Medicine

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ORAL PRESENTATIONS

Basics Research

OPI-1 DISRUPTION OF REGULATORY ELEMENTS IN THE CDH1 VICINITY AS A POTENTIAL HDGC CAUSE, INCREASED PENETRANCE AND EARLY ONSET CANCER

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Hereditary diffuse gastric cancer (HDGC) predisposes for diffuse gastric cancer (DGC) and lobular breast cancer (LBC). CDH1 and CTNNA1 single nucleotide variants and copy number variants (CNVs) are the main HDGC triggers, explaining <50% of cases. We used whole exome/genome sequencing (WES/WGS), CNV analysis and MLPA to identify the missing heritability in HDGC families. CRISPR-Cas9, 4C-seq, RT-PCR, flow cytometry and RNA-seq were used to characterize potential causing mechanisms in cell lines. We found and validated a 39bp CDH1-TANGO6 intergenic deletion (WGS) in a HDGC family, and a highly penetrant CDH1-TANGO6 116kb deletion (WES) in a 2nd HDGC family with extremely early onset DGC. 4C-seq showed chromatin interactions between the CDH1 promoter and the CDH1-TANGO6 intergenic region affected in both families, suggesting cross regulation between regulatory elements. We hypothesized that the intergenic CNV causes HDGC in the 1st family, and the CDH1-TANGO6 intergenic region with the CNV aggravates disease presentation in the 2nd family. By CRISPR-Cas9, we mimicked each deletion, in parallel with CDH1 or TANGO6 CNV portions independently, all in homozygosity. CDH1 mRNA downregulation relative to the wild-type, was 3.5-fold for deletion of CDH1-TANGO6, 1.5-fold for the intergenic region, 1.6-fold for CDH1 deletion alone, and unchanged for the TANGO6 deletion. Deletions of both CDH1-TANGO6 and the intergenic region induced downregulation of CDH1-associated pathways, namely cell-cell junction, cadherin binding, cell substrate junction, and mitosis and nucleosome organization pathways. Herein we identify a novel regulatory region contributing as much as the CDH1 coding region for CDH1 downregulation. Deletion of this intergenic region alone may cause HDGC, and justify the extreme phenotype in the 2nd family when the deletion extends into the CDH1 coding portion. Ongoing enhancer activity in mouse embryos will add on to these findings.

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OPI-2 DDIT4 AND TRIM13 TRANSCRIPT LEVELS ARE BLOOD-BASED BIOMARKERS OF EARLY STAGES OF MACHADO-JOSEPH DISEASE/SPINOCEREBELLAR ATAXIA TYPE 3

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Machado-Joseph disease/Spinocerebellar ataxia type 3 (MJD) is a rare late-onset polyglutamine (polyQ) neurodegenerative disease. MJD is characterized by a long preclinical phase, preceding disease onset, during which some unspecific clinical manifestations, brain abnormalities and molecular alterations are known to be already present. MJD is caused by the expansion of a polyQ-encoding CAG repeat in the ATXN3 gene encoding the ataxin-3 protein that above a pathological threshold initiates a cascade of pathogenic events, such as transcriptional impairment. Importantly, some of these molecular alterations have the potential to be used as disease biomarkers, in the context of the ongoing and emergent clinical trials for MJD. The identification of biomarkers for early stages of MJD is particularly important as current clinical scales are insufficient to rigorously measure the efficacy of disease-modifying therapeutic agents, more so in the preclinical phase. Pursuing the overarching goal to identify novel blood-based transcriptional biomarkers for MJD and building on previously reported as well as novel data from a cross-sectional blood-based whole genome microarray of MJD carriers and controls, we identified DDIT4 (down-regulated) as well as TRIM13 and P2RY13 (upregulated) as being consistently dysregulated in MJD subjects from different cohorts. Our results showed that DDIT4 and TRIM13 are reliable blood-based transcriptional biomarkers for MJD, in particular for the early stages of the disease. Additionally, their respective protein abundance was also found altered in brains of MJD patients, linking these transcriptional changes with MJD pathogenesis. Our results further show that the MJD mechanism(s) in which TRIM13 and P2RY13 proteins are involved could be preserved in the central nervous system and the periphery of patients. In conclusion, DDIT4 and TRIM13 are blood-based transcriptional biomarkers for the early stages of MJD and, thus, we anticipate that they can be tested in a clinical setup to determine their value to complement clinical scales for the accurate monitoring of disease progression and measurement of the efficacy of therapeutics in MJD carriers.

OPI-3 DAB1-ANTISENSE EXPRESSION AT THE SPINOCEREBELLAR ATAXIA TYPE 37 (SCA37) LOCUS AND DISEASE

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Introduction: Spinocerebellar ataxias (SCAs) are neurodegenerative diseases, characterized by progressive speech, gait and limb incoordination. We discovered that SCA37 is caused by a noncoding (ATTTC)n insertion into a polymorphic (ATTTT)n in a 5'-UTR intron of the neurodevelopmental gene DAB1. The RNA transcribed from this mutation is toxic, leading to loss of Purkinje cells. Many repeat expansion diseases are bidirectionally transcribed. In DAB1-antisense strand, the (AAAAT) n is in a primate-specific AluJb middle poly(A), near a CpG island and a transcription factor binding sitezs, raising the hypothesis of a transcriptional regulatory role for the SCA37 locus.
Methods and Results: We generated zebrafish transgenic lines with the DAB1-antisense repeat and its flanking region, named Fragment T (FragT), fused with a GFP sequence. This FragT was able to drive GFP expression to the muscle. After generation of transgenic lines with fragments of FragT, Frag1 (upstream the repeat), Frag2 (AluJb with repeat) and Frag3 (downstream of AluJb), only Frag1 and Frag3 retained the ability to trigger GFP expression. This suggests that in the human sequence, in DAB1-antisense strand, there are 2 promoters, one upstream of the repeat, likely driving its expression, and another downstream; however, no corresponding transcripts are annotated in databases. Thus, we assess their (1) brain expression in our transgenic lines and (2) promoter activity in human cell lines. By confocal microscopy, we observed that FragT and Frag3 were also able to drive GFP expression to the eye and cerebellar precursors (midbrain and hindbrain). By in vitro reporter assay, after transfecting human non-neuronal and neural stem cells with the promoterless luciferase vector, pGL3-Basic, empty and cloned with FragT, Frag1 or Frag3, we observed a significant effect in luciferase reporter activity for FragT and Frag3, but not for Frag1. ConclusionWe discovered a human promoter downstream the SCA37 repeat, antisense to the DAB1 strand. The promoter able to drive pathogenic repeat expression was only active upon random integration into the zebrafish genome, suggesting it requires distal regulatory elements.

OPI-4 MODELLING MACHADO-JOSEPH DISEASE IN PATIENT-DERIVED ISOGENIC LINES BY CRISPR/CAS9-MEDIATED CORRETION OF ATXN3-CAG EXPANSION

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Introduction: Machado-Joseph disease (MJD) integrates the group of polyglutamine (polyQ) disorders as the most dominant form of spinocerebellar ataxia. Caused by an abnormal expansion of the CAG trinucleotide tract within the ATXN3 gene, MJD is characterized by the formation of toxic polyQ-expanded Ataxin-3 species that interfere with numerous physiological mechanisms and culminate in neuronal loss. Patient-derived induced pluripotent stem cells (iPSC) can recapitulate MJD disease mechanisms in a physiologically relevant context but are limited by the high phenotypic variability inherent to inter-individual comparison. To address this, we established a CRISPR/Cas9 genome editing approach to integrate a non-expanded CAG tract within the endogenous ATXN3 locus by homology directed repair and thus generate isogenic iPSC lines that share an identical genetic background.
Methodology: MJD iPSC reprogrammed from patient fibroblasts were nucleofected with CRISPR/Cas9 ribonucleoprotein (RNP) targeting the CAG-encoding exon 10 followed by transduction of adeno-associated virus (AAVs) packaging a donor repair template (ATXN3-D14Q). 5 days post-treatment, ATXN3 gene correction was monitored by Sanger sequencing and validated by western blot. Selected isogenic lines were differentiated into neuronal cultures by Neurogenin overexpression to characterize MJD disease mechanisms and validate phenotypic rescue.
Results: We found high levels of ATXN3-CAG correction (25-30%) in cells treated with Cas9-RNP and AAV-ATXN3-D14Q. Single-cell screening isolated a minimum of 4 clones from two patient lines with biallelic and seamless ATXN3-CAG correction, confirmed by the absence of mutant Ataxin-3 expression. Undesirable off-target genomic modifications were not detected. Homogenous neuronal populations were successfully obtained from isogenic iPSC lines. Characterization of MJD phenotype is currently ongoing.
Discussion: In summary, our work provides novel human in vitro models of MJD to accurately investigate therapeutic interventions and establishes a precise method to correct CAG trinucleotide expansions potentially translatable for autologous cell therapy of polyQ disorders.
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OPI-5 TRNA EPITRANSCRIPTOME REPROGRAMING IN ALZHEIMER'S DISEASE CORRELATES WITH PROTEIN AGGREGATION BURDEN

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In recent years, dysregulation of protein translation is emerging as a central mechanism in the pathogenesis of neurodegenerative disorders. Neurons are particularly dependent on spatial and temporal control of mRNA translation, and disruptions in translation components have a dramatic impact in neuronal survival. One of these components are transfer RNAs (tRNAs). These molecules are essential molecular adapters that are enzymatically modified by tRNA-modifying enzymes to guarantee tRNA stability and mRNA decoding efficiency. In the last years, mutations in genes that modify tRNAs and loss of tRNA modifications have been implicated in many neurological disorders, including familial dysautonomia, amyotrophic lateral sclerosis and intellectual disability, being correlated with the observed protein synthesis impairments. Quite surprisingly however, the impact of tRNA modifications (i.e tRNA epitranscriptome) in Alzheimer's disease (AD) pathophysiology is poorly characterized. After analysis of late-onset AD (LOAD) patient datasets within the Accelerating Medicines Partnership for Alzheimer's Disease Consortium (AMP-AD), our team found that a specific tRNA modifying enzyme is differentially expressed in LOAD patient brains. Remarkably, the same enzyme is also differentially expressed in AD cellular and animal models, and its expression levels correlate with tRNA hypomodification and proteostasis impairments, suggesting a link between tRNA modification levels and amyloid pathology. Additionally, SH-SY5Y cells incubated with cellular medium obtained from SH-SY5Y cells bearing the Swedish mutation that recapitulates AD in vitro (SH-SY5Y-SWE), recapitulated the SH-SY5Y-SWE phenotype, with increased protein aggregation, decreased tRNA modifying enzyme expression and tRNA hypomodification. Taken together, our data shows that reprograming of tRNA modifications occurs in AD and that tRNA modification levels can constitute an amyloid aggregationpredictor/biomarker.
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Clinical Research

OPII-1 IMPACT OF GENOMIC MEDICINE FOR CRITICAL ILL INFANTS: A RETROSPECTIVE STUDY OF A REFERENCE CENTER IN LISBON, PORTUGAL

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Introduction: Congenital malformations and genetic disorders are a leading cause of infant morbidity and mortality in the developed world, particularly in critical ill infants.
Aim: To investigate the prevalence of genetic disease in a level IV neonatal intensive care unit (NICU) by identifying and describing genetic diagnosis, testing methodologies, timing of diagnosis, and clinical utility.
Methods: A retrospective medical review of NICU patient referred for inpatient Genetics observation from 2019 to 2021.
Results: In total 50 patients were referred to genetic evaluation. The main clinical indication for referral was multiple congenital anomalies (19/50, 38%), followed by neurological disease (12/50, 24%), isolated congenital anomaly (7/50, 14%), single system condition (7/50, 14%), and multisystem disease (5/50, 10%). In total 18 patients received a genetic diagnosis (36%) using a variety of methodologies. In 42% cases (21/50), genetic evaluation is still ongoing and 22% (11/50) were clinical discharged without a genetic diagnosis. Cytogenetic techniques were the first-tier test in 42% cases (21/50) with a diagnostic yield of 10% (2/21). Whole exome sequencing (WES) was applied in 52% (26/50), of which 73% (19/26) as a first-tier test, and 27% (7/26) as follow-up investigation. Globally, WES had a diagnostic yield of 46% (12/26). Other methodologies included: Sanger sequencing (6/50, 12%), MS-MLPA (1/50, 2%), 7-dehydrocholesterol (1/50, 2%) and PCR (1/50, 2%). The age at molecular diagnosis ranged between 36 days and 34 months. A significant majority of diagnosis were made after inpatient discharge (13/18, 72%). Additionally, 28% (5/18) infants received a genetic diagnosis during hospitalization that impacted clinical decision making. All cases received genetic counselling.
Conclusion: Genomic medicine has high diagnostic and clinical utility in critical ill patients, as it allows for an appropriate management, early decision-making, esta-blishing patient prognosis and appropriate genetic counselling. Our study provides important data for further improvements to the genetic diagnostic odyssey of critical ill patients.
Declaration of interests: No.

OPII-2 EXPANDING THE GENETICS OF HEARING LOSS: THE LARGEST PORTUGUESE COHORT AND THE FIRST IN PEDIATRIC AGE

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Introduction: Hearing loss affects around 34 million children worldwide and can contribute to language acquisition delay, thus affecting cognitive development. Genetic heterogeneity of sensorineural hearing loss (SNHL) constitutes a major challenge in the detection of disease-causing variants. Therefore, early access to a molecular genetic diagnosis is essential to optimize clinical management and to offer better genetic counselling.
Methodology: We carried out a retrospective study on a cohort of 102 pediatric probands with SNHL, followed up in the deafness consultation at the Otorhinolaryngology Department of Hospital Dona Estefânia between December/2018-January/2021. Probands had no previous genetic studies and non-genetic causes were excluded. Blood samples were sent to CIMA Lab which performed a targeted NGS panel of 180 genes related to SNHL. Our team manually curated 3036 variants based on the Expert specifications of the ACMG/AMP Variant Interpretation Guidelines and ClinGen Hearing Loss Variant Curation Expert Panel.
Results: Probands were between 9 months and 16 years of age. We identified causative variants in 17 different genes (COL2A1, COL4A5, EYA4, GJB2, GJB6, HOMER2, LOXHD1, MITF, PCDH15, MYO6, MYO7A, MYO15A, PAX3, SLC26A4, STRC, TMC1 and USH2A) in 42 (41.2%) out of 102 probands. Of these variants, 11 had never been reported in the literature. No potentially causative variants were identified in 25 probands (24.5%). In the remaining 35 probands (34.3%), further studies are ongoing to clarify potential causal variants. Of these, we would like to highlight 15 cases in which presumptive causative variants were identified in CDH23, COL4A3, GJB2, MYO6, POU4F3, SLC26A4, TBC1D24, TECTA, USH1G and WFS1. Discussion We present the results of the largest Portuguese cohort subjected to targeted sequencing for SNHL to date and also the first cohort in pediatric age. The application of NGS technologies in SNHL proved to be an effective tool for ensuring an appropriate diagnostic yield. Our study provides new insights into the variants related to SNHL in the Portuguese population and allowed the identification of novel variants, which may contribute to expand the knowledge of the genetics of hearing loss.

OPII-3 THE CONTRIBUTION OF PRS FOR SMALL VESSEL DISEASE AND AGE-RELATED DNA METHYLATION AND GENE EXPRESSION CHANGES TO COGNITIVE AGING

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Introduction: Aging is a major risk factor for small vessel disease (SVD) and subsequent cognitive and non-cognitive impairments. However, the mechanisms by which age and SVD contribute to age-related cognitive alterations remains largely unknown. Here, the aim was to investigate the impact of polygenic risk scores (PRS) for SVD and age-related DNA methylation and gene expression changes on cognitive aging. Methods This study was conducted on 421 healthy individuals, aged between 50 and 89 from northern Portugal. Participants underwent a thorough neurocognitive evaluation conducted by a team of psychologists. Genotype (NeuroConsortium array, Illumina), transcriptomic (Sureprint G3 arrays, Agilent) and methylation (EPIC array, Illumina) data was acquired. PRS were constructed using GWAS summary statistics of MRI markers of cerebral SVD (white matter hyperintensities; WMH, fractional anisotropy; FA and mean diffusivity; MD) using the PRSice-2 software. Single omics supervised multivariate analysis and multi-omic data integration was performed using the mix-omics package. Results MD-PRS were negatively associated with long-term-storage and the polygenic signal was enriched in genes involved in cardiovascular system development, regulation of neuron projections, synaptic transmission and degradation of extracellular matrix. Age-related methylation changes were enriched for ontology term related to cellular component maintenance, cell adhesion and regulation of apoptosis. Additionally, an overlap between MD-PRS and age-related methylation and transcriptional alteration that localized to genes involved in developmental and immune system process was detected.
Discussion: These findings provide insights into the understanding of the biological mechanisms underlying the association between small vessel disease and cognitive aging and highlight how age-associated DNA methylation and gene expression changes contribute to cognitive aging.
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OPII-4 WHOLE-GENOME SEQUENCING ANALYSIS TO IDENTIFY CANDIDATE HDGC GENETIC MODIFIERS FOR THE CDH1 C.1901 VARIANT

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Background: Hereditary diffuse gastric cancer (HDGC) caused by CDH1 germline pathogenic, or likely-pathogenic variants predisposes to early onset diffuse gastric (DGC) and lobular breast cancer (LBC). Intriguingly, disease penetrance is incomplete, and clinical presentation or age of onset variable, suggesting a likely role for genetic modifiers. We aim to characterize the genome of CDH1 variant carriers and distinguish affected from asymptomatic carriers in a group of 11 families carrying the Northern Portuguese founder CDH1 c.1901C>T variant.

We seek to find a set of possible genetic modifiers acting as enhancers or protectors of the disease, in carriers of this pathogenic variant, and establish variant-specific lifetime-risk estimations for this disease.
Methods: 159 individuals from 11 families were screened for the CDH1 c.1901C>T variant. From these, 27 individuals were selected for WGS and analyzed with an in-house developed pipeline that previously proved feasible in prioritizing true-positive and disease-causing single nucleotide variants (SNVs), with the use of two different variant calling software (DeepVariant, Haplotype Caller). Statistical analysis was performed to find significant differences amongst Affected and Non-Affected groups, based on the SNV data retrieved from WGS across each chromosome.
Results: We found 71 carriers of the CDH1 c.1901C>T variant, across 11 families. Approximately a sum of 105 million variants were called from the genome-wide data on all patients, with 92% of these called by both DeepVariant and Haplotype Caller. The distribution across each patient was mostly uniform. Through non-parametric median tests, we found that the number of rare variants in chromosome 14 shows statistically significant difference between affected and asymptomatic carrier groups. Most of the rare variants are found in regulatory regions, and two SNVs in this chromosome (one intergenic and one intronic) were found to be exclusive to the Affected carriers' group.
Conclusions: This work represents a steppingstone in the development of lifetime-risk estimations for DGC and LBC patients. We aim to better predict the development of the disease according with the genetic background of individuals within affected families, in order to establish a more conservative approach in clinical prevention. Currently, we are applying the same analysis to copy number and structural variants collected with WGS, to help us expanding our findings.
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OPII-5 CTNNA1 GERMLINE VARIANTS: DISEASE SPECTRUM EXTENDING BEYOND HEREDITARY DIFFUSE GASTRIC CANCER

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Introduction: CTNNA1/αE-catenin germline pathogenic variants are a rare cause of Hereditary Diffuse Gastric Cancer (HDGC), and CTNNA1 missense variants cause Macular Dystrophy Patterned-2. The full disease spectrum and variant-type causality associated with CTNNA1 disfunction is understudied. Large cohorts and in vivo models are imperative to disclose genotype-phenotype correlations.
Methodology: Through collaborating ERN-GENTURIS partners, GENTURIS-external Institutions and literature search, we secured clinical data from 108 CTNNA1 germline variant carrier-families, 67 herein described and 41 yet to be analyzed. We classified variants, categorized families regarding HDGC-criteria, performed a genotype-first approach, and developed an αE-catenin humanized Drosophila melanogaster model to study CTNNA1 impairment in different tissues. Results From 67 families with rare CTNNA1 germline variants, 32% carried Pathogenic (PV) and 32% Likely Pathogenic (LPV), all truncating. Early-onset DGC and Breast Cancer of unknown-histotype (BC) were predominant. In PV-carriers, DGC prevailed (42%), followed by LBC (5%), Colorectal (6%), Prostate (5%) and Thyroid cancer (4%). LPV-carriers mainly developed BC (59%) or Melanoma (7%). Tumor spectrum at early age differed between PV- and LPV-families ($p<0.00001$). RNAi knockdown of Drosophila α-catenin in eye and wing primordial tissues caused severe fly lethality. Eye-RNAi surviving flies lacked eyes, while wing-RNAi cells invaded adjacent normal tissues in the larvae stage. Human wild-type αE-catenin expression in the eye rescued fly survival/organ development, while a human PV-bearing αE-catenin did not.
Discussion: Early-onset DGC/LBC are recurrent in CTNNA1 PV and rare in LPV-carriers. Disease spectrum extends beyond HDGC-classical phenotypes in PV-carriers and diverges in LPV-carriers. Phenotype-driven CTNNA1-specific variant classification guidelines, supported by robust in vivo models, as the humanized model herein described, is needed for CTNNA1 germline variants' interpretation.
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Clinical Cases

OPIII-1 BIALLELIC DYNC2H1 SPLICING VARIANTS CAUSING PRENATAL SHORT-RIB POLYDACTYLY SYNDROME: CLINICAL, RADIOLOGICAL, AND HISTOPATHOLOGIC FEATURES

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Introduction: Short-rib polydactyly syndromes (SRPSs) are a group of skeletal dysplasias, most autosomal recessively inherited, characterized by short ribs, short limbs, polydactyly, and extraskeletal involvement of major organs. SRPSs are caused by deleterious variants in genes involving the formation or function of cilia.
Clinical report: A 36-year-old women was referred to our institution for the 1st trimester ultrasound at 14 weeks. It was the first common pregnancy of a non-consanguineous couple. The ultrasound showed a polimalformed fetus with enlarged nuchal translucency, micromelia, polydactyly, heart defects, megabladder, and hypoechoic kidneys. Due to poor prognosis the parents decided to terminate the pregnancy. Cytogenetic analysis revealed a female fetus with triple X, which did not explain the phenotype. Post-mortem evaluation confirmed prenatal

findings, detecting additionally: craniofacial dysmorphisms, oral hamartoma, malformed uvula, bifid epiglottis, narrow thorax with short ribs, brachydactyly and polysyndactyly of all limbs, agenesis of a long bone of each forearm and leg, trident acetabulum, lung hypoplasia, hypoplastic left heart with ventricular septal defect and persistence of left superior vena cava, pancreas with cysts and fibrosis, multicystic dysplastic kidney, and megabladder with urethral stricture. A ciliopathies multigene panel was performed, revealing two variants of unknown significance in DYNC2H1, found to be in compound heterozygosity: c.7840-18T>G is a novel variant that is predicted to affect splicing by bioinformatic tools, and c.11070G>A is rare variant presumably resulting in a synonymous change, but previously shown to be pathogenic by affecting normal splicing. To better characterize their effect, we have carried out RNA studies which revealed that c.7840-18T>G generates an aberrant transcript lacking the first 124 nucleotides of exon 49, and c.11070G>A led to skipping of exon 76, both producing a truncated protein.
Conclusion: These results are consistent with SRPSs types I/III and illustrate the importance of a multidisciplinary approach to establish a proper diagnosis. Our findings enlarge the clinical and molecular knowledge about SRPSs, which ultimately facilitates

OPIII-2 MOHR-TRANEBJAERG SYNDROME: HEARING LOSS AS THE ONSET OF AN INSIDIOUS DISORDER WITH HIGH RECURRENCE RISK

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Introduction: Mohr-Tranebjaerg syndrome (MTS) is an X-linked recessive disorder caused by TIMM8A loss of function. It is characterized by sensorineural hearing loss in childhood, progressive optic atrophy in early adulthood, early onset dementia and psychiatric symptoms of variable expressivity. We present a family with 4 affected males, explore age-related and interfamilial variability and review the literature.
Case report: A 31yo male was evaluated due to psychiatric symptoms since 18yo and early onset dementia. He had sensorineural hearing loss since childhood. At 28yo he developed dysarthria, dysphonia, dysmetria, limb hyperreflexia, dystonia, and spasticity following an acute encephalopathic crisis. WES revealed a hemizygous novel likely pathogenic variant in TIMM8A, c.45_61dup p.(His21Argfs*11), establishing the diagnosis of MTS. Genetic counseling of the family allowed the diagnosis of 3 other symptomatic relatives - 3 nephews (11yo and two 6yo twins), children of a carrier sister. The oldest nephew had been followed since 4yo due to speech delay. Sensorineural hearing loss was diagnosed at 9yo, and hearing aids were prescribed. The two other nephews were monozygotic twins, and both had unilateral strabismus. One of the twins had macrocephaly and hypoplasia of the anterior temporal lobe disclosed by MRI done due to febrile seizures. The other twin developed cervical dystonia at 6yo. Both had developmental delays with striking speech delay, and audiograms confirmed hearing loss. All 3 nephews were hemizygous for the familial TIMM8A variant. DiscussionHearing loss, an early sign of MTS due to auditory neuropathy, can often be overlooked until more severe features of the disorder manifest. Recurrence risk is high for female carriers, and reproductive options should be offered, considering the neurological manifestations of the disorder. Early monitoring of hearing and vision loss and neurological impairment in MTS patients is important, as early interventions may positively impact their development. This family showcases the importance of performing timely etiological investigation of hearing loss and its impact on genetic counseling.

OPIII-3 NF2-RELATED SCHWANNOMATOSIS – AN ATYPICAL CASE PRESENTATION

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Introduction: The differential diagnosis between neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2), and schwannomatosis (SWN) is usually straightforward. There are however exceptions such as rare cases of NF1 with multiple spinal tumours and no or less than 6 café-au-lait (CAL) macules in the absence of other NF1 criteria. Additionally, distinguishing schwannomas or hybrid nerve sheath tumours from neurofibromas is challenging, leading to patients incorrectly diagnosed with NF1. Case report8-year-old male who had a left inguinal mass excised at age 7, revealing a plexiform neurofibroma (PN) on pathological examination. He had relative macrocephaly, 5 CAL spots >5mm, and left facial hypoesthesia. Investigation prior to surgery included normal ophthalmological evaluation, and head MRI documenting a spinal cord (C1-C2) lesion; cerebellar, VII and VIII cranial nerves (CN) bilateral schwannomas; and a left trigeminal nerve lesion, suggestive of a benign sheath tumour. Based on the presence of a PN, he was started on selumetinib, evolving with mild imagiological improvement of left V, VII and VIII bilateral CN lesions. Histological revaluation is ongoing.
Results: A paediatric tumour NGS panel was performed on a tumour sample identifying a pathogenic NF2 variant: c.1021C>T (p.Arg341*), with a mutant allele fraction of 69%. The variant was shown to be germline, confirming NF2-related schwannomatosis.
Discussion: Firstly, this case points to the challenges of benign nerve sheath tumour classification. In retrospect, the inguinal mass probably corresponds to a neurofibroma/schwannoma hybrid nerve sheath tumour (N/S HNST). The latter has been recently recognized as a distinct entity composed of schwannoma-like nodules within a neurofibroma-like tumour. In about 60% of N/S HNST patients an underlying tumour predisposing syndrome is confirmed (NF2 in 26%, SWN in 17%, NF1 in 9%). Secondly, this case illustrates the power of molecular genetics in reaching precise diagnosis, which is vital for clinical management and genetic counselling. Of note, there are ongoing clinical trials for selumetinib in NF2 patients, but only one other case reported in the literature.
Limit: 2.195/2.200

OPIII-4 ERF HAPLOINSUFICIENCY: EXPLORING VARIABLE EXPRESSIVITY, INCOMPLETE PENETRANCE, AND OVERLAPPING DIAGNOSIS

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Introduction: A recurrent missense variant in the ERF gene has been associated to Chitayat syndrome (MIM 617180). On the other hand, variants in ERF leading to haploinsufficiency were recently related to a completely different phenotype, namely craniosynostosis 4 (MIM 600775), with or without facial dysmorphisms.
Aim: To describe two additional cases of ERF haploinsufficiency in order to explore challenges in phenotypic recognition.
Case reports: P1: A 4-year-old male presented with developmental delay, facial dysmorphisms (hypertelorism, depressed nasal bridge, and short/upturned nose), short stature, sagittal synostosis, webbed neck, and brachydactyly, resembling Noonan syndrome. Rasopathies NGS panel was negative. Clinical exome sequencing identified a pathogenic variant in ERF (NM_ 006494.3):c.1201_1202del, p.(Lys401Glufs*10), inherited from an apparently unaffected father. P2: A 3-year-old male was referred for feeding difficulties, recurrent vomiting, and failure to thrive. He had development delay, craniosynostosis, and dysmorphisms (triangular face, hypertelorism, mild exorbitism, thin/long nose, and low-set/posteriorly rotated ears). Clinical exome sequencing identified a pathogenic variant in ERF (NM_ 006494.3):c.697C>T, p.(Arg233*), maternally inherited. The mother had mild Crouzon-like facial dysmorphisms. Since failure to thrive was not explained by the ERF mutation, further testing was performed. Microarray showed a homozygosity in 7q11.21q22.3, and polymorphic markers indicated a maternal uniparental disomy, confirming Silver-Russel syndrome (MIM 618905). DiscussionBoth cases do not have a fully recognizable phenotype associated with ERF gene variants. The first patient has a Noonan-like phenotype, which is not surprising considering that ERF is a negative transcriptional regulator of the RAS pathway. The second patient has a dual diagnosis: ERF-related craniosynostosis superimposed with the Silver-Russel syndrome, reinforcing the importance of revisiting cases when the full phenotype is not concordant with the genotype.

Additionally, this report reflects the wide intra and interfamilial variability, and incomplete penetrance of ERF mutations.

OPIII-5 TWO CASE REPORT OF A NEW RECOGNIZABLE SYNDROME – DEGCAGS (DEVELOPMENTAL DELAY WITH GASTROINTESTINAL, CARDIOVASCULAR, GENITOURINARY, AND SKELETAL ABNORMALITIES)

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Introduction: Genomics investigation and applied knowledge is constantly evolving. Every year researchers identify new and promising gene-disease associations. One of the most recent, in 2021, is a novel syndrome – DEGCAGS, caused by biallelic mutations in ZNF699 (PMID: 33875846), a gene with scarce previous studies. Only 14 cases have been reported and we describe two additional Portuguese cases.
Case report: A two year old boy (Pat1) was referenced to our genetics unit for investigation of global development delay, anaemia, neutropenia, feeding difficulties and retractile testis. He had a coarse facies, sparse hair, synofris, bilateral ptosis and low set posteriorly rotated ears. There was a background of shortened fetal long bones with normal prenatal CGH array. Haematological studies, including bone marrow biopsy, not reported in the literature, were inconclusive and brain MRI reported leukoencephalopathy, delayed myelination and vermis hypoplasia. Whole exome sequencing, plus segregation studies, detected a homozygous variant (c.1327C>T, p.Arg443*) in ZNF699. Evaluation of the literature confirmed the similarities to DEGCAGS patients, including one individual with compound heterozygosity for the same nonsense variant (PMID 35205213).A seven year old girl (Pat2), with prior inconclusive NGS multigene panel, was recognized as having a similar phenotype, by our colleagues in haematology. She had severe development delay, feeding difficulties, anaemia, dysmorphic facies, small kidneys, hypoplasia of C1 vertebra and congenital deafness. Molecular investigation identified the same homozygous variant, confirming the diagnosis of DEGCAGS. Further comparison with published cases will be presented.
Discussion: We report two infants with DEGCAGS, homozygous for the same nonsense ZNF699 variant. The families are not related but geographical origin is similar. ZNF699 variants are presumed as loss-of-function, but no functional studies have been reported. Few cases have been described and further phenotypic description is needed. This report portrays that DEGCAGS can be clinically recognized and highlights the importance of revaluating patients and their previous NGS studies.

OPIII-6 DESCRIPTION OF TWO NEW CASES OF WIEACKER-WOLFF SYNDROME (ZARD) ASSOCIATED TO DE NOVO ZC4H2 SPLICING VARIANTS

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Introduction: ZC4H2-Associated Rare Disorders (ZARD) are an X-linked Arthrogryposis Multiplex Congenita (AMC) disorder associated with a wide range of clinical features such as short stature, microcephaly, hypotonia, and broad neurodevelopmental delay. The spectrum of ZC4H2 defects comprises mostly missense variants in affected males, and de novo splicing, frameshift, nonsense, and partial deletions in affected females. Its wide phenotypic range goes from full manifestation in boys to milder features, such as mild contractures and foot deformities, in carrier females.
Methodology: Here we present two new Portuguese cases. The first one, a 10-year-old girl born with distal arthrogryposis and bilateral congenital hip dislocation that, at 3 years of age, was submitted to right femoral varus osteotomy. Although she suffers from gross and fine motor limitations in the involved joints, she has been developing well without apparent cognitive impairment. Secondly, a 17-year-old only girl born with claw hands, hypotonia, and posterior cleft palate that in the neonatal period suffered from respiratory distress due to laryngomalacia. During childhood and adolescence, she underwent cleft palate and clubfoot correction and was submitted to various orthopedic and ENT surgeries. Currently she presents with bilateral distal arthrogryposis, bilateral camptodactyly of 2-5 fingers, obesity with high appetite, short stature, non-progressive scoliosis, hypotonia with poor posture, gait impairment, mild intellectual disability, and hyperopia and astigmatism. Curiously, she is macrocephalic. In both cases, brain MRI was normal, and no relevant family history or parental consanguinity are known.
Results: NGS panel for distal arthrogryposis, in the first, and trio exome sequencing, in the second, identified two de novo splicing ZC4H2 variants, one of which was been recently described. X-inactivation studies were not conducted since that has not be shown to be able to predict clinical outcome.
Discussion: Through these cases, we hope to expand the known ZARD phenotype and to improve clinicians' recognition of this disease.
Declaration of Interests: Nothing to declare.

OPIII-7 46,XY DISORDER OF SEX DEVELOPMENT DUE TO LHCGR COPY LOSS IN AOH REGION

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Introduction: Sexual differentiation is a multistep process involving coordinated activation/ deactivation of multiple genes. Luteinizing Hormone/ Chorionic Gonadotropin Receptor (LHCGR) gene is a piece of this complex chain. It encodes a G protein coupled receptor that mediates chorionic gonadotropin signal transduction, culminating in testosterone production. Secreted testosterone stimulates differentiation of male external genitalia.
Case Repor: We report a 34-year-old woman referred to our Genetics Department for primary amenorrhea, lack of breast development, inguinal gonads, absence of Müllerian derivatives, and hypergonadotropic hypogonadism. Gonadal histopathology revealed absence of Leydig cells and spermatogenesis.
Results: Cytogenetic studies revealed a male karyotype in a phenotypically female individual, compatible with a diagnosis of 46,XY DSD (Disorder of Sex Deve-lopment).SRY deletion was excluded by MLPA. Further investigation was carried out by SNParray, revealing a region of Absence of Heterozigosity (AOH) with 6,44Mb in 2p16. A 102,79Kb homozygous deletion was detected within this AOH region, harbouring LHCGR gene and part of GTF2A1L gene. Parents were not available for genetic testing.
Discussion: Biallelic loss of function variants in LHCGR gene lead to Leydig cell hypoplasia and impairment of sexual differentiation in males, which is concordant with this patient's phenotype. LHCGR inactivation is primarily caused by point mutations. To the best of our knowledge, this is the first case of LHCGR whole gene homozygous deletion. Furthermore, this deletion is within a segmental AOH region that promoted the homozygous state. Although both parents are from the same small village, there is no parental consanguinity, as demonstrated by the detection of a single AOH region. This report highlights the hazardous potential of segmental AOH to expose recessive genes and conditions.

OPIII-8 TREACHER COLLINS SYNDROME ASSOCIATED WITH POLR1D – A MILDER PHENOTYPE? REPORT OF TWO FAMILIES

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Introduction: Treacher Collins syndrome (TCS) presents extreme clinical variability and incomplete penetrance. Heterozygous variants in TCOF1 (TCS1, MIM#154500) are responsible for about 85%. Variants in genes encoding for RNA polymerase I subunits POLR1D (TCS2, MIM#613717), POLR1C (TCS3, MIM#248390) and POLR1B (TCS4, MIM#618939) were documented in a minority. We report two familial cases of TCS caused by heterozygous variants in POLR1D, and compare these patients with those in the literature.
Case report: Case 1 is a 23-year-old girl referred for conductive deafness and mild microtia. She had a TCS gestalt, thus targeted NGS panel analysis was ordered, which identified a heterozygous c.164T>C (p.Leu-55Pro) variant in POLR1D gene classified as likely pathogenic, inherited from a healthy mother. Case 2 is a 12-year-old girl observed at birth due to microtia. She evolved with moderate conductive deafness and learning disability. This patient had a younger sister, stillborn with an unspecified cardiac defect, cleft palate and microtia. TCS gestalt was not recognized. WES identified an heterozygous pathogenic c.259C>T (p.Arg87*) variant in POLR1D, inherited from a healthy father.

Discussion and conclusions: Previously published cohorts1,2 of TCS2 patients (total of 17 patients, 5 familial cases) were reported as mild cases, having no major malformations, including cleft palate, choanal atresia or cardiac abnormalities, and in familial cases parents were described as asymptomatic. TCS2 is rare making it difficult to established genotype-phenotype correlations. Our cases corroborate extreme variability, including intrafamilial. However, assuming the stillborn sister of case 2 shared the familial POLR1D variant, and had no additional diagnosis, TCS2 cannot be considered a milder condition than the more common TCS1.

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OPIII-9 TAF2 RELATED TO THE TRANSCRIPTION FACTOR TFIID: A NEW FAMILY AND REVIEW OF THE LITERATURE

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Introduction: Initiation of RNA polymerase II-mediated transcription requires the assembly of a preinitiation complex, around the gene promoter region, containing general transcription factors, such as TFIID. TAF2 encodes for one of the TFIID critical components, the TATA-box binding protein associated factor 2. Causative variants in TAF2 are associated to Intellectual developmental disorder, autosomal recessive 40 (MIM #615599), phenotypically presenting with severe intellectual disability, pyramidal signs, postnatal microcephaly and thin corpus callosum. To our knowledge, only 11 cases, from 5 families, have been described.
Methodology: Characterization of a new family with 2 affected children, comparison to the previously reported cases and review of the TAF2 related pathophysiology.
Results: The first child was born from non-consanguineous healthy parents, following an uneventful pregnancy. Birth measurements were in the normal range (weight: -1SD; length: -0,5SD; occipito-frontal circum-ference: -1SD). For the first three weeks of life, feeding difficulties required nasogastric tube feeding. Initially interpreted as seizures, normal electroencephalogram and brain MRI showed that he had obstructive apneas since birth, due to severe hypotonia and laryngomalacia. At around 3 years old, he maintained hypotonia, along with pyramidal signs, strabismus, microcephaly (-3 SD), hypopigmented macules and severely delayed cognitive and motor development, with no autonomous walking or speech. At 7 years old, he acquired autonomous gait, but with frequent falls. Metabolic studies and CGH array showed no relevant alterations. Exome sequencing performed at 3 years old identified 2 novel variants of uncertain clinical significance in TAF2, c.2204T>G p.(Ile735Ser) and c.2933T>G p.(Leu978Arg), in confirmed compound heterozygosity. The parents decided for a new pregnancy and the second boy was born with similar clinical presentation, although with not so severe hypotonia and feeding difficulties. This boy was shown to have the same genotype.
Discussion: Our patients' phenotype, as well as the type of variants identified (missense), seem to match the previous literature reports. The consistent segregation of the variants in the family, with a second affected child, and the deleterious in silico predictions favour the plausible diagnosis related to this gene. We are currently aiming to perform functional studies in order to substantiate this hypotesis. To our knowledge, hypopigmented macules have not been previously described.

Basic Research

Poster Highlight 1

ATXN3 ALTERNATIVE SPLICING IN SPINOCEREBELLAR ATAXIA TYPE 3/ MACHADO-JOSEPH DISEASE: DIVERSITY AND ABUNDANCE OF TRANSCRIPTS IN THE CEREBELLUM AND BLOOD

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Spinocerebellar ataxia type 3 (SCA3)/Machado-Joseph disease is an autosomal dominant polyglutamine disease. The SCA3 causative gene, ATXN3, is known to undergo alternative splicing (AS) and 54 transcripts are currently annotated. However, the extent and regulation of ATXN3 AS, the abundance of alternative transcripts in health and disease conditions, and the existence of tissue-specific transcripts remain poorly understood. In this work we defined the abundance profiles of the annotated ATXN3 transcripts using RNA-seq datasets from cerebellum (n=12) and blood (n=60) samples of SCA3 subjects and controls, and performed the reads assembling/quantification using a standard pipeline for splicing analysis. ATXN3 transcripts were categorized in three biotypes: protein coding (n=16), nonsense mediated decay (n=19) and processed transcripts (n=19). Globally, transcript diversity and abundance were higher in the cerebellum than in blood, supporting, as expected, a putative key role of ATXN3 in this tissue that is affected with cell loss in SCA3. Interestingly, while the most abundant transcript in the cerebellum was a long non-coding RNA (lncRNA; ATXN3-208), the transcript with the highest abundance in the blood was the reference transcript (ATXN3-251) that is translated into an ataxin-3 protein isoform harboring three ubiquitin interactive motifs (UIM). In fact, the abundance of ATXN3-251 and ATXN3-214 transcripts, encoding ataxin-3 isoforms with, respectively, 3 and 2 UIM, in SCA3 subjects and controls was strongly related with tissue expression specificity: 3 UIM was expressed in blood 60x more than in cerebellum whereas 2 UIM was expressed in the cerebellum 20x more than in blood. While the abundance of the most frequent transcripts was similar in the cerebellum and in the blood of SCA3 subjects and controls, two transcripts (ATXN3-206 and 226) were expressed less frequently in cerebellum of patients than in controls. These findings provide new insights into the elucidation of ATXN3 AS in the cerebellum and blood which may lead to the development of future effective ATXN3 mRNA-lowering therapies, while contributing to a better understanding of SCA3 pathogenesis.

Poster Highlight 2

MESENCHYMAL STROMAL CELLS THERAPY RESCUES MITOPHAGY IN MACHADO-JOSEPH DISEASE MODELS

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Introduction: Machado-Joseph disease (MJD) is characterized by over-repetition of the CAG trinucleotide in the causative gene, translating into an overlong polyglutamine tail within the mutant ataxin-3 (ATXN3) protein. This protein causes toxicity and neurode-generation in specific brain regions. To tackle this issue, mesenchymal stromal cells (MSC) and their derived products have been investigated as possible therapies. However, the mechanisms by which MSC promote these neuroprotective effects remain unclear. In the present work we aimed at determining deficits in mitophagy and mitochondria dynamics in MJD, as well as the effects of MSC therapy in MJD mitochondria.

Methodology: We evaluated the levels of mitophagy markers and mitochondria dynamics in 8-month-old MJD transgenic mice treated with MSC. Protein levels from cerebellar extracts were determined by western blot. MSC effects over mitochondria morphology were assessed by immunocytochemistry of a neuroblastoma cell line stably expressing mutant ATXN3 upon transfection with mitoDSR ed. Mitochondria transfer from MSC to neuroblastoma cells was assessed by live imaging using Mitotracker-Red dye or mitoDSRed.
Results: MJD mice expressed abnormal levels of phosphorylated Parkin and mitochondrial PINK1 and MSC treatment was able to revert these alterations. Mitochondria number was found increased and presented evidence of fission in vitro. The effects of MSC over these parameters is under evaluation. Finally, we observed that MSC transfer mitochondria to MJD cells in direct co-culture experiments.
Discussion: This work highlights mitophagy dysfunction and impaired mitochondria dynamics in MJD models. Interestingly, we found that MSC transfer mitochondria to MJD cells in vitro and counteract alterations in mitophagy markers in vivo, denoting their therapeutic effects in mitophagy/mitochondria in MJD.
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Poster Highlight 3

DROPLET DIGITAL PCR FOR EPIGENETIC AGE ESTIMATION USING DNA METHYLATION IN ELOVL2 AND PDE4C GENES

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Introduction: In recent years, age estimation through the evaluation of DNA methylation (DNAm) levels has arisen as a promising biomarker in forensics. Several age prediction models (APMs) focusing on DNAm levels of many genes, including ELOVL2 and PDE4C, have been developed by different methodologies after bisulfite conversion. Droplet digital PCR (ddPCR) technology has also revealed promising results in DNAm age estimation. However, up to now, few studies have developed APMs based on this method.
Aim: In the present study, we describe a ddPCR-based assay to assess DNAm in ELOVL2 and PDE4C genes for epigenetic age prediction.
Methodology: Blood samples of 66 healthy Portuguese individuals (47 females, 19 males; aged 1-93 years) were analysed. The genomic DNA was subjected to bisulfite conversion and DNAm levels were assessed according the procedure described in Han et al. [1]. Droplet digital PCR was performed with a QX100™ Droplet Digital™ PCR System (Bio-Rad, CA, USA). The relationship between chronological age and DNAm levels of the selected CpGs were evaluated through simple and multiple linear regression using IBM SPSS software v.24.
Results and Discussion: Through simple linear regression analysis, PDE4C gene (N = 59) revealed the strong correlation between the chronological age and DNAm levels (R = 0.927, p = 6.089 × 10-26). A highly significant age correlation was also obtained for the ELOVL2 gene (N = 58) (R = 0.887, p = 2.099 × 10-20). Predicting age through the simple linear coefficients, allowed to obtain a mean absolute deviation between predicted and chronological ages (MAD) of 7.59 years for PDE4C and of 8.67 years for ELOVL2. The final multiple-linear regression model, using both the ELOVL2 and PDE4C DNAm data (N = 51), showed a high age correlation (R = 0.942), explaining 88.3% of age variation, highly significant (p = 1.765 × 10-23). The developed final APM, allowed to estimate age with a correlation between predicted and chronological ages of 0.952 and a MAD of 6.54 years.
Conclusion: This developed dual-locus APM seems promising to be used in forensics.
Reference
1. Han et al. BMC Biology, 2020;18:71.

Poster Highlight 4

UPF1 HAS ONCOGENIC FUNCTIONS IN COLORECTAL CANCER

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Colorectal cancer (CRC) is one of the most prevalent causes of death worldwide, especially in developed countries. There are many genes whose expression is misregulated in CRC, leading to the onset and development of the disease. Up-frameshift 1 (UPF1) is a multifaceted protein involved in mechanisms such as nonsense-mediated mRNA decay, cell cycle progression, or telomere maintenance and homeostasis. Importantly, UPF1 is also considered a tumour suppressor protein in most cancers. However, UPF1 plays an oncogenic role in CRC. Our in silico analyses using the Xena platform revealed that UPF1 is overexpressed in CRC contrary to several other analysed cancers. Besides, UPF1 expression levels are increased in CRC compared to the counterpart normal tissues. We confirmed these data experimentally and observed that UPF1 expression is maintained in different CRC cell lines under endoplasmic reticulum (ER) stress. Adding to this, we used a bicistronic reporter construct to test whether UPF1 5'UTR can mediate alternative mechanisms of translation initiation and we concluded that such sequence contains an internal ribosome entry site (IRES) that maintains UPF1 expression in both normal and stress conditions in a 5' cap-independent way. 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 in HCT116 (CRC) cells. Altogether, these results suggest that IRES-mediated translation help maintain UPF1 expression levels under stress conditions, such as those that mimic the tumour microenvironment, and that ASOs may be an upcoming therapy to target such alternative mechanism of translation initiation and prevent CRC development.

Poster Highlight 5

EXPLORING THE MISSING LINK BETWEEN PRIMARY CILIA AND NEURODEVELOPMENTAL DISORDERS: THE CASE OF A NOVEL MBD5 VARIANT IN A PATIENT WITH SEVERE EPILEPSY

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Abstract: Neurodevelopmental disorders (NDDs) are a growing health concern affecting millions of individuals worldwide. Multiple genetic factors are associated with NDDs, including variants in MBD5, the causal locus for 2q23.1 microdeletion syndrome. Recent studies of MBD5 haploinsufficiency showed altered expression of genes essential for brain development (Mullegama et al., 2021) and primary cilia function (Seabra et al., 2020). Despite these findings, little is known regarding the clinical and molecular aspects of 2q23.1 microdeletion syndrome. Stem cell-derived models are a valuable tool to further elucidate this condition, since their isogeneity and their ability to differentiate into every cell type in the body enable the creation of cellular models that recapitulate the genetic conditions of the patients.
Therefore, our goals were to report the only patient in Portugal known to harbor an MBD5 variant and to generate and characterize neural progenitor

cells (NPCs) derived from the patient's dental stem cells, to be used as future models to understand the impact of these alterations on primary cilia and neurodevelopment. We also used CRISPR/Cas9 to generate isogenic control lines. Our results indicate that the patient-derived NPCs present differences in the expression of MBD5 and RAI1, an autism-related gene, when compared to CRISPR-Cas9-edited NPCs. Furthermore, we observed that the primary cilia of the patient-derived NPCs are longer than the ones present in controls. With this project, it was possible to develop tools to shed some light on the contributions of MBD5 and primary cilia to NDDs. Though our understanding of neurodevelopment and NDDs is limited, new knowledge regarding this subject may bring us closer to obtaining more effective and specific therapeutic targets for these conditions.

Poster Highlight 6

BUB1B MONOALLELIC GERMLINE VARIANTS CONTRIBUTE TO PROSTATE CANCER PREDISPOSITION BY TRIGGERING CHROMOSOMAL INSTABILITY

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Prostate cancer (PrCa) is the most frequently diagnosed cancer in men. Variants in moderate- to high-penetrance genes associated with Hereditary Breast Ovarian Cancer (BRCA1/2, ATM, CHEK2, and PALB2) and Lynch (MLH1, MSH2, and PMS2) syndromes explain less than 5% of the early-onset/familial PrCa cases. Targeted next-generation sequencing revealed truncating variants in the BUB1B gene in 1.3% of 462 families. The BubR1 protein, encoded by BUB1B, is an essential component of the mitotic spindle assembly checkpoint (SAC), a surveillance mechanism securing accurate division of the genetic material in mitosis. Mosaic variegated aneuploidy syndrome 1 (MVA1) has been linked to biallelic mutations in the BUB1B gene, and characterized by a severe decrease in BubR1 abundance, the presence of premature chromatid separation (PCS) in more than 50% of the patients' lymphocytes, and early onset of cancer due to defects in SAC. Thus, we hypothesized whether a monoallelic in-frame recurrent variant in BUB1B, representing 3.4% of the PrCa patients with early-onset PrCa (<56 years) and family history of the disease, is sufficient to fuel chromosomal instability (CIN), potentially triggering PrCa development. We show that the variant c.1171_1173del; p.(Glu391del) is associated with strong reduction of BubR1 abundance in mitotic cells and, consequently, taxol-resistant proliferation due to disruption of SAC signaling, premature mitotic exit and CIN. Concordantly, higher rates of PCS were found in patients carrying different BUB1B germline variants, suggesting a common mechanism underlying carcinogenesis. This work highlights BUB1B as a PrCa predisposing gene and supports the likely pathogenic nature of the variant c.1171_1173del.

Poster Highlight 7

WHOLE-EXOME SEQUENCING RE-ANALYSIS OF FAMILIES WITH SUSPECTED GASTRIC CANCER TUMOUR RISK SYNDROMES STRENGTHENS THE ROLE OF PALB2 IN DIFFUSE GASTRIC CANCER AND LOBULAR BREAST CANCER PREDISPOSITION

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Background/ Objectives: Hereditary diffuse gastric cancer (HDGC), is the major gastric cancer tumour risk syndrome (GC-TRS) and is caused by loss-of-function germline variants in CDH1 or CTNNA1, which predisposes for diffuse gastric cancer (DGC) and/or lobular breast cancer (LBC). Overall, >50% of all clinically-diagnosed HDGC remain unsolved. We hypothesise that other tumour risk syndrome (TRS) genes explain unsolved GC-TRS cases.
Methods: We systematically searched the literature for individuals/families with cancer-history fitting clinical criteria for GC-TRS and bearing Pathogenic/Likely pathogenic variants (PV/LPV) in TRS genes. Within the SOLVE-RD project, we re-analysed germline WES from 61 unsolved GC-TRS suspected families from the Netherlands, the United Kingdom and Canada. A cohort of 23 unsolved Portuguese GC-TRS cases was used to validate actionable candidate single nucleotide variants (SNVs) or copy number variants (CNVs).
Results: The literature search returned 28 PVs affecting 16 TRS-genes in 27 GC-TRS families, with PALB2 pathogenic SNVs occurring in 7/27 (26%) families dominated by DGC and LBC. SOLVE-RD re-analysis identified 1/61 (1.6%) GC-TRS family bearing a PALB2 pathogenic CNV, and a further case with LBC (1/23;4.3%) was identified in the validation cohort, bearing a PALB2-PV, and no other candidate variants.
Conclusion: GC-TRS families may be explained by non-classical genes, with PALB2 as the most frequently mutated gene found here (2.4%) in families fulfilling HDGC criteria and unsolved by CDH1 or CTNNA1. This study supports the role of PALB2 SNVs and CNVs in the predisposition for DGC and/or LBC, and widening of genetic testing for GC-TRS suspected-families. Grants EU H2020 -779257 & PTDC/BTM-TEC/ 6706/2020

Poster Highlight 8

ANCESTRY OF THE A-MRE ASSOCIATED WITH THE 3.7KB A-THALASSEMIA DELETION IN THE PORTUGUESE POPULATION

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The α-major regulatory element (α-MRE), also known as HS-40, is located upstream of the α-globin gene cluster and has a crucial role in the long-range regulation of the α-globin gene expression. It is genetically polymorphic and six haplotypes (A to F) have been identified in different populations. The D haplotype was primary described in African populations and is nearly absent in other populations. The aims of this study were to identify the α-MRE haplotype associated with the common 3.7kb α-thalassemia deletion (-α3.7del) in the Portuguese population, and to investigate its ancestry. We searched for the -α3.7del in 111 selected Portuguese individuals by Gap-PCR. In addition, a DNA fragment containing the α-MRE was amplified by PCR and Sanger sequenced. Statistical analysis was performed using R software.Fifty individuals have the wild-type α-globin genotype (group I), 34 are heterozygous for the -α3.7del (group II) and 27 are homozygous (group III). Regarding the α-MRE, four haplotypes were found (A to D). The ancestral A haplotype is predominant in all groups. The B haplotype is the second most frequent in groups I and II, whereas in group III haplotype D is the second most prevalent. Concerning genotypes, the α-MRE AA and

AB are the most common in group I, while genotype AD is more prevalent in group III. In fact, 71.4% of AD individuals are homozygous for the -α3.7del. Moreover, the distribution of α-MRE haplotypes and genotypes are significantly different between groups with and without the -α3.7del ($p<0.001$). Furthermore, multiple correspondence analysis revealed that individuals without the -α3.7del are grouped with other European populations, while samples with the -α3.7del are split from these and found to be more closely related to the African population. This study revealed for the first time an association of a specific α-MRE haplotype with the common -α3.7del in the Portuguese population, and its likely African ancestry. These results may have clinical importance as the D haplotype has an alteration in the consensus sequence for the AP-1/NF-E2 binding site and in vitro experiments showed a decrease in its enhancer activity on α-globin genes.

Poster Highlight 9

EXOME SEQUENCING OF AFFECTED DUOS AND TRIOS UNCOVERS PRUNE2 AS A NOVEL PROSTATE CANCER PREDISPOSITION GENE

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Keywords: Germline variants, hereditary predisposition, prostate cancer, PRUNE2

Background: Prostate cancer (PrCa) is one of the most hereditable human cancers, however, only a small fraction of patients has been shown to carry deleterious variants in known cancer predisposition genes.

Methods: Whole-exome sequencing was performed in multiple affected members of 45 PrCa families to select the best candidate genes behind part of the PrCa missing hereditability. Recurrently mutated genes were prioritized, and further investigated by targeted next-generation sequencing in the whole early-onset and/or familial PrCa series of 462 patients.

Results: PRUNE2 stood out from our analysis when also considering the available data on its association with PrCa development. Ten germline pathogenic/likely pathogenic variants in the PRUNE2 gene were identified in 13 patients. The most frequent variant was found in three unrelated patients and identical-by-descent analysis revealed that the haplotype associated with the variant is shared by all the variant carriers, supporting the existence of a common ancestor.

Discussion: This is the first report of pathogenic/likely pathogenic germline variants in PRUNE2 in PrCa patients, namely in those with early-onset/familial disease. Importantly, PRUNE2 was the most frequently mutated gene in the whole series, with a deleterious germline variant identified in 2.8% of the patients, representing a novel prostate cancer predisposition gene.

Poster Highlight 10

PERFORMANCE OF THE ACMG-AMP CRITERIA IN A LARGE FAMILIAL RENAL GLUCOSURIA COHORT WITH IDENTIFIED SLC5A2 SEQUENCE VARIANTS

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Background: Familial Renal Glucosuria (FRG), MIM 233100, is a specific human trait characterized by persistent and isolated orthoglycaemic renal glucosuria. From 2003 to 2015 we have reported several cohorts validating SLC5A2 (16p11.2), encoding SGLT2 (Na+/glucose cotransporter family member 2), as the gene responsible for FRG. This research further established FRG as a co-dominantly inherited mendelian phenotype. More than 140 index cases have been published to date. Genetic heterogeneity is not relevant in FRG, with a single report of an affected individual displaying a homozygous splicing variant in the PDZK1IP gene (1p13), that codes for the SGLT2 accessory unit MAP17. The current work aims to evaluate the performance of the ACMG-AMP 2015 criteria in our international FRG cohort with identified SLC5A2 sequence variants performed in a research environment.

Methods: We have previously characterized FRG cases displaying mild (<10g/1.73m2/day) urinary glucose excretion (UGE) as heterozygotes, while compound heterozygosity or homozygosity are responsible for severe UGE (>10g/1.73m2/day). In order to apply the ACMG-AMP criteria (PM3 in particular) to a co-dominant inherited phenotype, we stratified our approach according to this threshold: for severe FRG cases an autosomal recessive model was assumed; for mild FRG, criteria for autosomal dominant were considered. In addition, the following were taken into account: i) although there are no clinically available in vitro or in vivo functional studies, the recent publication of the cryo-EM structure of the SGLT2-MAP17 complex enabled us to identify functional domains in SGLT2 (PM1); ii) whenever family members were available, co-segregation studies and phase determination were carried out (PM3, PP1); iii) Mutation Taster, Polyphen-2 and SIFT were selected for in silico evaluation, with 2 out of three defining the computational outcomes for tolerated vs. deleterious (PP3); iv) gnomAD exome database was used for populational allele frequencies (PM2), with variants considered rare (R) if 1e-2 to 1e-4 or ultra-rare (UR), if < 1e-4; v) in search for reported variants, a literature review was performed and HGMD as well ClinVar databases were accessed.

Results: 47 index cases were evaluated, including 13 previously unreported pedigrees with the latter accounting for 10 novel variants, A total of 46 different variants were characterized: 10 (21.7%) were LOF; 21 (46%) classified as LP, 13 (28%) as VUS and 12 (26%) as P. Most of the variants were UR or not present in the populational databases, but 3 R alleles were detected in multiple unrelated pedigrees: c.885+5g>a (P) and c.1961A> G,p.N654S (LP) in 7 pedigrees each, and c.1409 T>C;p.V470A (LP) in 3 families. The gnomAD frequen-cies for these were, respectively, 3.09e-4, 5.60e-3 and 2.45e-4. Computational evidence predicted lack of impact on gene/protein function (BP4) for 4 alleles: c.1750 C>A;p.P584T, c.500A>T;p.Q167L, c.26C>G;p.S9W and c.968C>G;p.T323R. However, when considering the remaining criteria only the c.1750 C>A;p.P584T variant was downgraded to VUS.

Discussion: While applying the ACMG-AMP 2015 criteria to the variants identified in our research cohort, 13 (28%) were classified as VUS, with 5 cases, nevertheless, displaying UGE >10g/1.73m2/day and compound heterozygosity together with LP or P variants. Signi-ficantly, in all of the VUS variants: i) no additional family members were studied; ii) they were either UR or absent in gnomAD; iii) they had not been previously published or submitted to HGMD/ClinVar databases. Being a FRG co-dominant disease, proving evidence for SLC5A2 variants' pathogenicity is challenging, particularly if no additional family members are studied or in the case of variants not previously reported. This work also confirms that missense changes are a common mechanism of disease in FRG. We highlight the importance of screening family members as well reporting/submitting all the variants identified either in diagnostic or research genetic studies.

POSTER

BASIC RESEARCHBIALLELIC BUB1 MUTATIONS CAUSE MICROCEPHALY, DEVELOPMENTAL DELAY AND VARIABLE EFFECTS ON COHESION AND CHROMOSOME SEGREGATION

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Abstract: BUB1 contributes to multiple mitotic processes. Here we describe the first two patients with biallelic BUB1 germline mutations, who both display microcephaly, intellectual disability and several patient-specific features. The identified mutations cause variable degrees of reduced total protein level and kinase activity, leading to distinct mitotic defects. Both patients' cells show prolonged mitosis duration, chromosome segregation errors, and an overall functional spindle assembly checkpoint. However, while BUB1 levels mostly affect BUBR1 kinetochore recruitment, impaired kinase activity prohibits centromeric recruitment of Aurora B, SGO1 and TOP2A, correlating with anaphase bridges, aneuploidy and defective sister chromatid cohesion. Interestingly, we do not observe accelerated cohesion fatigue. We hypothesize that unresolved DNA catenanes increase cohesion strength, with concomitant increase in anaphase bridges. In conclusion, BUB1 mutations cause a neurodevelopmental disorder, with clinical and cellular phenotypes that partially resemble previously described syndromes, including autosomal recessive primary microcephaly (MCPH), Mosaic Variegated Aneuploidy (MVA) and cohesinopathies.

GLIOBLASTOMA: GENOMIC AND EPIGENOMIC CHARACTERIZATION AND BIOMARKER IDENTIFICATION

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Introduction: Glioblastoma is the most aggressive and common form of astrocytic tumor, remaining incurable. This work aims to characterize through an in-silico pipeline and wet lab techniques the genetic makeup of glioblastoma by comparing the alteration profile of TCGA glioblastoma cohort, a Portuguese cohort, and a commercial cell line, as well as explore cell-free DNA (cfDNA) quantification after liquid biopsy as a progression biomarker.
Methodology: 595 patients' genetic data was retrieved from the TCGA database, a validation cohort from Portugal, and the U87 cell line, were used. The TCGA cohort was characterized through bioinformatics by identifying the genes and copy number alterations with known oncogenic roles in the most commonly altered regions. The Portuguese cohort tissue samples and the U87 cell line were analyzed with aCGH and MS-MLPA, adding conventional and molecular cytogenetics for the latter. cfDNA was extracted and quantified at several time points.
Results: Many known glioblastoma biomarkers, such as amplifications in chromosome 7 and deletions in chromosome 10, loci for the EGFR and PTEN genes, respectively, were found. Novel possible biomarkers arose, such as amplifications in PRKCA, CPA1/2, CPA4/5, ING3, KLF14 and several miRNAs; and deletions in PTCH1 and MMP21. Preliminary liquid biopsy results revealed that mean cfDNA plasma concentration was higher for patients' pre-maximum safe recession surgery than for controls, even if not significantly different ($p = 0.238$).
Discussion: In line with glioblastoma's heterogeneous nature, alteration profiles varied and have known roles in oncogenic pathways of interest, such as PI3K/PTEN/Akt/mTOR and TP53/MDM2/MDM4/CDKN2A-p14ARF. Some novel findings, such as alterations in PRKCA and PTCH1, suggest dysregulation in proliferation pathways not yet described for glioblastoma. A prospective study with a larger cohort is necessary to affirm the utility of these alterations in positively impacting clinical management. For liquid biopsy, results indicate that ctDNA passes to the wider bloodstream through the blood-brain barrier, making this a promising area to be further explored in diagnosis and follow-up.
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NUCLEOTIDE POLYMORPHISMS ASSOCIATED TO INCREASED RISK OF PANCREATIC CANCER MODULATE PANCREATIC ENHANCER'S FUNCTION

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Pancreatic cancer (PC) represents the 7th leading cause of cancer-related deaths worldwide. Genome-wide association studies have uncovered single nucleotide polymorphisms (SNPs) associated to PC in non-coding regions of DNA, many having potential cis-regulatory functions, essential to control the transcription of genes. So, alterations in these sequences might impact in gene transcription, contributing to an increased risk of PC development. Combining genome-wide resources with human pancreatic chromatin profiles, we found that 36 out of 115 risk SNPs associated to PC overlap with pancreatic enhancers. These results indicate that these SNPs could be affecting enhancers' function, changing the transcription of genes. So, to test this hypothesis, we explored the genomic landscape of NR5A2, a gene previously linked to PC development. We selected 4 putative enhancers, from the NR5A2 landscape, that overlap with PC risk SNPs. We tested the enhancer activity of these sequences containing wild type and risk variants, using in vitro and in vivo enhancer reporter assays. We found that these sequences are pancreatic enhancers and that the different SNPs alter the enhancer activity of these regions. Our results suggest that the modulation of pancreatic enhancers by SNPs might impact in gene function, contributing to the increased risk of PC development.

PREDICTED DAMAGING VARIANTS IN GENES REGULATING THE EFFECTS OF EXPOSURE TO XENOBIOTICS IN SUBJECTS WITH AUTISM SPECTRUM DISORDER

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Portugal; [6] Departamento de Física, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal.

Introduction: Heritability estimates support a role for gene-environment interactions in the etiology of Autism Spectrum Disorder (ASD). Detoxification pathways and physiological permeability barriers (e.g., blood-brain barrier, placenta and respiratory airways) regulate the effects of early life exposure to xenobiotics, when the immature brain is extremely vulnerable. We aimed to identify predicted damaging variants in detoxification and barrier genes (XenoReg genes), in subjects with ASD, and to understand their interaction patterns with ubiquitous xenobiotics previously implicated in the disorder.
Methodology: Large ASD datasets were inspected for predicted damaging Single Nucleotide Variants (N=2674) or Copy Number Variants (N=3570) in 519 XenoReg genes. We queried the Comparative Toxicogenomics Database (CTD) to identify gene-environment interaction pairs.
Results: We prioritized 77 XenoReg genes with high evidence for a role in ASD, according to pre-specified criteria. These include 47 genes encoding detoxification enzymes and 30 genes encoding barrier proteins, among which 15 are known ASD candidates. The CTD query revealed 397 interaction pairs between these genes and 80% (48/60) of the xenobiotics. The top interacting genes and xenobiotics were, CYP1A2, ABCB1, ABCG2, GSTM1, and CYP2D6 and benzo-(a)-pyrene, valproic acid, bisphenol A, particulate matter, methylmercury, and perfluorinated compounds. DiscussionIndividuals carrying predicted damaging variants in high evidence XenoReg genes may be particularly susceptible to early life exposure to xenobiotics, which elicit neuropathological mechanisms, such as epigenetic changes, oxidative stress, neuroinflammation and endocrine disruption. As exposure to xenobiotics may be mitigated, this work provides new perspectives to personalized prevention in ASD.
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Declaration of interests: The authors report no conflicts of interest.

ZEBRAFISH MUTANT FOR CDKL5 MIMICS PHENOTYPES ASSOCIATED WITH HUMAN CDKL5 DEFICIENCY DISORDER

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CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental condition caused by variants in the cyclin-dependent kinase-like 5 (CDKL5) gene that encodes a kinase important for normal brain development and function. Affected individuals display a broad range of clinical features that are characterized mainly by early-onset seizures, neurodevelopmental delay, and motor dysfunction. Other phenotypes including microcephaly and dysmorphic facial features have also been described. The pathophysiology underlying CDD are still not fully understood, and the developed mouse models do not completely mimic the human disorder. Thus, the generation of other animal models like zebrafish could represent a powerful tool to further study CDD. Therefore, our goal was to characterize a cdkl5 mutant zebrafish line (sa21938) to validate it as a model for CDD. Morphometric analysis showed that cdkl5 mutants with 5 days post-fertilization exhibit a smaller head size compared to wild type (WT), indicating a microcephaly phenotype. Craniofacial defects of cdkl5 mutants were analyzed by alcian blue/alizarin red double-staining. The craniofacial cartilage structures are shorter in the homozygous mutants suggesting an abnormal craniofacial development. The motor behavior of cdkl5 mutants was analyzed by the swimming behavior before and after treatment with PTZ, a seizure-inducing drug. The distance travelled by the homozygous mutants was decreased compared to the WT, indicating an impaired motor activity. A seizure behavior and a higher distance travelled were observed after treatment with PTZ.
In conclusion, cdkl5sa21938 zebrafish reproduce many features of CDD, thus validating its use to gain insights into the physiopathology of this condition and as an animal model for the screening of drugs to rescue CDD phenotypes.

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A SEX-BIASED ANCESTRY FOR FOUR ETHNIC GROUPS IN SÃO TOMÉ AND PRÍNCIPE

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Introduction: The Island of São Tomé and Príncipe (STP), uninhabited until the arrival of the Portuguese in the 15th century, soon became an outpost for the Atlantic Slave Trade and an important cultivation area. Its first inhabitants descend from slaves but also male Portuguese colonizers. Here we analyzed the maternal and paternal ancestry of four ethnic groups of STP: Forro, Angolar, Moncó and Tonga.
Methodology: 100 full mitochondrial genomes were sequenced using Illumina MiSeq. 101 male samples were typed for 17 Y-STRs (AmpFlSTR® Y-Filer®) complemented by Y-SNPs. Phylogenetic reconstruction of mtDNA variation and Y-STR diversity was performed using Network 10 (www.fluxusengineering.com) and the reduced-median algorithm.
Results: The complete mitogenome data allows two sources of ancestry of the studied ethnic groups to be discerned: Western Africa and Angola. No maternal European ancestry was detected. Among the best represented groups in our sample, Forro (n=45) display 18% of maternal lineages of Angolan ancestry (vs. 82% from West Africa), while the Angolar (n=34) show only 3%. Despite low sampling, we observed 28% and 18% of Angolan ancestry in Tonga (n=7) and Moncó (n=11), respectively. As for the paternal ancestry, European ancestry is present in all groups (13% in Moncó, 14% in Forro and Tonga and 11% in Angolar). However, the dual African paternal ancestry is remarkably different: Forro and Moncó show 27% and 23% Angolan ancestry; Tonga show 50:50% of Angolan and West African ancestry; over 90% of Angolar lineages trace back to Angola.
Discussion: Our results show for the first time a sex-bias within African sources in STP and confirm the documented sex-bias in European ancestry. This sex-biased ancestry scenario is maximized in the Angolar, with only a residual Angolan mtDNA ancestry but dominant male ancestry, likely due to a small but dominant founder group of male slaves. The dual ancestry of these populations was also explored in the genome-wide level that supported the average values of Angolan ancestry.
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Declaration of Interests: none.

INTEGRATING FUNCTIONAL GENOMIC DATA TO PRIORITIZE CANDIDATE NON-CODING VARIANTS IN MIGRAINE SUSCEPTIBILITY

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Migraine is a disabling and multifactoria[1] neurological disease, remaining unexplained most of its heritability and susceptibility. Recent studies suggest that non-coding variants contribute to the risk of migraine and phenotypic variability through gene expression and epigenetic regulation[1,2]. Thus, our aim was to find the best SNP candidates in the Portuguese population to study cis-regulation of genes potentially associated with migraine susceptibility: SYN1, SNAP25, VAMP2, STXBP1, STXBP5, SYN2, UNC13B, GABRA3, GABRQ, and STX1A[3,4,5]. We developed a protocol based on a candidate gene approach to prioritize functionally relevant non-coding tagging SNPs by incorporating annotation methods (VEP and SNPnexus), functional genomic databases (ENCODE and Roadmap Epigenomics), and predicting scores (e.g., CADD, GWAVA, and RegulomeBD). We further assessed the putative effect of the selected regulatory SNPs on transcription factors and miRNA binding sites using prediction algorithms (e.g., MEME SUITE tools, miRDB, and RNAhybrid) and functional genomics data. Our results suggested that rs6951030 (STX1A) and rs1150 (VAMP2) may affect the binding affinity of transcription factors (such as ZNF263) influencing the expression of TBL[2] and VAMP2 genes, respectively. Moreover, rs2327264 (SNAP25) was mainly predicted to be a target of miRNAs (has-miR-4528). Together these findings provide insights into the impact of non-coding variants and new possible susceptibility genes underpinning migraine risk. Currently, we are proceeding with the functional validation of the most promising regulatory SNPs through luciferase reporter assays using migraineurs' gDNA samples.1 - Neurogenetics. 2020; 21: 149-157. 2 - BMC Medical Genetics. 2010;1 1(1): 103. 3 - Headache. 2020; 60(10): 2152-2165. 4 - PLoS ONE. 2013; 8(9): e74087. 5 - Arch Neurol. 2010; 67(4): 422-427.This work was supported by "European Commission" and "European Regional Development Fund" under the project "Análisis y correlación entre la epigenética y la actividad cerebral para evaluar el riesgo de migraña crónica y episódica en mujeres" (Interreg V-A Spain-Portugal POCTEP 2014-2020: 0702_MIGRAINEE_2_E).

WHEN FOLKLORE MEETS SCIENCE: THE 21ST CENTURY BABY TEETH COLLECTOR THAT IS HELPING US SHED LIGHT OVER RARE GENETIC DISORDERS

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The development of adequate in vitro disease models is a major issue in Basic Research of Human Genetic Diseases. Those models allow for the initial screening of novel therapeutics and help us get an insight on the cellular mechanisms that underly pathology in each case. In fact, one of the best ways to get those insights is the analysis of patient-derived cells. Yet, not every cell holds potential to recapitulate relevant disease features. Mucopolysaccharidoses (MPSs), a Lysosomal Storage Diseases (LSDs) subgroup, are among the various diseases, which would benefit from the development of novel, disease-relevant models. With an inherited, metabolic, and rare pattern, these disorders may be characterized by a strong involvement of the brain and musculoskeletal symptoms. Access to those systems is challenging and, in addition to induced pluripotent stem cells (iPSC), which generation is laborious and expensive, while having significant limitations in production and subsequent uses, there are no alternative approaches to actually recapitulate the disease.Here we present an alternative to establish patient-derived MPS cells in a much more expedite way. We are taking advantage of the existence of a population of stem cells (SC) in deciduous (baby) teeth (SHEDs) to establish a new cell model for MPSs. So far, we have already implemented and optimized the protocol for collection, isolation, establishment and cryopreservation of those stem cells. Then, our rationale is simple: for those obtained from MPS patients suffering from multisystemic disease with musculoskeletal alterations, we are using a chondrogenesis differentiation protocol. For those derived from patients with neurological pathology, we will establish mixed neuronal/glial cultures. This is a total innovation in the field and we believe it holds potential to set a new trend for investigating the cellular/gene expression changes that occur in MPSs as it relies on a non-invasive, cost-effective approach that can be set as a routine in any lab with standard cell culture conditions. Ultimately, the same method may be applied for virtually any monogenic disorder with the same sort of "model issues".
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ALTERED TIGHT JUNCTION-ASSOCIATED PROTEINS SUBCELLULAR LOCALIZATION IN MACHADO-JOSEPH DISEASE/ SPINOCEREBELLAR ATAXIA TYPE 3

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Introduction: The blood-brain barrier (BBB) is a key cellular barrier that controls CNS homeostasis. Our group has shown that BBB is impaired in Machado-Joseph disease (MJD), a disorder caused by an expansion of the CAG repeat in the ATXN3 gene. BBB integrity was shown to be compromised in the cerebellum of MJD mice and human postmortem brain tissue of MJD patients. Here, we aimed at further investigating alterations in levels and subcellular location of tight junction (TJ)-associated proteins.
Methodology: Levels and subcellular localization of the TJ-associated proteins ocluddin, claudin-5, and the cytoplasmic adaptor zonula occludens (ZO)-1 were assessed by western blot of cerebellar tissue from a transgenic (Tg) MJD mouse model, expressing mutant and truncated human ataxin-3.
Results: Occludin levels were decreased, while claudin-5 monomeric form was increased in Tg mice compared with Wt controls. In the membrane fraction, there was also a decrease in ocluddin levels. Interestingly, claudin-5 oligomeric form was decreased, while levels of the monomeric form were increased in Tg animals, as compared to controls, suggesting that claudin-5 oligomeric assembly in the membrane is compromised. Finally, ZO-1 levels were decreased in the cytoskeleton in Tg mice relative to Wt, which may impair adequate BBB functio-nality.
Discussion: Our results suggest that in MJD there is a dysregulation in levels and subcellular localization of TJ-associated proteins, thus furthering our understanding of the mechanism(s) involved in BBB impairment.
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REGULATION OF ZNF687 DURING OSTEOBLAST DIFFERENTIATION

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ZNF687 gene translates a zinc finger protein involved in bone metabolism, although its function is poorly understood. ZNF687 was found mutated in individuals with severe Paget's Disease of Bone (PDB), which is a bone disorder characterized by abnormal bone remodeling. The osteoclasts are giant and hyperactive increasing the bone resorption that is followed by an excessive/aberrant osteoblast-mediated bone formation resulting in deformed bones susceptible to fracture. Previous studies showed that ZNF687 is highly expressed during the regeneration of zebrafish caudal fins and overexpressed in peripheral blood mononuclear cells of PDB patients, indicating that ZNF687 is implicated in bone metabolism. Therefore, this work aimed to analyze the expression of Znf687 and its regulation during osteoblastogenesis. MC3T3-E1 cells were differentiated into osteoblasts using an osteogenic cocktail for 28 days. The mineralization was detected by alizarin red staining and RNA and gDNA were extracted. The expression of Znf687 and several miRNAs were analyzed through qPCR while Znf687 methylation was determined by direct bisulfite sequencing. Our results showed a higher expression of bone markers and a significant calcium deposition in cells treated with the osteogenic cocktail compared to the non-treated cells, confirming the successful differentiation of osteoblasts. Znf687 was downregulated throughout osteoblast differentiation. To investigate the potential mechanism for the altered expression, we analysed the methylation profile of Znf687 and the expression of miR-142a, miR-122b and miR-124, that have putative binding sites in Znf687 3'UTR. Our results showed that Znf687 is hypomethylated in both osteoblasts and undifferentiated cells and the expression of the three miRNAs was found higher in mature osteoblasts. In conclusion, our work indicates that Znf687 may have a role in osteoblastogenesis and DNA methylation does not appear to be involved in Znf687 regulation, however it might be post-transcriptionally regulated by miR-142a, miR-122b and miR-124. Further studies are needed to elucidate the mechanisms involved in Znf687 regulation during this process.

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DNA METHYLATION PROFILES IN MACHADO-JOSEPH DISEASE: A PILOT STUDY IN WHOLE BLOOD SAMPLES FROM PATIENTS

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Machado-Joseph disease (MJD) is a late-onset neurodegenerative disease for which epigenetic studies are scarce. DNA methylation (DNAm) is the most widely studied epigenetic mechanism and plays a pertinent role in several neurodegenerative diseases, such as Alzheimer's disease and Huntington's disease, where a discrepancy between chronological age and age predicted from DNAm data – age acceleration- has been reported. Our pilot study aimed to obtain a MJD genome-wide blood-based DNAm profile and to explore the existence of epigenetic age acceleration in MJD. The DNAm profiling was performed using the Illumina EPIC array on whole blood DNA samples from eight MJD patients and eight controls, matched for age, gender, smoker status and DNA storage time. The DNAm data was analysed using different R Bioconductor packages. The list of genes presenting the most differently methylated CpGs was ranked by p-value, and an enrichment analysis was performed to identify overrepresented gene ontology terms and pathways. Two epigenetic clocks were then used to obtain DNAm age: Horvath Pan-Tissue and Hannum Blood Based clocks. We obtained a list of 1279 genes presenting differently methylated CpGs (nominal p-value < 0.005). Enrichment analysis showed an involvement of pathways previously related with neurodegenerative diseases, such as calcium signaling pathway. We were not able to find differences between MJD patients and controls for the estimated DNAm age obtained with both epigenetic clocks, nor for measures of DNAm age acceleration. These preliminary results can be influenced by sample size and the associated limited power of this study and need to be confirmed in further studies, with a higher number of DNA samples from individuals with detailed lifestyle factors. Moreover, the study of DNAm patterns in post-mortem brain samples of SCA3 patients is mandatory, since these patterns should be tissue specific; such studies are currently being developed by our group.

LOW COVERAGE DEPTH NANOPORE SEQUENCING IS AN ALTERNATIVE TO GENOMIC MICROARRAYS FOR DETECTION OF COPY NUMBER VARIANTS IN THE HUMAN GENOME

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Introduction: Copy number variations (CNVs) represent ~13% of the human genome1. These structural variations (SVs) can produce a genetic phenotype through a dosage effect or other mechanisms. High-resolution whole genome microarray analysis is a gold standard tool for detection of CNVs associated with genetic disorders. Paired-end short read sequencing is an alternative approach but lacks mapping accuracy in highly repetitive DNA regions, which are enriched in SVs2, and reduces genome contiguity, limiting the detection of large size SVs. We applied long read whole genome sequencing to call CNVs and compared the results with those obtained by microarray.

Methodology: Genomic DNA from the eosinophilic leukemia EOL-1 cell line was analysed using a CytoScan HD Array and CNVs were called using ChAS software (ThermoFisher). DNA was also sequenced on the portable MinION device (Oxford Nanopore Technologies) following a rapid library preparation method. Sequencing data were basecalled using Guppy and mapped with LRA. SVs were called using CuteSV. A minimum CNV calling size threshold of 35 Kb was used in both methods.

Results: Curated microarray analysis confirmed 22 CNVs in EOL-1 ranging in size between 35 Kb and 1.4 Mb. Twenty CNVs (91%) were correctly called in nanopore data at a mean genome coverage depth of 13X. One uncalled deletion was found to span a much smaller region (~12 Kb) compared to the size obtained in microarray analysis (~42 Kb). Furthermore, nanopore sequencing allowed the refinement of several breakpoint positions of deletions and duplications, thus accurately defining the gene sequences involved in each CNV.

Discussion: Nanopore sequencing technology proved to be technically effective in the detection of CNVs of different types and sizes. This approach may be an alternative to the use of genomic microarrays in the detection of pathogenic SVs associated with genetic diseases.

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GENERATION OF MICE EXPRESSING IN THE LIVER HUMAN SPLICING MUTATIONS: A GOOD MODEL TO TEST IN VIVO THE THERAPEUTIC EFFICACY OF MODIFIED U1 SNRNAS FOR MUCOPOLYSACCHARIDOSIS TYPE IIIC

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Mucopolysaccharidosis type IIIC (MPS IIIC) is a very rare Lysosomal Storage Disorder caused by mutations in the HGSNAT gene that encodes an enzyme involved in heparan sulphate degradation. Splicing mutations are one of the most frequent (~20%) genetic defects in MPS IIIC. Around 55% correspond to 5' splice-site (ss) mutations thus constituting a good target for splicing therapeutics. Previously, we have demonstrated that a modified U1 snRNA vector designed to improve the definition of the HGSNAT exon 2 5'ss can restore splicing impaired by the mutation

c.234+1G>A[1]. Currently, our goal is to evaluate in vivo the therapeutic potential of that modified U1 snRNA by testing it in mice expressing the human splicing defect. For this purpose, two full-length constructs were generated by cloning the wild-type (WT) or the mutant HGSNAT splicing-competent cassettes in the pcDNA 3.1 vector. Then, both constructs were transfected in Hep3B, COS-7 and HEK293T cells reproducing the WT and mutant splicing patterns. Therefore, these constructs were used to generate mice expressing the WT (c.234+1G) or mutant (c.234+1A) alleles in the mouse liver. So, these mice can be used for testing in vivo the modified U1 snRNAs efficacy. For the transient expression, WT and mutant constructs were administrated by hydrodynamic injection following a protocol described by Balestra et al. [2]. After 24h or 48h, animals were sacrificed, the liver was collected, and the molecular analysis was performed. Preliminary results showed the expression of both constructs in the liver of several animals. To obtain the sustained expression, presently we are also cloning both constructs in adeno-associated viral (AAV) vectors. Both methodologies could be used for the in vivo testing of splicing correction therapies, such as modified U1 snRNA vectors.

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EPIGENETIC CHANGES IN PACLITAXELRESISTANT BREAST CANCER CELLS

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Introduction: Breast cancer (BC) is the largest cause of cancer-related mortality in women. The acquisition of cancer drug resistance (CDR) is a hurdle to effective treatments. Epigenetic changes seem to have a relevant role in the establishment of CDR. Paclitaxel (PAX) is used as first-line therapy together with other agents to manage metastatic and early-stage BC or can be used alone as a second-line agent for the treatment of metastatic BC. Aims/Methodology: Since the mechanisms behind PAX resistance are not entirely known, our aim was to develop a metastatic BC cell line resistant to PAX, to study which mechanisms influence resistance acquisition. Resistance was monitored by measuring cell viability (MTT assay), changes in cell morphology (confocal microscopy), and cell cycle (flow cytometry). We assessed global DNA methylation patterns using a specific ELISA kit and global miRNAs expression profiles through microarray.

Results: We show that the PAX-resistant cell line had a change in its cell cycle, an altered cell morphology, specifically an increase in cytoplasm area, increased nuclear fragmentation, and an increased number of lysosomes. Intriguingly, these cells retain their viability and continue to proliferate. We show that the percentage of global methylated DNA does not increase linearly with PAX concentration. Furthermore, we detected 6631 miRNAs of which only 303 were differently expressed. Of these, 198 were up-regulated and 105 were down-regulated. A pathway enrichment analysis of these differently expressed miRNAs revealed that the most significant KEGG pathways were "Proteoglycans in cancer", "Hippo signalling pathway", "Pathways in cancer", "p53 signalling pathway", "Ubiquitin mediated proteolysis", "FoxO signalling pathway".

Discussion/Conclusion: We show that despite cell morphological changes, resistant cells are viable and that lysosomes may play an important role in the acquisition of PAX resistance. There is a non-linear behaviour in global DNA methylation patterns and analysis of differentially expressed miRNAs might help clarify which mechanisms are behind the acquisition of PAX resistance.

PATHWAYS INVOLVED IN THE DOWNSTREAM EFFECTS OF CHROMOSOME 17Q12 MICRODELETION SYNDROME: INSIGHTS ON POSSIBLE CAUSES FOR DIFFERENT SYMPTOMS

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Introduction: Chromosome 17q12 microdeletion syndrome results from a structural chromosomal alteration in the long arm (q) of chromosome 17. Patients with this syndrome show various signs and symptoms, however, abnormalities of kidneys/urinary tract and the development of type 5 diabetes (MODY5), are the most evident. Cytogenetic studies at the level of copy numbers variations (CNVs) have demonstrated a high incidence of neurodevelopmental disorders such as autism spectrum disorders (ASD) and intellectual disability. Understanding which genes are involved in this microdeletion make it possible to associate their absence with the different syndromic symptoms and the impact they have on neurodevelopmental diseases.

Methodology: PathVisio for chromosome 17q12 pathway was used; PathVisio is a biological pathway creation and curation software that in this case was used to describe all genes encoding in this region and all the proteins associated with them, as well as the functions that these proteins exert in our body. The information was obtained from the public databases Ensembl, UniProt, WikiPathway and HGNC as well as literature references.

Results and Discussion: The creation of chromosome 17q12 pathway allow us to verify that there are 99 coding genes in that region, but only 15 of these are involved in the 17q12 microdeletion syndrome. These 15 genes encode different proteins, most of them with different functions, which may explain the wide variety of symptoms associated with this syndrome. The dosage of these 15 genes is so sensitive and essential for normal brain function that a deletion in one or more of them possibly confers a high risk for ASD. The compilation of all this information allows the scientific community to access the pathway of chromosome 17q12 in a faster and clearer way, aiming to help research studies associated with this syndrome, as well as correlating them with other CNVs that are involved with neurodevelopmental diseases.

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BRCA1 VUS: THE LINE BETWEEN BENIGN AND PATHOGENIC CLASSIFICATION

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Introduction: The evolution of personalized medicine has opened the path to routine large-scale sequencing and increased the importance of genetic counselling for hereditary cancers, in particular hereditary breast cancer. Several high penetrance genes for breast cancer (e.g. BRCA1/2, ATM) belong to DNA repair pathways. Thus, genetic testing for susceptibility genes through Next Generation Sequencing (NGS) has become a standard, and a number of genetic variants have been identified in these genes, several of which are variants of unknown significance (VUS). These VUS can either be pathogenic or benign, but since their biological effect is unclear, functional assays need to be carried out to classify their mutational nature.

Methodology: The involvement of DNA repair genes in breast cancer development has the advantage of allowing us to assess the impact of genotoxic agents through various assays. Accordingly, a functional approach was performed in peripheral lymphocytes of 2 women with a VUS in the BRCA1 gene (NM_007294.3:c.1067A>G) and compared to results of 2 non-carriers. Several assays (chromosomal aberrations, cytokinesis-blocked micro-nucleus assay, comet assay, γH2AX, caspases and TUNEL assay) were conducted after a genotoxic challenge by ionizing radiation or doxorubicin in order to evaluate the functional role of the identified BRCA1 VUS.

Results: Our results revealed a higher DNA induced-damage in the non-carrier group compared with VUS carriers using as end-points the micronucleus (MN) and the TUNEL assays. These results suggest that

this BRCA1 VUS is probably benign, since the VUS carriers were even apparently protected from deleterious chromosomal rearrangements, reducing genomic instability and conse-quently the activation of programmed cell death.

Conclusion: Genome sequencing has revolutionized the diagnosis of genetic diseases, requiring a close collaborations between basic scientists and clinical geneticists to associate genetic variants with disease causation. We believe that the strategy followed here could be successfully applied to study other variants, helping in clinical counselling.

UNRAVELLING THE MOLECULAR MECHANISMS DEREGULATED IN LAP1-ASSOCIATED DISEASES USING A PROTEOMICS APPROACH

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Although rare, mutations in nuclear envelope (NE) protein-encoding genes are a major threat to cell homeostasis by compromising nuclear integrity and function, as well as nucleocytoplasmic signaling. Genetic alterations in the human TOR1AIP1 gene that codes for lamina-associated polypeptide 1 (LAP1), a ubiquitous NE protein, have been causatively linked to the development of several, often life-threatening, diseases (e.g. muscular dystrophy, myasthenic syndrome and multisystemic disorder). The pathogenic effects of TOR1AIP1 mutations hint at a physiologically important role of human LAP1, but its functional properties and the molecular repercussions of its deficiency remain unclear. In this work, we used the liquid chromatography with tandem mass spectrometry (LC–MS/MS) technology to perform a quantitative proteome analysis of patient-derived skin fibroblasts carrying a pathological TOR1AIP1 mutation that leads to strongly reduced LAP1 protein levels (p.E482A), previously reported in a case of severe dystonia, cerebellar atrophy and cardiomyopathy. This proteomics approach permitted the identification of up-/down-regulated proteins in LAP1 E482A fibroblasts relative to age-matched control fibroblasts. A functional analysis of these differentially expressed proteins using bioinformatics tools revealed various signaling pathways deregulated in LAP1-deficient cells, such as DNA repair, neurodevelopment and myogenesis. Our results provide novel insights on LAP1's biological relevance for the maintenance of cell homeostasis and unveil potential molecular mechanisms to be targeted in future therapies for LAP1-associated pathologies.

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RAMP1 PROMOTER METHYLATION STATUS IN PORTUGUESE AND SPANISH WOMEN WITH MIGRAINE

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Introduction: Migraine is a common neurological disorder, which predominantly affects females. Calcitonin Gene Related Peptide (CGRP) is a neuropeptide implicated in migraine and its receptor consists of 3 proteins: calcitonin receptor-like receptor (CLR), receptor activity modifying protein (RAMP1), and receptor component protein (RCP). DNA methylation is an epigenetic mechanism that adds a methyl group to DNA often at the promoter region, which can then modulate gene expression. Aberrant methylation levels have been associated with various diseases. Our aim was to investigate the RAMP1 promoter methylation status to find epigenetic biomarkers that can predict migraine risk in an accessible body fluid (blood samples).

Methods: We investigated the methylation state of the RAMP1 promoter in 126 blood DNA samples from Portuguese and Spanish women (76 migraineurs and 50 controls). We treated DNA with sodium bisulfite and performed PCR, Sanger Sequencing and Epigenetic Sequencing Methylation software analysis.

Results: In the RAMP1 promoter, a sequence of 493bp was amplified and 51 CpG dinucleotides were identified, with 5 of them showing methylation variability. Migraine cases showed a higher number of individuals with all five CpG sites methylated when compared to controls (26% vs 18%). We also found a CpG that showed significantly higher methylation levels in cases (p=0.006, OR=1.07; 95% C.I: 1.02-1.12).

Discussion: The herein presented results reinforce recent findings1 that methylation at this CpG may potentially play a role in migraine by affecting RAMP1 transcription.

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GENETIC VARIANTS IN THE IFNGR2 LOCUS ASSOCIATED WITH SEVERE CHRONIC Q FEVER

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Introduction: Q fever, caused by Coxiella burnetii, is a zoonotic disease that can manifest as acute disease (mainly self-limited febrile illness with hepatitis and/or pneumonia) or as a chronic disease (mainly endocarditis). The association between genetic variants in immune response genes and chronic Q fever remains practically unexplored. We sought to identify novel variants associated with chronic Q fever in these biological pathways.

Materials and Methods: The present case-control genetic association study included 60 Q fever patients (43 acute and 17 chronic cases) and 43 healthy individuals representative of the general Portuguese population. Long range Takara LA Taq® DNA polymerase was used for Nextera library construction and Illumina® NGS. Variant calling was performed using GATK. Genotype-phenotype association was performed using the PLINK toolset. Allelic and genotypic frequencies were also compared to those observed for the general population. SNPs in association with chronic Q fever were investigated for linkage disequilibrium (LD) patterns. Haplotype blocks were estimated following the default procedure in Haploview via PLINK v1.7. Variants in putative regulatory regions were analyzed using the GTEx functional genomics database.

Results: NGS provided 607 SNPs from 94 DNA samples. Of these, 405 SNPs and 82 DNA samples passed quality control. Altogether, 29 SNPs with statistically significant associations were identified, four of which

in the IFNGR2 passed the Bonferroni correction. These were referenced in GTEx as possible eQTLs. Three of the SNPs belonged to a 64.17kb haplotype, significantly associated with chronic Q fever. It was identified in 20% of the chronic Q fever cases, absent from the acute Q fever control group and detected in 7% of the general population samples. The entire sequenced region of IFNGR2 was also investigated for LD patterns with the associated variants.
Conclusion: The possible involvement of the associated variants with a higher expression of IFNGR2 could be in line with observations suggesting that IFN-γ production in chronic Q fever patients is significantly higher than in healthy controls. Further investigations are required to clarify the role of IFNGR2.

Y-STRS GENETIC DIVERSITY IN ETHNIC GROUPS FROM BENIN

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Introduction: Benin has a marked socio-cultural and linguistic heterogeneity reflected in its more than 60 ethnic groups. For centuries, its people have been under the influence of many African kingdoms and, between the 16th and the 20th centuries, have also significantly contributed to the North Atlantic Slave Trade. It is, therefore, of interest to characterize and deepen our understanding about the genetic variation in nowadays Beninese.
Methodology: We have genotyped 17 Y-STR markers in 435 unrelated males from several Beninese ethnicities, using the AmpFlSTR® Y-Filer® (Thermo Fisher Scientific). Allele and haplotype frequencies and molecular diversity indexes were calculated using the Arlequin software and multidimensional scaling (MDS) plots were built to represent the genetic relatedness among Beninese and other selected ethnic groups.
Results: A total of 368 different Y-STR haplotypes were observed, thus rendering a discrimination capacity of 84.6% and a haplotype diversity of 0.9981 (DYS385a/b not considered) in the male Beninese population. Among the best represented ethnic groups in our sample set, the Bariba and the Dendi exhibit the highest Y-STR gene diversity, while the Aizo are suggested as having the least diverse genetic profiles. MDS analysis showed that Aizo, Idatcha and Mahi are significantly distant from other Beninese. When comparing our data with published data for selected neighboring populations, most Benin ethnic groups cluster together with other Western Africans (Ghana, Nigeria and Burkina-Faso), with the exception of Aizo.
Discussion: The results of this study agree with historical records of long-term socio-cultural relationships and demographic events where Beninese have participated. For example, the high genetic diversity of Bariba likely reflects their contacts with Sahelian pastoralists and Islamic groups, while the low diversity of Aizo results from their isolation since the slave raids. In sum, this dataset contributes to our understanding of the patterns of male genetic variation and demographic events in Benin and sub-Saharan West-Central Africa, demonstrating their usefulness for population and forensic genetics.

MOLECULAR STUDY OF MODY: LATEST RESULTS FROM 2011 TO 2022

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Introduction: MODY diabetes is a monogenic condition caused by a genetic variant in one of 14 genes associated with this disease. This condition is often misdiagnosed as type 1 or type 2 diabetes, which can result in inadequate disease management. No particular biomarker or clinical sign allows to diagnose MODY and the patient´s phenotype is very heterogeneous. Thus, genetic testing is the only method that provides the correct diagnosis to patients and their clinicians. Here, we present the latest results of our 11-year project.
Methods: Blood samples were collected from 178 participants, 112 with a clinical diagnosis of MODY and their relatives, from which DNA was extracted for PCR amplification and DNA sequencing. The promoter region, coding sequences and adjacent intronic regions of GCK, HNF1A, HNF1B and HNF4A genes were screened for genetic variants. This was followed by in sílico analysis of the identified variants and they were classified according to ACMG recommendations. Large rearrangements studies were done by MLPA.
Results: A pathogenic or probably pathogenic variant was identified in 32 index patients and, through cascade screening, 24 relatives were also diagnosed with MODY. We found pathogenic or probably pathogenic variant in the four genes studied but most variants were identified in the GCK gene. It was not possible to identify a genetic aetiology for 41 index patients.
Discussion: Approximately 28% of the index cases were diagnose with MODY, in which most of the variants identified were in GCK gene. Although we didn´t find a pathogenic variant in 36% of the index patients, we suspect that some of them may have alterations in the rarer genes. In near future we hope to be able to study them with a NGS MODY panel that we are validating in the department. In conclusion, there are still many challenges that we want to overcome so that the concept of personalized medicine becomes an accessible reality for MODY patients in Portugal.

WHOLE GENOME SEQUENCING ANALYSIS: THE FUTURE IN HEALTHCARE

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Genomics medicine is a very important and largely active research field, driven by needs in healthcare. The complexity of biological systems and clinical problems has potentiated the emerging of high-throughput technologies for DNA sequencing to study different genomic areas (genome, transcriptome, proteome and metabolome). The genome is prone to genomic aberrations, such as single nucleotide variants (SNVs) or structural variants (SV). Whole genome sequencing (WGS) is one of the preferred methods to find inherited disease-associated variants. Complete loci sequencing of well-selected patients' cohorts, may be advantageous to identify coding and non-coding alterations in disease-causing genes. Among other considerations, a lack of user-friendly data analysis and interpretation tools are the major barrier to routine use of these techniques. We proposed a WGS analysis workflow for Alignment (BWA mem); Post-processing (GATK tools); CNV calling (results integration of LUMPY, Delly and GRIDSS) or SNV calling (results integration of HaplotypeCaller from GATK and DeepVariant); and Management of multiple called variants (overlap fractions of CNV calls and overlap analysis for SNV calls). Besides conventional alignment and post-processing, our proposed WGS analysis workflow uses a combination of multiple variant callers for both CNV and SNV, which were submitted to overlap analysis to improve the pick-up rate of true positives. The work that we have been developing allowed us to understand the main problems in analysing WGS and how to solve them. With this pipeline, we will be able to analyse WGS, fast and accurately, and give opportunity to integration into other pipelines. We also aim at producing readouts easy to understand for health-practitioners.
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DIFFERENTIAL EXPRESSION OF DRUG METABOLISM ENZYMES IN DOXORUBICIN RESISTANT BREAST CANCER CELLS

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Introduction: Altered expression and/or activity of drug metabolism enzymes (DMEs) is a hallmark of chemotherapy resistance. Doxorubicin (DOX), an anthracycline used in breast cancer (BC) treatment, is metabolized mainly by CYP3A4 and in a minor extent by CYP2D6, 2B6, 1B1, and several oxidoreductases (https://go.drugbank.com/). This study aimed to investigate the role of Phase I DMEs involved in the first stages of acquisition of DOXresistance in BC cells.
Methodology: The expression of 92 genes was assessed (RT-qPCR) of either sensitive (parental) (MCF-7/DOXS) or DOX-resistant (MCF-7/DOXR 25 nM or 35 nM) cells – engineered by stepwise exposures to increasing concentrations of DOX. The fold change difference in genes expression was determined by the 2 –ΔΔCt method. Microsomal fractions were used to measure CYP-complex enzymes activities, using standard probe substrates.
Results: Significantly altered levels of transcripts were detected in MCF-7/DOXR (12 CYPs and 8 oxidoreductases), albeit not for CYP3A4. The analysis of CYP-complex enzymes activities showed a significant decrease of coumarin 7-hydroxylation (C7H) activity (0.68 X; $P < 0.005$) and an increase of dibenzylfluorescein O-debenzylation (DBFOD) (1.29 X; $P > 0.05$) activity, in MCF-7/DOXR 25 nM.
Discussion: The genes detected to be differentially expressed in MCF-7/DOXR were indicated previously to be involved in tumor progression and/or chemotherapy response in BC (e.g., CYP2S1, 3A5, 4F12, 4V2, 26B1, cytochrome b5 reductase, kynurenine 3- hydroxylase, or phenylalanine hydroxylase). A discrepancy was observed between C7H or DBDOF activities (mediated particularly by CYP2A6, or by CYP3A4 and 3A5, respectively) and the corresponding levels of mRNA transcripts. This is indicative that the phenotype of DMEs is not linearly correlated with transcription induction responses, confirming the multifactorial complexity of this mechanism. Nevertheless, our results pinpoint the potential role of specific CYPs and oxidoreductases, involved in the metabolism of xenobiotics, fatty acids, sterols, and vitamins, in chemo-resistance and carcinogenesis of BC. Partly funded by the Research Center grant ToxOmics (UIDB/00009/2020).

GENETIC AND EPIGENETIC CONTRIBUTION OF THE BDNF AND NTRK2 GENES TO THE DEVELOPMENT OF LEIOMYOMAS

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Introduction: Leiomyomas are the most common benign tumors of the uterus in women at the reproductive age and leiomyoma susceptibility is strongly influenced by genetics1. BDNF neurotrophin together with its receptor, TrkB (NTRK2 gene), may contribute to tumor progression through characteristics such as inhibition of apoptosis and promotion of cell proliferation2. Thus, variants were selected in each gene, with the main goal of analysing the genetic and epigenetic contribution of the BDNF and NTRK2 genes to the development of leiomyomas. Additionally, we assessed if there were differences in the methylation patterns of the gene promoters between populations.
Methodology: 240 DNA samples derived from women with leiomyomas (120) and from healthy controls (120) were used. For rs6265 (BDNF) and rs2289656 (NTRK2) we performed an endpoint genotyping analysis. To assess differences between the methylation patterns, we performed a Melting Curve Analysis - Methylation assay. Statistical tests were performed with IBM® SPSS® Statistics 27.0. Statistical significance was defined as a p-value < 0.05.
Results: T allele of rs6265 (BDNF) was found to be protective (OR = 0,457, IC (95%) = [0,248 – 0,842]). As for the NTRK2 gene, there was no association found. Regarding the methylation assay, for both genes, all samples showed similar melting temperatures to Non-Methylated Control.
Discussion: rs6265, a missense mutation, changes the structure of BDNF, altering its interaction with SorCS2, a co-receptor, resulting in lower BDNF activity and plasma levels. Since higher BDNF expression is associated with tumor progression, the results were expected. No differences in the methylation patterns were verified between samples, so, epigenetics at this level, seems to be not involved in the development of this disease.
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MOLECULAR CHARACTERIZATION OF CONGENITAL ERYTHROCYTOSIS AND IDIOPATHIC ERYTHROCYTOSIS ANALYSED BY NEXT-GENERATION SEQUENCING

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Erythrocytosis or polycythemia is a pathology charac-terized by a significant increase in erythrocyte mass (>125%) as well as an increase in hemoglobin and hematocrit levels to reference values according to age and sex. Erythrocytosis can be congenital (CE) or acquired. According to pathophysiological mechanisms and based on the levels of erythropoietin (Epo), they can be classified as primary or secondary. Approximately 60% of patients still do not have an identified molecular etiology, being designated as having idiopathic erythrocytosis (IE). Next-Generation Sequencing (NGS) studies are essential to identify new pathogenic variants in genes already described or in candidate genes that can clarify the origin of the pathology.
Design and Methodology: In this study, 77 samples of patients with IE were analysed. Laboratory tests were guided by clinical and family history and Epo levels, using NGS and Sanger sequencing, with a NGS panel of genes dedicated to erythrocytosis to find pathogenic variations which justify the presented phenotype.
Results and Discussion: The study carried out allowed the identification of variants in 28 of the 77 individuals studied. In 5 patients, 4 pathogenic variants that were already described as associated with CE were detected in the SH2B3, HBB, and VHL genes. In 15 patients, 13 new variants in EPOR, JAK2, SH2B3, EGLN1, EPAS1, EPO, PKLR, and VHL genes were detected. In 8 patients we found 6 new variants in candidate genes, EGLN2, EGLN3, HIF1α, HIF3α, and PIEZO1. The degree of pathogenicity of the variants found was evaluated using in silico tools. Of the 18 variants analyzed: 6 were classified as Pathogenic, 1 as Likely Pathogenic, and 4 as Variants of Uncertain Significance. The remaining 7 variants were classified as Benign or Likely Benign.
Conclusion: This study allowed the identification of pathogenic variants in 5 of the individuals studied. In 18 individuals, variants classified by the in silico tools as probably pathogenic or of uncertain significance were detected. It will be necessary to confirm the pathogenicity of these variants with family and functional studies. When verifying that they are pathogenic variants, the study carried out allowed the identification of the molecular cause responsible for CE in 11 of the 18 samples studied by in silico tools.
KeyWords: congenital erythrocytosis, idiopathic ery-throcytosis, erythropoietin, hypoxia, Sanger, Next-Generation Sequencing (NGS).

FUNCTIONAL CHARACTERIZATION OF APOB VARIANTS FROM FAMILIAL HYPERCHOLESTEROLEMIA CASES

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Introduction: Familial hypercholesterolemia (FH) is clinically characterized by increased levels of circulating LDL cholesterol leading to premature coronary heart disease. It can be caused by variants in LDLR, APOB, and PCSK9 genes. APOB variants are responsible for 5-10% of

the FH cases, p.(Arg3527Gln) being the most common, and until recently only two small fragments of the gene were routinely studied. With Next Generation Sequencing being implemented in the last years, the whole APOB gene has been sequenced routinely by many labs, increasing the variant spectrum of APOB and with it the number of variants that need to be functionally assessed. Most of these variants are missense variants but nonsense and small indels in exon 29 are also being identified and can be the cause of disease. This project aimed to characterize 5 novel APOB variants identified in patients included in the Portuguese FH Study to confirm if they are the genetic cause of hypercholesterolemia in these patients.
Methodologies: We performed the analysis of APOB fragments from index cases to confirm the variants previously detected by NGS and performed cascade screening in families. To access if the variants under study affect the binding of the apoB to the LDL receptor, LDL from index cases and relatives with the variants was separated using sequential ultracentrifugation, and proliferation assays were performed with U937 cells. These cells do not synthesize cholesterol but need it to grow, they depend on apoB: LDL receptor binding.
Results: U937 proliferation is reduced when there are defects in the APOB gene, meaning that these variants affect LDLR activity.
Discussion: Functional studies are crucial to increase the scientific knowledge about the effect of the variants in protein function, being one of the most important criteria to be able to classify variants as pathogenic. Functional studies of FH associated variants can confirm the clinical diagnosis by highlighting the cause of disease, contribute to stratify patient associated cardiovascular risk and optimize treatment.

ANALYSIS OF R1B-M269 SUBHAPLOGROUS IN THE CENTRAL REGION OF PORTUGAL

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Introduction: The most frequent Y-chromosome haplogroup in West Europe is R1b-M269, representing frequencies of about 60% in Portugal. Several reports have gone deeply into the study of the R1b-M269 subhaplogroups. R1b-M269 is split into geographically localized subhaplogroups, showing the S116-DF27 branch as the most common in the Iberian Peninsula (40-48%). This study aimed to characterize the main branches of R1b-M269 in the central region of Portugal and, in particular, to examine the DF27 sublineages.
Methodology: The sample analyzed comprised a total of 90 individuals carrying the derived allele at the M269 SNP (haplogroup R1b-M269), mainly from districts of Viseu, Coimbra, Guarda, and Aveiro. The main immediately known subhaplogroups for R1b-M269 were analyzed, including U106, S116, DF27, U152, and M529. The six known subbranches of DF27 were also analyzed, including Z196, L617, L881, A431, F1343, and Z2571, following the ISOGG 2019-2020 Y-DNA Haplogroup R Tree. Genotyping was made by Sanger sequencing or PCR-RFLP using primers previously described or designed according to DNA templates.
Results: The subhaplogroup DF27, belonging to paragroup S116, is the most common (70%) of the M269 individuals. The S116 subtypes U152 and M529 reached frequencies of 3.3% and 12.2%, respectively, and five (5.6%) individuals were assigned to belong to the S116* paragroup (×U152, ×M529, ×DF27). Four (4.4%) individuals belong to the subhaplogroup U106, and four (4.4%) were assigned to belong to the M269* haplogroup (xU106, xS116). The dissection of the Y chromosome subhaplogroup DF27 for its known sublineages revealed a high frequency for F1343 (41.1%). From the other DF27 sublineages, only the Z196-derived allele was present with a frequency of 5.6%.
Discussion: The frequencies of M269 sublineages obtained in our study are similar to other results reported for the Iberian Peninsula regions. For the first time, we highlight F1343 as the most common DF27 sublineage found in Portugal and an Iberian Peninsula population.

BUILDING ATAY SACHS VARIANT B1 CELLULAR MODEL

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Tay Sachs disease variant B1 (TSD B1; OMIM 272800) is a neurodegenerative lysosomal storage disease (LSD) which, although rare, is the most frequent form of TSD in Portugal. The mutation p.R178H (c.533G>A; rs 28941770), associated with TSD B1, leads to a mutant HexA protein with altered kinetics and reduced residual activity. The mechanism leading to this variant is not well established, but the variant protein is unlikely to be degraded by the endoplasmic reticulum (ER)-associated protein degradation (ERAD) since it has residual activity in lysosomal-like conditions. The availability of disease-relevant cell types derived from induced pluripotent stem cells (iPSCs) provides a model for clarifying the pathogenic mechanisms and, eventually, test therapeutic approaches for TSD B1 patients. The main objectives of this project are: to generate a neuronal TSD B1 specific cellular model using iPSCs and to implement genetic profiling by Next Generation Sequencing (NGS) to examine potential changes in the manipulated cells. In this work, we present all the steps from the iPSC reprogramming, and initial differentiation into neural progenitor cells (NPCs), up to the preliminary NGS results obtained with the donor fibroblasts. Using non-integrative episomal vectors we obtained iPSCs from TSD B1 patient and control fibroblasts. The iPSCs require multiple characterisation steps to ensure that they mimic the donor background at genetic and protein levels. A lysosomal-related gene profiling of the DNA from the original cell lines, using an in-house customized NGS panel, was performed. The results of the cells in a "naïve" state will later be compared with TSD B1 iPSCs and NPCs. Due to the extensive handling of iPSCs, multiple checkpoints and repetitions are required to ensure the accuracy and reproducibility of the cell models. Such work will be continued in the near future.
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DOPA-RESPONSIVE DYSTONIA: THE FIRST CONFIRMED CASE OF TYROSINE HYDROXYLASE DEFICIENCY IN PORTUGAL

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Background: Dopa-responsive dystonia (DRD) is a rare movement disorder associated with defective dopamine synthesis. This impairment, if due to a primary deficiency, may have at least four genes at its origin. Differential diagnosis is made through detailed clinical assessment, analysis of cerebrospinal fluid (CSF) neurotransmitter metabolite patterns and molecular investigation to confirm the diagnosis. Among DRDs is tyrosine hydroxylase deficiency (THD), a rare autosomal recessive and treatable neurometabolic disorder. Worldwide, less than 100 cases have been reported.
Research and case report: We describe a 7-year-old female presenting a sudden onset of symptoms at six months of age with episodes of global dystonia, hypotonia with poor movements and absence of cervical control, tremor, hyperreflexia in lower limbs, striatal toe and developmental delay. An early CSF biochemical analysis showed alterations in dopaminergic pathway metabolites, with a normal serotonergic pathway, suggestive of TH deficiency. L-DOPA treatment was initiated and clinical improvement was significant, including normal motor development. A first molecular genetic analysis revealed a single heterozygous pathogenic variant in the TH gene, c.698G>A, p.(Arg233His). Exome sequencing was later performed and an additional previously undescribed 43-bp deletion was identified, TH: c.1071-1_1112del. In silico analysis suggested that this variant could affect pre-mRNA splicing. Genetic testing of the proband's healthy parents revealed that both are carriers of a TH variant, c.698G>A (father) and 43-bp deletion (mother). Since the TH gene is mainly expressed in the brain and adrenal medulla, we are currently evaluating the functional consequences of the novel deletion using a splicing minigene reporter assay.
Conclusions: The CSF metabolites signature guided a timely therapeutic intervention with significant clinical improvement. The analysis of neurotransmitters and confirmatory molecular investigation, allowes the

distinction between primary and secondary deficiencies, but constitutes a challenge. Here we present the first patient in Portugal with TH deficiency confirmed at the molecular level.

A MACHINE LEARNING TOOL TO PREDICT GENE-PHENOTYPE ASSOCIATIONS

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In recent years, technological developments in DNA sequencing techniques have resulted in drastic improvements in speed and cost. This has made these techniques increasingly relevant for clinical diagnosis, and the approaches to genetic diagnosis are shifting from gene panels to whole-exome sequencing (WES) and to whole-genome sequencing (WGS). However, despite steady improvements, diagnostic yields can still be significantly increased, in particular for WES and WGS, which can generate large numbers of variants, thereby providing a large number of possible candidates. Recent approaches to tackle this issue have involved the use of gene-phenotype association algorithms to prioritize candidate variants. However, there is still room for improvement in this area. With this in mind, we developed a machine learning based method to determine gene-phenotype associations using data from publicly available databases. Our methodology is based on the use of Knowledge Graph Embedding methods to rank associations between genes and Human Phenotype Ontology (HPO) terms. We show that our method improves the identification of pathogenic variants in the ClinVar database, when compared to recent tools, such as Exomiser, GADO, Phen2gene and Phenolyzer.

THE MISSING HERITABILITY OF COMPLEX TRAITS/DISEASES: A REVIEW

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Introduction: The availability of dense genome-wide genetic maps allows for inferring individual relatedness of genetic data with phenotype similarity in humans and allows the estimation of the so-called genomic heritability (h2) from genomic wide association studies (GWAS). Comparison of h2 values calculated by the latter studies and earlier estimates estimated from phenotype correlation between studies of relatives (the "true" heritability) unveiled a major h2 gap that needs to be clarified.
Methodology: A bibliographic review was carried out focusing on human genetic heritability to identify putative reasons of the gap mentioned above.
Results: Four different hypothesis were addresses and tested, namely:1. The common-trait – common-variant hypothesis. Many loci containing SNPs with small effects contribute to h^2. A recent study[1] shows that it is possible to project the true heritability with an expected number of samples of ~ 500k from results with 100-200k N.2. The rare-variant – large-effect hypothesis. Some of these variants were studied recently, and their contribution was small.3. Structural variants are a part of genomic variability. Most common variants are well tagged by SNPs and thus unlikely to explain the missing heritability. 4. Interactions at play in common diseases. Up to now, the contribution of gene-gene and gene-environment interactions is scarcely established.
Discussion: will address the following questions and hypothesis. 1. Can true h^2 be calculated from GWAS? A plateau may arise instead, at larger N.2. More rare variants are expected to be found, but they probably won't be the principal component in heritability of common traits.3. and 4. More structural variant studies and larger N samples are needed to truly understand their contribution to h^2 of common diseases.
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GENE EDITING IN FABRY DISEASE: A STRATEGY DELINEATION

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The use of iPSCs, in the last years became wide spread, even in our group at INSA, the use of iPSCs to develop models of disease is now envisaged for various Lysosomal Storage Diseases. Such cell models are being used to experiment several types of therapeutic methodologies, as well as approaches that interfere with normal pathways to provide understanding about pathologic mechanisms, and gene editing is particularly interesting among the latter strategies. Recently, a new CRISPR-based method – Prime Editing (PE) – provides all-possible base-to-base conversions, "indels", and combinations; the human genome can be edited without the need of double-strand breaks (DSBs) or donor DNA templates (1). This method proved its efficacy to correct a pathogenic insertion that causes Tay-Sachs disease (HEXA 1278+TATC; OMIM 606869) (1). In this work, our aim is to correct one of the Fabry Disease (FD) causing mutations, the p.W287X, located on the GLA gene (OMIM 300644). For this purpose, our strategy is to use a construct that uses a one-step golden gate digestion-ligation cloning that is called Prime Editing All-in-One (PEA1) plasmid, consisting in a cassette for expression of all PE3 components and a selection marker (2). A few years ago we developed iPSCs from skin fibroblasts of patients (3). The present correction approach will be tested in our FD iPSC line (3). At this moment, we are initiating the work but we hope to achieve positive results soon. The use of new genetic engineering tools, like PE, and its use as possible therapeutic strategy (4) should provide further comprehension of FD and act as a potential therapy. 1 - Anzalone, A. V. et al. Nature 576, 149-157, doi:10.1038/s41586-019-1711-4 (2019). 2 - Adikusuma, F. et al. Nucleic Acids Res. 11;49(18):10785-10795, doi: 10.1093/nar/gkab792 (2021). 3 - Duarte, A. J. et al. Stem Cell Res 45, 101794, doi:10.1016/j.scr.2020.101794 (2020). 4 - Savić, N. & Schwank, G. TranslRes 168, 15-21, doi:10.1016/j.trsl.2015.09.008 (2016).

Clinical Research

ESTABLISHING AN OBJECTIVE CLINICAL SPECTRUM AND GENOTYPE-PHENOTYPE CORRELATIONS IN THE ELLIS-VAN CREVELD SYNDROME: THE FIRST SYSTEMATIC REVIEW OF EVC AND EVC2-ASSOCIATED CONDITIONS

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Introduction: Ellis-van Creveld (EVC) syndrome is a skeletal ciliopathy caused by biallelic pathogenic variants in EVC or EVC2 genes. Monoallelic pathogenic variants in those genes can lead to an apparently milder form of the condition – Weyers acrofacial dysostosis. While this syndrome was first described in 1936, and its genetic cause identified in 2000, there is currently no published work that objectively characterizes the frequency of manifestations and genotype-phenotype correlations regarding the affected gene, type of variant and allelism. We performed the first systematic review of all published cases of EVC syndrome to address this gap.
Methodology: we selected 725 papers for assessment by two independent researchers. The inclusion criteria were clinical reports of EVC syndrome with confirmed genetic diagnosis. A total of 54 studies between 1964 and 2022 and 310 subjects were obtained. Phenotype frequencies between different groups were statistically assessed using Chi-square models.
Results out of the total sample, 190 were affected patients carrying biallelic variants (40 prenatal, 150 postnatal cases): 56% on EVC, 41% on EVC2, and 4% affecting both genes in cis. In 86% of cases both variants were truncating. The frequency of single nucleotide and copy-number

variants was 86% and 14%, respectively. We identified novel recurrent features of this syndrome (ventricular septal defect, brachydactyly and syndactyly) and found genital manifestations to be less common than classically reported. In prenatal cases, skeletal findings and heart conditions were more commonly identified. While most features were equally recurrent in EVC and EVC2 variants, patients with EVC2 were more affected by low weight, microcephaly and shortening of tubular bones. Subjects with non-truncating variants had less common facial features and were less affected in terms of anthropometry. Regarding affected patients with monoallelic variants, they had a smaller frequency of several phenotypes when compared to the biallelic group, with the exception of postaxial polydactyly of the feet, which was more common. Monoallelic EVC variants showed incomplete penetrance, while monoallelic EVC2 variants are likely to have higher penetrance, especially if occurring on exon 22.

Discussion: this is the first systematic review of clinical and gene-phenotype findings in EVC syndrome, which establishes a framework for the objective understanding of the phenotype linked to the EVC and EVC2 genes.

FIRST YEAR OF LDLR VARIANT CLASSIFICATION FROM THE CLINGEN FAMILIAL HYPERCHOLESTEROLEMIA VARIANT CURATION EXPERT PANEL

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Introduction: Familial hypercholesterolemia (FH) is the most common disorder of lipid metabolism associated with increased cardiovascular risk. Over 3000 different variants in LDLR have been identified in FH patients. In 2016, the ClinGen FH Variant Curation Expert Panel (VCEP) was created with the goals to (1) increase the number of FH-associated variants in ClinVar, (2) adapt the general ACMG variant interpretation guidelines to the FH context, and (3) curate all FH variants in ClinVar at the 3-star level. In this work, we will present the results of the first year of LDLR variant curation.

Methods: Each variant was assessed independently by 2 curators using ClinGen's Variant Curation Interface (VCI) and approved by 3 reviewers. Associated labs were asked for de-identifiable case data, which were uploaded into the VCI prior to the curation round. Variants with conflicting classifications and all variants in the same codon were prioritized.

Results: We are currently on our 7th curation round and have trained 4 cohorts of biocurators. The FH VCEP is currently composed of 13 reviewers, 17 curators and 12 associated labs. Training of biocurators started in August 2021 and sustained curation in November 2021.So far, 316 LDLR variants have been evaluated by the FH VCEP, of which 172 are already published in ClinVar at 3-star status, 48 are approved and in the process of being published, 37 are nearly approved and 59 are still under evaluation. Prior ClinVar classifications were 156 conflicting, 106 Pathogenic/Likely pathogenic (P/LP), 38 Uncertain Significance (VUS), 15 Benign/Likely benign (B/LB) and 1 not provided, totalling only 38% of variants with definite classifications. Within the 257 variants already with an FH VCEP classification, 124 were classified as VUS, 113 as P/LP, 16 as B/LB and only 4 remained conflicting, improving the number of definite classifications to 50% and decreasing about 30-fold the number of conflicting classifications.

Discussion: Accurate genetic diagnosis relies on correct variant interpretation. FH VCEP's guidelines improve LDLR variant interpretation, which will expedite FH diagnosis and ultimately improve patient management and prognosis.

LDL-C GENETIC RISK SCORES IN FAMILIAL HYPERCHOLESTEROLEMIA PHENOTYPE

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Introduction: Familial Hypercholesterolemia (FH) is the most common inherited lipid metabolism disorder. Genetic diagnosis includes the study of 3 genes (LDLR, APOB, PCSK9), but 50-60% of clinical FH patients present a negative result. Some of the FH-negative patients may carry a high burden of polygenic small-effect SNPs that in interaction with the environment increase LDL-C levels and produce an FH phenotype. This work aims to determine if the cause of hypercholesterolemia in FH-negative individuals can be explained by a polygenic contribution and compare 3 different LDL-C genetic risk scores (GRS).

Methodology: A total of 69 index cases (ICs) with clinical diagnosis of FH (Simon Broome criteria) were analysed by a NGS panel and were negative for FH genes. Specific SNPs associated with raising plasma LDL-C were included in the panel and used to construct 3 different GRS described for hypercholesterolemia: 12-SNPs[1], 6-SNPs[2], and 10-SNPs[3]. High polygenic risk score (PRS) was considered for scores ≥1.23, ≥0.76 and GRS≥1.96, respectively.

Results: Among the 69 FH negative ICs (26 children and 43 adults), a high PRS was identified in 3 (12-SNPs GRS), 29 (6-SNPs GRS), and 12 ICs (10-SNPs GRSs). In addition, 34 (0.98≥ 12-SNPs score <1.23) and 60 (0.51≥ 6-SNPs score <0.76) FH negative ICs were considered to have an intermediate PRS and 9 ICs a 6-SNPs GRS<0.51 (low PRS).

Discussion: There are large differences in the identification of polygenic hypercholesterolemia. The 6-SNPs GRS identifies 42% of all FH negative ICs with a high PRS compared to 4% (12-SNPs) or 17% (10-SNPs). Using a less conservative approach, 87% (6-SNPs) and 49% (12-SNPs) of the FH negative ICs have intermediate polygenic risk score. The 6-SNPs GRS additionally identifies 13% of the FH negative ICs with a low PRS, highlighting that they can have an unidentified monogenic cause. Population specific cut-offs should be determined for these GRS. There is still space to develop other GRS for polygenic hypercholesterolemia.

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ABC SYSTEM USED AS AN ADD-ON TO CLARIFY GERMLINE VARIANTS PREVIOUSLY CLASSIFIED AS VUS ACCORDING TO ACMG GUIDELINES

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The increasing number of patients screened by NGS to identify germline variants associated with hereditary breast/ovarian cancer (HBOC) syndromes, is leading to a growing number of variants classified as Variants of Uncertain Significance (VUS) according to ACMG guidelines1. Since the ACMG system merges functional and clinical data into a one-dimensional system, it is not always clear how the classification was obtained. The ABC system (ABCs) of variant classification2 splits functional and clinical grading and aims to give a better guide to variant significance. The main goals of this work were i) to apply the ABCs to a group of previously classified ACMG-VUS and ii) to evaluate the potential clinical impact of this review/classification. Germline variants (36 - 29 missense, 1 synonymous and 6 intronic) detected in 5 genes (BRCA1, BRCA2, ATM, CHEK2, PALB2) previously classified as ACMG-VUS, were selected from our database of patients with HBOC, to be reclassified with the ABCs. Variant assessment included: query of clinical and population databases, literature and in silico tools (VEP, HSF, Alamut, Varsome).Eleven variants were classified as Class 0 (functional - fVUS); 17 as class E (functional - HFE (Hypothetical Function Effect), and 8 as Class D (functional - LFE (Likely Functional Effect). fVUS are not clinically graded. Considering that ACMG-VUS are not actionable, it is still an ongoing debate if they should be reported or not. Since the ACMG merges functional and clinical data, it might be difficult for clinicians to understand how VUS classification is achieved. The ABCs allowed us to distinguish between VUS classified due to lack of data from those that might have a functional impact. Class 0 variants (11) should not be reported and class E (17) reporting is optional. The use of ABCs highlighted 8 variants (class D) which might be a susceptibility factor with functional impact and should be reported. Functional and segregation studies are of major importance to clarify the clinical significance of these variants. 1- PMID: 25741868. 2-PMID: 33981013. Support: FCT/MCTES, ToxOmics and Human Health (UIDB/00009/2020). GenomePT(POCI-01-0145-FEDER-022184).

THE IMPORTANCE OF CLINICAL GENETICS IN THE ASSESSMENT OF CRANIOSYNOSTOSIS - FINDINGS FROM A CASE SERIES

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Introduction: Craniosynostosis (CS) constitutes a major cranial malformation, occurring in 1 per 2500 live births. It can be classified as simple or complex and its etiology is frequently multifactorial, with studies showing a genetic cause in around 20% of cases. In our center 45 patients were diagnosed with CS between Jan 2011 and Jun 2022 and a database (CRASY) was created to characterize our patient's features and define multidisciplinary follow-up. We report our findings regarding the genetic etiology of CS.
Methodology: Descriptive analysis of primary CS cases included in the CRASY registry (n=45). We reviewed clinical files and set clinical appointments to collect data (19 patients were referred, and 11 had an appointment). A molecular test for the detection of hotspot variants in FGFR2 and FGFR3 genes was implemented using Sanger sequencing for patients with unknown etiology. A second tier WES-based NGS panel was performed.
Results: A total of 45 patients were diagnosed with primary CS. 13 (29%) were complex and 32 (71%) simple. 31 patients underwent genetic investigation, with 11 achieving an etiological diagnosis: 3 were diagnosed with Saethre-Chotzen Syndrome, 2 with FGFR2-related craniosynostosis, 1 with Apert Syndrome, 1 with Crouzon Syndrome, 1 with Muenke Syndrome, 1 with Greig Syndrome, 1 with Craniosynostosis 4, and 1 with TLK2-related Intellectual developmental disorder. In 2 cases the variant was inherited from an affected parent. In 1 patient a pathogenic deletion on chromosome 2 was found, but its relation to CS is still to be ascertained. 14 patients were referred to clinical genetics following the start of the CRASY registry, 7 were tested for genetic-variant hotspots in FGFR2 and FGFR3 and a diagnosis was achieved in 2.
Discussion: An etiological genetic diagnosis was achieved in 24% of patients, allowing for a specific follow-up plan and genetic counseling for families. A majority of patients without a diagnosis underwent investigation and clinical genetics was able to provide counseling and recurrence risks based on empiric evidence. We conclude that the assessment of patients with CS must include a clinical genetics evaluation.

CONGENITAL HEART DEFECTS DETECTED IN PRENATAL CARE AT HOSPITAL SANTA MARIA – 6 – YEAR RETROSPECTIVE ANALYSIS

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Introduction: Congenital heart defects (CHD) have an estimated prevalence of 8.3/1000 births in Portugal. Most are multifactorial but about 18-22% correspond to chromosomal anomalies (CA).
Methodology: Retrospective observational study using medical records of CHD cases diagnosed from January 2016 to December 2021 in a tertiary hospital.
Results: A total of 2172 echocardiograms were performed, revealing 150 fetuses with in-house confirmed CHD (gestational age range, GAR: 16-35w): 52 left-to-right shunts, 19 critical and 5 non-critical right obstacles, 17 critical and 1 non-critical left obstacles, 8 ventricular disproportions, 8 complex cardiopathies of transposition physiology, 6 complex cardiopathies of univentricular heart physiology, 5 other complex heart defects, 12 systemic venous malformations, and 17 other defects. 43% had additional ultrasound fetal markers/ anomalies. Reasons for performing fetal echocardiogram were: fetal factors in 130, maternal problems in 6, family history in 1, other factors in 6, and multiple factors in 7.61% underwent invasive prenatal testing for karyotyping (27%) or ArrayCGH (73%). 19 numerical CA (10 T21; 6 T18; 1 X monosomy, 1 triple X, 1 mosaic T22) and 10 structural chromosomal disorders were identified: an unbalanced translocation leading to partial 9q trisomy, and 9 microdeletions: one each 7q11.23, 12q21.31, 14q32, 15q13.3, 16p11.2, Xq26.3-q27.3 and three 22q11.2. One fetus was diagnosed with Russell-Silver syndrome and another with achondroplasia.31 pregnancies were terminated. In most cases, fetal autopsy confirmed prenatal findings. Of the remaining 119 pregnancies, 6 resulted in stillbirth and there were 113 live-borns (GAR: 26-40 w; birth weight: 990-3840g). Prenatal findings were corroborated in 83%. 44 were admitted to NICU and 5 died in the neonatal period.
Discussion: Overall, our results are in agreement with the literature. CA were uncovered in 20%. Left-to-right shunts were the most represented defects in fetuses with CA, followed by critical left obstacles. ArrayCGH should be first line once aneuploidies are excluded by qPCR. Multidisciplinary work is essential in early identification, management, and follow-up.

MISSENSE MED12 VARIANTS IN 22 MALES WITH INTELLECTUAL DISABILITY: FROM NON-SPECIFIC SYMPTOMS TO COMPLETE SYNDROMES

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In the past decade, next generation sequencing (NGS) has revealed several unique variants in MED12 in male patients with intellectual disability, and there is an urgent need to assess whether the phenotype of those patients is compatible with either FGS1, Lujan-Fryns or Ohdo syndromes. Nevertheless, the more variants are identified in this gene, the more difficult is the patients' classi-fication into the different related syndromes due to the high phenotypic variability related to MED12 variants. Because of

this phenotypic variability in the increasing number of MED12 variants that are found by NGS, it is essential to gain more knowledge about the different effects of MED12 variants and possible phenotypes. We describe the phenotype of 22 male patients (20 probands) carrying a hemizygous missense variant in MED12. The phenotypic spectrum is very broad ranging from non-specific intellectual disability (ID) to the three well-known syndromes: Opitz-Kaveggia syndrome (FGS1), Lujan-Fryns syndrome, or Ohdo syndrome. The identified variants were randomly distributed throughout the gene (p=0.995, X2 test), but mostly outside the functional domains (p=0.011; X2 test). Statistical analyses did not show a correlation between the MED12-related phenotypes and the locations of the variants (p=0.591; Pearson correlation), nor the protein domain involved (p=0.070; Pearson correlation). In conclusion, establishing a genotype-phenotype correlation in MED12-related diseases remains challenging. Therefore, we think that patients with a causative MED12 variant are currently underdiagnosed due to the broad patients' clinical presentations.

GENETIC AND CLINICAL CHARACTERIZATION OF PATIENTS WITH CYP1B1 GENE BIALLELIC VARIANTS

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Introduction: Primary congenital glaucoma (PCG) is an ocular disease, with tremendous impact in vision. It is commonly inherited in an autosomal recessive manner and CYP1B1 gene was the first and more frequently associated. The goal of this study was to characterize the phenotype and genotype of 14 Portuguese patients diagnosed with glaucoma and biallelic variants in CYP1B1 gene. MethodsThis study was based on the consultation of clinical and genetic data from 14 patients with biallelic variants in CYP1B1 gene from the Medical Genetics Unit of the CHUC, Portugal. The genotype of CYP1B1 was obtained through Sanger sequencing or WES, from genomic DNA.
Results: All patients presented bilateral compromise, and most were diagnosed at birth or early after birth. Except for one, all patients were submitted to at least one glaucoma correction surgery. Ten different CYP1B1 gene variants were identified, namely 5 frameshift, 4 missense and one in-frame mutation. Three of these mutations had not been previously described.
Discussion: Given the observational and retrospective nature of this study, some clinical parameter values are missing, and for some criteria, different evaluation methods were used, precluding the direct comparison between patient values. The most frequent variants found was p.Ala179ArgfsTer18 (V1) and p.Thr404SerfsTer30 (V2) which were also the most common for the other studies involving Portuguese patients. Although functional study is lacking, the three novel variants identified in this study were predicted to be pathogenic or likely pathogenic, according to ACMG guidelines.
Conclusion: The frameshift variants V1 and V2 were the two most frequent. Despite the small number of patients included in this study, three novel pathogenic mutations were identified, expanding the spectrum of mutations associated to PCG. Although biallelic variants in CYP1B1 gene were mostly identified in PCG, in our small cohort of 14 patients one had secondary congenital glaucoma related to Peters anomaly, and one had congenital glaucoma with suspicion of secondary glaucoma, reinforcing the importance of CYP1B1 gene in etiology of childhood glaucoma (primary or secondary).

DIAGNOSTIC UTILITY OF NGS PANEL TESTING INCLUDING NON-CODING AND MITOCHONDRIAL DNA VARIANTS IN PATIENTS WITH MONOGENIC DIABETES

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Introduction: Identifying the molecular etiology for diabetes can guide treatment, allow early screening and supportive therapy for associated features, and inform familial recurrence. Most panel testing historically performed has not included non-coding regions or mitochondrial genes. We retrospectively assessed the diagnostic utility of NGS panels containing both nuclear and mitochondrial genes, including selected non-coding variants, associated with monogenic diabetes.
Methods: Clinical reports of 507 patients with suspected monogenic diabetes who underwent panel testing at a CLIA laboratory were examined (MODY Panel, n=387; Comprehensive Monogenic Diabetes Panel; n=120). Testing included sequence and copy number variant (CNV) analyses of NGS data from a validated clinical exome assay, including established non-coding variants. Mitochondrial genome was included for 199 patients. Molecular diagnosis was defined as the identification of pathogenic or likely pathogenic variant(s) consistent with the patient's phenotype and known associated disease inheritance.
Results: A molecular diagnosis was established in 24.9% (126/507) of patients in 11 genes. Most molecular diagnoses were identified in GCK (n=78, 61.9%), HNF1A (n=22, 17.5%), and HNF1B (n=7, 5.6%). Diagnostic CNVs were reported in ten patients. A diagnostic non-coding variant in INS was identified in one patient. The mitochondrial MT-TL1 m.3243A>G variant was identified in six patients.
Discussion: Nearly 25% of patients in this cohort received a molecular diagnosis, including a non-coding variant in one patient and mitochondrial variant in six patients, demonstrating the diagnostic utility of panel testing with concurrent analysis of both nuclear and mitochondrial genes including selected non-coding variants for individuals with suspected monogenic diabetes.

GENETIC CONTRIBUTIONS OF HPSE1 AND TGFB1 GENES IN LEIOMYOMAS DEVELOPMENT

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Introduction: Leiomyomas are uterine benign tumors with higher prevalence in reproductive age, along with severe symptomatic conditions. The pathophysiology is complex and have risk factors involved: African ancestry, menarche at an early age, family history and nulliparity. Trans-forming growth factors-ß and Heparanase (HPSE) are involved in the remodeling of the extracellular matrix and angiogenesis. making them interesting to investigate a possible involvement in leiomyomas onset.
Methodology: DNA samples were used to perform genetic analysis, being the analysis of the TGFB1 polymorphisms obtained by PCR-RFLP and HPSE1 through Endpoint genotyping. Variants under study are: TGF-ß-509 C/T, TGF-ß-29 T/C and HPSE1A/G. For this project we use 2 populations: 136 women with leiomyomas (median age of 55.7 years-old) followed in St. Louis Hospital, and a control group, with 100 women (median age of 40 years-old). Statistical tests were performed with IBM® SPSS® Statistics 27.0. Statistical significance was defined as a p-value < 0.05.
Results: TGF-ß-29 T/C and HPSE1 A/G show no difference in the genotype or allele distribution between populations. Regarding TGF-ß-509 C/T, we found a significant difference (p=0.007) in the genotype distribution between populations with T allele appearing associated with protection [p= 0.004; OR= 0.39 (0.203-0.738)] against the development of leiomyomas.
Discussion: Since this factor promotes extracellular matrix fibrosis formation that may contribute to leiomyomas development, the association of the TGF-ß gene is expected. We hope that this study could be a contribution the pathophysiology knowledge of this disease.

NEXT GENERATION SEQUENCING OF LIQUID BIOPSIES IN ORAL CANCER: A SMALL PILOT STUDY

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Introduction: In the clinical management of oral cancer there is a lack of biomarkers available for relapse and metastasis detection and treatment selection. The aim of this pilot study was to explore the applicability of liquid biopsies to detect the mutational landscape of oral tumors as well as to monitor the disease course.
Methodology: A total 24 samples of plasma and tissue were collected from 5 oral cancer patients before and at several timepoints after initiation of treatment. After cell-free DNA (cfDNA) isolation, concentrations were determined, compared between patients and controls, and monitored throughout the patients' clinical course. The ctDNA mutational profile was compared with the profile of corresponding tumor tissues and monitored at different timepoints using Next Generation Sequencing (NGS). The data was correlated with the patients' clinicopathological features.
Results: The levels of plasma cfDNA seem to increase right after initiation of treatment in all patients, before decreasing. 4 patients developed metastasis/relapse and a slight increase in plasma cfDNA levels was observed in some of these patients during clinical follow up, being needed more patients and studies to correlate this data with the prognosis capability of liquid biopsies. Sequencing of ctDNA showed not only pathogenic variants in pivotal genes for carcinogenesis process such as TP53, EGFR, KRAS, and PIK3CA but also the identification of new alterations that seems to arise during the treatment course, like in SMAD4. Interesting, some variants were found both in tumor tissue and biofluid samples, while others were only detected in biofluid samples, which suggest the capability of liquid biopsies to reflect individual tumor heterogeneity.
Discussion: Our results suggest that NGS of liquid biopsies may evolve into a useful tool in the clinical management of oral cancer patients.
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MOLECULAR DIAGNOSIS OF CYSTIC KIDNEY DISEASE WITH NGS PANELS COVERING DIFFICULT-TO-SEQUENCE REGIONS

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Introduction: Inherited cystic kidney disease (CKD) is a heterogeneous group of conditions commonly leading to end stage kidney disease. Identifying the molecular etiology of CKD can have implications for clinical management. However, genetic testing using NGS is complicated by segmental duplication in several genes. A strategy that addresses these challenging regions is needed to maximize diagnostic potential. Here, we assessed the diagnostic yield of NGS panels in a cohort of CKD patients.
Methods: We examined test results from 1007 patients tested for CKD at Blueprint Genetics. Testing was done with the Polycystic Kidney Disease Panel (up to 12 genes) or the Cystic Kidney Disease Panel (up to 41 genes). The panels included 4 genes challenged by regions of segmental duplication. Copy number analysis was done bioinformatically using two different pipelines, including a proprietary pipeline for the detection of small CNVs.
Results: A genetic diagnosis was established in 557 patients (55%). Diagnostic variants were identified in 17 genes. The most frequently implicated genes were PKD1 (364 patients, 65%), PKD2 (75 patients, 13%), PKHD1 (40 patients, 7%) and HNF1B (37 patients, 7%). Of patients with a diagnostic PKD1 variant, 77% (280/364) were located within the pseudogene regions. Diagnostic CNVs were reported in 9% (49/557) of all diagnosed patients.
Discussion: These results demonstrate the clinical utility of genetic testing for CKD using NGS panels. Most diagnoses are due to variants in PKD1 emphasizing the importance of including tailored approaches for challenging genomic regions. Given the frequency of CNVs, including high-resolution CNV detection significantly improves the diagnostic potential.

NOVEL INTRAGENIC REARRANGEMENT IN CFTR HIGHLIGHTS THE IMPORTANCE OF THOROUGH MOLECULAR ANALYSIS

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Cystic fibrosis (CF; MIM# 219700) is one of the most common life-shortening autosomal recessive disorders in Caucasians, with a frequency of about 1 in 2,000-3,000 live births. It is caused by mutations affecting the CF transmembrane conductance regulator (CFTR) gene, with over 2000 different variants documented to date. We report a case of a six year-old female with clinical findings consistent with CF, including severe respiratory insufficiency and elevated sweat chloride concentration. In a previous study carried out elsewhere, with multiplex ligation-dependent probe amplification (MLPA) and sequencing of all CFTR exons, only the presence of the common CF variant NM_000492.4:c.1521_1523del (p.Phe508del) was reported. The clinical suspicion of CF was maintained, leading to a repeated request for molecular testing. We confirmed the presence of the common variant but also detected an abnormal MLPA profile, suggestive of a large genomic rearrangement. This prompted further molecular investigation, beginning with cDNA analysis of mRNA obtained from nasal epithelial cells. An aberrant transcript was identified, in which exon 16 was replaced by an intact copy of exon 19 (NM_000492.4:r.2620_ 2657 delins2989_3139). Genomic long-range PCR of this region revealed the duplicated exon 19 within intron 15, along with sequence structures typifying the mutational mechanism, such as a repetitive element and flanking inverted short sequences. An almost intact exon 16 was also present, missing the first three exonic base-pairs, which are required for MLPA probe recognition (explaining the altered MLPA signal) and with no recognizable upstream intronic splicing consensus sequences (explaining exon skipping). Compound heterozygosity for the two variants was ascertained by testing the parents. Although the identification of large rearrangements in CFTR is challenging, clinical input may justify extensive molecular studies, so as to avoid underdiagnosis of CF cases with these rare variants. Moreover, accurate genotyping has become crucial for therapeutic decision-making, given the continued development of drugs targeting specific classes of CF-causing variants.

THE ROLE OF SIGNALLING PATHWAYS AND PRKCB IN GLIOBLASTOMA MULTIFORME PATIENTS' SURVIVAL

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Introduction: Glioblastoma multiforme (GBM) is the most common primary tumor of the brain. Despite advances in treatment, median survival has not seen improvements, having settled at around 15 months. Low survival rates can be attributed to late age of onset, treatment limitations and the lack in understanding of the pathophysiology of GBM. The main objective of this work was to characterize the genetic makeup of GBM in order to identify genetic predictors of survival.
Methodology: Methylation, gene expression and clinical data from GBM patients was retrieved from The Cancer Genome Atlas. After data reduction, signaling pathway analysis was performed separately for each omic data, resulting in the most overrepresented pathways ($p < 0.05$). From there, genes present in, at least, 30% of these pathways were selected, resulting in two sets of seven genes, one for each omic approach. One gene found in both sets was further investigated by performing survival analysis using the Kaplan-Meier method based on its methylation profile.
Results: A total of 61 and 112 signaling pathways were found to be overrepresented in methylation and gene expression datasets, respectively.

They were majorly related to carcinogenesis, cell cycle progression and neurobiological processes. From the two sets of genes present in the majority of identified pathways, PRKCB is worthy of notice. This gene is involved in pathways related to neurological function and carcinogenic processes, including the glioma, multiple synapse, MAPK and Rap1 signaling pathways. Furthermore different methylation PRKCB profiles exhibited a difference of 2.6 months in median survival time ($p < 0.01$), with those that were under-methylated performing better.
Discussion: A deeper understanding of the genetic and epigenetic background of GBM may lead to novel therapies and new ways to improve prognosis, survival time and life quality of the patients. Also, the identification of PRKCB as a possible indicator of prognosis may positively affect the clinical management of GBM patients. The protein encoded by this gene is known to be involved in diverse cellular processes and being a major receptor for tumor promoters.

COPY NUMBER VARIATIONS ON CHROMOSOME X AND IMPACT IN NEURODEVELOPMENT DISORDERS

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Background: Copy number variations (CNVs) of the chromosome X are associated with several human disorders, especially impairment of the neurodevelopment. Several disease susceptibility regions have been identified in X chromosome, some already associated with known genetic syndromes, nonetheless others remain to be clarified. Array comparative genomic hybridization (aCGH) is a useful method to access pathogenic or likely-pathogenic CNVs in a clinical population. The main goal of this study was to provide a genotype-phenotype correlation focused on CNVs present on chromosome X.
Methods: A cross-sectional study was performed using the aCGH database of the Genetic Department of the Faculty of Medicine of University of Porto and the patient´clinical records from hospitals database. Patients with pathogenic or likely-pathogenic CNVs on chromosome X were included in the study. Databases and related literature were used for a better understanding of the genotype-phenotype correlation.
Results: From 2852 patients studied using aCGH, 51 presented clinically relevant CNVs on chromosome X: 52,94% (27/51) were classified as likely-pathogenic and 47,06% (24/51) as pathogenic. Among the 27 likely-pathogenic CNVs 11,11% (3/27) were identified in the prenatal context, while 88,89% (24/27) were postnatal. Concerning to the 24 pathogenic CNVs, 25% (6/24) were prenatal cases and 75% (18/24) were postnatal cases. Chromosomal regions Xp22.31, Xp22.32 and Xq28 exhibited a higher incidence of pathogenicity. Other regions that frequently showed clinically relevant CNVs were the Xp11.22, Xp11.3 and Xq11.2. In two patients, additional CNVs involving other chromosomes (chromosomes 3, 6 and 17) were also observed.
Conclusions: With this study, we contributed for a better understanding of the disease CNVs associated with chromosome X, especially the ones associated with neurodevelopment disorders, allowing a more accurate diagnosis. This can be important to improve genetic counseling namely in prenatal diagnosis setting.

IMPACT OF HIGH BODY MASS INDEX IN NONINVASIVE PRENATAL TESTING

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Introduction: Non-invasive prenatal testing (NIPT) is now widely used to detect common fetal chromosome aneuploidies (T13, T18, T21 and sex chromosomes). Pregnant women with a high body mass index (BMI) tend to have a lower fetal fraction (FF), leading to inconclusive results in non-invasive prenatal testing (NIPT) for common aneuploidies based on cell-free DNA. According to some studies, the failure rate of the NIPT test can reach values close to 25%, leading to guidelines that do not advise screening for aneuploidies by NIPT in patients with significant obesity. Given the high sensitivity and specificity of the NIPT, many pregnant women with a high BMI do not accept an alternative test and insist on trying the NIPT first. This study aimed to evaluate the impact of high BMI on FF and NIPT results in a cohort of approximately 1100 pregnant women undergoing screening for the most common aneuploidies and redefine the test exclusion criteria by determining a maximum BMI cutoff point.
Methodology: We evaluated the relationship between FF, aneuploidy, BMI, and failure rate in approximately 1000 pregnant women screened with a NIPT based on cfDNA NGS sequencing. The expected analytical sensitivity due to the absence of results and the BMI value was determined by the Mann-Whitney U test, while the association between BMI subgroups and qualitative data was performed using Fisher's exact test. Thresholds and cutoffs for positive discrimination between groups were determined using ROC curves.
Results: Among women who presented NIPT inconclusive results, 76 (58.9%) were overweight. All subgroups with BMI > 25 had higher failure rates than general sampling The statistical analysis of the results confirmed the expected correlation between the low FF and the increased BMI. A theoretical BMI cutoff for performing the NIPT test was calculated for the population studied. Women with BMI > 27.34 are more likely to present inconclusive tests due to low fetal fraction.
Discussion: Among the various factors that prevent a conclusive result in the NIPT, FF is the most important. Weight gain and high BMI negatively affect fetal fraction. The possibility of performing the NIPT in pregnant women with a high BMI and with lower failure rates will force the definition of tighter cutoffs and tests of greater sensitivity in low FF. Another alternative would be the use of FF enrichment strategies, aimed at pregnant women with high BMI or pregnant women with early pregnancy loss.

CREB1 CONNECTS FMR1, OVARIAN INFERTILITY, AND DNA DAMAGE RESPONSE PATHWAYS

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Introduction: Recent studies in mouse granulosa cumulus cells revealed that Cyclic AMP response element-binding protein 1 (Creb1) inhibited the Has2, Ptgs2, and Igfbp4 mRNA levels, suggesting its implication in follicu-logenesis, ovulation, and luteinization. In humans, attempts to establish a correlation between female infertility and DNA damage response in cumulus cells are still subject of discussion. CREB1 has never been implicated in human female infertility. Despite the extensive literature showing that variants in this and other genes hamper biological processes, namely oogenesis, hormone signaling, reproductive organs development, ovarian reserve preservation as well as chromosome abnormalities. Conversely, FMR1 gene is implicated in Fragile X-associated primary ovarian insufficiency (FXPOI; OMIM #311360), seen in ~20% of females carrying 55–200 CGG repeats (premutated alleles). Aiming to boost the knowledge on increased risk for premature ovarian aging, infertility in FMR1 premutation carriers and DNA damage response, possible interaction(s) between the different pathways were analyzed.
Methodology: Cytoscape (v3.9.1) software was used to visualize molecular interaction networks, and integrate them with gene expression profiles. Pathways for the molecular networks were provided by WikiPathways, an open community created, expert curated database for molecular pathway information.

Results: The Fragile X Syndrome (FXS) and ovarian infertility, functional, and regulatory pathways were compared showing an overlap of fourteen genes. Then the FXS and miRNA response DNA damage pathway were compared showing a single gene in common, CREB1. Interestingly, this gene was also found in ovarian infertility pathways.
Discussion: Considering the function of CREB1 gene product, it is possible to recognize an association with female infertility, as revealed by the in silico bioinformatic tools. The unexpected interaction between FXS, ovarian infertility and DNA damage pathways provides grounds for the molecular analysis of the CREB1 gene in infertile females, particularly in FMR1 premutation carriers. This work warrants further research towards understanding the synergy between these pathways.

MUTATIONAL SPECTRUM OF GENES RELATED TO HEREDITARY NEUROPATHIES – DATA FROM A MOLECULAR DIAGNOSTICS LABORATORY

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Hereditary neuropathies constitute a large, heterogeneous group of diseases with a prevalence of ~40/100,000 (or 1:2,500). More recently, the use of whole-exome sequencing (WES) lead to the identification of new genetic causes of hereditary neuropathy. We aimed to characterize the mutational spectrum of genes related to hereditary neuropathy, identified either by WES or conventional targeted approaches. We reviewed our database of patients tested at our lab (2016-2021), with pathogenic (PAT), likely-pathogenic (L-PAT) or variants of unknown clinical significance (VUS), in genes related to hereditary neuropathy (n=890). For this study, only index patients with a "pure" hereditary neuropathy and PAT or L-PAT variants were considered. Single nucleotide variants (SNVs) or small insertions/deletions, in 109 genes, were identified among 706 patients, either by single-gene (n=252) or WES-based panels (n=454) approaches. TTR-related familial amyloid polyneuropathy comprised most of the cases associated with SNVs (n=125). In addition, 31 patients carried variants in neuropathy-causing genes corresponding to 27 SNVs already reported and 8 novel variants in the MME, MTMR2, SACS, PRX and PLP1 genes. Intragenic copy number variants (CNVs) were more frequently (but not exclusively) associated with Charcot-Marie-Tooth disease (29 patients; 13 deletions, 16 duplications); 2 patients were compound heterozygotes for a SNV and a CNV in genes associated with Charcot-Marie-Tooth disease, axonal, type 2X, and spinocerebellar ataxia with axonal neuropathy. CNVs encompassing the entire PMP22 gene were identified in 155 patients (49 whole-gene deletions, 106 duplications) with suspected phenotypes of hereditary neuropathy with liability to pressure palsies or Charcot-Marie-Tooth type-1A. Diagnostic yield in hereditary neuropathies is relatively high, when compared to other neurological diseases, reaching 80% in CMT1A, but significantly lower in other types. Many PAT/L-PAT variants were identified in this cohort, particularly CNVs. This knowledge has both significant scientific and clinical impact.
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IDENTIFICATION OF DE-REGULATED CIRCULATING MICRORNAS AS PUTATIVE BIOMARKERS IN AMYOTROPHIC LATERAL SCLEROSIS

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Introduction: Amyotrophic Lateral Sclerosis (ALS) is a heterogeneous and progressive neurodegenerative disease. Diagnostic delays are a challenge in the management of ALS, thus sensitive diagnostic and prognostic biomarkers are needed. Blood-based biomarkers, such as microRNAs (miRs) are ideal for first-line screening tests. We previously identified 10 dysregulated miRs in pooled plasma samples of ALS patients. We here verified these de-regulated miRs and combine these results with clinical phenotypes. Additionally, a longitudinal assessment of the miRs signatures in ALS patients was performed.
Materials and Methods: The study population consisted of 3 groups: ALS (36), mimic disorders (16), and healthy controls (15). Total RNA in each sample was purified according to the miRNeasy® Serum/Plasma Advanced Kit (QIAGEN) protocol. A quantitative real-time PCR (qRT-PCR) with TaqMan miRNA assay kits (ABI) was used to quantify miRs expression. The results were analyzed by manufacturer-advised software and combined with a set of clinical variables in a multivariate analysis.
Results: 3 miRs were significantly overexpressed in ALS patients compared with healthy controls: miR-93-3p (p=0.0430); miR-206 (p=0.0424); miR-152-3p (p=0.0379). When comparing the miRs expression in ALS samples with the mimic disorders group, miR-9-5p was significantly underexpressed in the ALS group (p=0.0027).
Discussion: Our results indicate that miR-206 is a promising biomarker of ALS, in concordance with the literature. In contrast, miR-152-3p and miR-93-3p have not been associated with ALS. These results may be indicative of a possible role in neurodegeneration in general. Moreover, miR-9-5p, which regulates a pathway with a protective effect on motor neurons, was also reported to be downregulated in ALS.
Conclusion: Although promising results were obtained, we should analyze them with caution. More studies with miRs as biomarkers for ALS are needed to validate our results. Even though miRs seem to reveal predictive potential, most complex diseases cannot be explained by single biomarkers. Nevertheless, microRNA signatures might shed light on ALS pathology.

GENETIC HETEROGENEITY IN MUSCULAR DYSTROPHIES AND CONGENITAL MYOPATHIES: DATA FROM MULTIGENE WES-BASED GENETIC STUDIES

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Hereditary myopathies comprise a group of genetically heterogeneous diseases, characterized by anomalies in muscle structure and/or function, with estimated prevalence of ~4:100,000 for congenital myopathies (CM) and 19.1-25.1:100,000 for muscular dystrophies (MD). We present an overview of the mutational spectrum of genes related with myopathies, derived from diagnostic studies by WES-based multigene panels. The information from our laboratory database from patients (tested between 2016-2022) with clinically relevant variants in genes associated with muscular dystrophies (MD, either congenital or "progressive" forms) and CM was reviewed. A total of 106 patients with at least one variant classified as pathogenic or likely-pathogenic (PAT/L-PAT), harboured variants in 28 different genes. Among these patients, 24 were possibly compatible with CM (most frequent genes: RYR1 and NEB) and 13 with congenital MD (most frequent genes: COL6A2 and COL6A1); whereas 69 had a progressive form of MD (most frequent genes: SGCA, ANO5, DYSF, LAMA2 and TCAP). Overall, 76 patients had autosomal recessive forms (44 compound heterozygotes and 32 homozygotes), whereas 16 patients showed autosomal dominant inheritance. A total of 8 hemizygotes were identified in our cohort, all associated with variants in DMD. Three patients presented challenging diagnoses, given the presence of PAT/L-PAT variants in more than one gene. Interestingly, the pathogenic variant NM_000426.3 (LAMA2):c.2461A>C (p.Thr821Pro), previously associated to "atypical" late-onset LAMA2-related MD, was identified in 6 out of 106 patients (one homozygote and 5 compound heterozygotes). This report increases the total number of cases with this variant to 24, so far exclusively found in patients of Portuguese ancestry. These results illustrate the genetic heterogeneity typically found in these diseases and the interplay of genes underlying both muscular dystrophies and myopathies. In such patients, particularly those having no pathognomonic (clinical or neuropathological) signs, analysis by WES-based multigene panels is recommended to improve diagnostic yield and reduce time to diagnosis.

PARADIGM SHIFT IN PHENYLKETONURIA NEURODEVELOPMENTAL OUTCOMES – RETROSPECTIVE COHORT ANALYSIS

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Introduction: Phenylketonuria is an inborn error of metabolism in which exposure to high levels of phenylalanine (Phe) in childhood has a neurotoxic effect. Early diagnosis by newborn screening and early dietary intervention prevents these effects. In Portugal, the paradigm of PKU diagnosis and treatment has been improving and we aim to review with unbias computations the timing of the improvement in neurodevelopmental performance.
Methodology: It was performed a retrospective analysis of 89 PKU patients from Centro Centro Hospitalar Universitário do Porto - Centro de Genética Médica Dr. Jacinto Magalhães. Patients were stratified in low vs high performance in the Griffith's Mental Development Scales (GMDS) age 6 (cut-off GMDS ≤ 89) and at the Wechsler Intelligence Scale for Children III (WISC-III, cut-off WISC-III ≤ 89), and their features were compared. Multivariable logistic regression was computed with a top-down strategy to predict low vs high performers. Cut-offs were defined using the regression model using Youden's method.
In this cohort, 14/89 patients had a lower performance in the GMDS and 25/65 patients in the WISC-III. For the GMDS at age 6 the year of birth and the median annual level of Phe at age 3 were the best predictors of performance and for the WISC-III the sociocultural level of the family and median annual level of Phe at age 5. On the unbias definition of a cut-off for the year of birth, it was possible to hypothesize that the patient born after 1993 had a better performance on the GMDS at age 6 (specificity: 74.7%; sensitivity: 78.7%) and those born after 1993 for the WISC-III (specificity: 92%; sensitivity: 48.7%).
Discussion: Being born after 1993 marks a paradigm shift in the performance of PKU patients. We hypothesized that the definition of safety Phe levels ≤ 6 mg/dL up to the age of 12 years, diversification of special low protein foods, available for free, and establishment of a dedicated multidisciplinary team to follow these patients, contributed to this improvement.

NEXT GENERATION SEQUENCING – A KEY TOOL FOR DIAGNOSIS AND INVESTIGATION OF FAMILIAL DYSLIPIDEMIAS

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Introduction: Dyslipidemia, a clinical condition defined by abnormal lipid concentrations in blood, can have a genetic etiology. Familial dyslipidemias are a group of genetic diseases, the majority being rare, associated with several serious conditions. Raised triglyceride levels are associated with pancreatic/ hepatic complications. Eleva-ted cholesterol levels promote atherosclerosis and increase patients' cardiovascular risk, and low levels of this particle are associated with neurological manifestations and poor weight progression. Nowadays, with the advance in genome sequencing technologies, the investigation and diagnosis of these disorders is expanding. This study aimed to identify the molecular cause of dyslipidemia in 96 Portuguese individuals with a clinical diagnosis of hypercholesterolemia, hypo-cholesterolaemia, or hyper-triglyceridemia.
Methodology: The lipid profile of 96 cases was determined for biochemical characterization. The mole-cular study was performed by an NGS target panel (customised for monogenic disorders of lipid metabolism with 57 genes). Molecular analysis was performed considering three smaller panels including 18 genes strongly associated with the three distinct lipid traits. Rare variants detected were confirmed by PCR and Sanger Sequencing.
Results: A definite cause of dyslipidemia was identified in 35 patients: 22 individuals were diagnosed with familial hypercholesterolemia, 3 with familial hypobetalipopro-teinemia, 2 with familial chylomicronemia syndrome, 7 with multifactorial chylomicronemia, and one with transient infantile hypertriglyceridemia. During this project, NGS allowed the identification of numerous variants with uncertain significance (VUS), the majority lacking functional data. Since these variants may constitute the cause of dyslipidemia in some cases, the performance of functional studies is crucial to assess the variant effect on protein.
Discussion: The application of NGS as a diagnosis/research tool allowed a correct and early identification of Portuguese patients suffering from different familial dyslipidemias, thus providing guidance for personalized pharmacological treatment and lifestyle changes to avoid disease complications.

CYTOGENETIC FINDINGS IN INFERTILE COUPLES FROM TRÁS-OS-MONTES AND ALTO DOURO REGION: A GLIMPSE INTO THE GENETIC BASIS OF INFERTILITY

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Introduction: A major contributing cause of infertility is chromosomal abnormalities. The production of unbalan-ced gametes during meiosis leads to a history of recurrent pregnancy loss or adverse delivery outcomes. Thus, it is not surprising that the prevalence of chromosomal abnormalities is 10 to 15 times higher amongst infertile couples. Our study aims to determine the incidence and patterns of chromosome abnormalities among infertile couples from the Trás-os-Montes and Alto Douro Region.
Methodology: Peripheral blood lymphocyte karyotype analysis was performed on 883 patients who attended the Trás-os-Montes and Alto Douro medical center for infertility consultation between January 2010 and July 2022. Karyotyping was done by conventional cytogenetics.
Results: Chromosomal abnormalities were found in 24 couples (2,7%), 11 female (1,2%) and 13 male (1,5%) partners. Balanced translocations, all involving autosomes, were the most common aberration (41,7%). Sex chromosome aneuploidies (37,5%), the presence of marker chromosomes (12,5%), and inversions (8,3%) were next in frequency. Five patients exhibited low-grade mosaicism.
Discussion: The incidence of chromosomal anomalies in infertile couples varies considerably, country and region-wise, with studies describing rates ranging from 1,3 to 15%. For the sampled region, we report a 2,7% incidence of chromosomopathies in infertile couples, nine times the estimated birth rate of chromosome abnormalities assigned to the European population. Our research highlights the significance of chromosomal structural and numerical aberrations and their associated effect on reproduction. Karyotype analysis, despite its known drawbacks, is essential to the diagnostic process. The presence of cytogenetic abnormalities allows for proper counseling and management of the affected couple.

MOLECULAR CHARACTERIZATION OF GENES ASSOCIATED WITH CILIOPATHIES IN INFERTILE MEN WITH ASTHENOZOOSPERMIA BY NEXT-GENERATION SEQUENCING

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Introduction: Ciliopathies (CP), autosomal recessive genetic disorders, arise from ciliary dysfunction resulting in a range of symptoms as cerebral abnormalities, respiratory problems, liver and renal disease, and male infertility. Male infertility can be associated with primary ciliary dyskinesia (PCD), which is associated with defects in motile cilia affecting the respiratory tract, abnormal positioning of organs within chest and abdomen, and spermatozoa motility. However, the individual clinical diagnosis of CP does not facilitate genetic testing since PCD is associated with at least 45 genes, hence the need to validate gene panels by next-generation sequencing (NGS). This study aimed to evaluate

the association between infertile men with asthenozoospermia (Atz) and a gene panel traditionally associated with PCD.MethodsThe genes DNAAF2, DNAH5, DNAH11, DNAI1, DNAI2, NME8, RSPH9 and RSPH4A (223 exons) were analyzed in 48 men with Atz by NGS using Illumina's MiSeq platform and AmpliseqTM methodology. Variants annotation included DNA Amplicon, VarAFT, Variant Interpreter, HGMD, Alamut Visual Plus, VarSome, ClinVar and IGV. PCR and DNA sequencing were performed for regions with coverage <20x.
Results/ Discussion: 57 variants were identified in heterozygosity/ homozygosity, comprising 1 pathogenic, 2 likely pathogenic, and 9 variants of uncertain significance (VUS). Two pairs of VUS are more relevant, namely DNAH11:c.2909A>G and c.9935A>T, that most probably are in trans (found in a man with oligoasthenozoospermia - OAz), and a double heterozygosity for DNAH5: c.3614C>T and DNAAF2:c.2239G>A (man also with OAz). These four variants are all VUS, and therefore we cannot establish a direct correlation between them and the patients' phenotype. Furthermore, in two OAz men two variants, DNAH11:c.8990G>A and c.10739G>A, were found and described with opposite classification, pathogenic and benign. We emphasize the need of functional and family studies to properly classify these variants and understand their impact.
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ARE X-CHROMOSOME INACTIVATION PATTERN AND TELOMERE LENGTH UNDERLYING RECURRENT PREGNANCY LOSS?

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Introduction: Recurrent Pregnancy Loss (RPL) affects about 1-5% of women in reproductive age worldwide and is characterized by the loss of two or more clinically recognized pregnancy before 24 weeks of gestation, according to the European Society of Human Reproduction and Embryology (ESHRE). RPL is a multifactorial and heterogeneous disorder that has some wellaccepted etiologies. Nevertheless, it has been estimated that about 50% of cases remain idiopathic (iRPL). We tested a possible association between extreme skewed X-chromosome inactivation (ES-XCI) patterns and also shortened telomeres with RPL since both are hypothesized to underlie RPL.
Methodology: For this purpose, two groups of women were analyzed and compared: a group of 23 RPL women, composed of women aged between 23 to 42 years old (RPL group), and a group of 27 age-matched healthy women with proven fertility (CTRL group). Then, both Xchromosome inactivation patterns and telomere length were measured in DNA extracted from peripheral blood. The X-chromosome inactivation pattern was obtained through the human androgen receptor (HUMARA) assay and the average telomere length was measured using the Absolut Human Telomere Length Quantification qPCR Assay Kit (AHTLQ) by ScienCell™. ResultsOur data showed no statistically significant differences between groups, suggesting no association between ES-XCI and RPL, for both cut-offs of ≥85% and ≥90%. Additionally, for telomere length, no statistical differences were observed between the RPL group (7,24 ± 2,57 kb per chromosome end) and the CTRL group (7,37 ± 1,94 kb per chromosome end). Additionally, the effect of maternal age on both X-chromosome inactivation pattern and telomere length was tested, but no correlation was found.
Discussion: Although no associations were found, an increased number of samples and new approaches are planned in the future. Moreover, considering its clinical relevance, a continuous effort to better understand this condition is mandatory.

GLOBAL BLOOD DNA METHYLATION LEVELS IN MACHADO-JOSEPH DISEASE (MJD)/SPINOCEREBELLAR ATAXIA TYPE 3 (SCA3): AN EXPLORATORY STUDY

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DNA methylation (DNAm) is the most well understood epigenetic modification, impacting the pathophysiology of neurodegenerative disorders, by regulating gene expression. Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative polyglutamine (polyQ) ataxia, caused by the expansion of a CAG tract in the coding region of the ATXN3 gene. MJD has a marked clinical heterogeneity, including a wide variation in the age at onset (AO) that only partially correlates with the size of the CAG tract. Although variants in known genes contribute to the explanation of AO, a large part of the variation unexplained by the expanded allele needs to be further clarified, and a role for epigenetic modifiers can be hypothesized. We explore, for the first time, the global 5-methylcytosine (5-mC) levels in blood samples of MJD subjects. The Zymo 5-mc kit was used to quantify global 5-mC levels in blood samples from 23 MJD subjects (13 patients and 10 preclinical carriers) and 23 healthy controls, matched by age, gender, and smoking status. For a subset of nine patients, measurements in two collection points were also available. Although not reaching significance, the mean global 5-mC levels were higher in the overall MJD subjects compared to controls and were also higher when considering separately the two groups of MJD subjects (patients and preclinical carriers) and their respective matched controls. No differences were found in the 5-mC global levels between patients and preclinical carriers. The comparison of 5-mC levels in samples from two collection points denoted a decrease in the global 5-mC levels in six of the nine patients with follow-up data. No correlations were found between 5-mC levels and AO or expanded allele. Our results evidencing higher 5-mC global levels in MJD samples and needs to be further confirmed in a larger sample of MJD subjects and matched controls.

PRELIMINARY ASSESSMENT OF USING DNA FRAGMENTATION IN CUMULUS CELLS AS A MARKER OF INFERTILITY

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Introduction: Cumulus cells (CC) play a key role in oocyte developmental competence. These cells surround the oocyte and are important for signal and nutrient transfer through gap junctions. In male infertility, sperm DNA fragmentation is used as a marker for the prediction of fertilization success. No similar link has been established for female infertility, mainly due to contradictory results. We hypothesize that CC DNA fragmentation can be used as a marker of female infertility. We aim to analyze CC DNA damage by the comet assay and correlate the results with basic parameters evaluated in medically assisted reproductive, as well as genetic data, to assess a possible impact on female infertility.
Methodology: DNA damage was assessed in twelve CC samples, recruited through our fertility and donation center: six oocyte donors (mean age 25.2 ± 4.4 years) and six females with fertility issues (mean age 33.7 ± 3.4 years). CC DNA damage from both groups was correlated with the number of cumulus-oocyte complexes (COCs) retrieved, hormonal levels, total dose of FSH, stimulation days, age and genetic parameters such as allelic complexity of FMR1 alleles. In females with fertility issues, the CC DNA damage was further correlated with the endometrial thickness and fertilization percentage.
Results: Overall, in both groups, no significant correlation between the CC DNA fragmentation and the parameters tested was observed. The reduced number of samples (6) strongly influenced the absence of statistically valid correlations. Evidence have already been provided that a minimum sample size of 25 may be required for valid values for the estimation of Pearson correlation. The allelic complexity of both FMR1 alleles was the parameter that shows higher, although non-significant, correlation.

Discussion: Our results allowed us to assemble a well-defined strategy to verify the possible impact of DNA damage in infertility. We were able to identify the parameters with analytical viability to be used in the short term with larger samples. In addition, we reinforce the importance of understanding the impact of the FMR1 gene on female infertility.

RESTRICTIONS IN CARE FOLLOWING THE COVID-19 PANDEMIC SEVERELY IMPACTED MACHADO-JOSEPH DISEASE PATIENTS: A STUDY IN SÃO MIGUEL, THE AZORES

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The COVID-19 pandemic exacerbated existing difficulties and created extra hazards for rare disease patients. No studies have reported, so far, how the COVID-19 pandemic impacted the Machado-Joseph disease (MJD) communities. The island of São Miguel, in the Azores, is one of the clusters with the highest MJD prevalence rate worldwide (37.72/100.000, or 1/2.651).This qualitative study describes how restrictions imposed by this pandemic impacted on MJD patients and their care, in São Miguel. It draws on empirical data from a larger study on experiences surrounding MJD in São Miguel, from the perspective of patients, relatives, health-care professionals, and care providers. We present the sub-corpus of data focusing on the impact of the pandemic, a theme that emerged during that analysis. In-person semi-structured interviews were conducted with 11 participants (8 female), out of 28, including 6 patients, 1 family member, 2 healthcare professionals, and 3 care providers (range: 30-54 years), recruited through the Azorean MJD patients' association. Interviews were audio-recorded, fully transcribed, and analysed thematically. Main findings highlighted the key role played by the local association in psychosocial and healthcare for MJD patients and families, and the adverse effects on their care following the onset of the COVID-19 pandemic. In particular, hindered access to the day-care centre increased isolation and had a negative impact on mental health, but also in disease progression. For persons with a progressive, severe neurological disease, there is no "back to normal." Future restrictive measures ensuing need to be accompanied by a careful definition of daily-care routines for patients, which reduces isolation and promotes adequate mental health and wellbeing.

Declaration of Interests: The authors declare no competing interests.

INCIDENCE OF TP53 MUTATIONS IN PATIENTS WITH CANCER IN CENTRO HOSPITALAR DE TRÁS-OS-MONTES E ALTO DOURO: A PRELIMINARY STUDY

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Introduction: TP53 is a tumor suppressor gene located on chromosome 17 that encodes the p53 protein. This protein is involved in the control of cell proliferation, preventing cells from growing and proliferating too fast or in an uncontrolled way. Most TP53 mutations change single amino acids in the p53 protein, which leads to the production of an altered protein that cannot regulate cell proliferation effectively and is unable to trigger apoptosis in cells with mutated or damaged DNA. Therefore, tumors with TP53 gene mutations tend to have a poorer prognosis being more aggressive, resistant to treatment and relapsing after treatment. Somatic mutations in this gene are the most frequent genetic alterations found in human cancers, with an incidence of 45% to 50% in all cancers.

Methodology: Between August 2021 and September 2022, 103 samples of several cancer types were received at the Genetic Laboratory of Centro Hospitalar de Trás-os-Montes e Alto Douro. DNA was extracted from FFPE sections and libraries were prepared according to the manufacturer's instructions.

Results and Discussion: In the 103 samples analysed, 42.7% were lung cancer (LC), 12.6% colon cancer (CC) and 2.9% breast cancer (BC). TP53 mutations were present in 50.5% of total cancer cases and detected in 52.3% of LC, 69.2% of CC and 66.7% of BC. Regarding LC, our results are in agreement with literature data where somatic mutations in the TP53 gene have been found in almost half of all LC. In CC and BC, our incidence were slightly higher than described in the literature (nearly 50% and 30%, respectively) but only a few samples were analysed. In the LC, BC and CC samples, most TP53 mutations occurred in exons 5 to 8.

Conclusion: Despite the small number of samples in this study, TP53 mutations were the most frequent. Its detection is critical for specific clinical orientation, since is a mutation that responds poorly to therapy and has an aggressive disease course.

CDKN1C GENE: IS BECKWITH-WIEDEMANN SYNDROME UNDERDIAGNOSED?

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Introduction: Beckwith-Wiedemann Syndrome (BWS; OMIM # 130650) is an autosomal dominant rare human genomic disorder with variable clinical expression and complex molecular aetiology. In addition to an increased propensity for embryonal and Wilms' tumour, the most common signs of BWS include macroglossia, hemi-hypertrophy, overgrowth and omphalocele. The main causes of BWS, accounting for more than 75% of cases, are alterations in methylation pattern and copy number variations in the 11p15 locus. Variants in genes within this region account for most of the remaining cases. One of these genes is CDKN1C, where pathogenic variants in the maternal allele account for ~5-10% of sporadic and ~40% of familial BWS cases.

Aims and Methodology: In an attempt to improve the existing guidelines for BWS, we have elaborated a scheme towards a more effective diagnostic workup. Based on the literature and databases (PubMed, HGMD, ClinVar and LOVD) we collated the documented pathogenic and likely pathogenic variants in CDKN1C gene together with positive phenotypic correlations between these and specific clinical traits.

Results and Discussion: CDKN1C sequencing should always be conducted as a second-tier test when MS-MLPA reveals no methylation or structural alteration in 11p15. In cases with family history or some specific clinical traits such as omphalocele and cleft palate, it should be considered as the first molecular approach, followed by karyotyping. Additionally, as this syndrome has a high prevalence of mosaicism, investigations should be conducted on different tissues, including fibroblasts from hypertrophied limbs, tumour samples and buccal swabs, this highlights the relevance of providing the laboratory with detailed clinical data. With the elaboration of this flowchart, we propose a stratified approach according to phenotype, which should lead to an increase in diagnostic yield.

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FAMILY STUDY OF A PERICENTRIC INVERSION IN CHROMOSOME 7 AND THE ANALYSIS IN SILICO OF THE BREAKPOINTS

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Introduction: It is estimated that the frequency of pericentric inversions in the general population is 1:600. These are intrachromosomal structural rearrangements that may be pathogenic if it disrupts a coding sequence, depending on the precise location of the double strand breaks (DSB). However, when occurring in heterochromatic regions, are clinicallly considered as polymorphisms with no phenotypic consequences. Despite the phenotypical implications the carrier, an inversion may have impact on meiotic recombination and can lead to infertility, miscarriages, and chromosomal unbalanced offspring.
Clinical repor: A family study of a rare pericentric inversion involving a large chromosome 7 segment, 7p13 to q22.1 region, complemented with an in silico analysis.
Results: According to the in silico analysis, both chromosome bands 7p13 and 7q22.1 are extremely rich in coding sequences. However, the analysis also revealed a marked presence of repetitive sequences, namely, transposable elements at most of the intergenic regions in these bands.
Discussion: Chromosome 7 pericentric inversion is a rare event with only four cases described in the literature, all presenting different breakpoints. Since no clinical phenotype was detected in this family, despite of the presence of a large number of coding sequences, we suggest that the DSBs preceding the inversion event have occurred at the transposable elements' sequences.
Every new case of a rare inversion should be reported in order to obtain a more precise genotype/phenotype correlation, important for genetic counseling and risk evaluation.

Clinical Cases

A NOVEL HOMOZYGOUS DELETION IN CCDC32 GENE CAUSING CARDIOFACIO- NEURODEVELOPMENTAL SYNDROME

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Introduction: In 2020, CCDC32 gene has been associated with a human disorder by the identification of homozygous pathogenic variants in three patients from two consanguineous families presenting mainly with cleft lip and palate, cardiac defects, and neurodevelopmental anomalies. Since this initial description, only one additional patient has been reported exhibiting a similar phenotype. The biologic function of CCDC32 is still unknown. Studies in zebrafish suggested that ccdc32 is required for normal cilia formation, while recently Wainberg et al (2021) demonstrated that CCDC32 is involved in clathrin-mediated endocytosis.
Clinical Report: We describe a female patient born to consanguineous parents after a poorly supervised pregnancy. Family history was unremarkable, except for a second cousin with cerebral palsy of unknown etiology. At birth, the patient presented microcephaly and bilateral cleft lip and palate. During infancy and childhood, she had feeding difficulties, global developmental delay that progressed to intellectual disability, and recurrent respiratory infections and otitis. Cardiac evaluation revealed an atrial septal defect, and an abdominal ultrasound showed a bicornuate uterus. Brain magnetic resonance imaging was normal. At her last physical examination, at age 15, she presented intellectual disability, short stature, microcephaly, craniofacial dysmorphic features (hypertelorism, thick eyebrows, upslanting palpebral fissures, wide nasal bridge, large prominent ears), small hands, brachydactyly, nail clubbing, large halluces, and hypoplastic toenails. Clinical exome sequencing identified the likely pathogenic variant c.(244+1_245-1)_(*872_?)del in the homozygous state in CCDC32, causing deletion of exons 3 and 4, thereby establishing the diagnosis of cardiofacioneuro-developmental syndrome (OMIM #619123). The patient's mother is a heterozygous carrier of the variant; the father was not available for study.
Conclusions: This study further expands the molecular and clinical spectrum of this rare and still poorly known disorder. The description of additional patients is needed to establish the complete phenotype and will provide valuable insights on the CCDC32 function and interactions in biologic pathways.

COPY NUMBER VARIATIONS DETECTED BY ARRAY COMPARATIVE GENOMIC HYBRIDIZATION IN FETUSES WITH CARDIAC DEFECTS

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Introduction: Congenital heart defects (CHDs) are among the most common birth defects, contributing to neonatal morbidity and mortality. Different chromosomal abnormalities have been related to CHD. The prenatal diagnosis and evaluation of the disease can be important to optimize the neonatal treatment strategies. Array comparative genomic hybridization (aCGH) has been an important diagnostic methodology for the detection of submicroscopic chromosomal imbalances missed by conventional cytogenetics, being recommended as the first tier genetic test when fetal ultrasound abnormalities are identified.
Methods: A cohort of 212 prenatal cases with abnormal cardiac ultrasound findings were investigated – 125 with isolated cardiac defects and 87 with cardiac defects associated with other abnormalities. Rapid aneuploidy detection (RAD) was performed by quantitative fluorescent PCR (QF-PCR), 22q11.2 deletion was screened for some cases by multiplex ligation-dependent probe amplification (MLPA) and genomic imbalances were studied using Agilent 4x180K array-CGH platform.

Results: QF-PCR detected an aneuploidy in 9% of the cases with abnormal cardiac ultrasound findings and 22q11.2 deletion was detected by MLPA in 1% of the cases. Samples without the most common aneuploidy or 22q11.2 deletion were studied by aCGH: pathogenic copy number variations were detected in 4.7% of the samples and 3.1% revealed a likely pathogenic CNV. Additionally, variants of unknown significance (VUS) were observed in 9% of all cases studied by aCGH. Considering that classical karyotype analysis can detect deletions and duplications greater than 5-10 Mb in size, among the 15 pathogenic/likely pathogenic CNVs detected by aCGH, about 52% of CNVs would be missed by conventional cytogenetics analysis.
Discussion: The obtained results highlights the importance of aCGH to detect submicroscopic chromosomal imbalances which would not be found using conventional cytogenetic technics, namely in fetuses with CHD. Our results are in accordance with previous reports which refer clinical relevant CNVs are found in 3-12% of the fetus with CHD studied by aCGH.

URINE CFDNA FOR ORAL CANCER MONITORING: A CASE REPORT

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Introduction: Oral cancer is typically diagnosed in an advanced stage and associated with high recurrence and metastasis. The available histopathological methods based on tissue biopsies fail in the classification and prognosis of these tumors. Tumor DNA can be evaluated in easily accessed biofluids and its levels tend to reflect the tumoral burden. This study aimed to evaluate the potential of urine for the diagnosis and follow-up of oral cancer.
Methodology: We report a 62-year-old male man with pT1N2b tongue tumor. A total of 5 urine samples were collected from this patient before and at several timepoints after initiation of treatment. After cell-free DNA (cfDNA) isolation, concentrations were determined and monitored throughout the patients' clinical course. The ctDNA mutational profile was compared with the profile of corresponding tumor tissue and monitored at different timepoints using Next Generation Sequencing.
Results: The levels of urine cfDNA decrease right after initiation of treatment and increase in the following two clinical timepoints during follow-up. This patient developed a second primary tumor in the hypopharynx. The urine cfDNA levels at the time of diagnosis of this second tumor, were the lowest of the entire clinical follow up, which was not expected and requires evaluation in more patients. Sequencing of tumor tissue DNA and ctDNA revealed mutations in important genes such as in EGFR and KRAS that keep present during the treatment course.
Discussion: Our results reveal that it is possible to isolate ctDNA from urine and monitoring the oral cancer patients during clinical follow up. Moreover, following the levels of cfDNA and the mutational profile during treatment might have value to monitor disease evolution and treatment response. More studies including more patients and a longer follow-up period will be crucial to validate the potential of liquid biopsies for the diagnosis and monitoring of this cancer.
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THE CHALLENGE OF SOMATIC VARIANTS IN FOCAL CORTICAL DYSPLASIA: A CASE REPORT

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Introduction: With the advent of next generation sequencing (NGS), low-level brain somatic variants in postsurgical tissue of focal cortical dysplasia (FCD) can now be detected. The genetic background of FCD type I remains elusive, while the mTOR signaling pathway appears to play a significant role in FCD type II pathogenesis. Aiming to unravel the molecular basis of FCDs, we performed whole genome sequencing (WGS) in postsurgical tissue to detect candidate brain-specific somatic variants and evaluated their clinical significance.
Methodology: Pathological brain specimens were obtained from two patients with drug-resistant focal epilepsy, who underwent neurosurgical treatment. WGS was performed using matched blood/postsurgical brain DNA samples. Libraries were prepared using the Roche KAPA HyperPrep PCR free library preparation kit. Paired-end 150 bp reads were generated on the Illumina NovaSeq platform. To call somatic variants, the FASTQ files were processed using the nf-core sarek pipeline (version 3.0). Somatic single-nucleotide variants (SNVs) were annotated using ANNOVAR.
Results: The study included two female patients with drug-resistant focal epilepsy. The histopathological diagnoses revealed an FCD type Ia and an FCD type IIa. Five non-synonymous SNVs were detected, three in FCD Ia tissue (WDR24 p.Trp259Gly; MICAL1 p.Lys1036Arg; and KATNB1 p.Leu566Ile) and two in FCD IIa tissue (MATN4 p.Phe91Val; and ANKRD6 p.His386Gln). Several tools (PolyPhen-2, Sorting Intolerant From Tolerant, and MutationTaster) predicted all variants to be potentially pathogenic, but they were classified as variants of uncertain significance by ACMG.
Discussion: The analysis of postsurgical tissue by NGS may contribute to identify somatic missense variants in new candidate genes to further understand the genetic background of FCD. All the reported genes were previously related with epilepsy and/or malformations of central nervous system and cortical development. However, the pathogenicity assessment of these variants and, consequently, its impact in clinical practice still poses an important challenge.

NEW VARIANTS CAUSING OKUR-CHUNG SYNDROME AND MACROCEPHALY

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Introduction: Okur-Chung syndrome (OCS) (MIM #617062) is an autosomal dominant disorder with less than 40 patients reported so far. It causes neuro-developmental delay, variable dysmorphic features, growth retardation, immune deficiencies, gastrointestinal disorders and brain abnormalities, among other findings. OCS is caused by pathogenic loss-of-function variants in the CSNK2A1 gene.
Methodology: We report two Caucasian patients with OCS and new likely pathogenic variants, describe their features and compare with previous reports in the literature.
Results: The first patient is a 14-year-old male born to non-consanguineous parents. He had acute adrenal crisis in the first month of life requiring high dose hydrocortisone and fludrocortisone, that recurred upon every reduction attempt. He was born with atrial septal defect, developed post-natal growth retardation, macrocephaly and moderate intellectual disability. The patient has mild facial dysmorphisms (deep-set eyes and wide and depressed nasal bridge) and persistent fetal fingertip pads. An etiology for the adrenal insufficiency was not found. Genetic testing for genes related to Congenital Adrenal Hyperplasia did not reveal possibly pathogenic variants. Array comparative genomic hybridization (CGH array) showed a deletion in band 20p13, encompassing 12 genes, among which the CSNK2A1 gene and others not related to genetic disease to date. The deletion was inherited from the father, also with short stature and history of learning difficulties. The second patient is a 9-year-old

male born to non-consanguineous parents. He was born premature and had neonatal respiratory distress. He presented hypotonia at birth and developed post-natal macrocephaly and intellectual disability. Physically he displays normal stature, scoliosis, butterfly vertebrae and mild dysmor-phisms (deep-set eyes and depressed nasal bridge) and persistent fetal fingertip pads. He has low IgG levels. The CGH array did not show any possibly related variants and clinical exome sequencing revealed the heterozygous likely pathogenic variant c.723+1dup r.(spl?) in the CSNK2A1 gene.
Discussion: In conclusion, we report two novel variants causing OCS. In the cohorts of patients with OCS, gross deletions have not been reported. As for splice-site variants, this is the third reported to date. Both patients have macrocephaly, unlike previously reported patients, and some dysmorphic features. We also include the first patient with unexplained adrenal insufficiency. New descriptions are essential to establish the clinical spectrum of OCS.

A NOVEL LIKELY PATHOGENIC VARIANT IN KIF1A – A NEW CASE OF NESCAV SYNDROME

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Introduction: Pathogenic variants in KIF1A are associated with NESCAV syndrome (OMIM#614255), characterized by neurodegeneration and spasticity, dystonia, cortical visual impairment, and global neurodevelopmental delay/intellectual disability (DD/ID).
Methodology: We describe a 6-year-old boy born to non-consanguineous parents with no relevant personal or family history. Pregnancy and neonatal period were unremarkable. He showed early moderate to severe DD and generalized hypotonia. On dysmorphological exa-mination, relative macrocephaly, frontal bossing, hyper-telorism, epicanthus, anteverted nares, low-set ears, uplifted ear lobes with skin creases, long smooth philtrum, thin upper lip, wide intermammillary distance, and bilateral fetal finger pads were noticed. Ophthalmological assessment revealed bilateral optic atrophy. On neurological follow-up, he eventually developed pyramidal signs, with mildly hyperkinetic deep tendon reflexes, bilateral Babinski sign, and foot dystonia. Additional evaluations included cardiac and abdominal ultrasounds and brain MRI, which were normal.
Results: Pathogenic copy number variations (CNVs) and Mowat-Wilson syndrome were excluded by array-CGH and targeted molecular analysis of ZEB2 gene, respectively. Whole exome sequencing detected a novel heterozygous variant in KIF1A: c.926C>T, p.(Ser309Phe), classified as of uncertain clinical significance. This variant was confirmed to be de novo by subsequent parental studies. It is located in the kinesin 1 motor domain of the KIF1A protein, where other missense variants have already been reported, including one located in the same amino acid residue reported as pathogenic. Also, it is absent from controls, and multiple lines of computational evidence support a deleterious effect. It was thus reclassified to probably pathogenic, fitting the patient's phenotype.
Discussion: Our case underlines the importance of clinical follow-up in patients with unexplained DD/ID since diagnostic approaches evolve over time due to technological improvements in the field. Also, novel signs and symptoms may develop, serving as clues to suggest a diagnosis or be valuable in variant interpretation.

TRISOMY 6 IN B-CELL LYMPHOMA: A RARE EVENT

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Introduction: Cytogenetic studies play an important role in the diagnosis and prognosis of lymphomas. However, apart from recurrent cytogenetic abnormalities, the role of other uncommon abnormalities is not well understood.
Case Report: A 59-year-old female referred to hematology consultation in 2019 due pancytopenia in routine analyses. She was asymptomatic, denying B symptoms or severe recurrent infections. The blood count revealed Hb 11.4g/dL, leukocytes 2.5x103/µL, neutrophils 1.9x103/µL, lymphocytes 0.3x103/µL and platelets 95x103/µL. She also had severe hypogammaglobulinemia, without associated monoclonal gammopathy. At June 2021 consultation, she remained asymptomatic, with a small left supraclavicular adenopathy and palpable splenomegaly. About 2 weeks later, an hemaphagocytic syndrome was diagnosed due to worsening of pancytopenia, massive hepatosplenomegaly, fever, hypertriglyceridemia, hypo-fibrinogenemia, hyperferritinemia and identification of hemophagocytosis on the myelogram. The etiological study identified a high-grade B-cell lymphoma in the cytometric evaluation of bone marrow and peripheral blood. Conventional cytogenetic analysis revealed trisomy chromosome 6 in three metaphases out of 20 analyzed. She was treated with cyclophosphamide, vincristine, dexa-methasone and rituximab. She presented an unfavorable clinical and analytical evolution. Due to severe thrombocytopenia associated with disseminated intra-vascular coagulation, she developed an extensive cerebral hemorrhage that led to her death.
Discussion and Conclusion: To our knowledge this is the first case described of a trisomy 6 in B-cell lymphoma. In literature the cases described only had partial trisomy of the short arm of chromosome 6 in a subset of B-cell non-Hodgkin's lymphomas, and these gains were linked to a poor prognosis. The few cases described in the literature with trisomy 6 are associated with the myeloid lineage. In the present case, although the low incidence of the clone with trisomy 6, its presence may be associated with a poor therapeutic response with a poor and rapid evolution of the patient.

A MYOPATHY CASE REPORT ASSOCIATED WITH A TRUNCATING VARIANT IN MLIP GENE

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Introduction: Rhabdomyolysis is a medical condition characterized by loss of muscle homeostasis causing muscle weakness and increased serum creatine kinase (CK) level. Abath Neto et al. (2021) reported the first evidence of an autosomal recessive myopathy presenting episodes of rhabdomyolysis with elevated serum CK level associated to MLIP (MIM 614106), a gene currently without OMIM entity. Here we report a patient with a phenotype overlapping the ones recently described in patients with MLIP gene variants.
Methodology: A 15 years old woman with a metabolic myopathy presenting multiples episodes of high serum CK level and rhabdomyolysis was studied. Whole exome sequencing was performed (Illumina Novaseq 6000®), in house bioinformatics pipeline and Agilent Alissa Interpret® software were used for variant calling and analysis.
Results: The homozygous nonsense variant NM_ 001281747.2: c.2530C>T p.(Arg844*) was detected in MLIP gene.
Discussion: Here we report a patient with a myopathy profile carrying a homozygous nonsense variant in MLIP gene. The detected variant is not described in ClinVar and is present in gnomAD (AF: 0.0013%). Abath Neto et al (2021) reported this variant in homozygosity and speculated that it results in the disruption of MLIP's binding capacity. This clinical case supports the previous description of an autosomal recessive chronic myopathy associated to MLIP gene and emphasizes the importance of similar reports to consolidate variants pathogenicity and a concrete spectrum for the disease.
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DIPLOID/ TRIPLOID MOSAICISM – A RARE DYSMORPHIC SYNDROME IN AN 8 YEARS OLD BOY

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Introduction: Triploydy is one of the most frequent causes of pregnancy loss. In contrast, diploid/triploid mosaicism (or diploid/triploid mixoploidy), a rare condition with fewer than 90 cases reported in literature, seems to be less severe with a survival beyond the neonatal period. The phenotypes share many features with about 50% of the mixoploid individuals presenting mental retardation, growth retardation, body and/or facial asymmetry, hypotonia, syndactyly, clinodactyly, malformed lowset ears and, in male cases, a small phallus.
We present an 8 years old boy with severe cognitive impairment, hypotonia, obesity, limbs asymmetry with shortening of the left arm and leg and brachydactyly and whose cytogenetic analysis on cultured skin fibroblasts showed a diploid/ triploid mosaicism. Previous tests for X-fragile and Beckwith-Wiedemann syndromes had normal results.
Methodology: Two samples of skin biopsy, one of each arm, were collected, cultured, harvested and banded according to the standard procedures. ResultsThe cytogenetic analysis of both arms showed diploid/ triploid mosaicism, of about 50%.
Discussion: Although no significant difference between the right and left (more affected) arms has been observed, the boy's clinical features are in accordance with the ones reported for the same alteration. It is suggested in literature that the frequency of myxoploid individuals may be underestimated. Most of the postnatal tests are performed in blood samples with 75% of them showing normal results. Additionally the diploid/triploid mosaicism might mimic different imprinting syndromes with, in many cases, extensive investigations prior to the identification of the condition. In our case Beckwith-Wiedemann and X-fragile syndromes were previously excluded. It is the combination of features, namely, mental and growth retardation, truncal obesity, asymmetry, hypotonia, syndactyly, clino/camptodactyly, malformed lowset ears and a small phallus that should suggest the diploid/triploid mosaicism, being the skin fibroblasts karyotype the most suitable test to achieve the diagnosis and consequently an earlier accurate genetic counselling.

NOVEL KANSL1 GENE VARIANT IN GIRL WITH PHENOTYPIC KOOLEN-DE VRIES SYNDROME: A CASE REPORT

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Introduction: Koolen-de Vries Syndrome (KdVS; OMIM #610443) is a clinically heterogeneous disorder with autosomal dominant inheritance, caused by a 17q21.31 microdeletion encompassing KANSL1 gene in 95% of cases. Heterozygous pathogenic variants in KANSL1 gene are responsible for less than 5% of the cases. KdVS is characterized by neonatal hypotonia, developmental delay, intellectual disability, facial dysmorphisms. friendly behaviour, epilepsy, musculoskeletal anomalies, conge-nital heart defects, urogenital malformations, and ectodermal anomalies.
Case Report: We report an 11-year-old (yo) girl, first child of a nonconsanguineous couple, born at 40th week of gestation, pregnancy complicated by oligoamnios. At birth, weight was at 25th centile, length at 14th centile and head circumference at 42nd centile. At 12-months-old, she was referred to our outpatient clinic due to failure to thrive, development delay and hypothyroidism. At observation, she presented a triangular face, epicanthus, bulbous nasal tip, protruding ears, long philtrum, brachydactyly and small umbilical hernia. At 2yo, she presents short stature (-2.2 SD), strabismus, no speech, abnormal gait and accentuated coarse facial features. At 9yo, she has moderate intellectual disability, seizure, myopia and prognathism. Brain MRI revealed hypopituitarism, with adenohypophysis hypoplasia and ectopic neurohypophysis. Karyotype and chromosomal microarray analysis were normal. Exome Sequencing analysis revealed a heterozygous likely pathogenic variant c.(2666+1_2667-1)_(2724+1_2725-1)del at KANSL1 gene.
Discussion: KANSL1 gene haploinsufficiency causes KdVS and to the best of our knowledge, we hereby firstly describe this novel KANSL1 variant, associated with less common features of KdVS, including hypopituitarism and hypothyroidism, contributing to a broader genotype-phenotype correlation related to KdVS. This case report also shows the importance of exome sequencing in dysmorphic patients with intellectual disability, and a normal karyotype and microarray, increasing the diagnostic yield and allowing proper genetic counselling to the families.
Declaration of interest: None

ARRAY CGH FOR PRENATAL DIAGNOSIS, DIAGNOSTIC YIELD FROM ONE LAB IN THE LAST 32 MONTHS (CGC UNILABS)

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Introduction: In our routine, we receive cases of prenatal fetuses for array CGH studies, mostly due to ultrasound abnormalities or high risk prenatal screening. Array CGH allows the detection of copy number variants (CNVs), including their size and gene content, on a genome wide and high resolution scale. In the literature, array CGH diagnostics yields in these cases varies from 2.7% to 12% with a broader clinical indication [PMID: 22313859; PMID: 22034057; PMID: 22467166, PMID: 22865506, PMID: 23454632] and up to 37% with a more specific clinical indication [PMID: 34946970].We present a review of our prenatal diagnosis data from the last twenty months, with a focus on the different clinical information received, to determine the diagnostic yield and establish a relationship with the karyotype test.
Methodology: The aCGH is performed using Affymetrix Cytoscan 750K and the results are analyzed using ChAS software.
Results: In a global approach, our cohort consists of 817 fetuses, with aneuploidies and unbalanced aberrations detected in 105 (12.9%). If the karyotype were the test used for the diagnosis of these fetuses, the detection rate would be around 4%.These diagnostic yield varies with the clinical information: In the "ultrasound abnormalities" group the diagnosis yield was 14.6% (79 in 542 fetus), while the karyotype would reach 4.1%.In the "augmented nuchal translucency" group the diagnosis yield was 6.6% (12 in 181 fetus), while the karyotype would reach 1.7%.The "others" group diagnosis yield was 14.9% (14 in 94 fetuses), while the karyotype would reach 7.4%.
Discussion: Our diagnostic yield in ultrasound abnorma-lities are comparable to those described in the literature, as you will see in the poster. In the "augmented nuchal translucency", the array CGH and karyotype yields differ significantly, as was expected from the latest systematic reviews. The "others" group very high yield must indicate that important clinical information didn't arrive at the lab with the samples. In global, the difference in detection rates between array CGH and karyotype test is evident. This results and the financial cost and response time all support the strong trend to define array CGH as the first tier test after an invasive prenatal procedure (while exome is lurking in the near future).
The authors declare that they have no conflict of interest.

XQ28 AND BLUE CONE MONOCHROMACY: A HEMIZYGOUS DELETION INCLUDING OPN1LW AND OPN1MW

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Introduction: Xq28 CNVs are rarely described and have been associated with several X-linked cone opsin disorders, which display wide phenotypic variability depending on the underlying genetic mechanism. However, deletions and duplications involving OPN1LW and OPN1MW genes are commonly detected by routine genomic analysis, and considered polymorphisms of no clinical consequence. We discuss an Xq28 deletion comprising the OPN1LW/ OPN1MW cone opsin gene cluster in a young male presenting clinical features compatible with sporadic Blue Cone Monochromacy (BCM), a cone photoreceptor disorder associated with loss of both long and middle wavelength-sensitive cones.
Case Report: We report a 7-year-old boy referred to our Genetics Department due to isolated bilateral cone rod dystrophy and dyschromatopsia. Family history was unremarkable. He had no dysmorphic features. NGS multigene panel analysis was suggestive for OPN1LW deletion. To clarify this finding, genomic analysis was performed using array-CGH (180K CGX-HD, Genoglyphix v3.3 software, Signature Genomics, Perkin Elmer).
Results: Array-CGH detected an interstitial 180.23 Kb hemizygous pathogenic deletion on Xq28 [arr[GRCh37] Xq28 (153380336_153560564) x0]. The deletion encom-passes five OMIM genes including OPN1 LW/ OPN1MW gene cluster and its locus control region (LCR).
Discussion: OPN1LW and OPN1MW loss of function results in absence of functional red and green cone opsins in the human retina. Moreover, LCR deletion compromises expression, morphology, density and mosaic

organization of cone opsin. In this circumstance, vision depends on the preserved blue cone and rod photo-receptors, leading to severe impairment of color discrimi-nation, which results in BCM. Genetic investigation of causative variants for X-linked cone opsin disorders was crucial for a proper clinical diagnosis and genetic counselling, enabling predictions about the course and severity of the disease, as well as recurrence risk. Notwithstanding the central role of NGS in the routine of diagnostic genetic laboratories, array-CGH was critical to establish an etiological diagnosis in this patient.

RING CHROMOSOME 20 SYNDROME: TWO CASES REPORT WITH REFRACTORY FRONTAL LOBE SEIZURES AND LEARNING DISABILITIES

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Constitutional ring chromosomes (RCs) result from rare intrachromosomal fusions of unstable telomeres or subtelomere breaks that stabilise by circularising. Among RCs, ring chromosome 20 [r(20)] is one of the less understood. Between mosaic patients with r(20) syndrome reported in the literature, females seem to be the more frequent (64%), with a r(20) that maintain intact subtelomeric sequences with no genomic imbalances. Post-zygotic telomere fusion is thought to be the most probable mechanism for ring formation. The mechanisms through which the r(20) causes the typical clinical manifestations remains unanswered.

We report two unrelated patients, a 24 years-old female and a 24 years-old male, referred with refractory epilepsy and learning disabilities with the suspicion of r(20) due to their electro-clinical phenotype. High resolution cytogenetic analysis, on peripheral blood lymphocytes, revealed in both patients a mosaic with two cell lines. In the female patient the r(20)(::p13->q13.3::) was observed in 22% of the metaphases and in the male patient 27% of cells carries the ring. The r(20) was characterized by FISH, subtelomeric probes showed a balanced result. The epileptic phenotype of both patients is characterized by intractable focal seizures and non-convulsive status epilepticus (NCSE). The age of onset of seizures, 8 years in the female and 7 years in the male, inversely correlates with the degree of mosaicism in blood. The phenotype could be related to the ring intrinsic instability, with recombination or loss of the ring, having a deleterious effect on cellular function and proliferation. A silencing effect of peritelomeric genes could not be the pathogenic mechanism, as suggested in recent reports. The underlying genetic mechanisms are complex and may involve differential expression of genes not even located on the ring structure.

The report of more patients, with the investigation of genetic and transcriptional patterns could provide new insights into possible pathophysiologic mechanisms. Conventional cytogenetic analysis undoubtedly represent the gold standard for this diagnosis.

CAKUT SPECTRUM: RENAL HYPODYSPLASIA/APLASIA TYPE 3 PRENATAL DIAGNOSIS ACHIEVED BY WES. CASE REPORT

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Introduction: Renal hypodysplasia/aplasia tipo 3 (RHDA3) is an autosomal dominant disorder characterized by abnormal kidney development beginning in utero. The phenotype is highly variable, even within families, and there is evidence for incomplete penetrance. Some affected individuals have bilateral renal agenesis, which is usually fatal in utero or in the perinatal period, whereas others may have unilateral agenesis that is compatible with life, or milder manifestations, such as vesicoureteral reflux. Renal aplasia falls at the most severe end of the spectrum of Congenital Aomalies of the Kidney and Urinary Tract (CAKUT acronym).Methods We present a case which fetal ultrasound at 15 weeks, revealed kidney anomalies with oligoamnios. Chorionic villus biopsy was performed at 15 weeks, as well Aneuploidy screening and Array-CGH analyses. Whole-exome-sequencing (WES) was performed and the bioinformatic analysis was focused on our targeted panel with 113 genes, related with renal anomalies – CAKUT Spectrum.

Results and Discussion: Aneuploidy screening and Array-CGH analysis revealed a normal result. WES identified a heterozygous variant in GREB1L gene: 556T>C (p.Cys186Arg), classified as a variant of uncertain signifi-cance, according to ACMG recommendations. Since pathogenic variants in GREB1L gene are associated with dominant RHDA3 disorder, clinical reassessement of the fetus, as well as the parents study of the referred variant were performed. The finding that the variant was been inherited "the novo", along with the clinical reassessement of the fetus, allowed us to reclassify the variant as probably pathogenic and to conclude it is compatible with RHDA3 disorder (OMIM# 617805).Once again, WES technology revealed to be a highly useful tool to prenatal diagnostic.

AUTOSOMAL DOMINANT TUBB3-RELATED DISORDER DETECTED IN A PRENATAL CASE. THE IMPORTANCE OF WES IN THE DIAGNOSTIC OF PRE-NATAL ANOMALIES

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Introduction: TUBB-related (tubulin Pathology spectrum) disorder result predominantly in developmental brain malformations: microcephaly (impaired mitosis/ proliferation), cortical dysgenesis (impaired neuronal migration), anomalies of white matter pathways (impaired axonal pathfinding), anomalies of the cranial nerves (impaired axonal pathfinding), and malformations of the midbrain and hindbrain (possibly impairment of both neuronal migration and axonal pathfinding). We describe a case of an ongoing pregnancy where the ultrasound revealed brain malformations.

Methods: 27-year-old pregnant woman, amniocentesis at 19+5 weeks, due to corpus callosum hypoplasia, mild fetal ventriculomegaly, dilated third ventricle and abnormality of the fetal cardiovascular system. Microrray-CGH and WES analysis were performed. Bioinformatic analysis was focused on our targeted 60 genes panel, related with congenital anomalies of nervous system.

Discussion: The microarray-CGH analysis revealed a normal result, which lead to WES analysis. A heterozygous variant in TUBB3 gene, c.785G>A (p. Arg262His) was detected by WES. This variant causes an amino acid change from Arg to His at position 262. According to ACMG guidelines we classified this variant as Pathogenic. The clinical information provided allowed us to conclude that this variant is compatible with autosomal dominant cortical dysplasia, complex, with other brain malformations 1 (OMIM#614039). The whole-exome sequencing (WES) has proved to be very helpful to establish the definitive diagnosis and the result was given in time to, together with ultrasound information propose TOP. Prenatal whole-exome sequencing (WES) is becoming a very important tool to diagnose the aetiology of foetal malformations, increasing diagnostic rates.

AN UNEXPECTABLE CYTOGENETIC RESULT IN A CHRONIC MYELOID LEUKEMIA CASE

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Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by a t(9;22)(q34;q11). About 5% to 10% of CML patients show complex variant translocations that involved a third or more chromosomes in addition to chromosomes 9 and 22.

Case Report: A 68-year-old man with significant leukocy-tosis was referred to the CHTMAD Hematology consultation. The patient didn't have a fever, symptoms of an infection, constitutional symptoms, abdominal pain, or signs of an early satiety. Leukocytosis was confirmed, with no discernible alterations in the blood count. Abdominal ultrasound revealed an enlarged liver and a spleen that was above the usual range. Myeloid hyperplasia was evident on the myelogram, although there was no increase in the number of blasts. Conventional cytogenetic analysis

revealed a translocation involving chromosomes 9, 22 and a third chromosome, the chromosome 16 [t(9;22;16)(q34;q11;p13)], in the 20 metaphases analysed. Fluorescent in situ hybridization technique with t(9;22) probe showed BCR/ABL fusion signals in 80% cells. In terms of prognostic stratification, the patient had a low-risk ELTS score and an intermediate-risk Sokal score. Imatinib was started as treatment. After receiving treatment for three weeks, his hematologic response was fully restored.
Discussion and Conclusion: The present case had a complex variant translocation t(9;22;16) at diagnosis. There is no agreement on the implications of patients with t(9;22) translocation variants, with some papers reporting a worse outcome while others have shown that patients with these complex translocations have a similar outcome to patients with classic t(9;22) translocations when treated with imatinib mesylate. In the literature there are only eleven cases described with the involvement of chromosome 16. However, the impact of t(9;22;16)(q34;q11;p13) at diagnosis and its prognostic significance has not been fully clarified. In this case, the patient has tolerated the treatment with no proof of imatinib-related toxicity and an optimal response after 3 months of therapy according to the most recent European LeukemiaNet criteria with a complete cytogenetic response.

TEMPLE SYNDROME BY 14(Q32) DELETION DETECTED PRENATALLY BY ARRAY-CGH

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Introduction: Temple syndrome (TS) is a rare, genetic disease characterized by pre and postnatal growth delay, feeding difficulties, muscular hypotonia, motor develop-mental delay (with or without mild intellectual disability) and mild facial dysmorphisms, such as broad, prominent forehead, short nose with flat nasal root and wide tip, downturned corners of mouth, high-arched palate and micrognathia. TS is an imprinting disorder caused by disruption of a differently methylated region at chromosome 14(q32) that includes MEG3 (maternally expressed) and DLK1 (paternally expressed) genes. The majority of cases are caused by maternal uniparental disomy of chromosome 14 (upd(14)mat), but there is a crescent number of epimutations and deletions. Methodology: Agilent 180K oligonucleotide array-CGH was performed in a male fetus with growth restriction, evaluated at 27 weeks gestation.
Results: Array-CGH revealed a 1Mb deletion at chromosome 14(q32.2q32.31) including the MEG3 and DLK1 genes. The deletion was confirmed by Methylation specific Multiplex Ligation dependent Probe Ampli-fication (MS-MLPA) with probes for MEG3 and DLK1 genes. Study of the progenitors by MS-MLPA revealed the deletion to be de novo and the methylation profile revealed a non-methylated MEG3 and a fully methylated DLK1 compatible with absence of the paternal allele.
Discussion: The prenatal diagnosis revealed a de novo 14(q32.2q32.31) deletion of the paternal allele, compatible with TS. At six months age the boy was reevaluated and presented short stature, several facial dysmorphisms - almond-shaped eyes, epicanthus sketch, small nose with bulbous tip and depressed nasal bridge, smooth philtrum, small mouth, retrognathia – and the genetic diagnosis was confirmed on blood sample. In the first studies performed, upd(14) mat was the most common molecular alteration detected in TS patients, but deletions can be more frequent than expected and should be considered in growth retardation syndromes. Due to the clinical heterogeneity and overlap with other imprinting disorders, TS molecular diagnosis might remain underestimated, with aCGH and MS-MLPA being useful methodologies for diagnosis.

PRENATAL DIAGNOSIS OF WOLF-HIRSCHHORN SYNDROME WITH TWO DIFFERENT CELL LINES

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Wolf-Hirschhorn syndrome (WHS) is a rare genetic condition caused by distal deletion of the short arm of chromosome 4, characterized by phenotypic variability, leading to a wide spectrum of clinical signs that includes a pre and postnatal growth retardation, hypotonia and intellectual disability. Most of the reports of WHS in prenatal diagnosis are associated with large 4p deletions, that are identified by conventional cytogenetics. However, the use of molecular techniques has increased the detection rate of submicroscopic alterations and structural rearrangements associated with WHS. Fetal mosaicism is a challenge in prenatal diagnosis and occurs in approximately 1-2% of chorionic villus samples (CVS). Usually chromosomal mosaicism diagnosed prenatally involves common aneuploidies, nonetheless, a mosaic situation involving structural rearrangements is rarer. We report a 29-year-old woman referred to prenatal testing due to a fetus with prenatally diagnosed omphalocele and posterior fossa enlargement. Array analysis in uncultured chorionic villus cells revealed two genomic imbalances: a de novo 8.6 Mb terminal deletion on the short arm of chromosome 4, overlapping WHS region, and a de novo mosaic terminal deletion, with an extension of approximately 32.4 Mb, in about 40% of the cells, on the long arm of chromosome 4. Conventional cytogenetics confirmed the deletion in 4p, however the mosaic cell line, with both deletions, was not found. Non-specific ultrasound findings of WHS, due to heterogeneous expression, make prenatal diagnosis of this syndrome a challenge. Most cases of WHS are caused by a pure deletion of 4p but may also be caused by complex alterations. In the present case the ultrasound alterations are in accordance with the WHS but it is not possible to determine the possible contribution to the phenotype of the cell line with the two terminal deletions in 4p and 4q. Chromosomal microarray combined with cytogenetic analysis can more clearly elucidate genomic alterations present in these cases and can contribute to determine if the deletion is pure or part of a more complex rearrangement.

REPORT OF A RARE 18P11.32P11.21 TERMINAL DELETION IN A FETUS WITH MULTIPLE ANOMALIES

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Introduction: Ultrasound abnormalities in multiple systems in the fetus are usually related to chromosomal disorders. 18p deletion clinical features vary considerable among patients. Common features include developmental delay, hypotonia and specific facial features. Here we report the case of a 35-year-old woman who underwent a chorionic villus sampling (CVS) at 12 weeks of gestation, referred for prenatal diagnosis (PND) due to generalized subcutaneous edema, microphthalmia, holoprosencephaly, increased nuchal translucency (NT) and absent nasal bone. Chromosomal microarray analysis (CMA) is the recomended genetic test for pregnancies with ultrasound abnormalities.
Methodology: In the CVS sample, CMA study was performed using the CytoScan 750K (Affymetrix®). Subsequently, the karyotype of the fetus and the parents was performed. Results CMA identified, in a female fetus, a 13,14 Mb terminal deletion at 18p11.32p11.21 – arr [GRCh37] 18p11.32p11.21 (136228_13271319)x1. Fetal and parental karyotypes revealed a de novo terminal deletion in the fetus.
Discussion: The deletion encompasses 11 morbid OMIM genes, and the 18p microdeletion syndrome. Presentation of this syndrome is quite variable with dysmorphic features, growth deficiencies, mental retardation, and abnormalities of the limbs, brain, eyes, and heart. The identification of similar deletions in PND was rarely described, but the increased NT is one of the associated echographic markers. The deletion has a clinical significance considered pathogenic, although with a variable phenotype, and it is not possible to predict, in PND, which clinical features may manifest after birth. In the present case, the increased NT and holoprosencephaly, also present in other cases of PND, may be explained by the alteration found. The fetal karyotype allowed to identify the nature and origin of the chromosomal disorder and a reduced risk of recurrence.

The results emphasize that the combination of CMA and karyotype is, in many cases, essential for effective pregnancy management and genetic counseling. Given the varied and untypical clinical presentation of this syndrome, the PND of the syndrome still presents as a challenge.

PRENATAL DIAGNOSIS OF A RECOMBINANT CHROMOSOME 11 RESULTING FROM PERICENTRIC INVERSION O PATERNAL ORIGIN

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Introduction: Pericentric inversions can be found in 1–2% of the population. These balanced rearrangements are usually innocuous for the carrier but confer a risk for the formation of unbalanced gametes at meiosis, with duplication and/or deletion of segments involved in the inversion, as result of uneven crossing-over events. These unbalanced chromosomes can be transmitted and lead to miscarriages and/or affected offspring.
Methodology and Results: Amniocentesis was performed at 12w+6d due to: increased NT (6.7mm; >P99), cleft lip/palate, jugular sacs bilaterally, hyperechogenic bowel, permanently flexed legs and 4-chamber heart malformation. Fetal karyotype revealed additional material at 11qter [46,XY,add(11)(q24)]. Array-CGH study revealed a 34.1 Mb gain of the segment 11p15.5-11p13; and a 5.3 Mb loss of the terminal segment 11q24.3-11q25.Parental karyotypes were requested and a pericentric inversion of chromosome 11 was identified in the father [46,XY,inv(11)(p13q24.3)].
Conclusion: Unbalanced conceptions that arise from pericentric inversions can, depending on the size and nature of the segments/chromosome involved, be compatible with life. In the present case, an unbalanced recombinant chromosome 11 derived from a paternal balanced rearrangement was observed.
In 2019, Liehr et al found only 7 families with a recombinant chromosome 11 due to an inherited pericentric inversion, including at least one in the prenatal period. The duplication of the 11pter segment has been associated with two growth disorders with opposite phenotypes/syndromes: Beckwith-Wiedemann and Silver-Russel syndromes. Small terminal 11q deletions could be associated with some features of Jacobsen syndrome. Although it is difficult to establish if array results could correlate with the ultrasound findings, cleft lip and palate have been reported in some cases of BWS and SRS.The combined use of different techniques such as conventional cytogenetics and array CGH, enables the laboratories to deliver a more accurate and detailed report in the presence of an unbalanced rearrangement, so that clinicians can convey personalized counselling and follow-up for the present and future gestations.

A DE NOVO PRENATAL DIAGNOSIS 21 RING CHROMOSOME DETECTED IN THREE STEPS – CLINICAL CASE REPORT

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Introduction: We describe an unusual chromosome 21 ring with a complex cryptic rearrangement with a duplication together with a deletion, detected in a pregnancy of a healthy and non-consanguineous couple.
Methodology: A 29-year-old woman underwent chorionic villi sample collection at 12 weeks of gestation due to fetal hydrops, cystic hygroma (NT) (6mm) and subcutaneous oedema. Rapid molecular aneuploidy (MRA) test (QF-PCR), array-CGH and cytogenetic studies were performed.
Results: MRA revealed for both D21S1411 and D21S1446 STR markers, an abnormal ratio, compatible with a segmental deletion located on the 21(q22.3) region. Further characterization, performed by array-CGH analysis, not only defined the length of the deletion but also identified two additional unbalanced alterations on chromosomes 21 and 17, respectively. A deletion of approximately 4.9Mb on the terminal region of the long arm of chromosome 21 21(q22.3) was found, including 19 genes reported in the OMIM Morbid Map, and a duplication of approximately 2.9Mb on the region 21(q22.13q22.2), including 5 genes reported in the OMIM Morbid Map. Array-CGH also identified a duplication of the short arm of chromosome 17(p12), with a length of 1.3Mb, including 5 genes reported in the OMIM Morbid Map. Cytogenetic study of fetal cultured cells revealed mosaicism condition with two lines: mos45, XY,-21[2]/46,XY,r(21)[18]. Both parents were assessed by array-CGH and the results disclosed a paternal inheritance for the 17(p12) duplication.
Discussion: Chromosomic instability of the ring chro-mosome might explain not only the presence of two different cell lines but also the unusual conformation of this structural unbalanced r(21), with a duplication and a deletion. The fetal 17(p12) duplication may not be related with ultrasound findings, as the father has no phenotypic alterations and also this region (Charcot-Marie-Tooth type 1A disease (OMIM #118220)) is associated with a wide phenotypical variability. The duplication of 21(q22.13q 22.2) region, that houses the Down Syndrome (OMIM #190685) critical region, should be related with the ultrasound findings, namely hydropsis, as described in literature.

AN INTERSTITIAL TRIPLICATION OF 15Q11-Q13: A NEW CASE REPORT WITH HYPOTONIA AND FETAL GROWTH RESTRICTION

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Chromosomal abnormalities involving the proximal region of chromosome 15q11-q13, occur relatively frequently in the human population. However, interstitial triplications involving one 15 homologue, leading to tetrasomy of this region, are very rare with only 12 cases reported to date. We present a 15q11-q13 triplication in a chromosome 15 and an additional supernumerary marker chromosome (SMC) identified in a 1-month-old infant with fetal growth restriction, hypotonia, motor delay, retrognathia, ogival palate and hypogonadism. Array comparative genomic hybridization (aCGH), using a 180K Agilent oligonucleotide microarray, revealed a 6,1Mb interstitial gain of 15(q11.2q13), including the Prader Willi/ Angelman Syndrome critical region (PWACR). Cyto-genetic analysis with GTLbanding, carried out in the proband, identified an apparent duplication at 15q11.2 and a SMC in all the metaphases studied. Fluorescence in Situ Hybridization (FISH) analysis, using SNRPN probe to 15q11.2, showed a triplication in PWACR. The SMC has been characterized as being dicentric, bisatellite and did not contain the SNRPN probe. Both alterations were de novo, because the aCGH and cytogenetic analysis of the parents were normal. The severity of clinical effects is clearly related to the number of additional copies of genes within the PWACR. Patients with 4 copies of PWACR usually have a much more severe phenotype than patients with 3 copies. Their pathogenicity is independent of the parental origin of the rearranged chromosome and the affected individuals have usually mild craniofacial dysmorphism (arched eyebrows, hypertelorism, and a wide mouth), severe mental retardation and developmental delay, hypotonia, intractable epilepsy and autism. In our case report, the triplication size is similar to the others reported in the literature and includes 24 genes described in the OMIM database. The triplication is the most probably cause of the phenotype at birth (hypotonia, fetal growth restriction, retrognathia). This report demonstrates once more that the combination of aCGH, conventional cytogenetics and FISH is a powerful tool to provide a more accurate genetic counseling and to better predict the prognosis.

DEVELOPMENTAL DELAY IN A PATIENT WITH THREE INHERITED CHROMOSOME REARRANGEMENTS

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Introduction: Multiple chromosomal rearrangements are defined as more than one structural cytogenetic event in the same individual. Despite the low frequency, their identification is essential since carriers can display various phenotypes ranging from normal subjects to patients with intellectual disability and/or congenital abnormalities. The rearrangement can be de novo or familial. Region 1q21.1 is rich in low-copy repeats, genome elements that have highly similar sequences, thus prone to deletions and/or duplications. Recent reports of 1q21.1 duplication syndrome (OMIM # 612475) have demonstrated the phenotypic variability associated to this region.
Methodology: Family case report referred to our Centre for conventional/molecular cytogenetic studies. The family members are a healthy and non-consanguineous couple and a 5 yo daughter presenting with global developmental delay and a previous arrayCGH result of a 507Kb microduplication on the region 1q21.1. ResultsMother´s karyotype was normal. Both father and daughter´s karyotypes revealed two paracentric inversions involving the long arms of chromosomes 1 and 4; MLPA studies confirmed the daughter´s 1q21.1 microduplication and revealed the same duplication in the father.
Discussion: Cytogenetic studies play an important role in a variety of human disorders, since they are key in the provision of significant diagnostic and prognostic outcomes to patients. Paracentric inversions are appa-rently balanced rearrangements that usually remain undetected and are not usually associated with abnormal phenotypes. However a high incidence of mental retardation and congenital malformations has been reported in the inversion carrier offspring of phenotypically normal parents with apparently identical chromosomal rearrangements. Genotype-phenotype corre-lation between these patients and previous family cases was established and the presence of significant genes associated with 1q21.1 duplication syndrome is discussed. The minimum presumed risk of phenotypic abnormality for multiple chromosome rearrangements may be estimated as the additive risk of the number of chromosome breakpoints involved.

IDENTIFICATION OF PATHOGENIC CDH1 MUTATION IN BILATERAL BREAST CANCER – A CASE REPORT

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Introduction: Breast cancer is the most common cancer among women worldwide. Within this, bilateral breast cancer is uncommon and has a low incidence, only 1-2.6% of all patients with breast carcinoma. It represents a major challenge for oncologists because of the relationship between the two lesions.
Methodology: A 54-year-old women was referred to the genetics consultation of the Centro Hospitalar de Trás-os-Montes e Alto Douro, with a recent diagnosis of bilateral lobular breast cancer and family history of cancer. DNA was extracted from blood sample and library preparation was performed according to the manufacturer instruction. After enrichment, library was diluted, pooled, denatured and 8 pM library pool was subjected DNA sequencing.
Results and discussion: A heterozygous variant in exon 13 was identified in the CDH1 gene considered pathogenic: c.1955T>A (p.L652*). CDH1 (E-cadherin) tumor suppressor gene plays a pivotal role in maintenance of cell adhesion and cell proliferation. Germline mutations leading to loss of function increase the risk of developing several cancers, namely hereditary diffuse gastric cancer (approximately 80% for both sexes) and lobular breast cancer (approximately 50% for women). However, some families, like the one reported here, don't have individuals affected by gastric cancer.
Conclusion: The presence of the CDH1 mutation is rare in bilateral breast cancer. However, in this case, its identification is very important, since it will allow a better screening, medical surveillance and an early detection of hereditary diffuse gastric cancer, if it develops in the future. Genetic study of family members is underway.

ARRAYCGH HIGHLIGHTED A KARYOTYPE RARE STRUCTURAL CHROMOSOME REARRANGEMENT

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Introduction: Couple infertility is established after two years of incapacity to achieve conception, this condition affects 8 to 12% of reproductive aged-couples [PMID: 2955531, 2018.03.012}. If a member of the couple has an altered karyotype, this would be enough to justify their failure to reach pregnancy. Therefore, karyotype analysis is a routine test for infertile couples under study. We present a 38 years-old women, referred to karyotype analysis due to conjugal infertility, for subsequent in vitro fertilization. No additional phenotype was mentioned.
Methodology: The karyotype analysis was performed in metaphases stained with GTG bands from stimulated cultures of peripheral blood. The aCGH was performed using Affymetrix Cytoscan 750K and the results were analyzed using ChAS software.
Results: The cytogenetic analysis showed a female karyotype, with a chromosome 9 presenting an apparent unbalanced alteration, with unknown additional material in the long arm (breakpoint at 9q13). C-band technique was also performed in order to ensure that the material involved would not be heterochromatic. Once this was confirmed the result was reported as: 46,XX, add(9)(q13). Chromosomal array study was performed to clarify the cytogenetic alteration detected and to define the gene content involved. Normal result was obtained: arr(X,1-22)x2.
Discussion: The apparent unbalance detected at first, caused strangeness as clinical indication known was "conjugal infertility", and chromosome array study did not confirm an imbalance. Therefore, the alteration observed in karyotype analysis would correspond to a balanced chromosome rearrangement. Taking these two conflicting results, karyotype images were reviewed and led to conclud that the derivative chromosome should result from a rearrangement of its own material. A new report was made and the result was rewritten: 46,XX,ins(9)(q22.3q33q34.3).
Conclusion: Once again, the importance of using different analysis techniques to confirm/exclude an altered result is demonstrated: the first result obtained in karyotype would have had a less favorable impact for FIV treatment adopted by the couple, which turned out to be without increased risk after aCGH analysis.
The authors declare that they have no conflict of interest.

PRENATAL DIAGNOSIS OF PHELAN-MCDERMID SYNDROME BY ARRAYCGH: MULTIPLE BILATERAL RENAL CYSTS

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Introduction: Phelan-McDermid syndrome (PMS, OMIM #606232) is a rare neurodevelopmental disorder resulting from haploinsufficiency of the distal long arm of chromosome 22q13. The deletion size ranges from less than 100 kb to over 9 MB and the phenotype is highly variable. Kidney disorders are among the most frequent non-neurological features of the syndrome, reported in up to 40%. There are no specific prenatal indications for PMS. Diagnosis is usually suspected in childhood when the most typical clinical features manifest, and therefore prenatal diagnosis is rare and often incidental. Most fetal abnormalities observed by ultrasound were growth restriction and abnormalities of the heart, kidney, or bones.
Case report: We present the case of a 28-year-old primigravida, referred to our Centre at 21+6 weeks of gestation due to multiple bilateral renal cysts noted at ultrasound screening. The array comparative genomic hybridization (aCGH) analysis performed on amniotic fluid, showed a 5Mb loss at 22q13 region compatible to PMS. aCGH result was further characterized by cytogenetic analysis. The couple opted to terminate the pregnancy. An amniocentesis was performed and the microarray revealed a male genomic profile with a microdeletion on chromosome 22 compatible with Phelan McDermid syndrome An amniocentesis was performed and the microarray revealed a male genomic profile with a microdeletion on chromosome 22 compatible with Phelan McDermid syndrome
Discussion: Chromosomal-microarray analysis is the first-tier test in pregnancies with structural malformations. The presented case highlights the importance of the benefits of array-CGH in prenatal diagnosis compared to karyotyping and pointed out which candidate genes are responsible for renal changes.