men, in compliance with EAA/EMQN best practice guidelines for Y-chromosomal microdeletions testing1, and participated annually in the EMQN-EQA programs.

Genomic DNA was analysed by multiplex-PCR amplification of STS specific for each AZF region, and SRY and AR (in Xq12) as control markers. In case of AZFabc deletions, an extension PCR-analysis was performed for DYZ3 Y-centromere marker: if present other Yq centromeric proximal border markers were screened; if DYZ3 was absent (most cases of 46,XX males SRY-positive) other Yp specific genes were screened (RPS4Y, ZFY, AMELY, PRKY) in order to characterize the Yp segment.

Results/Discussion: We characterized 10 men with AZFabc deletions, 8 of which 46,XX SRY-positive, negative for the centromere and all the AZFabc markers. In these cases, screening for heterochromatin (e.g. sY160) and markers mapping between the centromere and AZFa is inappropriate, as it is highly unlikely that these patients have Yq material between AZFa and the centromere and terminal Yq sequences. Testing for the presence of additional markers besides those used in our methodology do not have any relevance for the diagnosis nor the clinical management of these patients. In such cases, karyotyping evaluation is recommended.

1. Krausz et al 2014, Andrology; 2(1): 5–19

P66 A CASE REPORT OF ACUTE MYELOID LEUKEMIA WITH T(11;21)(Q13;Q22) DEMONSTRATING THE RELEVANCE OF CYTOGENETICS ANALYSIS

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Introduction: Acute myeloid leukemia (AML) is a complex hematological disease characterized by genetic and clinical heterogeneity. Different subtypes of AML are associated with distinct genetic and molecular abnormalities, being the cytogenetic analysis crucial for diagnosis, prognosis and follow-up of AML patients.

Material and Methods: The authors present a case of an 83-year-old man diagnosed with prostate carcinoma, chronic obstructive pulmonary disease, obstructive sleep apnea syndrome and atrial septal aneurysm. In 2021 he was admitted with severe anemia, asthenia and anorexia and the medullogram revealed 44% blasts in the bone marrow. The patient presented pancytopenia associated to myelodysplastic syndrome (MDS) and was diagnosed with AML together with high-risk genetic alterations. Conventional cytogenetic was performed. Fluorescent in situ hybridization (FISH) technique with panel probes for MDS was applied.

Results: Cytogenetic analysis revealed monosomy of chromosome 7 and a reciprocal translocation between the long arm of chromosome 11 (q13) and the long arm of chromosome 21 (q22), in 15 metaphases. FISH analysis confirmed the monosomy of chromosome 7 that was observed in 81% of the analyzed cells. FISH showed a normal pattern for chromosome 5, 8 and 20.

Discussion and Conclusion: The present case had monosomy of chromosome 7 and a translocation t(11;21)(q13;q22), detected by conventional cytogenetics. Monosomy 7 is the most frequent adverse cytogenetic feature reported in AML. Concerning to the translocation t(11;22)(q13;q22), there are only three cases described in the literature and in one case the translocation was detected in acute leukemia evolving from MDS. Recent studies identified a novel fusion gene (RUNX1-LRP16) resulting from this translocation. This provides a valuable research tool to investigate the mechanism of leukemogenesis generated by the RUNX1 fusion gene described as one of the most frequently mutated genes associated with acute leukemia. These alterations justified the patient poor prognosis in AML, proving the importance of cytogenetics analysis.

P67 PARTIAL TRISOMY 8 IN MOSAIC DETECTED IN CGH-ARRAY AND CONFIRMED BY KARYOTYPE

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Introduction: Small supernumerary marker chromosomes (sSMC) are defined as very small additional centric chromosome fragments. These are difficult to identify or characterize only by conventional cytogenetics. sSMC can lead to distinct phenotypes in individuals, ranging from normal to severe abnormalities and occur due to different degrees of mosaicism, differences in euchromatin DNA content, or uniparental disomy (UPD) of the chromosomes homologous to the marker.

Partial de novo trisomy of chromosome 8 is a rare finding and the phenotypic consequences cannot be accurately predicted.

The association between corpus callosum agenesis and mosaic partial trisomy 8 has been described before in at least one case.

Methodology: A 34-year-old pregnant woman referred for amniocentesis at 23 weeks of gestation due to fetal agenesis of corpus callosum.

Aneuploidy screening (Multiplex-PCR), CGH-array and karyotype analysis were requested and performed.

Results: Multiplex-PCR revealed absence of aneuploidies in a female fetus.

CGH-array analysis showed an increased value in the mean log ratio of 8p21.38q21.3 region, suggesting a gain in this region, in mosaic, consistent with the presence of a sSMC derived from chromosome 8.

Fetal karyotype analysis (47,XX,+mar[4]/46,XX[30]) confirmed the presence of a sSMC in mosaic, in agreement with the CGH-array finding.

Discussion: This case reinforces the importance of combining several techniques for an accurate genetic diagnosis.

Not only the genetic content of the marker chromosome, but epigenetic effects and mosaicism, are variables that need to be considered when delivering genetic counselling on a prenatal diagnosis setting, which is very challenging.

In the present case, fetal conventional karyotype helped us to confirm the genotype-phenotype correlation. It remains essential to keep expert conventional cytogenetics in a prenatal diagnosis laboratory.

P68 BECKWITH–WIEDEMANN SYNDROME – DIAGNOSTIC CHALLENGE

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Introduction: Beckwith–Wiedemann syndrome (BWS) is an imprinting disorder associating macroglossia/macrosomia, abdominal wall defects, visceromegaly and high risk of childhood tumors. Aetiology is mostly epigenetic, but CDKN1C pathogenic variants are implicated in 8% of cases, both sporadic and familial.

Case Report: First child of unrelated parents, with prenatal diagnosis of macrosomia and omphalocele. Prenatal aCGH and 11p15.5 imprinting analysis were normal. He was born at 33-week of gestation by C-section secondary to breech presentation in the setting of preterm labor. The newborn Apgar scores were 1, 3 and 6 at 1, 5 and 10 minutes respectively. He required positive pressure ventilation and intubation for recurrent apnea and was admitted to NICU. He weighed 2700g (P97), heighted 41cm (P10-50) with head circumference 30.5cm (P50). He presented bushy eyebrows, macroglossia, omphalocele, cryptorchidism and hypospadias. At admission he developed hypoglycemia and hemodynamic instability. Cardiac evaluation was normal and cUS showed extensive bilateral intraventricular hemorrhage. The continuous EEG monitoring recorded refractory multifocal seizures with migrating pattern. Molecular analysis with an epileptic encephalopathy panel identified an unknown significance variant, c.3062C>A (p.Thr1021Lys), in heterozygosity in the KCNT1 gene. It was inherited from the mother harboring the variant in homozygosity. Subsequent evaluation revealed hypothyroidism, hypocalcemia, development delay with bilateral hearing loss and cataracts. Dysmorphic features were more marked with prominent eyebrows, long eyelashes, long philtrum, macroglossia and anterior ear lobe creases. CDKN1C gene was sequenced and a pathogenic variant c.710delC (p.Pro237Leufs*35), was detected in heterozygosity, confirming the diagnosis of BWS. This variant was also maternal inherited.

Conclusion: Although clinical criteria for BWS diagnosis were present, the neurological condition deviated from the path to diagnosis. We highlight the importance of segregation studies to rule out the pathogenicity of the KCNT1 variant. The role of CDKN1C gene in developmental delay and epilepsy needs further elucidation.

P69 CASE REPORT: A RARE PARTIAL TRISOMY OF CHROMOSOME 15

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Introduction: The dup15q syndrome (15q11-q13) is a rare condition. Characterized by a wide spectrum of severity and clinical heterogeneity, being the main clinical manifestations global developmental delay, hypotonia, fetal growth restriction, and craniofacial dysmorphisms. It is more common in those with autism spectrum disorders or intellectual disabilities, with an estimated prevalence of 1 in 5,000. The authors present a rare case of partial trisomy that involves the 15p13-q15 region.

Clinical report: A 35-year-old pregnant woman was referred to amniocentesis at 21 weeks of gestation after the detection of ventriculomegaly, single umbilical artery and cardiac abnormalities. Fluorescence in situ hybridization (FISH) technique analysis of uncultured amniocytes, demonstrated normal results for the aneuploidies tested and for CATCH. The cytogenetics analysis revealed a female karyotype with one extra partial chromosome 15, confirmed by FISH with Prader-Willi probe. The couple decided to terminate the pregnancy at 23 weeks and 6 days.

Discussion: The present case had a partial trisomy of 15p13 to 15q15 region, detected in prenatal diagnosis, in a fetus with ultrasound malformations. The most common cases with partial trisomy 15, more than 50% cases described, involve the region 15q11 to 15q13 that includes the critical region for the Prader Willi/Angelman Syndromes, being usually associated with growth restriction and craniofacial dysmorphisms. Others, without euchromatic material, are generally associated with a normal phenotype. To our knowledge, this is the first case described involving this region, being the q13 to q15 involving approximately 198 genes.

All new cases detected should be reported to obtain a more precise correlation between genotype/phenotype to be used in genetic counseling.

P70 EPIGENETIC ANALYSIS OF A GANGLIOGLIOMA BY MS-MLPA - THE INCREASING IMPORTANCE OF METHYLOME PROFILING

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Introduction: Ganglioglioma (GG) is a low grade glioneuronal tumor, causing medically refractory epilepsy. Over the past decade, methylome profiling has emerged as a powerful approach to central nervous system tumor classification, identifying key genes that may serve as diagnostic and prognostic biomarkers. We aim to identify such potential biomarkers through a genetic and epigenetic analysis of a GG.

Methodology: DNA copy number alteration and methylation status were analyzed by Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MPLA) using fresh frozen post-surgical pathological brain tissue.

Results: A 27-year-old male presented his first focal motor seizure with impaired awareness. The neurological exam was normal. Brain magnetic resonance imaging revealed a right mesial temporal lesion. His seizure frequency increased despite antiepileptic treatment and two years later he underwent surgery. Two more surgeries were performed years later due to an increase of the residual tumor. Histopathological analysis, according to 2021 WHO criteria, was compatible with a GG harboring BRAF:p.V600E mutation. Copy number losses were detected in chromosomes 1p (TP73), 2p (MSH6), 3p (VHL), 10p (CREM), 11q (GSTP1), 12q (CHFR), 14q (MLH3), 16p (PYCARD), 17p (TP53), 17q (BRCA1) and 19p (STK11). Copy number gains were detected in chromosome 11p (CD44). The MGMT (58%) and CD44 (51%) genes were methylated. The patient has an Engel surgical outcome of IA at 2 years of follow-up.

Discussion: A high number of chromosomal aberrations were detected in our brain tissue sample, among which deletions dominated, reinforcing the large spectrum of complete and partial chromosomal abnormalities previously described. We found that none of the genes evaluated exhibited both copy number loss and methylation. In contrast, we observed copy number gain and methylation in CD44 gene. Expression CD44 is virtually ubiquitous amongst glioma cell lines, and glioma tumor specimens, with a lower gradient of expression amongst lower grade lesions. The methylation of MGMT in our GG tissue is concordant to previous studies, reporting that 20% of GG harbored MGMT promoter methylation.

P71 ISCN RULES IN FISH ANALYSIS: HOW TO REPORT A RARE RECOMBINANT CHROMOSOME?

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Introduction: Unbalanced chromosomes are often the result of inheritance of a balanced chromosome rearrangement from one parent. During meiotic segregation, the chromosomes with balanced alteration in the parent may ´´unbalance´´in the offspring, resulting in a partial gain and a partial loss of different regions of that chromosome.

Two peripheral blood samples, from mother and daughter, were analysed by FISH to confirm the chromosome 4 alteration detected in the daughter´s chromosome array study – dup4p16.3 and del4q35- and to confirm the pericentric inversion of chromosome 4 in the mother.

Method: Both FISH studies were performed on 10 metaphases hybridised with probes specific to the subtelomeric regions of chromosome 4 (Vysis TotelVysion).

Results: In the metaphases analysed on mother's sample, the observation of the subtelomeric regions allowed to identify that one of the chromosome 4 presents a pericentric inversion. In contrast, inthe metaphases analysed on the daughter's sample, it was observed that one of the chromosomes 4 had no FISH signal for the subtelomeric region of the long arm (4qter) and, in its place, one signal was observed for the subtelomeric region of the short arm of the same chromosome (4pter).

Discussion and Conclusion: The pericentric inversion of chromosome 4 in mother was confirmed and the result was reported: ish inv(4)(pter-qter)(D4S3359+,D4S2930+); However, the nomenclature of the daughter's result was not straightforward and there were no examples in ISCN for this specific alteration; we have than followed the available examples for other distinct cases and a formulation was achieved, presumed to describe what was observed by FISH:ish rec(4)dup(4p)inv(4)(pter+;qter-,pter+)(D4S3359+;D4S2930-,D4S3359+)dmat.

Considering that the scientific community is a place for discussion, mutual help and sharing, a proposal was submitted to ISCN team. After their internal discussion and consideration, the answers were:

ish inv(4)(pter)(D4S2930+)(qter)(D4S3359+) and

ish rec(4)dup(4p)inv(4)(pterqter)

(D4S3359+,D42930-,D4S3359+)dmat

P72 REPORT OF A RARE 16Q23.1Q23.2 INTERSTITIAL DELETION IN A GIRL WITH MULTIPLE ANOMALIES

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Partial monosomies of chromosome 16q are uncommon. The severity of the signs and symptoms depend on the size and localization of the deletion and genes content.

The 16q23 deletion is a rare genetic condition. Reported clinical features include poor growth, global developmental delays, cataracts and craniofacial anomalies.

Here we report a case of a 16-year-old girl referred to karyotyping due to short stature, overweight, hirsutism, myopia, syndactyly and scoliosis. We were recently informed that she also has mild psychomotor developmental delay, especially language difficulties, and a family history of cognitive delay, namely a sister with a similar phenotype.

The karyotype was performed. Additional chromosomal microarray analysis (CMA) was carried out using CytoScan 750k to characterize the chromosomal findings.

Karyotype revealed an interstitial deletion in region 16q23.

CMA identified a 5.36 Mb deletion at 16q23.1q23.2 - arr[GRCh37] 16q23.1q23.2(75922170_81280700)x1

This region encompasses 16 OMIM genes including MAF, associated to pathologies with an autosomal dominant inheritance.

Few cases of interstitial deletions in 16q23 are described in the literature and presentation of the clinical features shows variability.

Short stature, some degree of psychomotor developmental delay, language difficulties, eye and skeletal anomalies are usually present, as in our case. Overweight and hirsutism present in our patient have not been describe to date.

Pathogenic heterozygous mutations in MAF are causal for Aymé-Gripp syndrome (AYGRP), with features including congenital cataracts, hearing loss, intellectual disability and skeletal anomalies. Some of those features overlap the ones present in our patient. Nevertheless there are no large deletions encompassing these gene reported in AYGRP patients. It is expected that the phenotype with this deletion results from the complexity of the genetic factors involved and not just the MAF, and other genes within or outside the deleted region, or their interaction, may contribute to the observed phenotype.

Recent studies in the sister revealed a similar loss in 16q23 while parental studies are still pending.

P73 18P DELETION SYNDROME: A CASE REPORT

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Introduction: The 18p deletion is a rare syndrome with an estimated incidence of 1/50000 live births. There is a phenotypic variability associated to this syndrome, but the main clinical featuresinclude mild to moderate mental retardation, short stature, skeletal and cardiac malformations and facial dysmorphism like round face with short philtrum, palpebral ptosis and large ears with detached pinnae. Most cases are de novo deletions.

The authors presented a case of a complete deletion of 18p.

Clinical Report: 57 years old woman with intellectual disability, short stature, obesity and craniofacial dysmorphisms: brachycephaly, large arched eyebrows with lateral rarefaction, epicanthal folds, flat midface, and small mouth with thick lips. No other major health issues were referred except for hypercholesterolemia.

Cytogenetics analysis revealed a deletion of the short arm of chromosome 18, which was confirmed by a CGH, at 18p11.32p11.21.

Discussion: The present case has a deletion of almost the entire short arm of chromosome 18 (18p11.32 to 18p11.1). To our knowledge there are more than 150 cases described.

The phenotypic characteristics that the patient presented included intellectual disability, short stature and relatively nonspecific facial dysmorphisms, which are consistent with 18p deletion phenotype.

Since the clinical features vary among individual cases, phenotype-genotype correlation is still a challenge, so it is important to report every new case of a rare chromosomal alteration.

P74 2Q32-Q33 DELETION SYNDROME: A DISTINCT SUBTYPE

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Introduction: Deletion of 2q32q33 region has been proposed as a new microdeletion syndrome, also called Glass syndrome (OMIM 612313). Clinical features comprise intellectual disability, moderate to severe developmental delay, significant speech delay, growth retardation, persistent feeding difficulties, hypotonia and dysmorphic features including microretrognathia, downslanting palpebral fissures, cleft palate, and crowded teeth.

The authors present a prenatal diagnosis case with an interstitial deletion of 2q detected by conventional cytogenetics.

Clinical Report: 35-year-old pregnant woman referred to prenatal diagnosis due to ultrasound anomalies, including bilateral ventriculomegaly and retrognathia. It was the fourth pregnancy of a non-consanguineous couple with no familial or personal story of anomalies.

FISH analysis in uncultured amniocytes were normal. Cytogenetic analysis revealed an interstitial deletion in the long arm of chromosome 2, involving 2q32 to 2q33 region that were confirmed by Array Comparative Genomic Hybridization (aCGH). The segment deleted had 10.168 Mbp and comprised SATB2 and 57 more genes.

The couple elected to terminate of pregnancy.

Discussion: In the present case, conventional cytogenetics detected an interstitial deletion on chromosome 2q32-q33. The array clarifies the amount and the segment involved. The deletion includes SATB2 gene, located at 2q33.1, that has been suggested to be a candidate gene for most of the clinical phenotype in Glass syndrome.

Comparing the clinical characteristics of our patient with those described in Glass´s syndrome, they share the facial dysmorphic features but also not yet described, bilateral ventriculomegaly.

To our knowledge this is the twenty-sixth case with 2q32-q33 deletion.

P75 FREQUENCY AND TYPE OF CHROMOSOMAL ABNORMALITIES DETECTED IN INFERTILE COUPLES – CASUISTRY

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Introduction: Infertility is the inability to achieve conception or to sustain a pregnancy until birth, and it affects 8 to 12% of reproductive aged-couples [PMID: 2955531, 2018.03.012].

Chromosomal alterations in the karyotype of one member of the couple could be enough to explain their infertility.

A retrospective study is presented with a review of the frequency and type of chromosomal abnormalities detected on karyotypes made in infertile couples, without known previous history of chromosomal abnormalities.

Method: Retrospective analysis of 2078 peripheral blood karyotypes studies results from couples referred for infertility.

Results: Fifty results with alterations were detected, representing 2,4% of the karyotypes studies: 33 structural alterations corresponding to 1,6% and 17 numerical alterations, corresponding to 0,8%.

Conclusion: These results shows that the karyotype analysis allows the detection of alterations that may justify infertility of couples. Additionally, it is shown that the karyotype was indispensable in 1,6% of the cases because it detects balanced alterations with a cost efficient analysis.

P76   OUR CASUISTRY OF ARRAY CGH SINCE 2019

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Introduction: In our routine we receive cases of children with intellectual disability (ID), autism spectrum disorders (ASD), multiple congenital anomalies (MCA) and developmental delay (DD), for array CGH analysis. Array CGH allows the detection of submicroscopic imbalances (copy number variants – CNVs) including their size and gene content. In the literature, array CGH diagnostics yields in these cases varies from 8% to 20% [PMID: 20466091; PMID: 31872051; PMID: 27271878, PMID: 23765050].

We present a review of our data from 2019 to present, to determine the diagnostic yield.

Methodology: The aCGH is performed using Affymetrix Cytoscan 750K or Cytoscan HD and the results are analyzed using ChAS software.

Results: Our cohort consists of 3445 patients with the mentioned indications (ID, ASD, MCA, DD). We found pathogenic CNVs in 293 patients, corresponding to 8,5% of the cohort.

Discussion: The literature refers higher diagnostic yields, maybe because our data included less severe indications such as isolated language delay or mild learning difficulties. These indications are not highly specific and reduce the detection rate.

Array CGH continues to correspond to a significant rate of diagnosis which, along with the lower cost and turnaround time, justifies the fact that it is still so often requested.