28th ANNUAL MEETING

ABSTRACT BOOK

SOCIEDADE PORTUGUESA DE GENÉTICA HUMANA

28Th

ANNUAL MEETING

Sociedade Portuguesa de Genética Humana

5 - 7 December 2024

Instituto Superior de Engenharia

PORTO

Caros colegas,

Caros participantes,
Caros convidados,

É com grande entusiasmo que a Direção da Sociedade Portuguesa de Genética Humana (SPGH) vos acolhe no Porto, para a **28ª Reunião Anual da SPGH**.

Estamos muito satisfeitas pela oportunidade de organizar o maior evento na área da Genética Humana em Portugal, que este ano, conta com um total de 300 participantes registados, 19 palestrantes (7 estrangeiros e 12 Portugueses), 237 participantes e 44 delegados de empresas que apoiam a reunião anual da nossa sociedade. Queremos agradecer a todos, e em especial aos oradores convidados que aceitaram partilhar a sua ciência e conhecimento connosco.

A organização do Programa da reunião ficou a cargo da Comissão Científica (CC) da SPGH, que este ano é coordenada pelo António Sebastião Rodrigues e co-coordenada pela Paula Jorge, com a contribuição de um conjunto de colaboradores da CC, das restantes comissões da SPGH, nomeadamente a Comissão de Bioética, e de outros grupos de trabalho, nomeadamente a SPGH-Jovem. A todos o nosso bem-haja pelo excelente trabalho, e por terem conseguido um programa científico extremamente relevante para a Genética Humana, diverso, inclusivo e que, esperamos, vá de encontro aos interesses e às expectativas dos muitos membros da nossa Sociedade.

O interesse na conferência anual da SPGH, fica demonstrado pelo elevado número de resumos científicos submetidos por Investigadores, Médicos, Técnicos e/ou Especialistas em Genética Laboratorial, Estudantes e outros profissionais que dedicam a sua vida ao trabalho nas diversas áreas da Genética Humana.

Este ano recebemos 120 resumos para avaliação pelos elementos da CC e dos seus colaboradores. Destes, 19 foram selecionados para apresentação oral em 4 sessões e 100 para apresentação sob a forma de poster.

Este ano temos novidades em relação à apresentação e discussão dos posters, por considerarmos que os autores dos posteres merecem a nossa atenção. Teremos 3 sessões diferentes de posters, cada uma delas com posters de interesse para todos. Esperemos que apreciem esta mudança!

Temos ainda pela primeira vez um pré-meeting que inclui para além dos tradicionais clubes, 2 Sessões Educacionais e workshops feitos por empresas que apoiam a nossa reunião.

Queremos também agradecer à ASIC, que coordenou a organização do evento em estreita colaboração com a Direção da SPGH, aos membros da Comissão Local, que atrás dos bastidores, ajudaram com a logística da reunião, ao ISEP que nos deu uma casa, e ao i3S que gentilmente cedeu os suportes dos Posters sem custos. Por último, o nosso agradecimento a todas as empresas ligadas ao setor, pelo seu notável e recorrente apoio.

Pretendemos que esta reunião seja o momento maior da SPGH e da Genética Humana em Portugal, que seja o ponto de encontro para partilhar conhecimento, para estimular colaborações, estreitar relações entre diversos setores, e o local onde encontramos amigos e colegas, novos e experientes, que admiramos e estimamos, e com quem aprendemos sempre mais.

A Direção efetiva de 2024

Carla Oliveira

Ana Grangeia

Cláudia Oliveira

Dear colleagues,
Dear participants,
Dear guests,

It is with great enthusiasm that the Board of Directors of the Portuguese Society of Human Genetics (SPGH) welcomes you to Porto for the **28th Annual Meeting of the SPGH**.

We are delighted to have the opportunity to organize the largest event in the area of Human Genetics in Portugal, which this year has a total of 300 registered participants, 19 speakers (7 foreign and 12 Portuguese), 237 participants and 44 delegates from companies that support the annual meeting of our society. We would like to thank everyone, and especially the invited speakers who agreed to share their science and knowledge with us.

The organization of the meeting program was carried out by the Scientific Committee (CC) of the SPGH, which this year is coordinated by António Sebastião Rodrigues and co-coordinated by Paula Jorge, with the contribution of a group of collaborators from the CC, from the other SPGH committees, namely the Bioethics Committee, and from other working groups, namely SPGH-Young. We would like to thank everyone for their excellent work and for having achieved a scientific programme that is extremely relevant to Human Genetics, diverse and inclusive and that, we hope, meets the interests and expectations of the many members of our Society.

The interest in the annual SPGH conference is demonstrated by the high number of scientific abstracts submitted by Researchers, Medical Doctors, Technicians and/or Specialists in Laboratory Genetics, Students and other professionals who dedicate their lives to working in the various areas of Human Genetics.

This year we received 120 abstracts for evaluation by members of the CC and their collaborators. Of these, 19 were selected for oral presentation in 4 sessions and 100 for presentation in poster form.

This year we have new features regarding the presentation and discussion of posters, as we consider that the authors of the posters deserve our attention. We will have 3 different poster sessions, each with posters of interest to everyone. We hope you enjoy this change! For the first time, we also have a pre-meeting that includes, in addition to the traditional clubs, 2 Educational Sessions and workshops held by companies that support our meeting.

We would also like to thank ASIC, which coordinated the organization of the event in close collaboration with the SPGH Board of Directors, the members of the Local Committee, who helped with the logistics of the meeting behind the scenes, ISEP, which provided us with a home, and i3S, which kindly provided the poster stands free of charge. Finally, we would like to thank all the companies linked to the sector for their remarkable and ongoing support.

We hope that this meeting will be the greatest moment for SPGH and Human Genetics in Portugal, a meeting point for sharing knowledge, stimulating collaborations, strengthening relationships between different sectors, and a place where we meet friends and colleagues, young and experienced, whom we admire and value, and from whom we always learn more.

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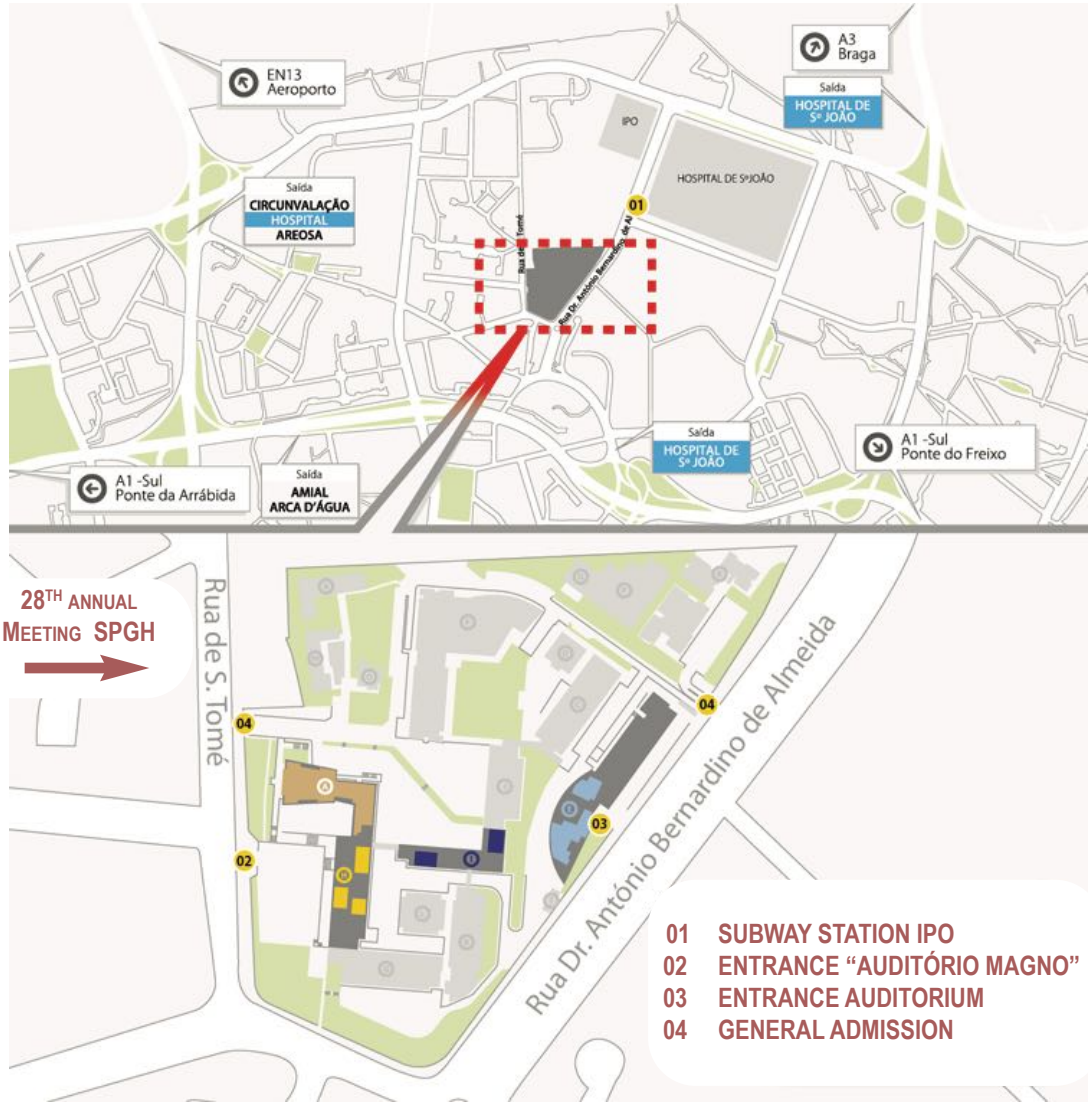

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28TH ANNUAL MEETING

SOCIEDADE PORTUGUESA DE GENÉTICA HUMANA

5 - 7 DECEMBER 2024

INSTITUTO SUPERIOR DE ENGENHARIA
ISEP PORTO

Rua António Bernardino Almeida, 431
4200-529 Porto

SPGH

Programme

Thursday 5th December

09h00-14h00 › Registration

10h00-12h00 › PRE-MEETING CONCURRENT SESSIONS

› **EDUCATIONAL SESSION 1:** *Scientific Writing*
Helder Maiato – *i3S, Porto*

› **CLUB 1:** *Cytogenetics and Molecular Genetics – Case discussion*
Sofia Dória – *FMUP, Porto* | **Natália Salgueiro – *Synlab, Porto***

› **EDUCATIONAL SESSION 2:** *Classification of variants – Rules and criteria*
Sónia Sousa | Sofia Quental – *Ipatimup Diagnósticos, Porto*

› **CLUB 2:** *Dysmorphology and Clinical Genetics*
Pedro Louro – *ULSSJ, Porto* | **Ana Rita Soares – *ULSSA, Porto***

12h30-13h30 › CORPORATE MEETINGS (concurrent in 2 rooms)

› **QUILABAN -** "*Unlocking genomic insights for rare and hereditary disease with Emedgene*", **Duarte Oliveira**

› **LIMBUS MEDICAL TECHNOLOGY -** "*Exome diagnostics and beyond: How to navigate the NGS universe with confidence using the varvis® software*"

Chair: Bruno Pescara

Part 1: *How standardized filtering strategies accelerate exome diagnostics,* **Ximena Escalera-Fanjul**

Part 2: *Cabinet of curiosities: Detecting and interpreting extraordinary variants*, **Ben Liesfeld**

13h30-14h00 › Lunch break – *Lunch offered to attendees of corporate meetings*

14H00-14h15 › OPENING AND WELCOME

Carla Oliveira | President SPGH 2024 **& Sebastião Rodrigues** | Chair Scientific Committee

14h15-14h50 › KEYNOTE LECTURE 1

Chair: Peter Jordan | Sebastião Rodrigues

"*Single cell human genetics: understanding human disease one cell at a time*"

Malte Spielmann *(Institute of Human Genetics, Lubeck, Germany)*

14h50-15h50 › Invited Symposium I (IS-I): *Genetics of Neurological Disorders*

Chair: João Freixo | Jorge Oliveira

14h50-15h20 › Invited talk ISI-1: "*Genetics and emerging treatments for neurometabolic diseases*"

Fanny Mochel *(Sorbonne Université – Hôpital de la Pitié-Salpêtrière, Paris, France)*

15h20-15h50 › Invited talk ISI-2: "*Trinity of neuroscience, genetics, and bioinformatics*"

Friederike Ehrhart *(Maastricht University, The Netherlands)*

15h50-16h10 › CORPORATE SYMPOSIUM (CSI): AstraZeneca/MSD

"*Genetic Testing and PARP Inhibitors in Portugal: assessing the present and charting the course forward*"

Breast cancer | Cláudia Vieira *(IPO Porto)*

Prostate cancer | Ana Faria *(ULS Loures Odivelas)*

16h10-17h30 › *Coffee-break* | Poster viewing and Discussion | Session I

17h30-18h20 › SELECTED ORAL PRESENTATIONS I: *Basic Research*

Chair: Bibiana Ferreira | João Vinagre

17h30-17h40	Ana Raquel Soares	tRNA MODIFICATIONS ARE POTENTIAL ALZHEIMER'S DISEASE BIOMARKERS AND THERAPEUTIC TARGETS THAT CAN BE REPROGRAMMED THROUGH tRNA MODIFYING ENZYME EXPRESSION MANIPULATION TO RECOVER CELLULAR FITNESS
17h40-17h50	Ana Catarina Nunes	GENOME.WIDE CRISPR SCREEN IDENTIFIES RAB14 AS A NEW TARGET FOR GASTRIC CANCER TREATMENT
17h50-18h00	Susana Valente	RUNS OF HOMOZYGOSITY: BIOINFORMATIC APPROACHES FOR DIAGNOSTIC PURPOSES AND POPULATION ANALYSIS USING A SAMPLE OF 12,167 EXOMES
18h00-18h10	Liliana Matos	A 5' SPLICE-STE MUTATION CAUSING MUCOLIPIDOSIS TYPE III CAN BE EFFICIENTLY RESCUED BY U1 snRNA-BASED THERAPY
18h10-18h20	Maria João Pinho	THE IMPACT OF BALANCED CHROMOSOMAL REARRANGEMENTS IN PGT-SR

18h30 › SPGH GENERAL ASSEMBLY

Friday 6th December

09H00-10h00 › SELECTED ORAL PRESENTATIONS II: *Clinical research*

Chair: Célia Soares | Ana Luísa Carvalho

09h00-09h10	Luís Nunes	CO-OCCURRING MUTATIONS IDENTIFY PROGNOSTIC SUBGROUPS OF MICROSATELLITE STABEL COLRECTAL CANCER
09h10-09h20	José Garcia-Pelaez	CLINICAL CRITERIA FOR CDH1 GERMLINE TESTING ACROSS DIFFERENT WORLD REGIONS: SHALL WE KEEP THEM GLOBAL?
09h20-09h30	Filipe Alves	CLINICAL EXOME CONCEPTUALIZATION: A BIOINFORMATICS FRAMEWORK FOR SEMI-AUTOMATED REVIEW OF GENE-PHENOTYPE ASSOCIATIONS
09h30-09h40	Domingos Roda	CLINICAL IMPACT OF DYNAMIC CHANGES IN CFDNA DURING CHEMO/RADIATION IN GLIOBLASTOMA PATIENTS
09h40-09h50	André M. Travessa	IS MILD BONE FRAGILITY GENETIC? - GENETIC FINDINGS IN A COHORT OF PATIENTS WITH EARLY-ONSET FRACTURES AND/OR OSTEOPOROSIS AND NO EXTRASKELETAL SIGNS OF OSTEOGENESIS IMPERFECTA

10h00-11h00 › BUILDING BRIDGES: *Hereditary Cancer Network in Portugal*

Chair:Carla Oliveira

Guests: Carla Pereira (*Department of Quality in Health, Ministry of Health of Portugal*) | **Fátima Vaz** (*ERN Genturis Reference Centre IPOL*) | **Manuel Teixeira** (*ERN Genturis Reference Centre IPOP*) | **Luzia Garrido** (*ERN Genturis Reference Centre ULSSJ*)

11h00-12h00 › Coffee-break / Poster Viewing and Discussion, Session II

12h00-13h00 › INVITED SYMPOSIUM II (ISII): *Update on preimplantation genetic testing and prenatal diagnosis in Portugal*

Chair: Ana Grangeia | Rosário Pinto Leite

12h00-12h30 › INVITED TALK ISII-1: "*Preimplantation genetic testing in Portugal*"

Filipa Carvalho *(Faculty of Medicine, University of Porto, Porto, Portugal)*

12h30-13h00 › INVITED TALK ISII-2: "*Prenatal diagnosis in Portugal*"

Inês Carvalho *(Unidade Local de Saúde São José, Lisbon, Portugal)*

13h00-14h00 › Lunch break

14h00-15h00 › POSTER VIEWING AND DISCUSSION, Session III

15h00-16h00 › Invited Symposium III (ISIII): *Cancer Genetics*

Chair: Joana Xavier | Sofia Fernandes

15h00-15h30 › Invited talk ISIII-1: "*Genetics of Li-Fraumeni*"

Gaëlle Bougeard *(Universitè de Rouen, France)*

15h30-16h00 › Invited talk ISIII-2: "*The urothelial gene regulatory network: understanding biology to improve bladder cancer management*"

Francisco X. Real *(CNIO, Madrid, Spain)*

16h00-16h30 › Coffee-break

16h30-16h50 › SELECTED ORAL PRESENTATIONS III: *Clinical Cohorts*

Chair: Marisa Silva | Joana Salgado

16h30-16h40	Luís Miguel Pires	CLINICAL UTILITY OF DNA METHYLATION EPISIGNATURES TESTING - A PORTUGUESE MULTICENTER COHORT STUDY
16h40-16h50	Rita Barbosa-Matos	PERSONALIZED REGION-SPECIFIC LIFETIME CANCER RISK ESTIMATES FOR AN ANCESTRAL CDH1 VARIANT

16h50-17h45 › ROUND TABLE: *Bridging imaging and genetics*

Chair: André Travessa | Marta Soares

João Soares Fernandes *(Hospital de Braga, Braga, Portugal)*

José Almeida *(Champalimaud Foundation, Lisbon, Portugal)*

17h45-18h45 › Invited Symposium IV (ISIV): *Understanding and treating disease*

Chair: Ilda Ribeiro | Sebastião Rodrigues

17h45-18h15 › Invited talk ISIV-1: "*Genotype-phenotype correlations in Parkinson Disease*"

Joana Damásio *(Universidade do Porto Instituto de Ciências Biomédicas Abel Salazar, Portugal)*

18h15-18h45 › Invited talk ISIV-2: "*Ataxia with giant axonopathy in Acbd5-deficient mice halted by adeno-associated virus gene therapy*"

Pedro Brites *(I3S, Universidade do Porto, Portugal)*

20h00 GALA DINNER

Saturday 7th December

08H40-09h30 › SELECTED ORAL PRESENTATIONS IV: *Clinical cases*

Chair: Ana Raquel Silva | Susana Fernandes

08h40-08h47	Sara Pinho	EXPANDING THE PHENOTYPE ASSOCIATED WITH IRF2BPL GENE VARIANTS: A REPORT OF 4 NEW CASES
08h47-08h54	Catarina Macedo	PRENATAL AND POSTNATAL PHENOTYPING AND MOLECULAR CHARACTERIZATION OF THREE CASES OF ZTTK SYNDROME: A POTENTIALLY RECOGNIZABLE CLINICAL ENTITY
08h54-09h01	Ana Miguel Capela	CTCF-RELATED INTELLECTUAL DISABILITY – FOUR PATIENTS SUGGESTING A RECOGNIZABLE FACIAL GESTALT
09h01-09h08	Isabel Serra Nunes	FRAXE INTELLECTUAL DEVELOPMENT DISORDER – WARNING FOR A FORGOTTEN DIAGNOSIS
09h08-09h15	Nuno Teixeira	A NEVER-ENDING RACE: A MULTILOCUS CAUSE OF AN INBORN ERROR OF IMMUNITY ASSOCIATED WITH COMPLEX NEUROLOGICAL DISEASELETAL SIGNS OF OSTEOGENESIS IMPERFECTA
09h15-09h22	Lígia Lameiras	A CHALLENGING DETECTION OF A NOVEL SPLICING DELETION IN THE DMD GENE IN A PATIENT WITH DUCHENNE MUSCULAR DYSTROPHYSKELETAL SIGNS OF OSTEOGENESIS IMPERFECTA
09H22-09H29	Sofia Fragoso	BEYOND THE AFFECTED: THE IMPORTANCE OF TESTING NON-AFFECTED INDEX INDIVIDUALS IN CANCER HIGH-RISK FAMILIES

09h30-10h30 › BIOETHICS DEBATE: "*Lack of access to healthcare in rare diseases*"

Chair: **Lina Ramos | Joaquim Sá**

"European point of view" **Dorica Dan** *(EURORDIS/ePAG)*

"A Portuguese approach" **Mariana Neves** *(Serviço Genética Médica, ULSSM)*

10h30-11h00 › CORPORATE SYMPOSIUM II (CSII) – QUILABAN | Illumina

"Non-invasive prenatal testing for fetal aneuploidies by Cell-Free DNA whole-genome sequencing"

Luís Miguel Pires and Oscar Bolanos

11h00-11h25 › Coffee-break

11h25-12h00 › KEYNOTE LECTURE 2

Chair: **Sérgio Sousa | Celeste Bento**

"Genoprotective interventions for healthspan extension"

Elsa Logarinho *(i3S, Porto, Portugal)*

12h00-12h10 › SPGH in 2025 | **Sérgio Sousa,** *President SPGH 2025* | **Janet Pereira, Cláudia Oliveira**

12h10-12h35 › SPGH Award Lecture

Chair: **Sebastião Rodrigues | President Elected (tba)**

12h35-12h55 › SPGH Awards Ceremony

Chair: **Sebastião Rodrigues | Paula Jorge**

12h55-13h10 › Closing Session

Carla Oliveira | Ana Grangeia | Cláudia Oliveira

SPEAKERS

Dorica Dan

My name is **Dorica Dan**, I am president of organizations for people with rare diseases in Romania and vice-president of EURORDIS – Rare Diseases Europe. My daughter has Prader Willi Syndrome, and I am ePAG chair in ERN ITHACA.

I was part of the EUCERD and CERD, Work Package Leader for Specialized Social Services for Rare Diseases on the EU-CERD Joint Action for Rare Diseases and member of the National Council for Rare Diseases.

I collaborated on Inclusion strategy for people with disabilities in Romania and Moldova. I am a member of the WHO Technical Working Group for Mapping toolkit for community-based care resources and co-chair for the Disability and Health WG.

I established NoRo Center 13 years ago in Romania: www.centrulnoro.ro.

Abstract

BIOETHICS DEBATE: Lack of access to healthcare in rare diseases

Summary and methodology:

Our goal is to assess the healthcare access for RD patients in Europe since 2008 and to present the remained disparities and challenges in the CEE Region and a case study from Romania

Despite overall improvements in healthcare for rare disease in Europe over the past 15 years, these achievements have not been distributed equally and there are still strong inequities within EU countries, especially in the CEE region.

Most of our MS experience challenges and huge lack of equity in the distribution of resources and an even greater lack of listening to the needs of patients and families.

The European Project for Rare Diseases National Plans Development (EUROPLAN) promoted National Plans or Strategies to tackle rare diseases, to share relevant experiences within Countries, linking national efforts with a common strategy at European level. This “double level” approach ensured that progress is globally coherent and follows common orientations throughout Europe.

Eurordis organized national conferences, involving all stakeholders to assess the needs of the rare diseases community and to establish priorities. Through JARDIN project, Member States will organize national networks to ensure better access for patients with rare diseases according to their needs.

Rezults

Since 2008 rare diseases are a priority area for action in Public Health funding programs.

Considering rare diseases as a whole, and not singularly, helps highlighting and recognizing a series of healthcare problems and planning focused public health actions involving groups of population with common needs, safeguarding at the same time their peculiarities and differences.

Dorica Dan [1] , Maria Puiu[2, 3], Ioana Streață [4], Dan Alexandru-Tiberiu [5]

1.Romanian National Alliance for Rare Diseases, Romanian Prader Willi Association (NoRo Center for RD), Ro-NMCA-ID- ITHACA ERN;
2.Centrul Regional de Genetică Medicală Timiș, membru in Rețeaua Europeana de Boli rare ERN ITHACA, Centru de expertiză în boli rare.
3.Spitalul Clinic de Urgență pentru copii " Louis Țurcanu ", Timisoara,
4.Centrul Regional de Genetică Medicală Dolj, membru in Rețeaua Europeana de Boli rare ERN ITHACA, Spitalul Clinic Județean de Urgență din Craiova
5.Dan Alexandru Tiberiu, CEO Centrul NoRo, Romanian Prader Willi Association

Mariana Neves

- 2012-2018: Integrated Masters in Medicine (MIM) at the Faculty of Medicine of Lisbon, Portugal.
- 2020-present: Specific training in Medical Genetics at ULS Santa Maria, Lisbon, Portugal.

Special interest in the area of Bioethics in Medical Genetics. From an early stage of internship, she sought training opportunities in this area, namely:

- February-June 2021: attendance at postgraduate studies: "VI postgraduate course in Bioethics", Private Law Research Center.
- December 2021: short-term internship in Bioethics at the Faculty of Medicine of Porto (MEDCIDS).
- April 2022: short course on ethical issues in the practice of Medical Genetics integrated into the Masters in Genetic Counseling at Cardiff University (supervised by Professor Angus Clarke).
- Participation in Genethics UK Forum meetings.

Elsa Logarinho

E. Logarinho concluded her PhD in Biomedical Sciences by the University of Porto (UP) in 2002. After PhD, she was endowed with full-time professorship positions, first at ISCS-N/CESPU (2002-2007) and then at Medical School/University of Minho (2007-2009). In 2009, the IR shifted to a full-time research path under the 'Ciência 2008' program. Her work on mechanisms ensuring mitotic spindle-pole integrity was acknowledged with the Pfizer 2011 and SPGH 2013 national prizes. In 2013, she was awarded a Junior Researcher position to launch innovative research lines at IBMC (now part of i3S), accomplishing an independent research group status in 2015 whilst granted with an FCT Investigator competitive position.

Since 2017, E. Logarinho leads the Ageing and Aneuploidy group at i3S. Cellular and animal models of ageing and aneuploidy, such as the Hutchinson-Gilford Progeria and Down syndromes respectively, share common features including genomic instability. Novel molecular targets whose modulation restores genome stability and delays senescence have been identified (Nat Commun 2018, Embo Rep 2020, Cell Death Dis 2021, Nat Aging 2022). The originality of her work has caught international recognition as attested by the Maximon Longevity Prize 2022, the collaborative network, the delivery of invited talks in prestigious conferences, the evaluation of international grants as expert and journal peer review. She has secured >3.5M€ competitive funding, including grants from the Progeria Research Foundation and Jeróme Lejeune Foundation. Besides her core scientific activities, she was vice-coordinator of the Cancer Program and member of the Restrictive Scientific Council at i3S during 2019-2022, and she has been continuously involved in MSc/PhD lectures and examinations and in outreach activities. She is also a Research Consultant at the Insparya Hair Company. E. Logarinho co-authored a total of 45 publications with >2500 citations and an h-index of 27 (Google Scholar). Career breaks and unconventional path: 3 maternity leaves (2004, 2011, 2012) and academia (2002-2009).

Abstract

Genoprotective interventions for healthspan extension

t is becoming increasingly clear that genomic instability, caused by many external and internal insults leading to DNA damage, plays a central role in the ageing process. All human syndromes exhibiting premature aging features, are caused by mutations in DNA repair genes or in lamin genes (required to maintain nuclear integrity). Also, there is accumulating evidence that DNA damage accrual, affects most, if not all, aspects of the ageing phenotype, making it a potentially unifying cause of ageing. For instance, increased levels of DNA damage cause sequestration of chromatin modifiers, precluding them from their epigenetic remodelling function. This leads to epigenetic erosion, changes in 3D genome architecture and gene expression, that lead to loss of cell identity and function, eventually triggering a pro-inflammatory state know as senescence, as well as its secretory phenotype causing local chronic inflammation and age-related diseases. Given that DNA damage primarily drives all other ageing hallmarks, its inhibition appears the logical rationale for a unified intervention against age-related diseases. However, since DNA damage comprises many distinct chemical alterations, the repair of which depends on the delicately balanced activities of at least seven core multi-enzyme pathways, it has been challenging to target therapeutically. Our group has been exploring two novel genoprotective interventions and their geroprotective role: the FOXM1 gene therapy and the KIF2C pharmacological targeting. Our most recent results will be presented.

Fanny Mochel

Fanny Mochel is a professor of genetics at Sorbonne University. She received her MD in Genetics in 2005 at the University Paris Descartes, her PhD in Neuroscience in 2010 at Sorbonne University and is board certified in inborn errors of metabolism. Professor Mochel leads the French reference center on Neurometabolic diseases and Leukodystrophy in adults and runs a Neurometabolic research group at Paris Brain Institute of La Pitié-Salpêtrière University Hospital in Paris. She is chair of the adult section of the Society for the Study of Inborn Errors of Metabolism (SSIEM), and co-chair of the French society for inborn of errors of metabolism in adults. Her research is focused on the characterization and treatment of neurometabolic disorders and metabolic leukodystrophies in adults. Her major areas of expertise are the identification of neurometabolic biomarkers in vitro (metabolomics) and in vivo (metabolic imaging) as well as therapeutic approaches targeting the Krebs cycle.

Abstract

Contribution of metabolism and physics in the understanding of brain functions and diseases

In July 2024, Prof. Fanny Mochel (ICM/Sorbonne University) and Prof. Angela Garcia-Cazorla (Barcelona University) organized an international symposium that led to a groundbreaking encounter between physicists and neurobiologists to address the central role of physics and metabolism in brain functions and diseases. Among the 2,000 inherited metabolic diseases identified so far, almost 400 affect cellular trafficking and biophysics - i.e., vesicular trafficking, autophagy, membrane contact sites between organelles, and cytoskeleton trafficking.

My talk will address the principles of mechanotransduction (i.e., mechanisms by which cells convert mechanical stimulus into biochemical activity), biophysics of neurotransmission and application to neurogenetic disorders, as well as new methods combining physical and genomic approaches (e.g. spatial mechano-transcriptomics) to understand how pathogenic variants may not only affect cellular pathways but also cellular forces. The greater understanding of the interplay between metabolic and mechanical cues in neurogenetic disorders may also provide novel therapeutic approaches through mechanomedicine.

Filipa Carvalho

Filipa Carvalho is graduated in Biology in 1992, Faculty of Sciences, University of Porto and has obtained the Master degree in Oncobiology in 1994, Faculty of Medicine, University of Porto. The PhD degree in Human Biology was obtained in 1999 the Faculty of Medicine, University of Porto. Habilitation in Biomedicine was obtained in 2011.

Filipa Carvalho is a Full Professor of Genetics, Department of Pathology, Faculty of Medicine, University of Porto and is the technical supervisor of the molecular biology laboratory. She is responsible for the preimplantation genetic testing.

Filipa Carvalho is the supervisor of several graduate and PhD students and is involved in national and international research projects. Her main area of research is the genetics of infertility and preimplantation genetic testing.

Filipa Carvalho is author and co-author of several papers published in peer reviewed journals.

Abstract

Preimplantation genetic testing in Portugal

Preimplantation genetic testing (PGT) is a diagnostic method that has become a powerful complement to assisted reproduction techniques. It is currently widely used to detect genetic and chromosomal abnormalities in embryos (PGT-M: preimplantation genetic testing for monogenic disorders; PGT-SR: preimplantation genetic testing for structural rearrangements; PGT-A: preimplantation genetic testing for aneuploidy). The technique allows the selection of the embryos that are genetically transferable, thus avoiding the transmission of a disease to the offspring.

An update of PGT in Portugal, reviewing the indications for PGT, methods of biopsy and diagnostic technologies, will be presented.

Friederike Ehrhart

Friederike (Freddie) Ehrhart is an assistant professor in the Department of Translational Genomics at Maastricht University. Her main research interests are systems biology of rare genetic diseases, especially neuronal and metabolic diseases, which she investigates using neuroscience, genetics, and bioinformatics methods. Friederike studied Biology in Würzburg, Germany, with focus on Biotechnology. After graduating in 2004 she continued with a PhD thesis at Fraunhofer Institute for Biomedical Engineering in cooperation with Saarland University, Germany. After receiving the PhD title in 2009 she continued working as a postdoc researcher, later as group lead and head of laboratory at Fraunhofer before moving to Maastricht University in 2015. Since 2019 on 22q11.2 deletion syndrome with Therese van Amelsvoort. Since March 2019 she holds a tenured position as assistant professor on systems biology of rare diseases.

Abstract

Stepping stones in rare disease pathways

Rare diseases are defined by their prevalence, which is less than 2 per 1000 in the EU. However, there are currently about 6200 rare diseases known and their pooled abundance in the population is estimated to be about 5% [1]. In short, they are rare but many. Research of rare genetic diseases struggles with different specific problems of availability of patients, samples, and data for development and improvement of diagnosis and treatment. Lack of data availability and interoperability restrict the possibilities for data re-use. Fragmentation of data and information in different un-connected resources as well as limited awareness of researchers about the available resources hamper progress additionally.

In this presentation, I will show several recent works on how to de-fragmentise rare disease information - with sometimes surprising results - and show how to get the most out of rare (multi) omics data with specially adapted workflows. Within the European Joint Programme on Rare Diseases (EJP RD) and the follow-up European Researche Alliance on Rare Diseases (ERDERA), the largest research programs in Europe, we are integrating several of these rare disease specific and non-specific resources to create a virtual platform where researchers and clinicians can find information [2].

[1] Nguengang Wakap S, Lambert DM, Olry A, Rodwell C, Gueydan C, Lanneau V, Murphy D, Le Cam Y, Rath A. Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. Eur J Hum Genet. 2020 Feb;28(2):165-173. doi: 10.1038/s41431-019-0508-0. Epub 2019 Sep 16. PMID: 31527858; PMCID: PMC6974615.

[2] http://vp.ejprarediseases.org/

Francisco X. Real

Paco Real trained as a medical oncologist at Memorial Hospital, New York, where he became staff physician. He returned to IMIM, Barcelona, in 1988 where he set up a new research program in cancer cell and molecular biology. In 2003, he became Professor of Cell Biology at UPF. In 2007, he joined the CNIO where he is a Senior Group Leader. Over the last 30 years, his laboratory has focused on the study of the cell and molecular biology of pancreatic and bladder cancer. In pancreatic cancer, his group pioneered that notion that cell differentiation is first tumor suppressive mechanism using genetic mouse models, focusing on the identification of early mechanisms that favor tumor development. His group has provided a thorough understanding of the role of several transcription factors in normal pancreas and PDAC. They have uncovered that lineage-specific transcriptional programs suppress intrinsic inflammatory signals. In bladder cancer, his group has discovered novel tumor suppressor genes, has contributed to the understanding of transcriptional regulatory networks, and plays a key role in translational studies in the context of clinical and epidemiological studies. The group combines the use of genetic mouse models, organoids, and multi-omics data analysis to study the function of relevant genes/pathways. Paco loves collaborating and he has participated in several European and worldwide consortia. He is a member of the Scientific Advisory Board of several institutions and charities and, most notably, a member of CRUK Discovery Research Committee and of the ERC Consolidator grant LS7 panel (until 2023).

Abstract

The urothelial gene regulatory network: understanding biology to improve bladder cancer management

Francisco X. Real, Spanish National Cancer Research Centre-CNIO, Madrid, Spain

The urothelium is composed of basal, intermediate, and umbrella cells. Most bladder cancers are urothelial carcinomas. Loss of urothelial lineage fidelity results in altered differentiation, highlighted by the bladder cancer taxonomic classification into basal and luminal tumors. The genes encoding several TFs involved in urothelial differentiation display genetic alterations in bladder cancer, likely contributing to tumor development. We have systematically identified transcription factors relevant for urothelial identity and have defined their role in normal bladder and in tumors. We have also identified novel candidates to be involved in these processes that might provide opportunities to improve the management of patients with bladder cancer.

Gaëlle Bougeard

Gaëlle Bougeard is an Assistant Professor at the University of Rouen Normandy, in the U1245 Inserm unit (Cancer and Brain Genomics, CBG / team 1: Genetic Predisposition to Cancer), and at the Rouen University Hospital (CHU), where she leads the Li-Fraumeni group. She has worked in Professor T. Frébourg's team since 1998, with her scientific interest centered on understanding the molecular basis of Li-Fraumeni syndrome (LFS) and its clinical variability, by meticulously cataloging the personal and familial clinico-biological and genetic data of the French LFS cohort. Dr. Bougeard's team is currently focused on the clinical interpretation of TP53 variants identified in routine diagnostics, as well as on identifying modifier factors in LFS that affect TP53 and other genes. The Rouen team has established expertise in Li-Fraumeni syndrome research over the past 30 years.

Abstract

Genetics of Li-Fraumeni syndrome

Univ Rouen Normandie, Inserm U1245 and Rouen University Hospital, Department of Genetics, Rouen, France

Li-Fraumeni syndrome (LFS) is an autosomal dominant disorder characterized by a predisposition to a wide spectrum of cancers that can develop from early childhood to late adulthood, caused by germline mutations in the TP53 tumor suppressor gene, which encodes the transcription factor p53. For over 30 years, the Rouen genetics laboratory has dedicated efforts to developing molecular techniques to detect various types of inactivating TP53 alterations across the gene. With the centralization of national analyses, we have been able to study genotype-phenotype correlations and develop functional assays to examine p53 activity, initially in yeast and cell lines, and later in the genetic context of patients. TP53 was among the first genes to benefit from high-throughput functional assays, with results published in the literature providing critical support for interpreting TP53 variants. However, access to functional assays tailored to each patient is essential, as this approach enables the interpretation of private variants and the unmasking of loss of function in missense variants that may be masked by overexpression in transfection-based assays. Using a functional assay to analyze the transcriptional activity of p53 in normal blood cells, we demonstrated the loss of function of in-frame variants, low-penetrance variants, and splicing variants. Additionally, we revealed the potential modifying effects of polymorphisms and identified a new TP53 isoform, whose modifying effect on LFS remains to be explored. Identifying p53 loss of function is critical for tailoring treatment, follow-up, and genetic counseling. The p53 functional assay is part of a personalized care offering for LFS patients, given the increasing number of variants identified in diverse phenotypes, with the aim of advancing toward stratified medical management.

Inês Carvalho

Inês Carvalho, Inês Carvalho, graduated in Medicine in 2011. Specialist in Medical Genetics since 2018 and is currently a hospital assistant at the São José Local Health Unit in Lisbon and responsible for the Medical Genetics consultation in prenatal diagnosis at the Center for Integrated Responsibility for Surgery and Fetal Medicine at Maternity Dr. Alfredo da Costa. She has been a member of the National Technical Commission for Prenatal Diagnosis of the General Directorate of Health since 2018. She is the author and co-author of several scientific communications.

Abstract

Prenatal Diagnosis in Portugal

Prenatal diagnosis in Portugal has undergone significant changes in recent years, resulting in a decrease in infant mortality due to preventable diseases, with anomalies and genetic diseases becoming more important for health services and for society itself. Order No. 5411/97 (2nd series) defines prenatal diagnosis as "the set of procedures that are carried out to determine whether or not an embryo or foetus is a carrier of a congenital anomaly". Portugal has some technical and human resources in place this area, whose geographical distribution is not optimized and which are still insufficient to allow the full satisfaction of existing needs. Organization of the health care network includes the following levels of intervention:

a) Level I "Primary health care";
b) Level II "Prenatal diagnostic centers" in perinatal support hospitals;
c) Level III "Centres for prenatal diagnosis and therapy" in the hospitals with differentiated perinatal support.

The National Technical Commission is constituted by specialists of recognized scientific merit in the areas of medical genetics, obstetrics and obstetric ultrasound, appointed by the Director-General of Health.

The Portuguese Association of Prenatal Diagnosis (APDPN), among the various objectives, intends to: Promote the evaluation, in a multidisciplinary way, of the various procedures in DPN and standardize them at national level; To promote and facilitate contact and meeting between professionals who work in the area of TLD or who share this interest and are dedicated to related areas; To promote and facilitate the relationship and exchange with other national and international societies, with areas of common interest.

In this presentation, the Study of the activity of prenatal diagnosis in the hospitals of the National Health Service (SNS) will be presented, a document prepared based on the survey sent by the National Technical Commission for Prenatal Diagnosis to the hospitals of the SNS, to evaluate the activity developed in the year 2018 published in January 2024. For a more current perspective, preliminary survey data applied in July 2024 to six representative DPN centers at national level will be presented.

Joana Damásio

Joana Damásio completed her medical degree at the Faculdade de Medicina da Universidade de Coimbra and pursued her Neurology residency at Hospital de Santo António. She completed a clinical research fellowship at the National Hospital for Neurology and Neurosurgery in London, at Prof. Kailash Bhatia's team. Joana Damásio obtained her PhD in Medical Sciences from the ICBAS School of Medicine and Biomedical Sciences, under the supervision of Prof. Jorge Sequeiros and Prof. José Barros. Her primary research focus is on genetic movement disorders, particular hereditary cerebellar ataxias.

Currrently a consultant in Neurology at Hospital de Santo António, she coordinates the Neurology Outpatient Clinic and takes part in the movement disorders and deep brain stimulation groups. Additionally, she is also part of the clinical team at Centro de Genética Preditiva e Preventiva, Instituto de Biologia Molecular e Celular and is a member of the Genetic Epidemiology and Epigenetics Group at UMIB, ICBAS School of Medicine and Biomedical Sciences.

She is the President-Elect of the Sociedade Portuguesa das Doenças do Movimento and a member of the Movement Disorders Society Ataxia Study Group; serving on the Web Site Editorial Board and the Bylaws Committee.

Abstract

Genotype-Phenotype Correlations in Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the cardinal features of bradykinesia, rest tremor, rigidity and postural instability, and a wide range of additional motor and nonmotor symptoms. While its exact etiology remains unknown, significant strides have been made in understanding the genetic underpinnings contributing to its phenotypic spectrum. This talk will delve into the genotype-phenotype correlations observed in PD, which have provided insights into disease mechanisms, onset, progression, and response to treatment.

Disease-causing variants in SNCA (alpha-synuclein) are rare, but typically lead to autosomal dominant early-onset PD with severe motor symptoms and a high risk of dementia, reflecting the toxic aggregation of alpha-synuclein protein. Pathogenic variants in LRRK2 are associated with an autosomal dominant later onset disease with variable clinical manifestations, including mild cognitive impairment.

PARKIN and PINK1 are linked to autosomal recessive early-onset PD, generally presenting with slower disease progression and a more favorable response to dopaminergic therapy. These genes highlight the critical role of mitochondrial function and proteostasis in PD pathophysiology. Other genetic contributors, such as VPS35 and DJ-1, further emphasize the multifaceted nature of PD, illustrating diverse pathways from autophagy-lysosomal deficits to oxidative stress.

The interplay between these genetic mutations and the environment also shapes PD phenotypes, underscoring the importance of epigenetic regulation and gene-environment interactions. This correlation enriches our understanding of PD heterogeneity and aids in stratifying patients for tailored therapeutic approaches and potential gene-targeted treatments.

Future research integrating multi-omic technologies promises deeper insight into how genetic variations modulate clinical phenotypes and treatment responses. Unraveling these connections will pave the way for personalized medicine strategies improving clinical outcomes and disease management.

João P. Soares-Fernandes

João P. Soares-Fernandes, MD

Staff Neuroradiologist at Hospital de Braga, Braga, Portugal, with current practice in Fetal, Neonatal and Pediatric Neuroradiology.

Specialist training in Hospital de Braga and Hospital de Santo António, Porto, Portugal (General Neuroradiology, 2005-2009) and Hospital for Sick Children, Toronto, Canada (Pediatric Neuroradiology, 2008-2009).

Abstract

Genetic correlations in fetal brain MRI

Fetal brain MRI is an increasingly used technique in prenatal diagnosis as a complementary second-line imaging study, usually performed after the routine anatomic ultrasonography. 3-Tesla scanners have increasingly progressed from research to clinical tools for fetal MRI providing images with higher signal-to-noise ratio and speeding up the acquisition, necessary to overcome sudden fetal movement or improve spatial resolution for a more precise fetal brain depiction. T2-weighted images provide the best contrast for highlighting the anatomical differences in the fetal brain, specifically the water and lipid content in the mature brain. Ventriculomegaly is the most common indication for fetal brain MRI. The causes of ventriculomegaly include developmental, destructive, and obstructive processes, or a combination of them. MRI improves diagnostic accuracy and can be used to suggest genetic correlations which we will detail in this talk, with an emphasis on hydrocephalus, neurocutaneous syndromes, midline and cortical development malformations and posterior fossa abnormalities.

José Guilherme de Almeida

José Guilherme de Almeida is a researcher in the Computational Clinical Imaging Group at Champalimaud Foundation. After a Bsc. in Biochemistry and an MSc. in Cell and Molecular Biology at Universidade de Coimbra, José pursued a PhD in computational biology at Cambridge University and the European Bioinformatics Institute on histological image analysis and statistical modeling of longitudinal genetic sequencing. In 2022, he started working at Champalimaud Foundation on the development of methods and algorithms for clinical imaging analysis with machine - and deep-learning that can improve and accelerate routine diagnostic tasks.

Pedro Brites

Pedro Brites graduated with a degree in Biology from the Faculty of Sciences, University of Porto, and was awarded a Ph.D. in Medicine from the University of Amsterdam, The Netherlands. During his doctoral studies, the research was instrumental in identifying the gene associated with the peroxisomal disorder Rhizomelic Chondrodysplasia Punctata (RCDP), published in Nature Genetics, and in unraveling its molecular mechanisms, resulting in two publications in the American Journal of Human Genetics. This work underscored the critical role of plasmalogens—a class of ether-phospholipids—as essential cellular components whose deficiency drives the clinical presentation of RCDP. Subsequently, Brites contributed significantly to the characterization of mouse models for two peroxisomal disorders, shedding light on the effects of impaired peroxisomal function (published in Human Molecular Genetics and Proceedings of the National Academy of Sciences, USA). The findings, published in Brain, revealed that plasmalogen deficiency profoundly affects nervous tissue pathology.

In 2009, Brites joined the Nerve Regeneration group at the Instituto de Biologia Molecular e Celular (IBMC), Porto, where he launched independent research exploring plasmalogens' roles in nervous tissue and the cellular mechanisms controlled by these phospholipids. His team discovered that ether-phospholipids regulate AKT activation, essential for Schwann cell development and myelination (Journal of Clinical Investigation) and protect myelin from oxidative damage (Free Radical Biology and Medicine). The translational research led to the first proposed replacement therapy for plasmalogen deficiencies (PLOS ONE) and identified alternative agents that bypass plasmalogen requirements (Journal of Clinical Investigation, Brain Pathology).

Since 2017, Brites is Principal Investigator and group leader of the Neurolipid Biology group at IBMC and i3S, focusing on the roles of (phospho)lipids in cellular function, the lipid-driven pathophysiology of rare diseases, and the development of effective therapeutic strategies. Brites also serves as Scientific Coordinator of the Histology and Electron Microscopy Sciences platform and leads the CRISPR-Cas9 mutant mouse generation unit at the i3S Animal Facility.

Abstract

Ataxia with giant axonopathy in Acbd5-deficient mice halted by adeno-associated virus gene therapy

Mouse models have proven invaluable for studying rare human diseases, providing insights into pathogenesis, disease mechanisms and treatment approaches. The Acyl-CoA binding domain containing 5 (Acbd5) gene plays a crucial role in the metabolism of very long chain fatty acids (VLCFA) by facilitating their peroxisomal β-oxidation. Mutations in ACBD5 lead to VLCFA accumulation, manifesting in patients as retinal dystrophy, ataxia, psychomotor delay, and a severe form of leukodystrophy. To investigate the pathological mechanisms underlying ACBD5 deficiency, we generated and characterized an Acbd5 Gly357* mutant mouse model using CRISPR/Cas9 technology. These Gly357* mice faithfully recapitulated several key aspects of the human disorder, including shortened lifespan, impaired locomotion and reflexes, photoreceptor degeneration, and central nervous system demyelination. Ataxic symptoms in the Gly357* mice were associated with a notable loss of cerebellar Purkinje cells and the presence of giant axons, indicating widespread axonopathy. Lipidomic analyses revealed substantial disruptions in lipid composition due to VLCFA accumulation, while proteomic studies and functional assays in VLCFA-exposed neurons indicated cytoskeletal instability, reduced actin dynamics, and excessive neuronal filopodia formation. Importantly, an adeno-associated virus (AAV)-mediated gene therapy approach alleviated gait abnormalities, reduced giant axonopathy, and improved both myelination and astrocyte reactivity. This model thus provides a valuable platform for understanding the neuropathological consequences of VLCFA accumulation and evaluating potential therapeutic strategies. Our findings underscore the significance of the Gly357* mouse model for studying VLCFA-related disorders and its utility in preclinical therapeutic assessments.

Malte Spielmann

Malte Spielmann is the director and chair of Human Genetics at the University of Lübeck and a research group leader at the Max Planck Institute for Molecular Genetics in Berlin. The main focus of his work is to understand the role of non-coding mutations and structural variants as the cause of human disease and their influence on the 3D architecture of the genome. He has extensive experience with the clinical application of NGS technologies and the analysis of genome data. Recently, the lab has also pioneered the development and application of single cell technologies to study human disease. Dr. Spielmann was a Heisenberg fellow of the German research council (DFG) and is a member of the DFG Cluster of Excellence Precision Medicine in Chronic Inflammation. He also serves on the program committees of the German and European Society of Humane Genetics. He has published more than 120 articles in peer-review journals with an h-index of 40 and over 12000 citations.

Abstract

Single cell human genetics: understanding human disease one cell at a time

Over the last decade, single-cell sequencing has transformed many fields. It has enabled the unbiased molecular phenotyping of even whole organisms with unprecedented cellular resolution. In the field of human genetics, where the phenotypic consequences of genetic and epigenetic alterations are of central concern, this transformative technology promises to functionally annotate every region in the human genome and all possible variants within them at a massive scale. In my talk I will we describe the current status of the field of single-cell sequencing and its role for human genetics, including how the technology works as well as how it is being applied to characterize and monitor diseases, to develop human cell atlases, and to annotate the genome.

Manuel R. Teixeira

› Director of the Department of Laboratory Genetics of IPO Porto;

› Coordinator of the Cancer Genetics Group of IPO Porto Research Center;

› Guest Full Professor (Medical Genetics and Cancer Genetics), School of Medicine and Biomedical Sciences (ICBAS), University of Porto;

› Representant of ERN GENTURIS at Porto Comprehensive Cancer Center.

Luzia Garrido

Registered Nurse at the Medical Genetic Service & Breast Centre of Unidade Local de Saúde São João;

Specialist in Community Nursing;

EBMG (European Board of Medical Genetics) Registered Genetic Counsellor;

Member of the ERN-GENTURIS Team at the Unidade Local de Saúde São João;

Researcher at the "Expression Regulation in Cancer Group", I3S – Institute for Research and Innovation in Health at the University of Porto.

Dedicated to monitoring and guiding patients and families with Hereditary Cancer syndromes in the context of the Oncogenetics Consultation at Unidade Local de Saúde São João since 2010 and collaborates in works and research projects in the area of hereditary cancer, some published and of which she is co-author, notably in the context of SCGDH (Hereditary Diffuse Gastric Cancer Syndrome). Author of a research work presented and awarded by SPGH (in 2017) and ESHG (European Society of Human Genetics) (in 2018). Member of SPGH (Portuguese Society of Human Genetics), ASPIC (Portuguese Association for Cancer Research), EARC (European Association for Cancer Research), SPOncologia (Portuguese Society of Oncology) and APPAcGen (Portuguese Association of Counseling Professionals Genetic).

Fátima Vaz

- Director, Medical Oncology Service Instituto Português Oncologia, Lisboa- IPOLFG,EPE, Lisbon Portugal
- Coordinator, Molecular Group Breast, Ovarian, Prostate (IPOLFG,EPE)
- President, Ethics Committee IPOLFG,EPE
- Representative of ERN GENTURIS at Instituto Português Oncologia, Lisboa - IPOLFG,EPE, Lisbon, Portugal

Carla Pereira

Head of the Planning and Quality Improvement Division of the Health Quality Department of the Directorate-General for Health, Portugal

Master in Health Services Management from the University Institute of Lisbon, Portugal

PhD in Public Health, Health Policy and Management and Administration, Universidade Nova de Lisboa, Portugal

Technical and Scientific Coordinator in the Department of Health Quality of the Directorate-General for Health of the following actions:

a) coordination of the Inter-ministerial Commission of the National Strategy for Rare Disease (EDIR) 2015-2020

b) monitoring international work for the development of the European platform for Rare Diseases

c) management of the Rare Disease Person Card (CPDR)

d) coordinator of the Orphanet National Team

e) representation of the Ministry of Health and DGS in international working groups, namely European Joint Program on Rare Diseases – EJP RD, Policy Board, EJP Mirror group, Board of Member States in the European Reference Network, European Joint action for Integration Rare Diseases.

ORAL PRESENTATIONS I

Basic research

TRNA MODIFICATIONS ARE POTENTIAL ALZHEIMER'S DISEASE BIOMARKERS AND THERAPEUTIC TARGETS THAT CAN BE REPROGRAMMED THROUGH TRNA MODIFYING ENZYME EXPRESSION MANIPULATION TO RECOVER CELLULAR FITNESS

Marisa Pereira[1], Júlia Lacerda[1], Laura Fernandes[1], Stephany Francisco[1], Miguel Moutinho[3], Ana R. Soares[1]

1Department of Medical Sciences, iBiMED, University of Aveiro, Portugal; 3 Stark Neurosciences Research Institute, Indiana University School of Medicine, USA.

Ana R. Soares

Introduction

tRNA modifications, collectively known as the tRNA epitranscriptome, play a key role in translation. Impairments in tRNA modifications occur in several neurological disorders and are collectively known as modopathies. However, little is known about their impact in the context of Alzheimer's disease (AD). We have recently shown that tRNA modifications and corresponding tRNA modifying enzymes are altered in AD. Here we aim to understand the molecular mechanisms underlying these alterations and explore the potential of tRNA modification modulation as a therapeutic approach in AD.

Methods

We performed a combination of tRNA epitranscriptomics, small non-coding RNA-Seq, and molecular biology experiments including western blotting, immunoprecipitation and protein aggregation assays in AD cellular models.

Results

We found that the expression of several tRNA modifying enzymes and the levels of tRNA modifications are impaired in AD and the expression of specific tRNA modifying enzymes negatively correlates with the amyloid plaque burden. Additionally, we found that accumulation of beta-amyloid aggregates is the trigger for tRNA modifying enzyme expression disruption and for tRNA epitranscriptome alterations in AD cellular models, and that microRNAs and tRNA derived fragments (tRFs) are also dysregulated in line with epitranscriptome alterations, indicating that additional layers of regulation are associated with epitranscriptome modulation. Manipulation of the expression of two specific tRNA modifying enzymes that were found disrupted alleviated the ER stress and inhibited the accumulation of toxic beta-amyloid aggregates.

Conclusions

Our data suggests that tRNA modifications, miRNAs and tRFs can constitute promising AD biomarkers and that correcting tRNA epitranscriptome deficiencies triggered by alterations of tRNA modifying enzymes may represent a valid therapeutic strategy to recover translation efficiency and proteostasis in AD. We are now confirming these findings in mouse models of the disease, as well as in patient derived iPSCs.

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GENOME-WIDE CRISPR SCREEN IDENTIFIES RAB14 AS A NEW TARGET FOR GASTRIC CANCER TREATMENT

Ana Catarina Nunes[1,2,], Carla Oliveira[1,3,4], João M. Fernandes Neto[1,5]*

1 i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto. 2 ICBAS School of Medicine and Biomedical Sciences, Universidade do Porto. 3 IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto. 4Faculdade de Medicina, Universidade do Porto. 5 Division of Molecular Carcinogenesis, The Netherlands Cancer Institute, The Netherlands, Amesterdam. * anan@i3s.up.pt

Ana Catarina Nunes

In Gastric Cancer (GC) around 20% of the patients have HER2 overexpression. HER2-positive GC patients can undergo treatment with HER2 inhibitors, such as trastuzumab. However, due to the high heterogeneity of these tumours only 47% of patients benefit from trastuzumab, and a majority of initial responders eventually develop resistance.

This project aims to uncover the biology behind the lack of efficacy of anti-HER2 therapies in HER2-positive GC. We hypothesize that there might be genes whose loss may enhance (or hinder) the efficacy of anti-HER2 therapy. To test this hypothesis, we have performed a genome-wide CRISPR screen. This project's main goal is to validate the top hits from the screen. We did this genetically for RIC8A and RAB14 and pharmacologically for RIC8A and PTEN. We used two cell lines as models: NCI-N87 - to mimic the patients that respond to the treatment and MKN74 - to mimic the patients that have primary resistance to the treatment and do not respond.

Based on our findings, we have successfully validated the candidate gene RAB14 in two-dimensional cell cultures, demonstrating its synergy with HER2 inhibition. Interestingly, loss of RAB14 was synergistic with HER2 inhibition not only in the HER2-positive NCI-N87 cell line but also in the HER2-negative MKN74 cell line. Inhibition of RIC8A has shown promising results in the pharmacological validation but the genetic validation has been more challenging and additional studies in both cell lines are still ongoing. Similarly, inhibition of PTEN showed encouraging results in the pharmacological validation regarding its antagonistic effect with HER2 inhibition.

In summary, we have identified three potential biomarkers (PTEN, RAB14 and RIC8A) whose expression could be used to predict response to anti-HER2 therapy in HER2-positive GC and we have identified 2 new targets (RAB14 and RIC8A) for combination therapy. Importantly, loss of RAB14 is also synergistic in HER2-negative GC tumours, which represent 80% of GC patients and which currently do not have any targeted treatment options.

RUNS OF HOMOZYGOSITY: BIOINFORMATIC APPROACHES FOR DIAGNOSTIC PURPOSES AND POPULATION ANALYSIS USING A SAMPLE OF 12,167 EXOMES

Susana Valente[1,2], Mariana Ribeiro[1,2], Jennifer Schnur[3], Filipe Alves[1], Nuno Moniz[3], Jorge Sequeiros[1,4], João Parente Freixo[1], Paulo Silva[1], Jorge Oliveira[1,4*]*

1. Centro de Genética Preditiva e Preventiva (CGPP), Instituto de Biologia Molecular e Celular (IBMC), Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal. 2. Departamento de Ciências Médicas, Universidade de Aveiro, Aveiro, Portugal 3. University of Notre Dame, Indiana, United States of America 4. ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.. *- equally contributing

Susana Valente

Introduction

Runs of homozygosity (ROH) are continuous homozygous regions across the genome, often linked to consanguinity, as their size and frequency depend upon the degree of shared parental ancestry. Homozygosity mapping (HM) is a valuable technique for identifying genes associated with autosomal recessive diseases, and it is based on the presence of ROH. Whole-exome sequencing (WES) improves HM by allowing the simultaneous detection of ROH and disease-causing variants.

Methodology

To automate personalized multigene panel creation, based on WES data and ROH, we developed a bioinformatic solution integrating two algorithms: ROHMMCLI (hidden Markov model) and HomozygosityMapper (sliding-window), with the option to incorporate Human Phenotype Ontology (HPO) terms, and implemented it in a Django Web application. Additionally, we performed an extensive analysis of ROH using 12167 WES samples, producing the first ROH profiling of the Portuguese population. Clustering models were also applied to predict consanguinity from ROH features and assist diagnostic algorithms.

Results

Following the successful implementation of the new strategy for multigene panel creation, previously undiagnosed cases from WES-based genetic testing were reanalyzed. In two siblings with epilepsy, myoclonus and dystonia this strategy pinpointed the CSTB gene as the cause of this disease. Based on the population distribution from the 2021 Census, the initial dataset was reduced to 3941 WES samples distributed per municipality to establish a representative sample and measure genome-wide autozygosity from ROH (F_ROH). Portalegre, Viseu, Bragança, Madeira and Vila Real districts had the highest F_ROH scores. Through multidimensional scaling representations, the count and sum of ROH provided the bulk of the predictive power for consanguinity classification, with additional features the test F1 score was 0.96.

Discussion

This study marks significant progress in ROH analysis leading to new bioinformatics tools development and providing unprecedented population-level data for Portugal. Future research will enhance diagnostic capabilities by exploring gene discovery using ROH analysis in genomic data.

A 5′ SPLICE-SITE MUTATION CAUSING MUCOLIPIDOSIS TYPE III CAN BE EFFICIENTLY RESCUED BY U1 SNRNA-BASED THERAPY

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Liliana Matos

Introduction

A significant number of splicing mutations have been identified in Lysosomal Storage Disorders (LSDs). Mucolipidosis III (ML III) is a LSD caused by GlcNAc-1-phosphotransferase deficiency, which impairs the trafficking of lysosomal hydrolases. 10% of the genetic defects in ML III are splicing mutations, and around 45% affect 5′ splice-sites (ss) thus constituting a good target for mutation specific therapies. The use of engineered U1 snRNA (either modified U1 snRNAs or exon-specific U1s - ExSpeU1s) has been applied as a potential therapeutic strategy to correct 5′ss defects. Here we used engineered U1 snRNAs to correct the GNPTAB exon 17 skipping caused by the 5′ss mutation (c.3335+6T>G) found in a ML III patient.

Methodology

First, we performed transfection of exon-trapping minigenes expressing exon 17 surrounded by a portion of introns - pGNPTAB_WT and pGNPTAB_+6, in HEK293T cells to analyze if they reproduce the WT and mutant splicing patterns. Then, to evaluate the potential of 2 modified U1′s, 3 ExSpeU1s and 2 modified U6′s to restore mRNA splicing, these vectors were cotransfected into HEK293T cells along with the mutant +6 minigene as well as electroporated in patient's fibroblasts. Then, cells were harvested, and RT-PCR analysis was performed.

Results

Both minigenes reproduced the control or ML III patient cDNA's splicing patterns, thus, different concentrations of the modified U1′s and ExSpeU1s were tested together with the mutant minigene. The cDNA analysis showed almost 100% of exon 17 inclusion when one of the ExSpeU1s, was overexpressed in HEK293T cells.

The combination of the 2 modified U6′s with the modified U1′s or the ExSpeU1s allowed exon 17 inclusion at some extent, but not as effectively as with the best ExSpeU1 alone.

The electroporation of the 2 modified U1′s and of the 3 ExSpeU1s was done successfully, but the cDNA analysis is still ongoing.

Conclusion

We have developed an RNA therapy based on engineered U1 snRNAs for a ML III 5′ss mutation. We showed that an ExSpeU1 (binding downstream of the mutated 5´ss) can restore proper exon 17 definition in vitro, opening the opportunity for a personalized therapeutic intervention.

THE IMPACT OF BALANCED CHROMOSOMAL REARRANGEMENTS IN PGT-SR

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Maria João Pinho

Introduction

Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR) increases the probability of successful implantation and pregnancy by selecting embryos without chromosomal imbalances. This study aimed to analyze the chromosomal constitution of embryos from PGT-SR couples with balanced structural rearrangements and assess also other chromosomal abnormalities due to interchromosomal effect.

Methodology

The study included 72 couples with Reciprocal translocations (RecT), 20 couples with Robertsonian translocations (RobT), and 11 couples with inversions (Inv), referred for PGT-SR. A total of 559 trophectoderm biopsies from 176 cycles were analyzed at the Genetics Unit of FMUP using array-Comparative Genomic Hybridization (March 2015 to January 2018) or Next Generation Sequencing (February 2018-).

Results

The global percentage of normal/balanced embryos was 26.8% for RecT, 41.1% for RobT, and 31.1% for Inv. The percentage of normal embryos according to maternal or paternal origin was 40.4% and 59.6% for RecT, 36.4% and 63.6% for RobT, and 57.1% and 42.9% for Inv.

Most unbalanced embryos had abnormalities derived from the parental rearrangement, 66.4% in RecT and 57.1% in RobT, whereas the opposite is observed in inversions, with 77.4% of the embryos showing abnormalities not related with the original rearrangement.

The maternal origin has a greater negative impact in the abnormalities related with the rearrangement: in RobT (70.8% maternal vs. 48.7% paternal) and in Inv (29.2% maternal vs. 0% paternal). Abnormalities in chromosomes not related with the rearrangement were 33.6% for RecT, 42.9% for RobT, and 77.4% for Inv.

Discussion

The percentage of normal/balanced embryos varies with the rearrangement type, with RobT yielding more normal embryos. The parental origin affects the outcome: paternal rearrangements give rise to more unbalanced embryos in RecT and RobT, and maternal rearrangement in Inv. The most notable finding is the interchromosomal effect with a high percentage of abnormalities in chromosomes not related with the rearrangement, highlighting the importance of comprehensive chromosomal analysis.

ORAL PRESENTATIONS II

Clinical research

CO-OCCURRING MUTATIONS IDENTIFY PROGNOSTIC SUBGROUPS OF MICROSATELLITE STABLE COLORECTAL CANCER

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Luís Nunes

Introduction

Co-occurring mutations in pairs of genes can pinpoint clinically relevant subgroups of cancer. Most colorectal cancers (CRCs) are microsatellite stable (MSS) and have few frequent mutations. Large patient cohorts and broad genomic coverage are needed for comprehensive co-mutation profiling.

Methodology

Co-mutations were identified in a population-based Swedish cohort analyzed by whole-genome sequencing (n = 819 stage I-IV MSS CRCs)1. Prognostic value was further evaluated in a publicly available dataset of clinically sequenced metastatic CRCs (MSK-IMPACT; n = 934 MSS)2. Multivariable Cox proportional hazards analyses with clinicopathological parameters were performed for locoregional (stage I-III) and metastatic (stage IV and recurrent) cancers separately.

Results

Prevalent co-mutations (frequency above 5%) were detected in 23 gene pairs, 20 of which included APC, TP53, KRAS and/or PIK3CA. Several co-mutations involving APC were associated with good overall survival in locoregional CRC, including APC-TCF7L2 (multivariable HR: 0.49, 95% CI 0.27-0.89). This co-mutation was prognostic also in metastatic cancers (multivariable HR: 0.49 and 0.37, 95% CI: 0.24-0.98 and 0.17-0.82 in the Swedish and MSK cohorts, respectively). APC-SOX9 co-mutations were mutually exclusive with APC-TCF7L2, and the co-mutations combined had stronger prognostic associations than APC alone in both metastatic cohorts. BRAF p.V600E-RNF43 co-mutations were associated with poor overall and recurrence-free survival in locoregional CRC (multivariable HR: 4.13 and 3.2, 95% CI: 1.78-9.54 and 1.53-8.04, respectively).

Discussion

We report genome-wide profiling of co-occurring mutations in MSS CRCs, and suggest that co-mutations can improve the prognostic stratification compared to single mutations alone.

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CLINICAL CRITERIA FOR CDH1 GERMLINE TESTING ACROSS DIFFERENT WORLD REGIONS: SHALL WE KEEP THEM GLOBAL?

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José Garcia-Pelaez

Background/Objectives

CDH1 germline Pathogenic/Likely Pathogenic variants (PV/LPV) predispose for Diffuse Gastric and Lobular Breast Cancer syndrome (DGLBCS). Sensitive and specific clinical criteria for testing, ensure clinical management of the correct population at risk. Herein, we systematically analysed the frequency of gastric and breast cancer in CDH1-PV/LPV carrier families, and its relationship with current clinical criteria, across different continents.

Methods

We compiled retrospective clinical information from 878 CDH1-PV/LPV carrier families, including 207 from internal communications (32 institutions) and 671 from a systematic review of the literature (151 original articles). Variants were classified with CDH1 ACMG-AMP guidelines. Phenotypical presentation, fulfillment of clinical criteria and continent of origin were analysed.

Results

From 878 CDH1 germline PV/LPV carrier-families, 314 (35.8%) were European, 512 (58.3%) American, 26 (3.0%) Asian, 24 (2.7%) from Oceania and 2 (0.2%) African. 57% of all families (503/878) fulfilled current IGCLC criteria. In Europe, there was twice as much families fulfilling criteria as compared to America (78% vs 41.2%). European families fulfilling gastric cancer-related criteria correspond to 68% (215/314) while this number decreases to 39% in America (200/512). Regarding breast cancer (BC), in Europe 9.5% (30/314) of families fulfill lobular breast cancer (LBC)-related criteria compared to 2.1% (11/512) in America. When considering the recently proposed LBC-centered criteria (Garcia-Pelaez et al, 2023), 18% (57/314) European and 18.7% (96/512) American families fulfill those criteria. However, the LBC-expanded criteria proposed by Garcia-Pelaez et al, are still insufficient to accommodate 42% of American and 13% of European families.

Conclusion

This systematic collection of CDH1 PV/LPV carrier families across continents highlight the different perception of the importance of family history and confirmation of diffuse and lobular histology of gastric and breast cancers, when selecting patients for CDH1 genetic testing. Additionally, it strongly supports further broadening of clinical criteria for CDH1 testing and adaption to cancer predisposition and incidence in different world regions.

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CLINICAL EXOME CONCEPTUALIZATION: A BIOINFORMATICS FRAMEWORK FOR SEMI-AUTOMATED REVIEW OF GENE-PHENOTYPE ASSOCIATIONS

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Filipe Alves

Introduction

The advent of whole-exome sequencing (WES) in medical genetics enabled the development of multigene panels for various disease groups, which may be extended to the clinical exome (CE, all genes known to be associated with Mendelian diseases. Designing and updating such a panel, however, is time-consuming due to the large number of genes and multiple relevant clinical databases, warranting an efficient bioinformatic approach.

Methodology

We propose a semi-automatic algorithm to generate a list of disease-associated genes, primarily drawing from a dedicated in-house knowledge-database compiling information from external sources such as OMIM, Orphanet, HGMD, HGNC, MANE, NCBI, and SFARI. This list is then manually curated by a medical geneticist and a clinical laboratory geneticist, to produce the final set of genes for the CE.

Results

Starting from ~44,000 HGNC entries, 13,076 clinically relevant genes were selected for further analysis, 5,653 being directly included in the list; 23 additional genes, initially filtered-out by the algorithm, but having clinically relevant variants in our lab database, were included. The remaining 7,423 were classified as “approved”, “rejected”, and “manual curation required”, by considering each gene-disease pair and the number of disease-causing variants in HGMD. This resulted in 3,819 genes recommended for inclusion, 3,347 for removal, and 257 loci to be manually curated. After this, a final list containing 9,326 genes was obtained. These were categorized into 3 groups, according to WES-based horizontal coverage (HC): white (9,266 genes with HC>80%), grey (31 genes with 80%>HC>10%), and black-listed (26 genes with HC<10%).

Discussion

We envision that our algorithm can help streamline the process of generating CE gene-lists in a clinical context, allowing these panels to be frequently and efficiently updated. This will be particularly valuable for clinical labs, where ensuring accurate and up-to-date results is crucial in the rapidly evolving field of medical genetics.

CLINICAL IMPACT OF DYNAMIC CHANGES IN CFDNA DURING CHEMO/RADIATION IN GLIOBLASTOMA PATIENTS

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Domingos Roda

Introduction

Glioblastoma (GBM) is the most aggressive form of cancer affecting the nervous system. The aim of this study was to conduct a cytogenetic and epigenomic characterization of GBM tumor tissue samples and explore the potential of liquid biopsies for diagnosing and monitoring patients.

Methodology

A total of 173 plasma samples were collected from 65 GBM patients, before and during treatment, along with plasma samples from 10 controls. After isolating cfDNA, its concentration was measured, compared between patients and controls, and monitored throughout the patients' clinical course. Primary tumor cell cultures from GBM patients were also established, and the molecular profile of tumor samples was analyzed using aCGH, MS-MLPA, and karyotyping techniques. Additionally, commercial GBM cell lines were used for comparison and correlation with the molecular profiles of the patients' samples.

Results

Using this comprehensive approach, we were able to analyze the entire genome for copy number variations and identify the structural rearrangements underlying these genetic alterations. Frequent gains and losses were observed in chromosomes 1, 4, 5, 6, 7, 8, 9, 10, 12, 15, 16, 17, 20, 21, 22, and Y, where several genes involved in cell signaling pathways are mapped, whose dysregulation is associated with GBM development and progression. Trisomy of chromosome 7 and monosomy of chromosome 10 were identified as the most prevalent alterations, with EGFR, CDKN2A, PTEN, RB1, and NF1 being common genes altered in our cohort. The most common rearrangements are translocations, some in the centromere/near-centromeric regions. Plasma cfDNA levels revealed that GBM patients have higher baseline cfDNA levels compared to healthy controls. In some patients, a significant increase in plasma cfDNA levels was observed during follow-up, correlating with clinical events.

Discussion

Discussion: The quantitative and qualitative dynamics of cfDNA can be associated with imaging and clinical progression, facilitating differential diagnosis between true progression, pseudoprogression, and radionecrosis. The integration of cfDNA can be crucial for future therapeutic decisions in oncology.

IS MILD BONE FRAGILITY GENETIC? - GENETIC FINDINGS IN A COHORT OF PATIENTS WITH EARLY-ONSET FRACTURES AND/OR OSTEOPOROSIS AND NO EXTRASKELETAL SIGNS OF OSTEOGENESIS IMPERFECT

André M. Travessa [1-3], Patrícia Dias [1,3], Silvia Modamio-Høybjør [4,5], José Carlos Romeu [3,6], Ana Paula Barbosa [3,7,8], Ana Rita Cruz-Machado [3,6], Rita Barros [3,6], Sérgio B. Sousa [9,10], Karen E. Heath [4,5,11], Ana Berta Sousa [1-3]

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André M. Travessa

Introduction

Bone fragility results from a reduction in bone mass and density and increases the risk of fractures.

Except for monogenic disorders with significant bone fragility and/or extra-skeletal features, such as osteogenesis imperfecta (OI), bone fragility is usually considered a multifactorial disorder.

Objective

To clinically and molecularly evaluate a cohort of patients with mild bone fragility and no extraskeletal signs of osteogenesis imperfecta in order to identify monogenic causes and/or genetic risk factors for its development.

Methodology

Clinical evaluation of a cohort of 35 patients with mild bone fragility and absence of extraskeletal signs of OI who were referred to our Medical Genetics Department. Genetic analysis using a skeletal dysplasias NGS panel and MLPA for COL1A1 and COLA1A2. Written informed consent was provided.

Results

This cohort included 21 females and 14 males. Ten patients were children or adolescents and 15 were adults.

No relevant variants were detected in 22 cases (62.9%). A monogenic cause was identified in three patients (8.6%), namely a tandem duplication of exons 6 to 9 of the PLS3 gene, a complete deletion in mosaic state of the COL1A1 gene, and a frameshift variant in the LRP5 gene.

In the remaining cases (10/35, corresponding to 28.6%), variants that have been previously associated with increased risk of recurrent fractures and/or osteoporosis (including common polymorphisms) were found, namely in the LRP5, WNT1 and DKK1 genes. Five patients carried more than one such variants.

Conclusions

These results highlight that mild bone fragility with no extraskeletal signs of OI can be, albeit in rare cases, monogenic. The identification of such cases is relevant for genetic counselling.

In addition, knowledge about genetic risk factors contributing to mild bone fragility is increasing, and results so far suggest they play a role in a significant proportion of cases.

Nonetheless, known causes and risk factors for mild bone fragility still fail to explain the majority of cases, which means that other genetic and environmental factors should be involved.

ORAL PRESENTATIONS III

Clinical cohorts

CLINICAL UTILITY OF DNA METHYLATION EPISIGNATURES TESTING - A PORTUGUESE MULTICENTER COHORT STUDY

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Luís M Pires

Introduction

Methylation arrays are a high-throughput and robust technique used to assess unique DNA methylation patterns (episignatures). A growing number of rare disorders are found to be associated with discrete episignatures. This has been shown to contribute to fast and definite diagnoses in a broad range of clinical applications.

Methodology

Retrospective national study describing the first group of Portuguese patients in whom DNA methylation testing was performed in routine diagnostic setting (Jan 2023 – June 2024). Illumina Infinium methylation EPIC bead chip arrays were performed and methylation data processed using the clinically validated EpiSign assay. Testing was offered through national Medical Genetics Departments, who initially gathered to define clinical and selection criteria. Two main groups were included: 1 - subjects with variants of unknown clinical significance in genes with previously defined episignature and significant clinical overlap; 2 - subjects with no conclusive genetic findings and a clinical diagnosis consistent with a group of syndromes with previously described episignature.

Results

In our cohort, 44% of the total 48 cases included, had positive results for episignatures or imprinting alterations, with most having a high confidence level. Considering group 1, in 50% of cases the episignature was positive for the previously detected genetic variant, allowing its reclassification as likely pathogenic and achieving a definite diagnosis. In group 2, 41% patients had an episignature consistent with the clinical diagnosis, reinforcing it and leading to individual recommendations for further testing.

Discussion

Our study revealed a high proportion of positive results when comparing to other described cohorts. This probably reflects the careful clinical selection. A clinical-laboratory multidisciplinary approach was essential for the accuracy of the conclusions. Overall, in all cases, episignature testing was considered useful for clinical practice and we consider that both laboratory and medical genetics departments in Portugal are well prepared to continue its use in routine practice and enlarge its indications.

PERSONALIZED REGION-SPECIFIC LIFETIME CANCER RISK ESTIMATES FOR AN ANCESTRAL CDH1 VARIANT

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Rita Barbosa-Matos

Families with Hereditary Diffuse Gastric Cancer (HDGC) carry heritable CDH1 germline variants, presenting increased risk for Diffuse Gastric Cancer (DGC) and Lobular Breast cancer (LBC) development. Previous lifetime cancer risk estimates for CDH1 carriers have been overestimated and used to apply a "one-size-fits-all" approach for surveillance and risk-reduction. Understanding the probability of cancer development in individuals carrying pathogenic variants is key to establish preventive strategies and optimize disease management. We aimed to estimate CDH1-related cancer risk for an ancestral Portuguese variant using families from the same region, monitored at a single institution.

The genotype restricted likelihood (GRL) method was used to estimate penetrance in a cohort with high-quality clinical data of 285 individuals from nine HDGC families carrying an ancestral Northern Portuguese variant. We conducted various analyses on the cohort to obtain personalized lifetime risk estimates, calculating cumulative and relative risks for both cancers. A 'leave-one-out' strategy assessed potential bias from each family.

From 33.3% (95/285) CDH1 carriers, 9.1% (16/285) were diagnosed with Gastric Cancer (both sexes), 147 were females and 8.1% (12/147) presented Breast Cancer. In our analyses, DGC cumulative risk differed from 53.4% to 8.8%, and relative risks from 58.6-fold to 24.4-fold at 60 years old. While for LBC cumulative and relative risks differed from 21.1% to 8.8% and from 5.0-fold to 22.9-fold at 60 years old, respectively.

In the analyses with higher detail and granularity of clinical, genetic and epidemiological data, DGC and LBC cumulative risk were 8.8% at 60 years.

This is the largest lifetime risk estimation study on HDGC families sharing the same and most recurrent CDH1 variant in Europe, being the first to use region-specific cancer incidences. We disclose the lowest cancer risk for CDH1 variant carriers published to date, contributing to the development of more precise and personalized lifetime cancer risk for Portuguese HDGC families.

ORAL PRESENTATIONS IV

Clinical cases reports

EXPANDING THE PHENOTYPE ASSOCIATED WITH IRF2BPL GENE VARIANTS: A REPORT OF 4 NEW CASES

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Sara Pinho

Introduction

In 2018, heterozygous loss of function (LOF) variants in IRF2BPL gene were first associated with a severe Neurodevelopmental Disorder with Regression, Abnormal Movements, Loss of Speech and Seizures (NEDAMSS, OMIM #618088), presenting in the first decade of life. Since then, more than 30 patients have been described in the literature carrying pathogenic variants in this gene, with a wide range of symptom severity and age of onset.

Methodoloy

Retrospective analysis of clinical files from 4 unrelated patients with novel heterozygous LOF variants in IRF2BPL gene.

Results

3 patients exhibit a neurodevelopmental phenotype with mild developmental delay (DD)/intellectual disability (ID), without regression, unspecific facial dysmorphisms and no movement disorder. Additionally, one has refractory epilepsy, with 2 previous episodes of status epilepticus. The 4th patient is a 36-year-old male with progressive ataxia and spasticity, starting at 16. Brain MRI showed cerebellar atrophy. Molecular diagnosis was established through whole-exome sequencing or multi-gene panel. In each patient, a new heterozygous nonsense or frameshift variant was identified in the intronless IRF2BPL gene: p.Gln167* in the adult patient, p.Lys371Glnfs*52 in the child with DD and epilepsy, p.Ser433Valfs*14 and p.Gln618* in the subjects with mild DD/ID and dysmorphic features.

Discussion

Few genes are known to be involved in both neurodevelopmental and neurodegenerative conditions. Although most published cases with IRF2BPL LOF variants have NEDAMSS, recent reports also link this gene to late-onset dystonia and ataxia. Our cohort highlights a less severe phenotype, consisting of mild DD/ID without regression or epilepsy. At the time of diagnosis, these patients did not show any signs of movement abnormalities, but follow-up into adulthood is necessary to better delineate the natural history of the condition. No genotype-phenotype correlation has been proposed so far, but the milder phenotype seems to associate with variants farther down the transcript.

PRENATAL AND POSTNATAL PHENOTYPING AND MOLECULAR CHARACTERIZATION OF THREE CASES OF ZTTK SYNDROME: A POTENTIALLY RECOGNIZABLE CLINICAL ENTITY

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Catarina Macedo

Introduction

SON gene encodes a protein crucial for pre-mRNA splicing and cell cycle regulation. Pathogenic SON variants were first linked to the autosomal dominant Zhu-Tokita-Takenouchi-Kim (ZTTK) syndrome in 2016. To date, over 60 cases have been reported, predominantly with de novo loss-of-function variants. Clinical features are variable but consistently include developmental delay (DD)/ intellectual disability (ID), often accompanied by multisystem malformations, short stature, and dysmorphic features.

Methodology

Retrospective analysis of clinical files from two children (ages 12 and 5) and a termination of pregnancy for fetal anomalies (TOPFA) with molecularly confirmed ZTTK syndrome.

Results

The TOPFA presented microphthalmia, cleft lip/ palate, micrognathia, ventriculomegaly, and cerebellar hypoplasia. Prenatal findings in the living cases consisted of isolated urogenital anomalies (bilateral fetal pyelectasis, and unilateral renal cysts). DD/ID was mild in one child and severe in the other, but both children had short stature, low weight, and relative macrocephaly. All cases exhibited supra- and infratentorial brain malformations, and eye anomalies. Dysmorphic features included abnormal cranial shape (3/3), facial asymmetry (3/3), down-slanting palpebral fissures (2/3), midface hypoplasia (2/3), and low-set ears (2/3). Whole exome sequencing identified three novel heterozygous truncating SON variants: c.4776_4779del, p.(Ser1594LeufsTer28); c.5713_5716del, p.(Lys1905Glufs*100); and c.631delA, p.(Thr211fs)], confirmed to be de novo in two cases.

Discussion

Our findings support ZTTK syndrome as a distinct DD/ID syndrome characterized by poor growth; multisystem malformations, particularly involving the brain, urogenital tract, and eyes; and a distinctive gestalt. DD/ID severity is variable. Prenatal findings ranged from isolated to complex anomalies. Case 3 is the fourth report of cleft lip/ palate, further linking this malformation to deleterious SON variants.

CTCF-RELATED INTELLECTUAL DISABILITY – FOUR PATIENTS SUGGESTING A RECOGNIZABLE FACIAL GESTALT

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Ana Miguel Capela

Background

CTCF-related intellectual disability (MIM #615502) was first described in 2013 and manifests with variable degrees of intellectual disability (ID), microcephaly, and growth retardation. We report four patients from three families, diagnosed with CTCF-related ID.

Methods

Review of clinical files.

Results

Patient 1 is an 18 yo male. He presented with congenital microcephaly and feeding difficulties and later developed behavioral issues and developmental delays (DD). At 17 yo he presented with moderate ID (Global IQ of 62 on WISC-III scale), microcephaly, small ears, telecanthus, thin upper lip, retrognathia, and short webbed neck. WES detected a likely pathogenic variant in CTCF c.1485_1486dup p.(Lys496Ilefs*16), in heterozygosity. Parental studies presumed de novo origin.

Patient 2 is a 4 yo male. Both parents and older sister reported learning difficulties. He presented with language delay at 18 months and was diagnosed with autism spectrum disorder at 3 yo. Array-CGH showed a 15q11.2 microdeletion. He has cupped ears, hypertelorism and thin upper lip, which raised suspicion for a concomitant second diagnosis. WES identified an heterozygous likely pathogenic variant in CTCF c.1025G>A p.(Arg342His), inherited from his mother, who shares a similar facial gestalt.

Patient 3 is a 38 yo female, with short stature who reported persistent learning difficulties. She shares a facial gestalt with her son.

Patient 4 is a 14 yo male who presented with Pierre Robin sequence, cleft palate and DD. He had moderate ID (Global IQ of 54 on WISC-III scale), protruding ears, long face and small mouth with thin lips. WES-based ID gene panel detected a likely pathogenic variant c.1094A>G p.(Lys365Arg) in heterozygosity in CTCF and parental studies presumed de novo origin.

Conclusion

CTCF-related ID has been described with nonspecific dysmorphic features however, our four patients share similar facial features that point towards a recognizable gestalt. Patients 2 and 3 underline the need for further investigations when clinical features are more complex, especially in a dysmorphology setting, as well as the importance of parental assessment and counseling.

FRAXE INTELLECTUAL DEVELOPMENT DISORDER – WARNING FOR A FORGOTTEN DIAGNOSIS

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Isabel Serra Nunes

Introduction

Silencing of the AFF2 gene, as a result of a (CCG)n trinucleotide repeat expansion located in its 5' UTR, is one of the known causes of a rare X-linked intellectual disability disorder, associated with the fragile site FRAXE (FRAXE-ID, MIM #309548). This X-linked intellectual disability is characterized by a variable phenotype, including mild to moderate cognitive impairment, speech delay, hyperactivity and autistic behavior. Despite an estimated incidence of 1 in 25,000–100,000, FRAXE-ID remains underdiagnosed. A CCG triplet number of up to 30 repeats is considered normal, while individuals with FRAXE-ID typically have >200 repeats and hypermethylation (full mutation) within the expanded region.

Methodology

We conducted a retrospective analysis of clinical and laboratory data from three patients presenting with intellectual disability and associated features. Comprehensive genetic testing, including karyotyping, array comparative genomic hybridization (aCGH), and fragile X syndrome molecular testing, was performed.

Results

All three cases were unrelated young males with global developmental delay, mild to moderate intellectual disability and non-specific dysmorphisms. Initial genetic testing was unrevealing. However, routine internal quality control for FMR1 testing, which co-amplifies the AFF2 trinucleotide repeat, revealed absence of an AFF2 amplicon. Further characterization by Southern blotting and hybridization with sequence-specific probes revealed the presence of a full mutation in all three cases, establishing the diagnosis of FRAXE-ID. The mothers of each patient carried a pre-mutated allele. Notably, one patient presented size mosaicism, having both premutation and full mutation repeat expansions.

Discussion

The AFF2 gene is a critical component of the RNA interference machinery, and its disruption leads to the pathogenesis of FRAXE. The large size of the expanded (CCG)n repeat often precludes its detection by next-generation sequencing (NGS) or aCGH, necessitating targeted testing approaches. Awareness and testing for FRAXE is essential for accurate diagnosis and management.

A NEVER-ENDING RACE: A MULTILOCUS CAUSE OF AN INBORN ERROR OF IMMUNITY ASSOCIATED WITH COMPLEX NEUROLOGICAL DISEASE

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Nuno Teixeira

Introduction

it is expected that 5% of patients with rare conditions present with at least two distinct genetic disorders. In many cases, clinicians and laboratory diagnosticians tend to look for a single cause that explains patient phenotypes, even if they are extremely complex and multisystemic. Here, we present a patient with four simultaneous monogenic conditions: two inborn errors of immunity, and two neurological/neurodevelopmental conditions.

Methodology

we retrospectively reviewed clinical/laboratorial information of a single patient after exome sequencing (ES).

Results

37-year old male with a complex, multisystem phenotype. Since his childhood, he presents with mild intellectual disability, non-specific dysmorphic features, treatment-resistant epilepsy, mild cortical atrophy, and long standing neutropenia, thrombocytopenia and hepatosplenomegaly. At 29 years of age, he was diagnosed with systemic lupus erythematosus, hemophagocytic syndrome (acute fever and pancytopenia) and pulmonary hypertension. At this time he had lymphopenia and mild hypogammaglobulinemia. At 35 years of age he presented with newly-onset chorea and dysarthric speech, and acanthocytes were observed in peripheral blood. ES revealed several (likely) pathogenic variants: heterozygous c.497C>T in SIK1, associated with developmental and epileptic encephalopathy; heterozygous c.300dup in ELANE, associated with cyclic neutropenia; apparently homozygous c.16C>T in PRKCD, causal for autoimmune lymphoproliferative syndrome due to PKCδ deficiency; two variants in VPS13A (c.4114+1G>A and c.5574+1G>A), underlying choreoacanthocytosis. For the latter variants, conventional phasing was not possible as the patient was adopted and had no known biological relatives. We opted for long-range PCR in the region encompassing the VPS13A variants, currently ongoing, to confirm compound heterozygosity.

Discussion

this work illustrates the complexity of cases that may present with more than one genetic cause for the clinical phenotypes, typically underlying multisystem disease. It also illustrates that a complex phenotype from a single organ system may be justified by more than one monogenic condition.

A CHALLENGING DETECTION OF A NOVEL SPLICING DELETION IN THE DMD GENE IN A PATIENT WITH DUCHENNE MUSCULAR DYSTROPHY

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Lígia Lameiras

Introduction

Duchenne Muscular Dystrophy (DMD) is a severe X-linked genetic disorder caused by mutations in the DMD gene. Genetic testing typically involves Multiplex Ligation-dependent Probe Amplification (MLPA) and Next-Generation Sequencing (NGS) to detect deletions, duplications, or point mutations. However, certain variants may escape detection through these standard approaches. We report a challenging case involving a 7-year-old male patient with a clinical presentation highly suggestive of DMD, where initial testing failed to identify a causative mutation.

Methodology

Initial testing using MLPA to detect large deletions/duplications in the DMD gene was negative. This was followed by a muscular dystrophy NGS panel, which also returned negative results. Due to the persistent clinical suspicion of DMD, single gene sequencing of DMD gene was requested. NGS revealed incomplete coverage in an intronic region near exon 7, raising suspicion of a possible deletion. To confirm this, quantitative PCR (qPCR) was performed, identifying a deletion in the intronic region. Sanger sequencing was then employed to further characterize the deletion and assess its impact on the splicing site of exon 7.

Results

qPCR confirmed the presence of a deletion within the intronic region adjacent to exon 7 but did not resolve its full extent. Subsequent Sanger sequencing identified the deletion NM_004006.3: c.649+4_649+27199del, which included part of the splice donor site of exon 7. This deletion disrupts the splicing process, providing the molecular basis for the patient's DMD diagnosis.

Discussion

This case illustrates the diagnostic challenges presented by intronic variants affecting splicing regions, which may not be detected by routine MLPA or NGS panels. The use of complementary techniques, such as qPCR and Sanger sequencing, was critical in confirming and characterizing the deletion. These findings emphasize the need for a multi-step approach in cases where clinical suspicion is strong but initial genetic results are inconclusive, particularly when splicing regions are involved.

BEYOND THE AFFECTED: THE IMPORTANCE OF TESTING NON-AFFECTED INDEX INDIVIDUALS IN CANCER HIGH-RISK FAMILIES

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Portuguese Institute of Oncology from Lisbon

Sofia Fragoso

Introduction

The Hereditary Breast/Ovarian and Prostate Cancer (HBOPC) group at the Portuguese Institute of Oncology in Lisbon, established in 2000, focuses on cancer prevention, early diagnosis and treatment options. While BRCA1/2 were the main genes associated with HBOPC, since 2014 we expanded our approach to include broader gene panels. This change recognizes that many families with a significant history of cancer test negative for BRCA1/2. Whenever possible, genetic testing is initiated in affected individuals, however testing in non-affected index individuals (NAIs) is increasing.

Methods

review of genetic and clinical data of NAIs tested from November 2000 to May 2024.

Results

Of the 8394 tested indexes, 340 were NAIs by the time the genetic test was requested. The average age for NAI testing was 43.8 years and mostly females (309; 91%). The main reasons for NAI testing were: absence of affected relatives due to death, test refusal or inability to contact. Twenty-two had a pathogenic/likely pathogenic (P/LP) variant, mainly in high-penetrance genes (17/22; 77%) such as BRCA1 (3), BRCA2 (9), PALB2 (4) and TP53 (1). The remaining 23% (5/22) of P/LP variants were found in moderate-penetrance genes (CHEK2 (2), BRIP1 (1) and BARD1 (2)). Regarding high penetrance genes, median follow up was 9 years [0.37 to 22.49] with most individuals (88%) adhering to active surveillance and risk management. Only 1 patient (pt) was lost to follow-up. During follow-up 3 pts were diagnosed with cancer: 1 man with refractory high-grade B cell lymphoma (PALB2) and 2 women diagnosed with early breast cancer, a ductal carcinoma in situ (PALB2) and an invasive carcinoma (BRCA2). All BRCA1/2 and a PALB2 NAI underwent preventive surgeries (bilateral mastectomy and bilateral anexectomy), including the 2 breast cancer pts who underwent risk reduction mastectomy alongside therapeutic mastectomy.

Conclusion

Given the number of P/LP variants identified in high-penetrance genes among NAIs, our work highlights the importance of genetic testing in high-risk families, even when affected members are unavailable. Long-term follow-up further highlights the benefit of risk-reducing surgeries.

POSTERS

SESSION I

BREAST CANCER GERMLINE GENETIC TESTING CONTRIBUTION TO PREVENTION AND SURVIVAL

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Introduction

The detection of pathogenic/likely pathogenic variants (PV/LPV) may influence treatment in BC patients, and impact surveillance and risk-reducing strategies in their relatives (1). To streamline the process of germline genetic testing (GGT), our center started applying adapted Mainstream Cancer Genetics (MCG) criteria in 2020 (2).

The aim of this study is to characterize the cohort clinically, histologically and genetically, and assess the success of this program.

Methods

All patients who underwent BC GGT between 01/03/2020 and 29/02/2024 were included in this study. All data were anonymized, and descriptive statistics were used to describe the cohort.

Results

This study encompasses 535 individuals, 506 (94.6%) of which are affected. The detection rate (DT) in affected individuals was slightly higher than in healthy ones (8.1% vs 6.9%). Forty-four PV/LPV were detected in 43 individuals and 20 PV/LPV (45.5%) were detected in high penetrance genes. The most frequently mutated gene was BRCA2 (25%), and the Portuguese founder mutation c.156_157insAlu was the most common PV (9.09%). One of the 111 detected variants of uncertain significance (VUS) was meanwhile reclassified as a LPV.

Discussion

This study shows a lower DT than what is usually described in the literature. Nevertheless, the DT was higher than or close to 10% for most MCG criteria.

The amount of VUS described in literature varies greatly, but was high in our cohort (3). One VUS was reclassified, highlighting the importance of reevaluation.

GT is also important to PV/LPV carriers' relatives, as cascade testing may influence surveillance protocols and risk reducing strategies, lowering morbimortality in hereditary BC (4). More retrospective/long-term prospective studies are necessary to explore this hypothesis.

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CYSTIC FIBROSIS MODULATOR DRUGS INHIBIT MIGRATION OF COLORECTAL CANCER CELLS

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CANCER GENETICS

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, driven by complex genetic, epigenetic, and microenvironmental factors. Recent findings implicate the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel in CRC progression, as CFTR levels are notably reduced in sporadic CRCs, particularly in advanced and metastatic tumors, correlating with poorer patient outcomes. Additionally, cystic fibrosis (CF) patients, who carry CFTR mutations, have a 6-fold increased risk of early-onset CRC. Given recent advances in small-molecule modulators that restore CFTR function in CF patients, this study explored the potential of repositioning these modulators to address CFTR downregulation in sporadic CRC.

Using a panel of CRC cell lines, we investigated whether CFTR modulators can increase CFTR functional expression in cells with various genetic backgrounds and whether such improvements could reduce their oncogenic properties. Our data show that treatment with the CFTR folding correctors VX-661 and VX-445 led to a significant, approximately three-fold increase in CFTR abundance in CRC cells expressing reduced but detectable levels of the channel. Additionally, these treatments significantly reduced the migratory behavior of Caco-2 and DLD-1 cells, particularly when combined with the CFTR potentiator VX-770.

Our findings suggest that CFTR modulators may hinder the oncogenic properties of CRC cells. Further in vivo studies are necessary to fully assess their potential benefits for repositioning as a CRC treatment.

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LOSS OF BCL6 TRANSCRIPTIONAL REPRESSOR IMPACTS MIGRATION BUT NOT VIABILITY IN MCF7 BREAST CANCER CELLS

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CANCER GENETICS

Breast cancer (BC) incidence has risen over the past two decades, now being the most prevalent cancer worldwide and the second leading cause of cancer-related deaths. Despite advancements in BC treatment, challenges like acquired resistance, recurrence, and metastasis persist. BCL6, a transcriptional repressor, acts as an oncogene in BC, being overexpressed in about half of primary tumors of all subtypes and associated with disease progression and poor patient prognosis. This underscores the need to better understand BCL6 role in BC development.

This study used RNA interference to explore the impact of BCL6 depletion on the oncogenic progression of MCF7 cells, a low-tumorigenic estrogen receptor-positive cell line. While BCL6 is known to regulate mammary cell proliferation and differentiation, its depletion did not affect MCF7 cell proliferation or viability but significantly reduced their individual and collective migratory properties. RNA microarray analysis identified a set of genes upregulated following BCL6 depletion, including S100A7, previously reported to inhibit MCF7 cell migration and invasion by reducing MMP9 secretion. However, our findings showed that S100A7 downregulation alone did not affect MCF7 migration. Moreover, simultaneous depletion of BCL6 and S100A7 failed to restore MCF7 cell migratory behavior.

Our results suggest that increased expression of BCL6 is linked to increased cell migration but independent on S100A7 upregulation. Further studies are required to clarify the role of BCL6 in BC, including disease progression.

Keywords: Breast cancer, BCL6, MCF7, S100A7, migration.

IDENTIFYING ALTERNATIVE SPLICING ISOFORMS IN BREAST CANCER CELL LINES THROUGH NANOPORE SEQUENCING

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Introduction

Breast cancer (BC) is one of the most prevalent diseases worldwide, significantly impacting millions of people every year, especially women. This study explores the potential of direct RNA nanopore sequencing protocols from Oxford Nanopore Technologies (ONT) for transcriptome profiling in BC-related cell lines — non-tumoral MCF-10A cells, luminal A MCF-7 cells, and triple-negative MDA-MB-231 cells.

Methodology

Cell lines were cultured, RNA was extracted and sequenced, and the resulting data was processed through a bioinformatic pipeline called FLAIR, which allowed us to assess alternative splicing (AS) events in several genes that are relevant in tumorigenesis. Due to the extensive amount of information obtained, genes for AS assessment were selected due to their involvement in mRNA splicing and translation initiation pathways.

Results

Several AS events were observed in RNA polymerase II subunit genes — POLR2G, POLR2H — and the splicing factor gene SRSF2. Additionally, EIF3A and EIF3E genes, which encode subunits of the eukaryotic initiation factor 3 (eIF3) complex, essential for translation, revealed several novel isoforms and distinct patterns across cell lines.

Discussion

The discovery of novel isoforms in the previously mentioned genes suggests potential functional implications in BC development and progression. For instance, intron retention in POLR2G and exon skipping in POLR2H could impair co-transcriptional splicing, affecting the cell's ability to regulate RNA processing effectively. Nonsense mediated decay (NMD)-sensitive isoforms found in SRSF2 might play a role in its autoregulation and consequent impact in cell survival. The identification of a novel EIF3A isoform, dominant in MDA-MB-231, could indicate alterations that contribute to BC progression. Similarly, through protein modeling via AlphaFold of several EIF3E isoforms, the likelihood of distinct functional properties became apparent. Our results can be used as a first step for several biologically relevant findings in the field of cancer transcriptomics, hopefully contributing to forthcoming clinical management, diagnosis and therapeutical approaches for BC patients.

DNA METHYLATION PROFILING OF BREAST CANCER CELL LINES THROUGH NANOPORE SEQUENCING

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Introduction and Aim

DNA methylation is a key epigenetic mechanism that regulates gene expression without altering DNA sequence. Several genes involved in key processes such as cell growth, apoptosis, and DNA repair might be disrupted by this mechanism leading to tumorigenesis. Breast cancer (BC) is the most common cancer diagnosed in women. In BC cells, DNA methylation can become dysregulated, altering normal gene expression and leading to BC development. Thus, this study focused on the sequencing of different breast cell lines, by Nanopore sequencing, to profile and compare DNA methylation patterns of DNA repair and drug resistance genes.

Methodology

Non-tumoral MCF-10A cells, Luminal A MCF-7 cells and TNBC MDA-MB-231 cells were cultured for subsequent DNA extraction, purification and Nanopore sequencing. DNA methylation was detected by Dorado basecaller and visualized using Integrative Genomics Viewer (IGV).

Results

Our analysis revealed that the CpG islands of all examined DNA repair-related genes were demethylated in MCF-10A and MCF-7 but methylated in MDA-MB-231. Moreover, ABCC1, ABCG2, and SLC29A2 showed similar patterns to those observed in DNA repair genes, while other drug resistance genes showed more diverse methylation patterns, with ABCB1 and SLC47A1 methylated in both MCF-10A and MDA-MB-231, and ABCB4, SLC22A1 and SLC15A1 methylated in all three cell lines.

Discussion

These findings suggest that DNA repair genes may be silenced only in MDA-MB-231, potentially resulting in the accumulation of unrepaired DNA, contributing to the more aggressive tumor phenotype. Regarding the drug resistance genes analyzed, their methylation patterns suggest that these genes might influence drug resistance in MCF-7 cells, but not in MDA-MB-231 cells, which contradicts existing literature. This could be due to 66% of the promoter regions being methylated in this cell line, indicating a general increase in DNA methylation that may not reflect the true TNBC environment. Additionally, the potential expression of ABCC1, ABCG2, and SLC29A2 in MCF-10A could be linked to their role in maintaining cellular homeostasis and protecting normal cells from endogenous substances.

A COMPREHENSIVE STUDY ON INORGANIC NANOPARTICLES TESTED IN PRECLINICAL CANCER MODELS USING MACHINE LEARNING

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CANCER GENETICS

Introduction

Nanomaterials have been extensively used as an effective carrier of nucleic acids, broadening the therapeutic and biomedical applications of these molecules. Specifically, inorganic nanoparticles have shown promise in preclinical cancer therapy due to their unique optical/magnetic properties. However, challenges in optimizing their design for therapeutic purposes persist. Decision support systems could address these challenges, but their use is limited by small or non-existent datasets and without clear reporting and research standards.

Methodology

This study intends to compile a comprehensive database of inorganic nanoparticles from 745 preclinical cancer studies, using machine learning models. This identifies key design patterns on physicochemical and preclinical data that impact treatment efficacy, measured by tumor reduction.

Results

Our findings highlighted that gene therapies (4.5%) are still substantially underexplored in inorganic nanoparticles. Recent studies employed a synergistic combination of therapies (e.g., chemotherapy and gene therapy or chemotherapy and magnetic hyperthermia) to increase the success rate of heterogenous tumors. We concluded that nanoparticle shape (i.e., rod-shape nanoparticles) and therapy type (i.e., multi-therapy strategies) are key determinants for in vivo efficacy.

Discussion: The openly accessible dataset serves as a valuable resource for machine learning applications in nanomedicine. This research study reinforces the importance of standardizing nanoparticle reporting to enhance the safety and efficacy of nanoparticle-based drug delivery systems, which will improve patient outcomes in clinical settings.

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MULTIGENE PANEL TESTING FOR HEREDITARY CANCER PREDISPOSITION IN PORTUGUESE BREAST AND OVARIAN CANCER PATIENTS. EXPERIENCE OF SYNLAB GENETICA MEDICA PORTO

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Introduction

Next-generation sequencing (NGS) allows for a more cost-effective and quickly detection of pathogenic and likely pathogenic variants. Identification of these variants in breast and ovarian cancer allows for early detection and surgical decision, prediction of the response to PARP inhibitors, and allows genetic counselling.

Methodology

To determine the pathogenic /likely pathogenic (P/LP) variants prevalence in hereditary and sporadic breast-ovarian cancer cases, we performed a retrospective review of 2920 samples received at Synlab Porto between January 2022 and June 2024. All samples were tested by a multigene panel, that included the following genes: ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, CTNNA1, EPCAM, MLH1, MSH2, MSH6, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11 and TP53. NGS was performed on an Illumina Platform, using a custom Twist kit (Twist Bioscience).

Results

Our study revealed 238 P/LP variants in all analysed genes (238/2920=8,2%). However, we noticed that in the subgroup with breast or ovarian cancer and family history of HBOC (n=612) the prevalence is higher (9,4%), while in the subgroup with no family history (n=2158) the prevalence is 7,6%. In the subgroup of healthy individuals with family history of breast and ovarian cancer (n=150) only 5,3% had P/LP variants. Within the P/LP variants, 41% of the variants were identified in BRCA1 and BRCA2. The non-BRCA1/2 genes variants represent 59% of the total P/LP variants detected. Additionally, were detected 5 cases with two P/LP variants (3 cases with no family history and 2 cases with family history).

Discussion

This retrospective review supports the relevance of using multigene panels as a standard approach for breast and ovarian cancer patients. It was also important to highlight the genetic heterogeneity found in these patients, with the majority of P/LP variants detected in non-BRCA1/2 genes.

WHOLE EXOME SEQUENCING IN 135 PATIENTS WITH INTELLECTUAL DISABILITY/AUTISTIC SPECTRUM – EXPERIENCE OF SYNLAB GENETICA MÉDICA PORTO

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

Intellectual disability (ID) and autism spectrum disorder (ASD) belong to neurodevelopmental disorders and occur in ~1% of the general population. ID affects the individual, its family, and the community, therefore being an important socio-economic problem in healthcare. Approximately 30–50% of ID cases are due to a genetic cause, and 25–30% of these are due to genetic variants in single genes.

Autism spectrum disorder (ASD) generally becomes apparent after the first year of life and it has been reported in an increasing number (2.2–2.7%) of children. Whole Exome Sequencing (WES) has increasingly become used to rapidly identify variants associated with these two entities that often co-exist. In this study, we retrospectively studied 135 patients referred to our center for genetic evaluation.

Methodology

WES analysis was performed in all cases. Bioinformatic analysis was focused on our panel for ID and ASD and mainly on the targeted phenotype. In all cases the bioinformatic analysis included copy number variants (CNVs) analysis.

Results

We identified 40 pathogenic/ likely pathogenic variants with a detection rate of 29,4% (41/136). Pathogenic/likely pathogenic mutations in the following genes among other, were found in our cohort of patients: ARMC9, BRAF, BFPT CASK, CHD8, CHD2, CNKSR2, DCHS1, EPB41L1, EHMT1, FGFR3, GNB1, GRIN1, HUWE1, H1-4, IFH1, KANSL1, KINDINS220, KMT2C, KMT2D, LINS1, MID2, MN1, NAA15, NARS1, SETD5, SLC13A5, SHMT2, TAOK1, TNRC6B and TUBA1A. In 69 cases (50%) variants of uncertain clinical significance (VUS) were also detected.

Discussion

Due to disease heterogeneity, identifying the aetiology of ID and ASD remains challenging. Our diagnosis rate is high and many of these patients were undiagnosed for a long time. The diagnosis rate may improve after segregation studies of VUS in families. This study highlighted the importance of WES in the diagnosis of ID/ASD. Our study strongly supports the value of this technology, especially WES, in solo or trios (proband-parent), as an effective first-choice diagnostic tool.

THE PORTUGUESE VARIOME FROM OVER 12,000 EXOMES: STREAMLINING CLINICAL DIAGNOSTICS AND TRANSLATIONAL RESEARCH

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Introduction

As sequencing technology evolved, genetic testing has progressed from small multigene panels to more comprehensive approaches such as whole-exome (WES) and whole-genome sequencing (WGS). This shift led to a surge in identified variants per case and increasing interpretation challenges. International initiatives such as the 1000 Genomes Project, the Exome Aggregation Consortium (ExAC), and the Genome Aggregation Database (gnomAD) have played crucial roles by publicly releasing variant frequency information. However, to date, no genetic variant database capturing the unique genetic composition of the Portuguese population has been implemented.

Methodology

By leveraging a dataset of 12,167 patient exomes processed at our centre, we created a representative reference set of the Portuguese population (by municipality) resampling our cohort to 3,972 individuals. From this subset, we focused on the variants in the list of 81 secondary-finding genes elaborated by the American College of Medical Genetics and Genomics (ACMG), known for their direct medical actionability.

Results

The ExAC open-source codebase was adapted to be run locally and display our dataset, showing the allele counts and frequencies of variants in the ACMG actionable genes, grouped by municipality, as well as the visualization of the distribution of each variant in interactive maps. We identified 643 (230 distinct) pathogenic or likely-pathogenic variants across 49 ACMG genes, with an overall frequency of 8.1%, approximately 5.4% of the Portuguese population. We further explored the geographic frequency distribution of hemochromatosis-causing NM_000410.4(HFE):c.845G>A p.(Cys282Tyr) variant, revealing a distinctive north-south gradient consistent with previous literature reports.

Discussion

This work establishes a reference set of common, rare, and ultra-rare genetic variants in a representative sample of the Portuguese population. It provides a comprehensive view of genetic variations in the 81 actionable ACMG genes, which can ultimately offer valuable information for patients, practitioners, and stakeholders involved in genomic medicine, aiming towards improving healthcare in Portugal.

DEEP MOLECULAR INVESTIGATION TO ELUCIDATE HIDDEN TTC37 VARIANTS AND CORRECTLY DIAGNOSE TRICHOHEPATOENTERIC SYNDROME

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

Trichohepatoenteric syndrome (THES) is a rare and under-diagnosed genetic disorder with autosomal recessive transmission, associated with pathogenic variants in either TTC37 or SKIV2L. THES is a congenital disease presenting with early-onset severe intractable diarrhea in born infants, characterized by facial dysmorphism, growth failure, imune disorders and early onset of severe liver cirrhosis in some patients. To date, <140 cases in the world and no epidemiological data are available, however, the prevalence can be estimated at around 1/1.000.000. The prevalence does not seem especially different among ethnic groups. There is a trend to a higher degree of consanguinity. Here, we present the case of a young female patient with suspicion of a mild clinic of THES.

Methodology

Whole genome and exome sequencing (WGS/WES) followed by Sanger validation detected a new stop codon variant (p.Gln1411*) in heterozygosis in the TTC37, inherited from the mother. In order to detect the second missing pathogenic variant, total RNA from white blood cells was evaluated and showed aberrant transcripts in the father's allele in the same gene.

Results

qPCR showed a high expression of TTC37 resulting from the normal allele in the white blood cells of the patient, a finding that could be linked to the mild clinic of the patient. Interestingly, a higher expression of the TTC37 protein was detected in the plasma of the patient, suggesting an increased secretion of the protein.

Discussion

Our results show that although the first pathogenic variant was relatively easy to detect, the second one was challenging, evidencing the fundamental value of seeking the correct diagnosis of THES by carrying out different molecular techniques to reach the final and certain diagnosis.

FUNCTIONAL ANALYSES OF COL4 GENETIC VARIANTS

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Introduction

The advances in variant detection achieved in the latest years generate great amount of data that require additional criteria to improve the classification of these variants and implicit clinical decisions. Functional studies of variants that possibly interfere with splicing can answer questions, like: Does a rare variant classified as VUS based on population studies and proximity to a splice-site creates an alternatively spliced mRNA? What about a deep intronic one? If it does, is the normal transcript also produced? Is the ratio mutated/normal transcript enough to cause disease?

The collagen IV network is assembled by interactions between triple-helical α3α4α5(IV) protomers, being each monomer respectively encoded by the autosomal COL4A3 and COL4A4 genes, and the X-linked COL4A5 gene. Mutations in any of these genes are in the origin a pathogenically related but genetically and clinically heterogeneous group of COL4 nephropathies.

Methods

We performed functional studies in mRNAs of the three COL4A3/4/5 genes by extracting total RNA from fibroblasts cultivated from 7 patients' skin biopsies. We tested 7 variants with minor allele frequency (MAF) <0.01 and without functional criteria described. Three of these variants are classified as pathogenic, based on their MAF and canonical splice-site location. The other 4 are classified as VUS, likely benign/benign.

Results

Although COL4A3/4/5 expression is not ubiquitous, the functional studies in cultivated fibroblasts were successfully performed and met the computational analysis classification of the 3 variants previously classified as pathogenic, by allowing the detection of one or more alternatively spliced transcripts, either in the presence or absence of the normal transcript. As for the other 4 no aberrant transcript were found.

Discussion

Our results contributed important information for interpreting the clinical relevance of these variants in patients with clinical suspicion of COL4 nephropathy. This protocol can be offered as a complement to diagnosis for any variant suspected to interfere with the assembling of the precursor mRNA in collectible tissues where these genes, and other, are expressed.

IMPLEMENTATION OF A WHOLE-EXOME SEQUENCING PIPELINE FOR UNDIAGNOSED FAMILIES

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

Whole-exome sequencing (WES) has become the gold standard for diagnosing genetic diseases, though its high cost has restricted its widespread use. This study marks the first step in establishing WES at the iBiMED-Aveiro Genome Medicine laboratory, with the goal of enhancing both research and diagnostic capabilities to provide a more comprehensive understanding of genetic disorders.

Methodology

Both manual and automated workflows were implemented to support scalability for large-scale sequencing. To validate the pipeline, five commercial DNA samples with known variants from donor lymphoblastoid cells were analyzed. A range of bioinformatics tools were used for variant prioritization and analysis, and their efficiency was compared throughout the validation process.

Results

The methodology was successfully implemented and validated in the Genome Medicine laboratory, enabling precise detection of variants in all five commercial samples.

Discussion

These results open opportunities to translate the herein implemented methodology for analyzing undiagnosed families, while also paving the way for future projects in the field of human genetics. In this study, WES has already been performed on 11 previously undiagnosed families, with variant analysis currently ongoing, aimed at further validating the clinical relevance of the methodology.

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INCORPORATING GENETIC HETEROGENEITY TO OUTPERFORM CURRENT DEEP LEARNING METHODS FOR SCHIZOPHRENIA PHENOTYPE PREDICTION

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Introduction

SCZ is a complex disease with unclear onset, posing challenges for accurate prediction and understanding of its genetic basis. Current models often fail to capture this complexity, highlighting the need for improved approaches. This study presents a novel deep learning (DL) architecture for classifying schizophrenia (SCZ) phenotype using genotype data.

Methodology

The proposed model, GenoSCZ, integrates an Attention-Based Neural Network (ABNN) with an Encoder framework and a parallel fully-connected branch to capture complementary gene effects. It was trained on a large-scale case-control sequencing dataset (4,969 SCZ cases, 6,245 controls) using a ten-fold leave-one-out-inspired approach to address genetic heterogeneity. Performance was compared against leading models, including GenNet, DiseaseCapsule, and DeepCOMBI.

Results

GenoSCZ outperformed existing models, showing higher sensitivity, accuracy, and AUC. The final architecture significantly improved on the stand-alone ABNN, demonstrating the benefits of addressing disease heterogeneity when designing knowledge-based DL architectures. Pathway enrichment analyses revealed associations with biological processes like neuroplasticity, microtubule function, and protein localization, all relevant to SCZ pathophysiology.

Discussion

GenoSCZ performance on case-control classification and ability to capture a broader spectrum of genetic contributors underscore its potential in advancing our understanding of SCZ. By identifying relevant pathways, this model provides valuable additional insights into the genetic architecture of SCZ, opening up more opportunities for personalised approaches in future research.

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FROM NATURALLY-OCCURRING STEM CELLS TO ENGINEERED IPSCS – THE COUNTLESS POSSIBILITIES OF STEMNESS TO MODEL RARE GENETIC DISEASES IN VITRO

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DISEASE MODELS

Introduction

Over the last decades, stem cells have become invaluable tools for the study of genetic diseases as they allow for the generation of functional cells of all the major organs. This is particularly relevant for rare, monogenic and multisystemic disorders, such as the ones we work on, at INSA's R&D group on Lysosomal Storage Diseases (LSDs), and a major breakthrough to allow for rational in vitro drug screening.

Here we present our results on the establishment of two different types of stem cell models: the state-of-the art iPSCs, and the novel, virtually unexplored multipotent stem cells from the dental pulp.

Methodology

Briefly, we established primary cultures of control- and Mucopolysaccharidoses (MPS)-derived stem cells from exfoliated deciduous teeth (SHEDs) and characterized them at molecular, biochemical and pathophysiological levels using numerous techniques, including qRT-PCR, multi-lineage differentiation, enzyme activity measurement, glycosaminoglycan (GAG) quantification and LAMP1 staining. We have also briefly tackled the secondary accumulation of lysolipids using a biomarker panel approach. Concomitantly, we reprogrammed two independent MPS fibroblast cell line into iPSCs, subjecting them to the same analyses.

Results

Overall, we managed to establish and fully characterize 6 independent MPS cell lines (4 SHEDs and 2 iPSCs), confirming not only their stemness but also their capacity to either display or replicate visible and measurable phenotypes.

Discussion

Here we present an overview of our findings, while discussing the pros and cons of each stem cell model. We will also share our tricks and tips on how to establish stem cell cultures from the dental pulp for modelling rare monogenic diseases in a time- and cost-effective way.

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EVALUATION OF POR GENE VARIANTS IN THE HEPARG MODEL: IMPLICATIONS FOR DRUG METABOLISM

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Introduction

Cytochrome P450 (CYP) oxidoreductase (CPR) is essential for sustaining microsomal CYP activities, crucial for metabolizing therapeutic drugs and other xenobiotics. Genetic variations in the POR gene, which encodes CPR, can alter its function via changes in protein stability, cofactor interaction, NADPH or substrate binding, and modulation of CYP interactions. The hepatic HepaRG cell line has emerged as a valuable in vitro model for pharmacokinetic and drug metabolism studies, offering advantages over traditional hepatic cell lines and primary human hepatocytes. This study aimed to evaluate POR gene variants in this human hepatocyte model.

Methodology

Thirteen primer pairs were designed and synthesized to amplify the POR gene of HepaRG, covering all exons and adjacent intronic regions. PCR conditions were optimized for each primer pair, followed by Sanger sequencing. Fragment analysis was performed using dedicated nucleotide sequencing software.

Results

Two heterozygous mutations were identified in HepaRG cells when compared to the Reference genome (NG_008930.1), leading to the amino acid variants V85M and A503V in the CPR protein. A comprehensive review of available databases and literature -including in vivo, in vitro, and in silico data- was conducted to assess the potential impact of these variants on activity and pathogenicity.

Discussion

The POR gene of the HepaRG cell model was previously sequenced, identifying the POR37 genotype (A503V, V631I) (Heintze et al., 2021, PMID: 33441761). Our findings differ, with V85M, a rare allele (< 0.0005%), identified alongside A503V. While A503V is known to influence CPR activity, V85M remains uncharacterized. However, in silico analyses suggested V85M to be highly disruptive, significantly impacting protein structure (Velazquez et al., 2023, PMID: 38136599). As total CPR activity depends on both alleles, a non-functional POR allele in HepaRG cells could limit their utility in pharmacokinetic studies. Ongoing work by our group includes whole-genome sequencing of HepaRG to fully characterize their genetic profile, along with activity assays to assess the functional impact of these alleles.

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INSIGHTS FROM A SMALL, YET CLINICALLY DIVERSE COHORT OF SEVEN PATIENTS WITH 18Q- SYNDROME

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DYSMORPHOLOGY AND NEURODEVELOPMENT DISORDERS

Introduction

18q- syndrome is a chromosomal disorder caused by deletions on the long arm of chromosome 18 and has significant phenotypic heterogeneity. This study includes seven patients and aims to better understand the correlation between deletion size and clinical manifestations.

Methodology

Retrospective analysis of clinical files from seven patients of varying ages diagnosed with 18q deletions ranging from 5.62 Mb to 17.85 Mb. Chromosomal analysis was performed using array comparative genomic hybridization (PerkinElmer, 180K). Fluorescence in situ hybridization and conventional karyotyping were performed to confirm deletions and determine inheritance patterns.

Results

Patients with smaller deletions (5.62 Mb to 8.58 Mb) presented milder phenotypes, while larger deletions (10.38 Mb to 17.85 Mb) associated with more severe phenotypes. Five were terminal deletions (18q21-qter) and two were proximal deletions (18q11.2-q21.2). Two cases were de novo, one was familial, and four are pending confirmation. Intellectual disability was present in all cases, and was mild to moderate in 2 and moderate to severe in 5. Six patients had speech delay, ranging from mild language impairment to limited speech. Hypotonia was a feature in 4 cases, with marked hypotonia seen in larger deletions. Microcephaly was present in 2 patients, and craniofacial dysmorphisms in 5, including hypertelorism, ptosis, broad nasal bridge, and maxillary hypoplasia. Short stature was observed in 4 cases, and musculoskeletal abnormalities, namely bilateral clubfoot and scoliosis, in 2. Two patients had bilateral moderate to severe sensorineural hearing loss.

Discussion and Conclusion

Our findings align with previously published cases, reinforcing the correlation between deletion size and phenotypic severity. Nonetheless, there is also significant variability between patients with similar deletion sizes, making it difficult to predict clinical outcomes on the basis of size alone, and further supporting the need to increase understanding on the genotype-phenotype correlation of 18q- syndrome to improve patient outcomes.

CHARACTERIZATION OF GENETIC DEFECTS IN A COHORT OF PATIENTS WITH SUSPICION OF IMPRINTING DISORDERS

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Introduction

Imprinting disorders are a group of rare diseases with overlapping phenotypes for which clinical diagnosis is often challenging and requires testing of a broad spectrum of molecular variants of imprinted loci. Imprinting disruption in certain chromosomal regions is responsible for specific syndromic phenotypes including Angelman syndrome (AS), Prader-Willi syndrome (PWS), Beckwith-Wiedemann syndrome (BWS), Silver Russell syndrome (SRS), Multilocus imprinting disorders (MLID) and Temple syndrome (TS). Recently, other phenotypes have been associated with imprinting disturbance.

Methodology

Retrospective analysis of a group of 20 patients with one of the following: clinically suspected SRS/BWS or PWS/AS or TS (with imprinting defects excluded, respectively, in chromosomes 7, 11 or, 15, or 14); or patients with a very severe neurodevelopment phenotype, extense genetic investigation but without diagnosis. In order to extend molecular investigation, we applied a multi-locus methylation-specific multiplex ligation-dependent probe amplification assay that targets eleven different imprinted loci in seven chromosomal regions: 6q24, 7p12, 7q32, 11p15, 14q32, 15q11 and 20q13.

Results

Analysis of the imprinted loci revealed a heterozygous deletion and hypermethylation in one of the two probes for the KCNQ1OT1 gene (KvDMR/IC2; KCNQ1 opposite strand/antisense transcript 1), located at 11p15.5, in a female patient. Previous studies had included Angelman syndrome MS-MLPA, whole exome sequencing (WES), array-CGH and karyotype. Sanger sequencing confirmed the deletion in KCNQ1OT1. This variant is not present in population databases and has not been reported in the literature. Further studies are now required, including co-segregation analysis in the family.

Discussion

Imprinting disorders can have overlapping phenotypes so clinical diagnosis is often challenging. Thus, multi-locus imprinting MS-MLPA can be an interesting instrument to use in patients with imprinting disorders suspicion or a severe phenotype with no diagnosis allowing to search for multiple imprinting locus in only one procedement. Results interpretation require further research.

CYTOGENETIC ANALYSIS OF A PATIENT: UNCOVERING THE IMPLICATIONS OF A SUPERNUMERARY MARKER CHROMOSOME 2

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DYSMORPHOLOGY AND NEURODEVELOPMENT DISORDERS

Introduction

Small supernumerary marker chromosomes (sSMCs) are rare chromosomal abnormalities, found in about 0.044% of the population. While 70% of carriers are asymptomatic, 30% exhibit clinical symptoms, such as intellectual disability and infertility. Phenotypic variability may result from mosaicism, gene content and chromosomal origin. sSMCs derived from chromosome 2 are very rare and often linked to dysmorphic and psychiatric features. sSMCs involving 2q11.1 region and MAL gene have not been previously described in the reviewed literature. The authors report a patient presenting with dysmorphic features and cholestasis, with a sSMC derived from a chromosome 2.

Methodology

A 37 yo male was referred for cytogenetic studies due to cholestasis and a webbed neck. Karyotype was performed on metaphase mitotic cells obtained from peripheral blood. Molecular cytogenetic techniques (Fluorescence "In Situ" Hybridization (FISH) using a centromeric probe (D2Z2, Cytocell) and Multiplex Ligation-Dependent Probe Amplification (MLPA) panel P181-C1) were used to identify the marker's origin.

Results

The karyotype obtained was 47,XY,+mar[46]/46,XY[12]. MLPA panel identified the duplication of MAL gene located at 2q11.1 and FISH confirmed the mosaicism of a marker of chromosome 2.

Discussion

This rare case highlights the occurrence of a supernumerary marker of chromosome 2. Despite extensive literature review, no directly link between 2q11.1 duplication to cholestasis was found. However, some studies associate the MAL gene with increased liver cholesterol synthesis.

In line with our case, dysmorphisms linked to the duplication of the 2q11.1-q12.1 region, such as webbed neck, have also been documented. Parental genetic studies are recommended to determine whether the chromosomal alteration is "de novo" or inherited, complemented with a referral for genetics counseling. Given the lack of evidence for causality, it is recommended that etiological investigations continue regarding cholestasis in this patient. While a potential link between MAL gene duplication and cholestasis exists, further research and additional evidence are needed to confirm this relationship.

EP300-RELATED DISORDERS: A PHENOTIPIC AND MOLECULAR CASE SERIES

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Introduction

Rubistein-Taybi syndrome (RTS) is an autosomal dominant disorder caused by mutations in CREBBP and EP300, characterised by neurodevelopmental delay (NDD)/intellectual disability (ID), short stature and distinctive facial features [1].

Although EP300 variants cause a phenotype resembling CREBBP-related RTS, they seem to correlate with softer dysmorphisms and milder ID or even normal intellect [2], as well as intrauterine growth restriction (IGR) and microcephaly [3]. Variants in exons 30 and 31 of both genes produce a phenotype very distinct from RTS, the Menke-Hennekam syndrome (MHS) [4]. Thus, the phenotypic spectrum of EP300-related syndromes remains unclear. In this context, a clinical and molecular characterization of patients with EP300 mutations is presented.

Methodology

Retrospective analysis of medical records of patients from our centre (2017-2023) with relevant EP300 variants and phenotypes overlapping with RTS or MHS.

Results

The authors identified 8 cases with EP300 variants: 4 males/4 females, mean age of 14.4. 6/8 were index cases and had whole exome sequencing (WES) performed in the context of syndromic NDD/ID investigation. All patients had dysmorphisms, 2/8 had microcephaly, 2/8 had IGR and 2/8 had short stature. 6 variants were identified: 3 missense [uncertain significance (VUS)], 1 duplication (VUS), 1 frameshift (likely pathogenic) and 1 nonsense (pathogenic). Of the 6 variants, 1 was de novo, 2 were inherited from an affected progenitor, and 3 could not be segregated with both parents. 4 patients had variants in exon 31, but only 2 patients (index and relative) had MHS features. Five of 6 were novel variants.

Discussion

This study adds to the evidence of clinical variability and genetic heterogeneity of EP300-related disorders. We hope these results contribute to a detailed clinical and molecular spectrum of these patients and to a better understanding of genotype-phenotype correlations.

References

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FOUR CORNERS OF FATE: OUTCOMES IN BALANCED RECIPROCAL TRANSLOCATION

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DYSMORPHOLOGY AND NEURODEVELOPMENT DISORDERS

Introduction

Balanced reciprocal translocations involve the breakage and exchange of fragments between two non-homologous chromosomes, leading to various meiosis segregation patterns. These patterns can yield normal, balanced, unbalanced, or non-viable gametes.

Here we describe a family with four different offspring outcomes of a familial balanced reciprocal translocation.

Methodology

A boy with microcephaly and global developmental delay (DD) was referred for SNP array analysis, which detected a gain and loss in 16p13.3 and 10q26.13q26.3 regions, respectively, compatible with an unbalanced rearrangement between chromosomes 10 and 16 [der(10)t(10;16)(q26.13;p13.3)].

Follow-up FISH test of the parents confirmed that those alterations were associated with an abnormal chromosome derivative resulting from a maternal balanced translocation involving chromosomes 10 and 16.

SNP array did not detect any alterations on two other children.

The mother's siblings, two affected sisters (with short stature and DD) and one unaffected brother, were subsequently studied by SNP array.

Results

The SNP array analyses of the mother's siblings revealed three distinct outcomes from the segregation of a familial balanced translocation between the long arm of chromosome 10 and the short arm of chromosome 16.

One sister had a terminal heterozygous loss in 16p13.3 and a terminal gain (3 copies) from 10q26.13q26.3, compatible with the presence of the derivative of chromosome 16: der(16)t(10;16)(q26.13;p13.3).

The other sister had a terminal heterozygous loss in 10q26.13q26.3 and a terminal gain (3 copies) from 16p13.3, compatible with the presence of the derivative of chromosome 10: der(10)t(10;16)(q26.13;p13.3).

No alterations were detected in the brother.

Discussion

The outcomes for offspring of a balanced reciprocal translocation carrier can range from normal to various kinds of genetic anomalies, emphasizing the complexity and variability of inheritance patterns associated with translocation events. Genetic counseling is always recommended for individuals with balanced translocations who are planning to have children, as this can help to assess risks and options for family planning.

RETT SYNDROME – CASE REPORT

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Introduction

A 6-year-old girl with clinical features of epileptic encephalopathy and dysmorphic traits, including thin lips and low-set ears, symptoms consistent with autism spectrum disorder, characterized by poor eye contact, stereotyped hand movements, giggling, and delayed motor development, raising suspicion of Rett syndrome. Rett syndrome (MIM312750) is a neurodevelopmental disorder predominantly affecting females (PMID: 12615169). This clinical case aimed to identify a genetic cause for the patient's phenotype.

Methodology

A sequential approach to diagnostic testing was utilized, encompassing NGS panels for Rett and related syndromes and epileptic encephalopathy, whole exome sequencing with CNV analysis, aCGH (Cytoscan 750K), and MLPA for the MECP2 gene.

Results

Except for MLPA, all tests were negative. A reduction in signal was noted in one probe binding to exon 4, prompting Sanger sequencing. The variant c.1159_1346del p.(Ser387Alafs*49) was detected, resulting in the loss of 62 aa and a frameshift affecting 37 terminal aa. This variant has not been reported in literature or population databases, but similar rearrangements have been noted in Rett syndrome patients (PMID: 31206249).

Discussion

Sanger analysis suggests a mosaic pattern. Based on the available information, the variant was classified as uncertain clinical significance (ACMG criteria: PVS1 moderate and PM2 supporting). Additional tissue analysis was proposed to clarify the alteration's extent. It should be noted that, due to its size, it was not possible to detect this variant using NGS method previously carried out. Although the outcome of this patient's diagnosis remains inconclusive, it should be noted that somatic mosaicism has already been described in both female and male patients with Rett's phenotype (PMID: 11768391, 11022934). This case highlights the importance of a multimethodological approach to clinical diagnosis.

RAS/MAPK/ERK AND PI3K/AKT/MTOR RELATED OVERGROWTH SYNDROMES

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DYSMORPHOLOGY AND NEURODEVELOPMENT DISORDERS

Overgrowth syndromes (OS) are characterized by abnormal, excessive growth of a tissue or organ, leading to anomalies that can either be focal or diffuse, manifesting in a generalized or segmental manner. While Beckwith-Wiedemann syndrome is the most common overgrowth syndromes, it is essential to consider other possible aetiologies during evaluation. A subset of OS is caused by germline or somatic pathogenic variants in the RAS/MAPK/ERK and/or PI3K/AKT/mTOR signalling pathways often resulting in regional overgrowth and/or vascular malformation (VM).

We conducted a retrospective study, aimed to identify individuals (IDs) with abnormal growth features and/or VM and a confirmed genetic diagnosis. We were able to identify 13IDs: PTEN (n=6; 47%); PIK3CA (n=4, 31%); RASA1 (n=2, 15%); EPHB4 (n=1, 7%).

Here we reported 4 (IDs) with distinct confirmed molecular diagnosis associated with OS, to describe the phenotypic spectrum and raise awareness to other causative aetiologies:

ID1:2-year-old (yo) female with hypertrophy of the right lower limb, umbilical hernia, capillary and arteriovenous malformations (CM-AVM). Genetic diagnosis of EPHB4 related disorder. This variant was inhered from her mother, who also presented with VM and family history of brain stroke at young age;

ID2:10yo male with macrocephaly, hypertrophy of the lower limbs, intramuscular haemangiomas, pigmented macules and a genetic diagnosis of PTEN hamartoma tumour syndrome. After the diagnosis, treatment with rapamycin was implemented with a favourable outcome;

ID3:3yo female with obesity, VM and family history of brain stroke at young age. A genetic diagnosis of CM-AVM syndrome due to a RASA1 variant was made. Her father was confirmed to carry the pathogenic variant and his brain MRI revealed an AVM;

ID4:2yo female with right-sided hypertrophy, AVM, hemimegalencephaly. A genetic diagnosis of PIK3CA-related overgrowth spectrum was established at skin biopsy.

OS can be attributed to diverse etiopathogenetic mechanisms, despite presenting with similar clinical phenotypes, as evidenced by these 4IDs. A confirmed genetic diagnosis is of the most importance, allowing personalized care.

EXPERIENCES OF STIGMATIZATION AND ITS IMPACTS IN FAMILIES WITH HEREDITARY DISEASES: AN EXPLORATORY MIXED-METHOD STUDY

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Introduction

Stigma is defined as the perception of an undesirable attribute that leads to discrimination against individuals and groups. Stigmatisation is often triggered due to visible physical or cognitive differences. Although the literature consistently highlights the (fear of) stigmatisation as a significant concern among individuals living with hereditary conditions, no studies in Portugal have specifically provided evidence on this issue. This study aims to address this gap by examining the experiences and impact of stigma on individuals and families affected by hereditary diseases in Portugal.

Methodology

After receiving ethics approval, a total of 216 participants, including affected individuals, asymptomatic carriers and family members from families with a range of hereditary conditions, were recruited through patient support associations. Participants completed an online questionnaire via Limesurvey. Data were analysed through Exploratory Factor Analysis (EFA), median comparison tests, and thematic analysis.

Results

Of the participants, 78.7% were women, 55.6% had a university degree, and 20.4% were aged between 42 and 47 years. Findings indicate that stigma impacts individuals across various domains, including social interactions, institutional settings, the workplace, and healthcare. EFA identified a bi-factorial model of stigma, comprising Stigma Experiences and Perceived Support subscales, and the overall scale demonstrated high internal consistency (α = .879). Women and younger participants reported higher levels of stigma. Religiosity and humor emerged as key coping strategies.

Discussion

This study is the first in Portugal to assess stigma among individuals living with hereditary conditions. Our findings contributed to validating a measurement instrument, identified sociodemographic variations, and examined the psychosocial dimensions of stigma among affected patients. These findings highlight the need for comprehensive strategies to address and mitigate stigma, improve support systems, and enhance the well-being and healthcare experiences of individuals and families impacted by hereditary diseases.

The authors declare no conflicts of interest

HOW IS FAMILY COMMUNICATION DISCUSSED IN GENETIC COUNSELLING? AN OBSERVATIONAL, PROSPECTIVE, MULTICENTRE QUALITATIVE STUDY

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GENETIC COUNSELLING / SERVICES / EDUCATION

Cascade testing and genetic counselling (GC) largely depend on family-mediated contact with at-risk relatives. While guidelines recommend encouraging and supporting the disclosure of genetic information to family in GC, they are often vague. Research on how this is addressed in GC is limited and mostly retrospective; few studies observe GC practices. This study aimed at exploring how family communication is framed and discussed upon GC.

Since January 2024, we have been conducting a prospective qualitative study at three public hospitals in Portugal as part of the FCT-funded DECIDE project. Data collection is via observation and audio-recording of pre-and post-test GC consultations, which are transcribed and analysed using inductive content analysis.

We observed and recorded 59 consultations, performed by 11 clinical geneticists, with 55 adults referred for pre-symptomatic testing for autosomal dominant conditions with high penetrance, such as late-onset neurological diseases, and hereditary cardiac and cancer syndromes. Our analysis suggests that genetic healthcare professionals (GHPs) addressed family communication through three frames; i) a cautionary framing, drawing on the Portuguese legal and regulatory framework regarding privacy and confidentiality of genetic information; ii) a procedural framing, in which health-related implications for relatives are discussed focusing on providing information; or iii) an actionable framing, where implications for at-risk relatives are mentioned and family disclosure encouraged. Consultands also addressed family communication within this actionable framing, with family disclosure being reported as a reason for testing or a potential outcome of it, for which they seek information.

To our knowledge, this is the first prospective study exploring how family communication is addressed during GC. Our findings may be used to develop resources to assist GHPs supporting family disclosure and cascade testing. Further studies are needed to explore how the decision-making trajectories and experiences of family communication by consultands may be influenced by the GC process.

No conflicts of interest to declare.

GENETIC COUNSELLING SUPERVISION WORLDWIDE: INSIGHTS INTO GLOBAL PRACTICES, IMPLEMENTATION CHALLENGES, AND FUTURE TRENDS

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Introduction

Genetic Counselling Supervision (GCS) is essential for maintaining the quality of genetic counselling services worldwide. This research aims to provide a snapshot of GCS globally, examining models, frequency, and funding mechanisms. By exploring these variations, the study seeks to understand GCS practices across regions and to identify challenges, including compliance, resource allocation, and stakeholder engagement.

Methodology

Online semi-structured interviews were conducted with genetic counselling professionals from 24 countries: Europe (11), Americas (4), Asia (5), Oceania (2), Africa (1), and 1 in the Middle East (Israel). Focus areas included (1) the existence and distribution of GCS, (2) characteristics of GCS such as frequency, mandatory supervision, funding mechanisms, and (3) implementation processes, highlighting successful strategies and challenges. Data were analyzed through thematic analysis to identify key trends.

Results

Global awareness of GCS is increasing, with some countries making notable advancements supported by regulatory frameworks and funding. Integrating GCS early into training programs for junior professionals, including medical students and residents, has shown positive outcomes across various contexts. However, significant variability persists in the adoption and effectiveness of these practices, reflecting global disparities in access and professional initiatives. Key challenges include the non-mandatory nature of GCS, leading to reliance on informal practices, and inconsistencies between self-funding and support from professional societies. Additionally, some regions fail to recognize the importance of GCS, and overwhelming workloads affect its effectiveness and acceptance.

Discussion

Findings highlight a global shift towards the adoption of GCS, with early integration identified as a key success factor. However, significant challenges persist, particularly in underdeveloped regions. To address disparities, increased support and resources are essential, especially in underserved areas. By implementing training programs and establishing regulatory frameworks, stakeholders can enhance the quality and accessibility of genetic counselling services, ensuring equitable care and improving the overall effectiveness of GCS worldwide.

GENETIC COUNSELLING FOR PSYCHIATRIC CONDITIONS: EXPLORING CURRENT PERCEPTIONS OF FAMILY PHYSICIANS AND PSYCHIATRIST IN PORTUGAL

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GENETIC COUNSELLING / SERVICES / EDUCATION

Genetic counselling emerged as a specialized healthcare discipline in the 1960s, and since then, various sub-specialties have developed globally, namely and more recently, psychiatric genetic counselling. This study provides a pioneering exploration of family physicians and psychiatrists' perceptions regarding genetic counselling provision and its potentialities in the context of psychiatric illnesses in Portugal. A qualitative methodology with semi-structured interviews was used. Among the participants, five were family physicians, and six were psychiatrists. Thematic analysis revealed three themes: (1) the role of genetics in healthcare, (2) barriers to psychiatric genetic counselling implementation, and (3) perceived benefits associated with its implementation. Results show that while the importance of genetics in psychiatric disorders is acknowledged, there is low literacy about genetic counselling practice from the professional groups interviewed. Also, the availability and mainstreaming of genetic testing seem to influence how genetic counselling is perceived and utilized. There is a perceived need for training and guidelines that foster the dissemination of genetics into healthcare, specifically mental healthcare. A holistic and patient-centred approach is considered essential in managing psychiatric disorders and, by extension, in psychiatric genetic counselling, as it addresses both medical and psychosocial factors. Although psychiatrists and family physicians are keen to integrate psychiatric genetic counselling into their patients' care, it seems that certain fundamental challenges still persist in genetic healthcare provision. Future research should contribute for a more comprehensive evaluation of the readiness for psychiatric genetic counselling implementation in the country.

Keywords: psychiatric genetic counselling; healthcare provision; qualitative research

ACCESS TO COUNSELLING SUPERVISION AS PART OF GENETIC COUNSELLING EDUCATION IN EUROPE: ARE WE PROMOTING REFLECTIVE PRACTICE?

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Introduction

Genetic Counselling Supervision (GCS) plays an integral role in professional development, stimulating a safe and reflective practice and preventing burnout. However, evidence shows insufficient access to this practice. This study aimed to understand the current state of counselling supervision access during MSc training, as well as understand the barriers and facilitators for its implementation.

Methodology

All MSc coordinators of the current EBMG accredited programmes were invited to participate in this study. An email with the link to a questionnaire was sent and attached was also an informative sheet compiling a brief description of the goals of the study, the definition of genetic counselling supervision and an explanation of the requested participation. Data review was performed by thematic analysis.

Results

All MSc directors/coordinators considered GCS relevant during education, citing professional development, safe practice, and emotional support for the professionals as main reasons for the attributed relevance. All programmes included GCS as part of their curricula. Five MSc programmes (62,5%) provide students access to counselling supervision during placements, however in a heterogenous and limited way due to lack of available professionals. Internal and external barriers to the implementation of GCS in the remaining programmes were identified, such as lack of regulations and their country not yet recognizing the genetic counselling profession.

Discussion

This study compiled evidence of the insufficient practice of GCS across Europe and its limited integration in MSc programmes. We recommend that educational pathways continue to prioritize awareness and access to genetic counselling supervision routines, equipping graduates with the necessary tools for reflective practice to consequently ensure care that meets the required standards of safety and quality.

25 YEARS OF GENETIC TESTING IN MOVEMENT DISORDERS: A COMPREHENSIVE ANALYSIS FROM A COHORT OF OVER 9,000 PATIENTS

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MOVEMENT DISORDERS

Introduction

Movement disorders (MD) encompass a wide range of neurological diseases affecting the speed (hyperkinetic or hypokinetic), fluency, and ease of movement. Recent advances in next-generation sequencing (NGS) have greatly expanded our understanding of its genetic causes. We aimed to explore the mutational spectrum in a large cohort of MD patients studied in our laboratory from 1998 to 2023.

Methodology

We reviewed MD genetic studies in our database: i) multigene panels (MGP) by NGS, ii) single-gene (SG) by Sanger sequencing and/or MLPA iii) repeats expansions by fragment analysis, focusing on disease-causing variants and variants of uncertain significance (VUS).

Results

For hereditary ataxias tested for repeat expansions (n=2,894 patients), the diagnostic yield was 28.7%: 593 patients with SCA (78.1% with Machado-Joseph Disease), 138 with Friedreich ataxia and 99 with CANVAS. For other causes of ataxias, SG (n=554 tests; 382 patients) yielded an 11% molecular diagnosis, 3% had VUS, and 86% were negative; MGP (n=603 patients) led to 10% molecular diagnoses, 48% VUS, and no reportable variants in 42%.

For patients suspected to have Huntington's disease (n=2,203), 44% had a molecular diagnosis, 54% were negative, and ~2% carried reduced-penetrance alleles.

Studies in patients with Parkinson's disease (n=1,151), MGP (n=318) provided 11% molecular diagnoses, 42% had VUS, and 47% were negative. Whereas, SG (n=1,013; 833 patients), 8% had a molecular diagnosis, 4% had VUS, and 88% were negative.

For spastic paraplegia (SPG) (n=1,104 patients), MGP (n=495) provided 21% diagnoses, 38% VUS, and 41% negative results. SG (n=1,375 tests; 638 patients) yielded 9% molecular diagnoses, 2% VUS, and 89% negative results.

In dystonia patients (n=846), MGP (n=292) led to 9% molecular diagnoses, 51% VUS, and 40% negative; SG (n=722 tests; 577 patients) returned 8% diagnoses, 2% VUS, and 90% negative results.

Discussion

MD's overall diagnostic yield was ~30%, mostly explainable by repeat expansions. MGP improved diagnostic yield of MD's, particularly in SPG. These findings are essential for the development of a comprehensive genetic testing leveraged by long-read sequencing.

ASSESSING DIAGNOSTIC YIELDS AND MOSAICISM ACROSS DIFFERENT APPROACHES IN NEUROCUTANEOUS SYNDROMES GENETIC TESTING

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Introduction

Neurocutaneous syndromes (NCS) entail long-term skin and nervous system involvement, exhibiting diverse phenotypes and multisystem manifestations. Diagnosis is often complicated by age-dependent symptoms and mosaicism. We aimed to reassess diagnostic yields and the prevalence of mosaicism using different targeted and multigene approaches.

Methodology

We reviewed our laboratory database for NCS patients tested (from 01/2020 to 08/2024) by i) targeted approaches (by Sanger or WES, n=465 cases), ii) multigene panels (WES only, n=59) and iii) deep sequencing panels (n=75, genes included: AKT1, HRAS, LZTR1, NF1, NF2, PIK3CA, PTCH1, SMARCB1, SPRED1, SUFU, TSC1 and TSC2).

Results

For targeted approaches, diagnostic yields were as follows: 59% (192/326) for NF1, 38% (20/53 patients) for TSC1/TSC2, 12% (4/33) for NF2, 4% (1/25) for PTEN, 4% (3/74) for SPRED1, 50% (1/2) for PTCH1 and a single patient tested for PIK3CA. Mosaicism was detected in 1.5% (7/465) of cases, one from tumour biopsy. No disease-causing variants were identified in the remaining loci.

The overall diagnostic yield of multigene panels was 25% (14/56) with disease-causing variants identified in PTEN, NF1, LZTR1, EPHB4, RIT1, ACVRL1, ENG, H1-4 and EIF2B5.

The application of deep sequencing enabled a diagnostic yield of 40% (30/75), with 10% of samples showing mosaicism, one from skin biopsy. Finally, testing with an extended gene panel that included selected non-coding regions successfully detected pathogenic deep intronic variants in 60% (3/5) of cases, that had previously tested negative at other laboratories.

Discussion

Selecting the optimal testing approach for NCS is challenging due to phenotypic/genetic heterogeneity and high prevalence of mosaicism. Multigene panels, particularly deep sequencing, demonstrated high diagnostic yield, contributing to reduce time-to-diagnosis and retrieving a 10% rate of mosaicism. Taking advantage of our data, we propose an algorithm to facilitate decision-making for NCS' genetic testing.

MINIMIZING INVASIVE DIAGNOSTIC PROCEDURES THROUGH UNCONVENTIONAL GENETIC TESTING IN MCCUNE-ALBRIGHT SYNDROME

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ORGAN-SPECIFIC GENETICS: BONE

Introduction

gain-of-function GNAS variants are constitutionally lethal, while somatic mosaicism of these variants cause McCune-Albright syndrome. This condition is characterized by fibrous polyostotic dysplasia, hyperpigmented macules and hyperpituitarism. Identification of causal variants in peripheral blood is possible in only 25% of cases, due to low mosaic expressivity. Therefore, lesional tissue (such as skin) is typically used for testing, identifying a variant in 80% of cases. However, this implicates invasive biopsy procedures that are not always feasible.

Methodology

narrative description of a clinical case, including diagnostic approaches and patient management.

Results

a male patient was referred for a Clinical Genetics consultation at 7 years old due to intellectual disability and fibrous dysplasia. He had non-consanguineous parents and no relevant family history. He was diagnosed with global developmental delay and autism spectrum disorder at 4 years. At 5 years he was evaluated due to claudication, with a radiographical screening revealing polyostotic fibrous dysplasia. Growth occurred in the 50th centile until 2 years , and ascended to the 99th centile until 6 years of age. Physical examination revealed a large, irregular hyperpigmented macule in the low left hemiface and left anterior cervical region, macrocephaly, hypertelorism and macrognathia. Blood tests was remarkable for subclinical hyperthyroidism, excess growth hormone and prolactin, and a clinical diagnosis of McCune-Albright syndrome was established. Exome sequencing revealed no relevant variant, with adequate coverage of the GNAS gene. We did not perform skin biopsy in the hyperpigmented macule due to its location. Instead, we opted for a digital droplet PCR in peripheral blood which detected the c.601C>T, p.(Arg201Cys) activating GNAS variant, at a 0.05% expressivity.

Discussion

this case illustrates that novel technologies can be useful not only to increase genetic diagnostic yield, but to avoid invasive procedures and optimize diagnosis in rare diseases with rich and specific semiologic findings.

COMPLEX GENE REARRANGEMENTS IN SENSORINEURAL DEAFNESS INVOLVING STRC, CATSPER2, CKMT1A AND CKMT1B GENES: EVIDENCE OF GENE CONVERSION

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Introduction

Sensorineural hearing loss (SNHL) is a heterogeneous condition often associated with deletions and duplications involving the STRC gene. Additionally, genomic alterations in the CKMT1B and CATSPER2 genes may also contribute to the phenotype, although their role in hearing loss remains under investigation.

Methodology

We present two distinct cases of SNHL that were referred for next-generation sequencing (NGS) deafness panel testing, including copy number variation (CNV) analysis. This analysis detected complex gene rearrangements involving the STRC and CATSPER2 genes. The results were confirmed by multiplex ligation-dependent probe amplification (MLPA), which demonstrated that the detected CNVs also affected the CKMT1A and CKMT1B genes.

Results

The first case revealed a heterozygous deletion of the CATSPER2, STRC and CKMT1B genes, as well as a homozygous deletion of exons 19, 20, 23 and 24 of the STRC gene. Additionally, it was also detected a heterozygous duplication of the STRCP1 pseudogene. In the second case, a more complex rearrangement was identified. A heterozygous deletion of the CATSPER2 and STRC genes was detected, as well as a homozygous deletion of both STRC (exons 19, 20, 23, 24, 25 and 28) and CKMT1B genes. As in the previous case, a heterozygous duplication of STRCP1 was detected; however, this duplication was shown to include the CKMT1A gene as well.

Discussion

These findings underscore the complexity of gene rearrangements in SNHL. Both cases suggest the occurrence of two different CNVs in compound heterozygosity, the first involving at least the CKMT1B, STRC and CATSPER2 genes, and the latter involving at least the STRC gene. Both cases also show a duplication of the STRCP1 in heterozygosity, which may correspond to a conversion of STRC into STRCP1. In the second case, the STRCP1 duplication seems to be contiguous with the CKMT1A duplication. This suggests a possible gene conversion between CKMT1B and CKMT1A, not described in the literature. Parental testing is decisive in both cases to clarify the inheritance patterns underlying these complex gene alterations and contribute to more adequate genetic counseling.

FINDINGS FROM NEXT-GENERATION SEQUENCING-BASED MULTI-GENE PANEL TESTING FOR INDIVIDUALS WITH NEPHROLITHIASIS

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ORGAN-SPECIFIC GENETICS: KIDNEY

Nephrolithiasis affects approximately 9% of the general population in the United States. In addition to biochemical testing, genetic testing for monogenic forms can be helpful in predicting stone composition and tailoring treatment. The molecular findings within an unselected referral population receiving genetic testing for an indication of nephrolithiasis have not been characterized to date. In this study, we provide an overview of the genetic findings from next-generation sequencing (NGS) multi-gene panel tests (MGPT) for individuals with nephrolithiasis.

We performed a retrospective review of clinical reports for 160 consecutive patients with an indication of nephrolithiasis who underwent NGS MGPT at Blueprint Genetics. MGPT included both sequence and copy number variant (CNV) analyses of NGS data. The patients' sex, age at testing, and clinical history were collected from test requisition forms. Variant interpretation was performed using modified ACMG/AMP guidelines. A positive result was defined as the identification of pathogenic or likely pathogenic variant(s) consistent with the patient's reported phenotype and known disease inheritance.

The median patient age at time of testing was 14 years (range, 3-months to 77 years). A positive result was reported in 21.9% of patients (35/160) across 14 genes. The most common positive results were CYP24A1-associated autosomal recessive hypercalcemia/hypercalciuria (n=7) and SLC7A9-associated autosomal dominant (n=4) and recessive (n=2) cystinuria. Six individuals received positive results in a PH-associated gene: AGXT, GRHPR, or HOGA1. Almost all positive results were sequence variants (47/48), apart from a pathogenic 21kb deletion of CLCNKB exons 1-3 identified in trans with sequence variant.

We describe the molecular findings in an unselected referral cohort undergoing NGS MGPT for an indication of suspected nephrolithiasis. Approximately 1 in 5 patients with nephrolithiasis who underwent NGS MGPT received a positive result, including 6 individuals with positive results in genes associated with primary hyperoxaluria for which clinical trials of RNAi agents are currently recruiting.

THE IMPORTANCE OF FAMILIAL COSEGREGATION IN THE CLASSIFICATION OF NEW PKD1 VARIANT ASSOCIATED WITH ADPKD

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a highly penetrant and clinically recognizable disorder, characterized by the presence of multiple bilateral kidney cysts. Deleterious variants in the PKD1 or PKD2 genes are respectively responsible for ≈85% and ≈15% of the ADPKD families, while ≈5-10% remain genetically unresolved or are due to rare deleterious alleles in other loci. We aim to illustrate the utility of segregation studies in the classification of genetic variants, as applied to a family with a severe presentation of ADPKD and a novel missense variant in the PKD1 gene.

Methodology

A portuguese family with multiple ADPKD patients of particular severity sharing a new variant in PKD1 is described. Four affected and one unaffected family members were tested. Genetic testing was performed using either Sanger sequencing (PKD1 and PKD2) or NGS panel (PKD1, PKD2 and PKHD1 genes). CNV were excluded using MLPA for the different exons of the genes PKD1, PKD2 and PKHD1. Variants were classified according to the 2015 ACMG-AMP Standards and guidelines. The full likelihood Bayes factor (FLB) was computed using the R-package 'segregatr' (Version 0.3.0).

Results

The index patient had bilateral enlarged kidneys, multiple cysts and early onset kidney failure (KF). His father had died at a young age with KF, and he has 13 family members of 3 consecutive generations with ultrasonographic confirmation of ADPKD. The VUS c.8999G>C p.(R3000P) in heterozygosity in the PKD1 gene was identified in four affected patients and excluded in one unaffected patient. The FLB is 54.5, which, using Jarvik and Browning's criteria for cosegregation, the variant was reclassified as likely pathogenic.

Discussion

This family is clinically very suggestive of PKD1-related ADPKD and share one VUS in the PKD1 gene. The FLB calculation allowed for a rapid reclassification of the variant as likely pathogenic. A precise molecular diagnosis offers prognostic information, allows presymptomatic familial screening, prenatal diagnosis and preimplantation genetic testing, inclusion in therapeutic clinical trials or off-label indications and kidney donation from family members.

ANALYSIS OF Y-SNPS AND Y-STRS IN HAPLOGROUPS E-M35 AND J-M304 FROM INDIVIDUALS OF SANTARÉM DISTRICT

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POPULATIONS GENETICS

Introduction

The Y-chromosome haplogroups E-M35 and J-M304 were commonly found in the current-day Portuguese population, reaching frequencies between 10% and 20% [1,2]. In the district of Santarém, these two haplogroups were found at frequencies of 8.4% and 15.8%, respectively [4]. This study focused on the subtypes of these two paternal lineages within population of Santarém district, shedding light on the demographic events that impacted the genetic diversity in the region.

Methodology

The study sample comprised eight individuals belonging to haplogroup E-M35 and 15 to haplogroup J-M304. A total of 20 Y-SNPs were used to examine the subtypes within these two haplogroups. Seven Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393) were used to examine the internal variation of the haplogroups.

Results and Discussion

The common subtypes of the E-M35 lineage (n=8), E-M81, E-M78, and E-M123, were found at frequencies of 62.5% (n=5), 25% (n=2), and 12.5% (n=1), respectively. Four E-M81 samples carry the derived allele for the marker M183, the dominant subclade within E-M81. The two E-M78 samples belong to the E-V13, subclade, similar to results found in other European populations. Regarding the 15 J-M304 samples, five were assigned to the subhaplogroup J1-M267 and 10 to J2-M172. From the J1 samples, three (60.0%) belong to the rare J1a1-M365.1 lineage, and two (40.0%) were assigned to the J1a2-P58 / L147.1, a predominant subclade in the Portuguese population. From the J2 individuals, seven (70.0%) belong to the J2a-M410 subtype, one to J2b-M12 (10.0%) and two (20.0%) were assigned to the J2-M172* clade. The E-M35 network constructed with the Y-STRs showed genetic relationships with North African populations for the E-M81 subhaplogroup, evidenced by shared haplotypes with these populations. The Y-STR networks for the J1-M267 and J2-M172 haplogroups displayed shared haplotypes with Sephardic Jewish ancestry people, some of which were compatible with the Cohen modal haplotype.

Conclusion

The analysis of Y-SNPs and Y-STRs within the two common Y-chromosome lineages E-M35 and J-M304 in the population of Santarém district, has provided valuable insights into our past, shedding light on the North African / Berber and Jewish presence in the region.

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MASSIVELY PARALLEL SEQUENCING IN PRENATAL DIAGNOSIS: DIAGNOSTIC YIELD AND MUTATIONAL SPECTRUM WITHIN A LABORATORY COHORT OF 253 FOETUSES

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Introduction

Foetal anomalies detected by ultrasound are major indications for invasive prenatal diagnosis (PND). In recent years, massively parallel sequencing emerged as a valuable tool for PND, particularly when chromosomal analysis fails to detect abnormalities.

Methodology

To assess diagnostic yield of an invasive PND cohort and characterize its mutational spectrum, we reviewed 253 foetal samples tested using multigene panels based on whole-exome sequencing (WES; 01/2017-09/2024). These included: (1) apparently isolated anomalies (n=171; 62 skeletal, 30 central nervous system (CNS), 23 foetal growth restriction, 15 increased nuchal translucency/hydrops, 13 renal, 8 hypomobility/arthrogryposis, 7 cardiac, 5 heterotaxy, and 8 cases present only once/twice; and (2) multi-organ anomalies (n=82).

Results

Overall, the diagnostic yield (pathogenic variants related to phenotype) was 24% (n=61); 52% (n=132) had uncertain clinical significance results; and 24% (n=60) were "negative". In group 1, molecular diagnosis was achieved in 42 (25%), with skeletal anomalies representing the largest subset (n=25), where FGFR3 was the most frequently mutated gene (n=12). In group 2, 19 cases (23%) received a conclusive diagnosis: 10 had autosomal dominant inheritance (8 defective genes, 2 large duplications in chromosomes 9 and 12); 8 had autosomal recessive inheritance (7 defective genes); and 1 had X-linked inheritance (OFD1-related).

Among 192 cases with no conclusive molecular diagnosis, 20 were reanalysed for another multigene panel, yielding 2 additional diagnoses: 1 apparently isolated CNS anomaly involving FGFR2 and a renal anomaly involving FREM2.

Discussion

The wide phenotypic and genetic variability, combined with limited prenatal phenotypic characterization, highlights the utility of WES-based multigene panels in PND. Also, they enable reanalysis of uncertain or "negative" results in a timely effective manner, critical in the context of PND. Given the high number of unresolved cases in our cohort, further clinical research and/or evaluation is essential to improve molecular PND.

RAPID PRENATAL WHOLE EXOME AS A FIRST TIER TEST FOR FETUSES WITH CNS ANOMALIES

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PRENATAL

Introduction

Around 3–5% of pregnancies show ultrasound detected fetal anomalies and many of these have an underlying genetic cause. Central nervous system (CNS) anomalies are severe congenital abnormalities and account for around 9% of fetal malformations. Since, CNS anomalies are often diagnosed on an advanced stage of pregnancy, developing a rapid diagnostic tool is crucial.

Next generation sequencing technology, such as targeted multigene panels or Whole Exome Sequencing (WES) have increasingly become used as the first diagnostic test option in selected cases of CNS fetal anomalies. Recent studies have proven that WES provides an additional diagnostic rate of 5–57% in prenatal cases, in a reduced time.

In this study, we retrospectively studied 46 cases referred to our center for genetic study due to CNS anomalies. Eleven of these cases were study after TOP and 35 were ongoing pregnancies.

Methodology

All fetuses were referred to our center for genetic evaluation. WES analysis were performed in all cases. Bioinformatic analysis was focused on our 59 gene panel for congenital anomalies of nervous system and mainly on the targeted phenotype. In all cases the bioinformatic analysis included copy number variants (CNVs).

Results

In 24% (11/46) of the cases, our study identified a genetic aetiology for the fetus phenotype. In these 11 diagnosed cases, 5 were X-linked diseases, 4 were “de novo” autosomal dominant (AD) condition and 2 revealed an inherited autosomal recessive (AR) condition.

Discussion

In our study, WES provided a high diagnostic rate (around 25%) in fetuses with CNS anomalies. These results demonstrated that an accurate identification of the ultrasound detected anomalies is crucial to establish a definite diagnosis and consequently leading to an increase in diagnostic rate. These data prove that WES is a rapid and an extremely efficient tool to study fetuses with CNS anomalies and attests that WES may be considered as a first tier test for the prenatal diagnosis. This rapid and accurate diagnosis is of utmost importance, given the time constraints of an ongoing pregnancy and stablishing the risk of recurrence in future pregnancies.

NON-INVASIVE PRENATAL TESTING FOR FETAL ANEUPLOIDIES BY CELL-FREE DNA WHOLE-GENOME SEQUENCING: TWO YEARS OF EXPERIENCE IN A PRENATAL DIAGNOSTIC CENTER

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Introduction

Non-invasive prenatal testing (NIPT) based on the analysis of free fetal DNA circulating in maternal blood is a powerful tool for aneuploidy screening, currently recommended for all pregnancies, including twins, with an increasing trend towards integration into universal prenatal screening. The introduction of the whole genome sequencing (WGS) technique has made it possible to expand the screening of alterations to all chromosomes, including microdeletions and subchromosomal microduplications. In this work, we report the results and experience of our prenatal diagnostic center after implementing WGS-based NIPT.

Methodology

Retrospective review of NIPT results and clinical information related to the follow-up of 2,000 pregnancies between March 2023 and October 2024, using the VeriSeq NIPT Solution V2 platform (Illumina).

Results

A total of 2028 NIPTs were performed (1594 for high-risk combined screening, 101 for a history of aneuploidy, 42 for ultrasound abnormalities, 73 for a history of chromosomal abnormalities in the couple, and 236 for other reasons). Of these, 16 pregnancies (0.79%) presented altered results for common aneuploidies (14 for T21, one T18 and one T13). The number of inconclusive exams was 11 samples (0.5%), a significant improvement compared to the previous NIPT. However, we had three false positives (one for T13 and two for T21) and two false negatives (a female triploidy and a monosomy X). After implementing the WGS-based NIPT test, we identified 14 suspected cases of other alterations, rare aneuploidies, partial monosomies, and partial trisomies, followed by an invasive examination, with 12 false positives and two true positives.

Discussion

The introduction of NIPT in aneuploidy screening represents a paradigm shift in prenatal diagnosis. Implementing WGS brought advantages, namely the detection of rare aneuploidies and structural changes at an early stage of pregnancy and an evident reduction in inconclusive results. However, undesirable results, such as incidental maternal findings, can also be found that require appropriate genetic counseling and medical follow-up.

GENE IDENTIFICATION IN PRIMARY OVARIAN INSUFFICIENCY: A CRITICAL REVIEW

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Introduction

Primary ovarian insufficiency (POI), characterized by cessation of ovarian function before the age of 40, is a complex condition affecting approximately 3.7% of women. The etiology of POI is heterogeneous, encompassing genetic, autoimmune, and various other factors, with a significant proportion of cases remaining idiopathic. While NGS has facilitated the identification of new genes associated with POI, inconsistencies in gene classification and reporting among laboratories have hindered the development of standardized gene panels for clinical practice.

Methodology

A comprehensive review was conducted using the PubMed and ClinVar databases, employing the search terms “primary ovarian insufficiency” and “premature ovarian failure”. The articles were meticulously assessed for their variant classification methods, functional studies, and the quality of supporting evidence.

Results

A total of 70 articles providing evidence related to POI were reviewed, leading to the identification of 227 genes potentially involved in the condition. Of these, 25% were identified through GWAS. Additionally, around 10% of the genes lacked sufficient supporting evidence due to the absence of functional studies (e.g. many studies focused on mouse models). Notably, 2% of the genes were listed in ClinVar as disease-causing, although most were reported by the same laboratory without corroborating evidence. Furthermore, when 3% of the genes were reassessed using ACMG criteria, their pathogenic classification was deemed inappropriate. Ultimately, only 60% of the published genes associated with POI had credible evidence supporting their involvement.

Discussion

The findings of this investigation highlight significant gaps in the identification and validation of genes associated with POI. Furthermore, only 60% of the 227 genes provided evidence of their involvement in this condition, which underscores the need for a standardized evidence system to ensure scientific reproducibility and accurate diagnosis of POI. The authors propose a uniform framework which might facilitate the integration of genetic testing into clinical practice, enabling more precise patient management.

MOLECULAR CHARACTERIZATION OF SPERM DNA AFTER CRYOPRESERVATION

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Introduction

Cryopreservation of human spermatozoa is a widely used technique in Assisted Reproductive Technology (ART) and is considered a safe technique. However, a detailed analysis of epigenetic modifications, namely DNA methylation, after cryopreservation is still lacking.

Methodology

After written informed consent, human spermatozoa samples (11) were collected from normozoospermic (NZ) individuals and analysed before and after cryopreservation for 1 month. TUNEL technique was used to evaluate sperm DNA fragmentation (SDF) and bisulphite-sequencing to analyse sperm DNA methylation (SDM) at Differentially Methylated Regions (DMRs) of H19 and MEST imprinted genes.

Results

Results evidenced a small but significant increase in SDF after sperm cryopreservation in 36.4% of the samples, after 1 month of cryopreservation, with the remaining 63.6% samples showing no significant changes. Results showed no relevant changes in SDM of the imprinted regions in either H19 or MEST/PEG1 genes, with no changes in the expected normal H19 DMR hypermethylation and MEST DMR hypomethylation.

Discussion

Although it was previously shown that imprinting errors occur in sperm from infertile patients [1,2,3], and an increased risk of imprinting syndromes, such as Beckwith-Wiedemann and Silver-Russell syndromes, is documented in children born after ART, this data provides important information regarding the safety of sperm cryopreservation concerning the stability of paternal imprinting marks. To confirm these results, we are analysing longer periods of cryopreservation, with a higher number of NZ and non-NZ individuals.

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POSTERS

SESSION II

SENSITIZING TAXOL-RESISTANT HUMAN CANCER CELLS BY FA-SAT NCRNA SILENCING

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CANCER GENETICS

Introduction

Despite being considered transcriptionally inactive for many years, it is now known that satellite DNA sequences (satDNAs) are transcribed into non-coding RNAs (ncRNAs), playing important roles in genomes and in diseases, such as cancer. FA-SAT is the major satDNA in domestic cats, being conserved and transcribed in humans. Functionally, the FA-SAT ncRNA interacts with the PKM2 protein in the cell nucleus, and the disruption of this complex—either through FA-SAT or PKM2 silencing—results in apoptosis in both cat and human cells. Furthermore, a relationship was demonstrated between the decrease in FA-SAT ncRNA levels and cell sensitivity to the anti-mitotic Taxol in cat mammary tumor cells. In this sense, to explore how FA-SAT ncRNA expression impacts the response to anti-mitotic drugs, we used two human breast cancer cell lines, MCF7 and MDA-MB-231, with different response profiles to Taxol (sensitive and resistant, respectively).

Methodology

The study had three main phases: 1) we analyzed the cellular response to Taxol treatment in human MCF7 and MDA-MB-231 cells; 2) we quantified FA-SAT, PKM2, and MYC transcripts via RT-qPCR in Taxol-treated cells; and 3) we evaluated the response in Taxol-resistant cells with reduced FA-SAT expression through silencing with antisense LNA GapmeRs.

Results

Our findings demonstrated that the MCF7 cell line is more sensitive to Taxol, while the MDA-MB-231 cell line is more resistant. A decrease in FA-SAT transcripts was also observed in sensitive cells after exposure to Taxol. The performance of a sensitization assay on Taxol-resistant cells using an antisense LNA GapmeR to silence FA-SAT highlighted the potential of this approach to restore drug sensitivity.

Discussion

The observed decrease in FA-SAT transcripts in sensitive human cells suggests a potential regulatory role of this ncRNA in mediating cellular responses to anti-mitotic drugs. Furthermore, the ability to sensitize Taxol-resistant cells through FA-SAT silencing indicates that targeting this ncRNA could be a viable therapeutic strategy. However, further studies are needed to deepen the function of this satellite ncRNA in anti-mitotic drugs response.

EXTRACELLULAR VESICLES: MICRORNA-MEDIATED SIGNALLING IN FINE-TUNING BREAST CANCER CELLS SURVIVAL FOLLOWING DOXORUBICIN EXPOSURE

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CANCER GENETICS

Introduction

Extracellular vesicles (EV) secreted by cancer cells have been suggested to contribute to chemoresistance. A previous study from our group, demonstrated that hepatic spheroids differentially alter expression profiles of genes involved in xenobiotic, fatty acid and cholesterol metabolism, when exposed to EV (derived from DOX-sensitive and DOX-resistant breast cancer (BC) spheroids). This suggests a role in drug resistance (DR) mediated communication by EV in the BC-liver axis. Building on these findings, the present study aimed to evaluate the molecular content of these EV and their role in intercellular communication, tumour survival and chemoresistance development.

Methodology

EV were isolated from DOX-sensitive (EVS) and DOX-resistant (45 nM) (EVR) MCF7 spheroids, using standard differential centrifugation method. EV were characterized using NTA and immunodetection of specific protein markers, and cargo was analysed through RNA seq.. MicroRNAs (MIR) were assessed and pathway analysis and enrichment was performed using mirtarbase and uniport software.

Results

Titer of EVR was significant higher, than EVS. From EV cargo analysis, only 5 specific MIR (e.g. MIR4492, MIR4516) were found significantly more abundant in the EVS, while 30 different MIR (e.g. MIR182, MIR200C, MIR141) were found more significantly present in the EVR. From a total of 209 genes targeted by the 35 significantly present MIRs studied, 38 are known to be regulated by two or more of these MIRs, interestingly found in the EVR.

Discussion

Higher concentrations of EVR, seem to be indicative of a signalling role in DOX chemoresistance development. In EVR, highly representative MIRs were encountered targeting specific genes, such as BCL2, FOXO1 or PTEN part of pathways in programmed cell death, differentiation, chemoresistance, oxidative stress, cell cycle, lipid, metabolism and protein synthesis. The larger number of MIRs present in EVR suggests that the fine-tunning of cellular adaptation to DOX cells resulted from a complex network of EV-based communication seemingly directed both to the cellular microenvironment, and systemically.

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EVALUATION OF STAR-SHAPED POLY(LYSINE) POLYPLEX NANOPARTICLES FOR GENE DELIVERY IN BREAST CANCER SUBTYPE CELL CULTURE MODELS

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Introduction

Breast cancer (BC) affected 2.3 million women in 2022 and is driven by dysregulated pathways controlling cell proliferation (PI3K/AKT, MAPK), and apoptosis (p53), promoting tumour growth, metastasis, and treatment resistance. While gene therapy offers a promising approach for BC treatment by targeting key oncogenic pathways through gene modification or inhibition, nuclease degradation remains a challenge. This study proposes polymeric nanoparticles (NPs) as gene delivery systems to treat BC. NPs are promising candidates for enhancing BC therapy due to their low toxicity and genetic material protection from degradation.

Methodology

Polypeptide NPs were analysed for their physicochemical properties by DLS method. NPs developed enhance the delivery of miRNA mimic into 2D and (3D) spheroids BC cell lines – Luminal B, Her2-enriched and triple-negative. The cell response and uptake analysis were assessed by flow cytometry and confocal microscopy. The miRNA expression levels were evaluated by RT-qPCR.

Results

The optimized NPs exhibited homogenous hydrodynamic diameter, around 300nm, and positive surface charge. Cell viability assays confirmed that the polyplexes were non-toxic. Transfection assays revealed high efficiency in delivering miRNA to 2D and 3D BC cell culture models, with the highest transfection rate in triple-negative cells. miRNA expression levels showed a transient increase, that can further inhibit the proliferation and migration of BC cells.

Discussion

The use of spheroids provides a more physiological model of in vivo tumour biology and therapeutic resistance. The observed heterogeneity in BC spheroids reflects the complexities of the in vivo TME impacting both tumour progression and treatment efficacy. Here, we fabricated stable NPs that protect the miRNA from nuclease degradation. The cellular uptake increased due to NPs positive charge that interacts with the negatively charged cell membrane. Thus, they become versatile platforms for the encapsulation and delivery of therapeutic agents and by targeting PI3K/AKT pathway with miRNAs, potentially modulating signalling mechanisms and inhibiting BC cell proliferation and migration.

This work was partly supported by the Research Center Grant ToxOmics (UIDB/00009/2020 and UIDP/0009/2020), from the Portuguese Fundação para a Ciência e a Tecnologia.

IMPACT OF UPDATED VARIANT CLASSIFICATION CRITERIA ON BRCA1 AND BRCA2 GENE VARIANTS: A RETROSPECTIVE ANALYSIS

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CANCER GENETICS

Introduction

Recent specifications from the ClinGen/ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) BRCA1 and BRCA2 Expert Panel (VCEP) have led to significant updates in the ACMG/AMP variant interpretation guidelines for both BRCA1 and BRCA2 genes. These updates refine the criteria for assessing pathogenicity and benignity and introduce new combinations to classify variants as pathogenic (P), likely pathogenic (LP), likely benign (LB), or benign (B), tailored to each gene's specific characteristics (PMID: 39142283).

Methodology

A retrospective analysis was performed to reclassify variants previously identified in BRCA1 and BRCA2 at the clinical lab Instituto de Patologia e Imunologia Molecular da Universidade do Porto (Ipatimup Diagnostics) from March 1997 to December 2023. Variants were categorized by type and reassessed based on a comprehensive review of evidence, including population databases, literature, in silico prediction models, and laboratory-developed tools.

Results

A total of 154 variants in BRCA1 and 322 variants in BRCA2 were reviewed. Although there were no major changes in the previously established molecular diagnoses, there was a notable decrease in variants of unknown clinical significance (VUS) and an increase in LB/B variants. This shift was largely due to the activation of the 'BP1_Strong' criteria, which allowed the reclassification of certain synonymous and missense variants as LB, provided they do not affect critical functional domains or splicing. Any reclassified variants with clinical implications prompted the issuance of updated reports to the requesting clinician.

Discussion

The adoption of the new ClinGen ENIGMA VCEP criteria for BRCA1 and BRCA2 variant interpretation guidelines has led to an estimated 60% reduction in the number of inconclusive results in genetic testing. This improvement is crucial for enhancing the accuracy of genetic diagnoses and ensuring more reliable patient management strategies. By continually improving these criteria and reviewing genetic variants, we can provide more precise and actionable information, ultimately advancing the quality of patient care.

Declaration of Interests: Nothing to declare.

PERFORMANCE OF DIGITALMLPA IN CNV SCREENING IN GENES ASSOCIATED WITH HEREDITARY CANCER

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Introduction

Germline Copy Number Variations (CNV) in genes associated with hereditary cancer (GAHC) may predispose to multiple cancer types. Although CNV account for a reduced proportion of hereditary cancer genetic variants, identifying a pathogenic/likely pathogenic CNV has a significant impact on the clinical management of both the patients and their families. DigitalMLPA (dMLPA, a novel technology) combines Multiplex Ligation-dependent Probe Amplification (MLPA) and Next-Generation Sequencing for detection of cancer-related CNV in multiple genes and samples simultaneously.

Methodology

dMLPA was performed using the Probemix D001-C1 Hereditary Cancer Panel 1 (MRC-Holland). For validation of dMLPA, we used 29 DNA samples: 16 without CNV, 12 with CNV in GAHC and one with the Portuguese founder mutation BRCA2: c.156_157insAlu, all previously detected by conventional MLPA (cMLPA). A total of 53 test samples from patients with personal and family history of cancer, previously studied by other molecular methodologies, were analyzed single-blinded.

Results

dMLPA identified all the genetic variants previously detected by cMLPA revealing 100% concordance between the two methodologies. Among the 53 test samples, 5 different variants in GAHC were identified, including a deletion of exons 12 and 13 in the APC gene, a deletion of exon 23 in BRCA1, a duplication of exon 12 in BRCA1, the c.156_157insAlu insertion in BRCA2 and the c.1100del in CHEK2. Sanger sequencing elucidated that the BRCA1 deletion was partial (c.5503_5564del) rather than a complete exon deletion. A false positive result for a deletion of exon 9 in EPCAM was caused by a single nucleotide variant in the probe's target sequence.

Discussion

The sensitivity and specificity of dMLPA were 100% and 98%, respectively. This study demonstrates that dMLPA is an advantageous alternative to cMLPA for the screening of germline CNV in GAHC, comprising more genes than cMLPA and providing a cost-effective analysis with a fast turnaround time. Nonetheless, confirmation of results using other methodologies remains necessary.

SMAD4 PROCESSED PSEUDOGENE MAY LEAD TO IN SILICO FALSE POSITIVE DETECTION OF LARGE DUPLICATION EVENTS

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CANCER GENETICS

Introduction

SMAD4 (18q21.2) encodes a signal transduction protein that acts as a tumor suppressor involved in transcription regulation. Pathogenic variants in SMAD4 are associated with juvenile polyposis syndrome, a hereditary cancer syndrome (HCS), therefore SMAD4 is included in several next-generation sequencing (NGS) gene panels for HCS molecular diagnosis. Although in silico copy number variants (CNV) analysis is now widely integrated into NGS bioinformatics pipelines, its performance remains challenging. In this work, we characterize an apparent SMAD4 large duplication due to the presence of a SMAD4 processed pseudogene (SMAD4-PΨ) leading to a false-positive result.

Methodology

SMAD4 screening for molecular variants was performed by NGS with TruSight Hereditary Cancer Panel (Illumina) followed by in silico CNV analysis with panelcn.MOPS and Dragen Enrichment and visualization of aligned sequence reads with Integrative Genomics Viewer (IGV). Digital-MLPA (dMLPA) (MRC Holland) was used to assess in silico presumed CNV. Sanger sequencing was performed to confirm the genomic localization of the SMAD4-PΨ.

Results

During HCS diagnostic routine, using in silico CNV analysis, we detected two patients with an apparent SMAD4 whole gene duplication. Visualization of aligned sequence reads revealed that only the coding regions of SMAD4 (exons 1-12, NM_005359.6) were duplicated, which was partially confirmed by dMLPA. Literature search revealed the existence of a rare SMAD4-PΨ comprising nucleotides c.-497 to c.*1310 (NM_005359.6). We confirmed the presence of this pseudogene by sequencing the breakpoints of its integration at the last intron of SCAI (9q33.3), as reported by Watson et al (2017).

Discussion

Pseudogenes commonly interfere with genetic diagnosis of HCS but once they are known, analytical improvements can be implemented to overcome this issue. We identified the presence of a rare SMAD4-PΨ in two HCS patients as the cause of a false-positive in silico call for SMAD4 whole gene duplication. The creation of a HGNC ID for the SMAD4-PΨ is recommended. The clinical and functional consequences of the integration of SMAD4-PΨ in SCAI should also be clarified.

DEVELOPING A PRACTICAL DECISION TREE FOR THE NEW CLINGEN PP1/PP4 FRAMEWORK: INSIGHTS AND CHALLENGES FROM UNILAB'S VARIANT INTERPRETATION TEAM

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Intoduction

Since their introduction in 2015, ACMG/AMP criteria for germline variant classification have undergone several revisions by the ClinGen Sequence Variant Interpretation workgroup. The most recent one targeted PP1 (segregation) and PP4 (phenotypic specificity), based on the recent understanding that they represent the same type of information, therefore should be taken into account as a pair on the risk of double-counting evidence. After months of applying this new framework, we at Unilabs have designed a PP1/PP4 decision tree, resembling the ones designed for the PVS1 criteria, in order to facilitate the comprehension and simplify the application of these rules in routine variant analysis.

Methods

The PP1/PP4 decision tree was designed after careful analysis of the original article (Biesecker et al, 2023), with iterative refinements based on expert feedback from Unilabs' variant interpretation specialists and exchanges with the authors of the article.

Results

The PP1/PP4 decision tree is a structured fluxogram showing key decision checkpoints for applying these criteria depending on inheritance patterns, as well as availability of diagnostic yield measurements and segregation data.

Discussion

After several months of applying the new PP1/PP4 framework, a few questions have arisen, namely if this framework can be applicable to every type of disease and inheritance pattern, potential limitations and pitfalls of the PP4_strong criteria, defining a minimal core of "phenotypic specificity" to apply this criteria for syndromic conditions, and whether NGS CNV screening has enough sensibility to allow the interpreter to confidently exclude alternative causes of disease. Nonetheless, the decision tree has proven to be a great addition to the variant classification toolkit as it summarizes complex information and standardizes its application.

INCREASING DIAGNOSTIC VALUE: HIGH IMPACT OF GENE-DISEASE RELATIONSHIP CURATION

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

One of the major challenges for diagnostic laboratories is the evaluation of the clinical impact of genetic variants, with one important step being the establishment of gene-disease relationship (GDR). With this study, we summarize our experience in the last 2 years applying the ClinGen framework in the day-to-day work of a diagnostic laboratory.

Methodology

The ClinGen Clinical Validity Framework for evaluation of GDR was applied. We systematically evaluated genes without OMIM entries or with entries indicating that the relationship between the phenotype and gene was provisional. The gene selection depended on the specific variants identified during the routine diagnostic evaluation of patients' genomic data. Internal CENTOGENE biodatabank with exome/genome data from previously tested individuals, in addition to data available in the literature or public databases were used for gene classification. According to the framework, definitive, strong, moderate, limited, or no known disease relationship can be reached.

Results

More than 500 genes were evaluated, and a strong/definitive level of evidence has been reached for 24% of the genes, moderate for 27%, limited for 43%, and no known disease relationship or disputed for 5% of the genes. A total of 86 genes (14.7%) without OMIM entry reached moderate or strong GDR.

Discussion

Our results demonstrate the importance of careful assessment of gene clinical validity data, along with the use of genetic data repositories. Implementation of ClinGen standardized scoring system for routine assessment of gene-disease association is relatively easy to apply and relevant in a clinical diagnostic setting, besides beneficial for patients, families, and clinicians.

Conflict of Interest

All authors are employees of CENTOGENE GmbH

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A COMPARATIVE STUDY OF ISO 15189 ACCREDITATION AND EU REGULATION 2017/746 IN THE IMPLEMENTATION OF WHOLE EXOME SEQUENCING (WES) IN CLINICAL DIAGNOSTICS

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Introduction

The European Union's In-Vitro Diagnostic Device Regulation (IVDR) establishes a stringent framework to ensure the safety and accuracy of diagnostic devices through risk- and purpose-based validation, traceability, and post-market surveillance. In the absence of suitable commercial devices, laboratories often rely on in-house Laboratory-Developed Tests (LDTs). NGS (WES), has emerged as a critical LDT for comprehensive genetic analysis in clinical diagnostics, as not commercially available fully integrated system devices under IVDR currently meet the complex requirements of clinical practice.

Methodology

This study outlines a strategy for implementing IVDR requirements in a medical genetics laboratory, building on ISO 15189 accreditation with a focus on WES as an LDT. Scientific validation was performed by assessing the databases used in routine operations and developing clinical area-specific guidelines. A representative validation method was applied to assess reproducibility, repeatability, and cross-platform performance as part of the analytical validation. Clinical validation involved the re-analysis of blinded samples by multiple analysts across various clinical areas, along with inter-laboratory comparisons.

Results

Analytical validation demonstrated strong reproducibility and cross-platform consistency, achieving 100% sensitivity and specificity for SNVs and Indels, and over 90% for CNVs. Clinical validation confirmed high sensitivity and specificity through blinded sample analysis and successful inter-laboratory comparisons.

Conclusion

While ISO 15189-accredited laboratories benefit from strong quality systems and skilled personnel, meeting the IVDR requirements demands further steps. The IVDR introduces more detailed and stringent performance evaluation criteria, such as comprehensive clinical evidence, precise risk classification and management, and continuous post-market surveillance, which go beyond the scope of ISO 15189.

UNVEILING THE EPIGENETIC SIGNATURE OF THE CENTRAL NERVOUS SYSTEM: EXPLORATORY ANALYSIS OF BUCCAL SWABS, NEUROTRANSMITTER GENE EXPRESSION AND NARRATIVE REVIEW OF STUDIES FOCUSING ON SURROGATE TISSUES

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

Research on epigenetic mechanisms of complex diseases involving the Central Nervous System (CNS) has increased over the past decade, opening new views on diseases like Schizophrenia, Epilepsy, Multiple Sclerosis, among others. However, the CNS remains as a virtually inaccessible tissue and most of the research has relied on the study of blood samples of live patients or post mortem brain samples. However, few studies have focused on the correlation of methylation patterns between the CNS and peripheral samples and some present buccal swabs as a promising surrogate tissue. Hence, the authors sought to analyze gene expression from peripheral tissues focusing on neurotransmitter pathways and epigenetic regulators.

Methods

The buccal samples were collected in RNA stabilization buffer and nucleic acid extraction was performed using the AllPrep DNA/RNA Isolation kit (Qiagen). Expression analysis of genes involved in neurotransmitter pathways and epigenetic regulation was performed using datasets available online and transcript levels were analysed by qRT-PCR in 10 blood samples, 5 buccal samples and one brain sample.

Results

Using publicly available datasets, we analyzed expression at the protein level of GABAergic (RELN, GAD1), dopaminergic (COMT) and serotoninergic (HTR2B, HTR2A) markers. The dopaminergic and serotoninergic markers were detected in the four brain regions and buccal cells whereas GABAergic markers were only detected in the brain. Blood cells only showed expression of COMT. Of the epigenetic regulators, DNMT1 and DNMT3A were present ubiquitously whereas TET3 was mainly present in the brain and TET2 showed highest expression in blood cells. As for mRNA analysis, we were able to detect transcripts from DNMT1, DNMT3A, TET1 and TET2 in blood cells, whereas buccal cells did not present detectable levels of these genes.

Discussion

These preliminary results shed light on the suitability of using peripheral tissues to address CNS diseases, namely neuropsychiatric disorders, suggesting that blood (and to a lesser extent, buccal cells) may be promising surrogates to address epigenetic changes and fluctuations in neurotransmitter peripheral levels.

NGS BASED DIAGNOSIS OF SINGLE MITOCHONDRIAL DNA DELETIONS

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Introduction

Mitochondrial DNA (mtDNA) disorders are associated with point mutations and single or multiple large-scale mtDNA deletions and duplications. Single large-scale mtDNA deletions manifest as overlapping clinical phenotypes: Kearns-Sayre syndrome (KSS), Pearson syndrome (PS), chronic progressive external ophthalmoplegia (CPEO). They vary in size and can be identified in DNA from blood, buccal cells, and urine in affected children, and skeletal muscle tissue in affected adults. The present work shows our experience in a clinical diagnostic setup highlighting the value of the NGS in the diagnosis of mitochondrial disease, namely mtDNA deletions.

Methodology

The study cohort included patients for whom a targeted mitochondrial panel or exome sequencing was performed in the last 2 years. Reasons for referral included: seizures, stroke, CPEO, lactic acidosis. DNA was extracted from blood and NGS was performed.

Results

In 15 cases a single large-scale mtDNA deletion was detected with heteroplasmy levels ranging from 21% to 81%. Age at diagnosis varied from eight months, youngest patient diagnosed with PS, to a 23-year-old patient with KSS spectrum. Considering that in the same group of patients, 67 cases with pathogenic/likely pathogenic mtDNA variants were identified, single large-scale mtDNA deletions represent 18% of mtDNA disease-causing variations.

Conclusion

MDs are increasingly recognized however their diagnosis remains a challenge due to their genetic and clinical heterogeneity as well as complexity of mitochondrial genomics and often limited coverage of quantitative mtDNA changes in standard NGS assays and related bioinformatic pipelines. However, NGS technologies allow for the identification of single large-scale mtDNA deletions in a single platform and workflow, providing highly accurate and cost-effective diagnostic tests as our data demonstrate.

SERVICE PROVISION OF GENETICS HEALTH CARE IN PORTUGAL

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

Genetics healthcare has seen rapid technological advances in recent decades, notably transitioning to genomics, impacting health systems globally. In Portugal, the demand for genetic services has surged, exacerbating existing limitations in service infrastructure. This study aims to characterize the current status of genetic services in Portugal, identify key challenges, and explore future opportunities for improvement.

Methodology

A qualitative methodology was adopted through semi-structured interviews with directors of public genetics services in Portugal. Five interviews were conducted, covering 83.33% of public service leaders. Thematic analysis was applied to derive key categories, namely: specialty and technical advancements, structural difficulties, potentialities, and future directions.

Results

The analysis revealed significant advancements in genetic testing, particularly through comprehensive gene panels and next-generation sequencing. Structural challenges include service overload, inadequate funding, and a lack of human resources. However, opportunities arise from multidisciplinary team formation and improved integration with other medical specialties. Future directions point toward better patient management, enhanced research opportunities, simplification of test request procedures, and establishment of specialized units within genetics services.

Discussion

The study underscores the pressing need for systemic reforms in Portugal's genetic healthcare services. Despite technological advancements, issues such as long patient waiting times, underfunding, and workforce shortages persist. Addressing these gaps requires public policy interventions focused on restructuring and expanding genetics services, with an emphasis on equitable access and multidisciplinary collaboration. Improved training and accreditation, alongside research investments, will be crucial for the future of medical genetics in Portugal.

UNCOVERING ALU INSERTIONS IN NF1: A NEW FRONTIER IN NEXT-GENERATION SEQUENCING DATA ANALYSIS

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Introduction

Neurofibromatosis type 1 (NF-1) is a genetic disorder with a highly variable clinical expression. Milder disease forms present cutaneous and ophthalmological features: café-au-lait macules (CALM), axillary and inguinal freckles, cutaneous neurofibromas (CN) and Lisch nodules. Severe phenotypes present a range of tumours and variable neurological and cognitive features. NF-1 follows an autosomal dominant inheritance caused by germline variants in the NF1 gene, with mosaic cases, resulting from postzygotic pathogenic variants.

Methodology

48-year-old male meeting clinical criteria for neurofibromatosis underwent NF1 genetic testing. His family history was unremarkable, and clinical features included learning difficulties, multiple CALM, and numerous CN on the trunk and back, that became apparent in his thirties. No Lisch nodules or other manifestations were noted. Targeted NF1 gene analysis was performed by exome sequencing to detect sequence and copy number variants (CNV).

Results

No single-nucleotide variants and insertions/deletions were identified in this case, only a potential exon 45 heterozygous deletion was suggested by CNV analysis, not confirmed by MLPA. Upon BAM file's manual inspection of this gene's region, a potential poly-T insertion (at position c.6852_6853) was identified in few sequencing reads (n=7). PCR and Sanger sequencing of this exon confirmed the presence of a, possibly mosaic, insertion at this position. Due to the low representation of the mutated allele, its full sequence could not be determined. Subsequent variant-specific PCR revealed an insertion of approximately 343bp. The Alu's full sequence was submitted to GenBank (PP943058). A similar likely-pathogenic variant was previously reported in the literature (Douben et al., 2022).

Discussion

Selecting optimal testing approach for NF1 is challenging due to the high prevalence of mosaicism and homology with other genomic regions. Next-generation sequencing, especially with deep sequencing, offers the potential to improve detection of these low-represented variants. Our case highlights that Alu insertions add complexity and may be overlooked by conventional bioinformatic pipelines.

PORTUGUESE PUBLIC ATTITUDES TOWARDS GENETIC RISK DISCLOSURE SUPPORT DIRECT CONTACT BY HEALTHCARE PROFESSIONALS WITH FAMILY MEMBERS

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

Hereditary diseases can have implications for both patients and their genetic relatives, including future offspring. This study explores the attitudes of the general Portuguese population regarding the receipt of genetic risk information and policies on genetic risk disclosure.

Methods

A quantitative, cross-sectional study was conducted through an online survey disseminated via social media and public spaces. Participants rated eight statements on a 5-point Likert scale (1 = completely disagree to 5 = completely agree). Data were analyzed using SPSS: frequencies and percentages for categorical variables, mean (M) with standard deviation for continuous variables. Mann-Whitney and Kruskal-Wallis tests were used for group comparisons, Spearman's test was used to measure correlations.

Results

A total of 1,034 adults living in Portugal completed the survey (completion rate: 78.68%), with a mean age of 38.58±14.91 years (range: 18-81). Most were women (75.4%), lived in urban areas (81.2%), and had higher education (74.4%). The key findings were: a) respondents prefer being informed about genetic risks by a doctor rather than not being informed (M=4.75± .67); b) they are less supportive of first learning about genetic risks from a close relative (M=3.94±1.11); c) there is strong support for laws allowing healthcare professionals to inform them directly, even if their relatives do not wish to (M=4.47± .87); and d) less support exists for laws requiring patients to inform relatives about genetic risks (M=3.88±1.27). Significant differences were found by gender, marital status, education level, and whether participants had a medical appointment for a hereditary condition.

Discussion

This is the first study on public attitudes towards genetic risk disclosure in Portugal. While there is broad support for healthcare-mediated approaches, there is less backing for laws requiring patients to inform relatives of genetic risks. These findings contribute to ongoing debates about cascade testing and the roles and responsibilities of healthcare professionals in conveying genetic risk information to at-risk family members.

The authors declare no conflicts of interest.

COMMUNICATION ABOUT INHERITED CONDITIONS FROM PARENTS TO YOUNG CHILDREN: EXPERIENCES OF PORTUGUESE GENETICS HEALTHCARE PROFESSIONALS

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Communicating with young children about inherited genetic conditions (IGCs) is challenging for parents, who often struggle with when and how to share this information. Genetics healthcare professionals (GHPs) play a key role in supporting parents during these conversations, yet their views and experiences in this area are under-researched. This study aimed to examine Portuguese GHPs' perceptions, attitudes, and practices regarding parental communication about IGCs with their young children.

This observational, cross-sectional study used nonrandom convenience sampling. An online survey was developed based on a scoping review, with face and content validity ensured through expert consultation, and distributed in all Medical Genetics services in Portugal and social media. Data were collected over 3 months and analyzed using SPSS v.29.0, with correlations (Spearman test), percentages, means (M), and standard deviations calculated.

Thirty-two GHPs completed the survey (75% women, 43.7% with 5-19 years of experience, 78.1% with specific training in medical genetics or genetic counselling). Main findings were: a) GHPs felt responsible for supporting parents in this communication (M: 3.56±0.62) and emphasized it should be adapted to the child's developmental stage (M: 3.56±0.50); b) GHPs noted parents' concerns about the emotional impact of discussing IGCs on their children's well-being (M: 3.53±0.51); c) GHPs saw their role as encouraging parents to communicate openly about IGCs (M: 3.47±0.57); d) GHPs assess parents' understanding of IGCs (M: 3.41±0.56), while stressing the importance of informing children (M: 3.28±0.52) and tailoring the information based on disease and inheritance pattern (M: 3.28±0.58).

This study is the first to explore the experiences of Portuguese GHPs regarding communication between parents and young children about IGCs. It highlights GHPs' sense of responsibility in guiding parents through the disclosure process. GHPs need more tools and strategies to better support parents in ensuring that communication is age-appropriate and sensitive to young children's emotional needs.

No conflicts of interest to declare.

MOLECULAR CHARACTERIZATION OF A COHORT OF MODY PATIENTS FROM THE NORTH OF PORTUGAL – THE NEXT LEVEL

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Introduction

Maturity-onset diabetes of the young (MODY) is a group of monogenic disorders of autosomal dominant transmission affecting differentiation and function of pancreatic β-cell dysfunction. Although it is the most frequent form of monogenic diabetes mellitus, it accounts for only 2% of diabetic patients; is frequently misdiagnosed as type 1 or type 2 diabetes.

Fourteen different genes have been implicated to date, but the most frequently affected are glucokinase gene, GCK, and genes coding for hepatocyte nuclear factor 1α, 1β and 4α (HNF1A, HNF1B and HNF4A, respectively).

In 2018 we presented a study of around 100 patients, where 1 or more of these genes was studied by Sanger sequence.

Methodology

Roughly 400 patients with criteria for MODY type diabetes, from several hospitals of northern Portugal, were studied in ULSSA. Most underwent molecular genetics testing at UBG by Sanger sequencing of one or more of the following genes, according to mutation frequency – HNF1A – or specific clinical features - GCK and HNF1B.

As second tier approach, an NGS panel study was carried out in approximately 150 cases in which the genetic cause was not found in the initial approach.

At risk family members were also studied, totalling 77.

An in-depth clinical study of the diagnosed cases was carried out to completely characterize the patients.

Results:

Molecular characterization of patients is presented, where a definite or probable causing mutation was identified.

Comparison of Sanger sequence and NGS approaches is performed for this condition's diagnosis, in relation to cost-effectiveness.

Some genotype-phenotype correlations were identified.

Conclusions

The joint prevalence of HNF1A-MODY and GCK-MODY in our cohort is similar to what is reported in other populations.

However, as the diagnostic yield is somehow low, the algorithm followed has revealed to be less cost-effective than universal NGS approach to all clinical suspicions.

The applicability of genetic information in clinical practice and patient management is also discussed.

XYLT1 INHIBITION WITH GAPMER ANTISENSE OLIGONUCLEOTIDES AS A PROMISING GENETIC SUBSTRATE REDUCTION THERAPY FOR MUCOPOLYSACCHARIDOSIS TYPE III: IN VITRO RESULTS

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Introduction

Mucopolysaccharidosis type III (MPS III, subtypes A-D), is a neurodegenerative lysosomal storage disorder (LSD) characterized by the accumulation of undegraded heparan sulphate (HS) due to the lack of enzymes responsible for its degradation. Classical treatments, currently applied to other LSD are not effective in MPS III. Therefore, we have been addressing the potential of genetic substrate reduction therapy (gSRT) to ameliorate the MPS III phenotype. gSRT relies on the use of synthetic oligonucleotides to downregulate genes involved in the biosynthesis of the stored substances, and that is our goal: to reduce the production of HS and, consequently, its accumulation. Here we describe our results on the use of antisense oligonucleotides (ASOs), particularly gapmers, to silence a gene involved in the biosynthetic pathway of HS by promoting targeted mRNA cleavage via RNase H.

Methodology

We tested 5 gapmers targeting the XYLT1 gene, in MPS III fibroblasts to evaluate their effect at mRNA level by qRT-PCR. Then, we choose the best performing gapmers and proceeded to a macroassay to further analyse the treatment's effect on xylosyltransferase 1 protein (XYLT1) protein levels (by western blot) and, ultimately, on HS accumulation (by LC-MS/MS).

Results

We observed that all gapmers downregulate XYLT1 mRNA (until 10 times less expression compared to non-transfected cells), but the effect was more pronounced with two of them ($p<0.0001$), and very promising results were obtained for MPS IIIC: a decrease on XYLT1 levels ($p< 0.05$) and on HS storage ($p< 0.05$).

Discussion

Our results indicate that two gapmers promote a high reduction in mRNA levels of target gene, a slight decrease on XYLT1 protein levels, and a slower HS accumulation rate in patients' cells. Even though further research is needed, gSRT seems a promising approach for MPS III.

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FUNCTIONAL CHARACTERIZATION OF APOB VARIANTS: EXPLORING THEIR ROLE IN FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction

APOB variants are responsible for about 5-10% of Familial Hypercholesterolemia (FH) cases, a condition characterized by increased LDL cholesterol levels. Only in recent years has the whole APOB gene been studied, and variants in this gene may be more common than initially supposed. Although the majority are missense variants, nonsense variants and small indels were also identified in individuals with FH phenotype and can be the cause of disease.

This project aimed to characterize 10 APOB variants identified in individuals with clinical diagnosis of Familial Hypercholesterolemia.

Methodology

LDL from index cases and relatives was isolated through sequential ultracentrifugation. ED-LDLR was purified from HEK293 cells transfected with the pcDNA3.1-EC-LDLR-His plasmid by affinity chromatography. Purified ED-LDLR fragments were coated onto 96-well plates and incubated with the different APOB variants. Antibodies were used for ligand detection, and absorbance was determined at 405 nm. CHO-ldlA7 cells were transfected with wt LDLR plasmid and incubated with FITC-labeled LDL to determine LDL binding and uptake by flow cytometry.

Results

Preliminary results for the variants p.(Ala1393Val), p.(Asp1456Asn), p.(Met2042Thr), p.(Val4295Leu), and p.(Arg4519Thr) indicate that they do not appear to impact apoB's binding to the LDL receptor, similar to the findings for p.(Asp2213del) and p.(Ile3374Thr). In contrast, the p.(Gln4316*) variant demonstrated reduced affinity for the LDL receptor, impairing the binding of apoB to LDLR. The analysis of all variants is still ongoing, including p.(Arg1689His) and p.(Glu4387Asnfs*7).

Discussion

Functional studies play a critical role in assessing the pathogenicity of genetic variants and are among the key criteria for variant classification. These in-depth analyses not only confirm clinical diagnoses but also provide essential insights for developing personalized treatment strategies. In the future, we aim to increase the number of studied variants, starting with 15 more variants from the Portuguese FH Study.

ONE STEP CLOSER TO MODELLING AND "CORRECTING" THE RARE GENETIC DISEASE MUCOLIPIDOSIS TYPE II

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Introduction

Mucolipidosis type II (MLII, MIM 252500) is an autosomal recessive lysosomal storage disease of hydrolase trafficking caused by mutations in GNPTAB. It is multi-systemic, has a fatal outcome in early childhood, and has no treatment. So, our group is developing RNA therapies for MLII, but to test them and accelerate translation into the clinic, new cellular and animal models are need, ideally carrying the disease-causing allele. Thus, we are generating induced pluripotent stem cells (iPSCs) and a zebrafish model for the most frequent pathogenic variant: homozygous frameshift c.3503_3504delTC [NM_024312.5(GNPTAB):c.3503_3504del (p.Leu1168fs)].

Methodology

In vitro, fibroblasts were reprogrammed into iPSCs and characterized. Using a CRISPR-Cas9 knock-in (KI) approach, MLII iPSCs were nucleofected with CRISPR components [a ribonucleoprotein complex and a single-strand DNA donor (ssDO) with the normal sequence] to correct the mutation and generate an isogenic line. In parallel, a zebrafish MLII model with a delTG gnptab mutation (orthologous to the delTC), is also being generated. The protocol is under optimization but, briefly, 1-cell stage embryos are microinjected with CRISPR components and, by day 2-4 post fertilization, larvae are genotyped.

Results

MLII iPSCs were generated, fully characterized and registered at "Human pluripotent stem cell registry" as INSAi003-A. Using CRISPR-Cas9, we had 11% of edited iPSCs, which we are now increasing by selecting colonies until we reach an isogenic iPSC line with only the normal genotype. In vivo, from 98 larvae analysed, 3 had the delTG in over 50% of editing events.

Discussion

Reprogramming MLII fibroblasts into iPSCs was successful. Besides, a mutation-corrected isogenic iPSC line and an in vivo model of MLII in zebrafish are being generated. These two complementary and valuable platforms will allow for high-throughput screens to assist preclinical testing of new therapies.

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GENE EDITING AS A TOOL FOR CELL MODELS OF A LYSOSOMAL STORAGE DISORDER

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Introduction

Fabry Disease (FD) is a Lysosomal Storage Disorder characterized by defective α-Galactosidase A (α-GAL A), associated to mutations on α-galactosidase A gene. Therefore, Gb3 and lyso-Gb3 accumulate into the lysosome, leading to this multisystem disease. Recently, our group developed induced pluripotent stem cells (iPSCs) derived from fibroblasts of a FD patient, and derived from human dermal fibroblasts (HDFa). The FD cell line present the c.860G>A (W287X) mutation, which leads to α-Gal A loss of function. Our aim was to establish a FD disease model using CRISPR/Cas9 system by knocking-out the HDFa iPSC line, and also correct (knocking-in) our nonsense mutation in iPSCs from a patient with FD.

Methods

Single guide RNAs (sgRNAs) were selected using Benchling to perform the knock-in (KI) and the knock-out (KO). The cells were transfected using the Neon™ Electroporation System. The editing efficiency was obtained using the Tracking of Insertion, DEletions and Recombination events (TIDER) tool for KI, and the Tracking of Indels by Decomposition (TIDE) tool, in the case of KO. Single cell cloning was done to obtain pure edited cell lines. The genotypes, GLA gene expression and α-Gal A protein were analyzed.

Results

We established and characterized two edited iPS cell lines. The cells accurately recapitulates the molecular and biochemical characteristics of a FD patient (KO), and normal control cells (KI). To fully validate the cells as disease models, karyotyping and off-targets analysis must be conducted.

Discussion

The development of innovative cell models of LSDs is beneficial to study the pathophysiology of the disease mechanisms. Here, we developed two cell lines through gene editing, and our ultimate aim is to use them as models to study FD disease. New cell models also give contribute for future therapeutic alternatives.

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RESTORING N-ACETYLGLUCOSAMINE-1-PHOSPHOTRANS-FERASE FUNCTION IN MUCOLIPIDOSIS II: AN ANTISENSE OLIGONUCLEOTIDE EXON-SKIPPING THERAPEUTIC APPROACH

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Background

Mucolipidosis II (ML II) is a Lysosomal Storage Disorder caused by GlcNAc-1-phosphotransferase (GlcNAc-PT) deficiency, which impairs the trafficking of lysosomal hydrolases. Of all ML II mutations, c.3503_3504delTC in GNPTAB exon 19 is the most frequent, making it a good target for a personalized therapy. Here, we explored an innovative therapeutic strategy based on the use of antisense oligonucleotides (ASOs). Previously, in ML II patients' fibroblasts, we tested ASOs to induce exon 19 skipping in pre-mRNA, successfully generating an in-frame mRNA. Now, our aim is to determine whether this in-frame transcript leads to increased GlcNAc-PT levels improving ML II cellular phenotype.

Methodology

First, the GlcNAc-PT activity was measured in fibroblasts by a radioactive assay, but activity levels were similar in ML II and control fibroblasts (treated and non-treated) showing that the assay is not proper to measure endogenous levels. To overcome this, we designed 2 constructs: a WT (full GNPTAB cDNA) and a mutant (without the exon 19) that were transfected in HEK293T cells. Then GlcNAc-PT expression will be analyzed by Western Blot (WB). Additionally, we have measured the activity of several lysosomal hydrolases after ASO treatment in control and patient cells. To further help in the validation of this therapy we are also generating a novel GlcNAc-PT antibody in rabbits.

Results

Our results demonstrated that HEK293T cells were able to express both the WT and mutant (without exon 19) constructs. Next, GlcNAc-PT expression for both constructs will be analyzed by WB. We also observed a slight increase in the activity of various lysosomal hydrolases in ML II fibroblasts treated with the ASO, particularly 24 and 48 hours post-treatment, suggesting some level of GlcNAc-PT activity. Regarding the novel antibody, 2 distinct antibodies were generated. However, preliminary results revealed a band of the expected protein size in both control and in ML II patient fibroblasts (unexpected). So, further assays are needed to assess their specificity.

Conclusion

Our ASO therapeutic approach has shown promising results, but further studies are needed for its validation.

UNRAVELING KRABBE DISEASE:TWO UNIQUE CASE DIAGNOSES ACROSS THE AGE SPECTRUM

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Background

Krabbe disease (KD), also known as globoid cell leukodystrophy, is a lysosomal storage disorder resulting from galactocerebrosidase deficiency (GALC). KD presents in three forms: infantile, juvenile, and adult-onset, with the infantile variant being the most severe and prevalent. This report examines two patients: one adult-onset diagnosed through reverse genetic testing and an infantile-onset through biochemical methods followed by molecular analysis.

Methodology

Pt1: A 78-year-old woman exhibiting progressive motor dysfunction and spasticity. MRI imaging revealed white matter abnormalities. A panel for spastic paraparesis identified a homozygous GALC variant, c.927A>C[p.(Leu309Phe)]. Deficient GALC activity determination in leukocytes confirmed variant pathogenicity.

Pt2: A 12-month-old male presenting severe psychomotor delay and marked white matter alterations, with onset at 6 months. The diagnosis followed the classic workup with GALC activity determination, followed by molecular testing using PCR. Additionally, multiplex ligation-dependent probe amplification (MLPA) was necessary to identify the second pathogenic variant.

Results

Pt1: reduced GALC activity confirmed an adult-onset KD. Pt2: Severely reduced GALC activity. It was detected heterozygous pathogenics variants c.896_897delinsAG, p.(Gly299Glu) and c.1162-?_*1718+?del(del30kb) confirming infantile-onset KD.

Discussion

These cases highlight the broad clinical spectrum of KD, with varying outcomes based on the age of onset. While Case 1 remains alive at 80 years, sadly, Case 2 has passed away. A timely diagnosis through enzyme assays is of easy access and vital for a prompt intervention. This is particularly important in instances where hematopoietic stem cell transplantation could potentially slow disease progression, though it is not a definitive cure. Genetic counselling, carrier testing, and prenatal diagnosis are critical for families affected by KD to prevent severe phenotypic recurrence. Although no cure currently exists, advancements in combinatorial and gene therapies provide hope for effective treatments for this devastating disorder.

AN ATYPICAL PHENOTYPE CAUSED BY THE C.-167G>T P.? VARIANT IN THE PMM2 GENE

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Introduction

Congenital disorder of glycosylation type Ia (PMM2-CDG) [MIM 212065] is caused by biallelic pathogenic variants in the PMM2 gene and is typically characterized by developmental delay, hypotonia, and seizures, with symptom severity varying based on age of onset. Recently, a distinct phenotype associated with the c.-167G>T p.? variant in the promoter region has been reported, characterized by prenatal detection of bilateral polycystic kidneys and, later, normal neurodevelopment, with or without hyperinsulinemic hypoglycemia. Here, we present a case of a two-year-old girl with prenatally diagnosed bilateral polycystic kidneys, normal development, and ocular manifestations.

Methodology

Whole exome sequencing (WES) was performed using Illumina Novaseq 6000®. In house bioinformatics pipeline and NGS software were used for an idiopathic renal insufficiency NGS panel variant calling and analysis.

Results

The heterozygous pathogenic variants NM_000303.3:c.-167G>T p.? and c.470T>C p.(Phe157Ser) were detected in the PMM2 gene.

Discussion

The c.-167G>T p.? variant when present in homozygosity or in compound heterozygosity with a pathogenic variant is associated with a phenotype very different from the known classic condition. The two variants established a genetic aetiology for the clinical presentation of this patient. This PMM2 gene pleiotropy was discovered in 2017, with one clinical case that was already published by this Medical Genetics Unit. Early diagnosis allows for timely and appropriate treatment, as well as correct genetic counselling of this pathology with a 25% risk of recurrence.

CONGENITAL DISORDER OF GLYCOSYLATION TYPE IA. DIAGNOSIS THROUGH WES. CASE REPORT

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Congenital disorder of glycosylation type Ia (CDG-Ia) is a rare autosomal recessive, multisystemic genetic disease, caused by mutations in PMM2 (phosphomannomutase 2) gene, that encodes a protein which plays a crucial role in the protein glycosylation process.

The type and severity of problems associated with CDG-Ia vary widely among affected individuals, and even among members of the same family.

Affected infants usually present hypotonia, cerebellar hypoplasia seizures, inverted nipples, abnormal subcutaneous fat distribution, strabismus, developmental delay and feeding difficulties. Distinctive facial features are sometimes present, such as a high forehead, a triangular face, large ears, and a thin upper lip. About 20 percent of affected infants do not survive the first year of life due to multiple organ failure.

Methods

We present a case of a two-year-old girl who presents ataxia, hypotonia, global developmental delay and ponto-cerebellosa atrophy. Whole-exome-sequencing (WES) was performed and bioinformatic analysis was based on clinical information and focused on genes associated with patient´s phenotype (HPO).

Results and Discussion

WES analysis identified two heterozygous missense variants in PMM2 gene: c.422G>A (p.Arg141His) and c.548T>C (p.Phe183Ser), both classified as pathogenic variants, according to ACMG recommendations.

According to the clinical instructions provided, the result was compatible with autosomal recessive Congenital Glycosylation Disorder, type 1a (OMIM #212065). The study of the parents and family members at risk, for the found variants, was recommended.

Subsequently, the younger brother with an identical phenotype, was studied and the same two variants were identified.

CDG may be difficult to diagnose. WES technology allowed us to confirm the diagnosis and to provide the appropriate genetic counseling to this family, proving to be an excellent diagnostic tool.

ILLUSTRATING THE IMPACT OF CLINICAL-LABORATORY INTEGRATION IN THE DIAGNOSIS AND TREATMENT OF INBORN ERRORS OF COBALAMIN METABOLISM

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Introduction

communication between clinicians and laboratory diagnosticians is key for the best outcome in genetic testing. Suspicion of a specific condition based on clinical findings, whenever possible, can help guide the laboratorial assessment, with direct impact on patient care and counselling. This is typically feasible in inborn errors of cobalamin metabolism (IECM), as clinical and analytical correlates may fit on a specific entity (or small group of entities).

Methodology

we describe a clinical case and respective laboratorial approach to diagnosis, including its impact on the direct treatment of the patient.

Results

a 3 year old male patient was referred to Clinical Genetics due to a history of pancytopenia and (vitamin) B12 deficiency. At 2 years old, he presented to the emergency department with acute viral gastroenteritis. Blood tests at this time reveled macrocytic anemia, hypersegmented neutrophils with leukopenia, thrombocytopenia, and proteinuria. Further workup for pancytopenia was remarkable for severe B12 deficiency, adequately treated with parenteral supplementation. Malabsorption was excluded, and correction of nutritional errors did not prevent a steep decrease in B12 levels after this episode. A WES-based NGS sequencing panel for IECM genes was requested, and was negative. After further clinical assessment, and based on the suspicion of Imerslund-Gräsbeck syndrome, WES-based NGS sequencing for the CUBN and AMN genes was requested. These genes had already been tested in the previous panel, and with adequate coverage. In this second test, the pathogenic homozygous c.1006+34_1007-31del, r.844_1006del variant was detected in the AMN gene, confirming the diagnosis. The patient started regular parenteral B12 supplementation with adequate response, without the need of additional measures.

Discussion

this case illustrates that clinical-laboratorial communication is essential in solving diagnoses, even if prior tests were negative for the main suspicion. In this case, discussion of the most likely implicated genes was the key. This molecular diagnosis was proven critical, as it allowed for specific treatment, avoiding unnecessary workup.

ASSOCIATION STUDY OF BCL11A, HBS1L-MYB, AND BETA-GLOBIN GENE CLUSTER POLYMORPHISMS WITH FETAL HEMOGLOBIN (HBF) LEVELS IN A SAMPLE OF WOMEN FROM S. TOMÉ E PRÍNCIPE

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Introduction

Fetal hemoglobin (HbF, α2γ2) represents less than 1% of total hemoglobin in healthy adults. Higher levels of HbF can improve the clinical course of patients with β-thalassemia or sickle cell anemia by reducing the number of symptoms and mortality. It is well established that HbF expression is influenced by three main quantitative trait loci (QTL), namely various polymorphisms in the BCL11A gene, the HBS1L-MYB intergenic region (HMIP) and the β-globin gene cluster. This study aims to explore how different genetic variations in these regions affect HbF levels in a group of healthy adult women from São Tomé e Príncipe, Africa.

Methodology

After extracting DNA from blood collected on filter papers, 99 samples were subjected to polymerase chain reaction (PCR) – restriction fragment length polymorphism (RFLP) or TaqMan PCR to analyze six genetic variants. The association between polymorphisms and HbF was tested using logistic regression in a case-control framework and comparing HbF levels between genotyping groups using non-parametric tests.

Results

The minor allele frequency (MAF) obtained in the total sample was 0.313 and 0.278 for the BCL11A polymorphisms rs11886868 and rs1427407, respectively; 0.035 and 0.045 for the HMIP polymorphisms rs66650371 and rs4895441, respectively; and 0.147 and 0.182 for the polymorphisms HBG2 rs7482144 (XmnI) and rs7924684 (BGLT3), respectively. The association results showed that the minor allele of the two BCL11A polymorphisms presented a significant association ($p < 0.05$) with increased levels of HbF. The major allele of the BGLT3 rs7924684 polymorphism showed a marginally significant association ($0.05 < p < 0.10$) with increased HbF levels, and the two HMIP polymorphisms showed no association ($p > 0.05$) with HbF levels. The rs7482144 (XmnI) polymorphism showed inconsistent results for the two statistical tests.

Conclusions

Concordant with previous studies in other populations of different origins, whether in healthy individuals or patients with β-hemoglobinopathies, this study reveals the significant association with HbF levels for two common BCL11A polymorphisms.

EXPRESSION OF RNF122 IS UPREGULATED IN IDIOPHATIC PREGNANCY LOSSES FROM SECOND TRIMESTER PLACENTAS

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Introduction

Pregnancy loss occurs in 15-20% of all gestations, with 2-5% being recurrent pregnancy losses (RPL). However, in approximately 50% of RPL, the etiology remains idiopathic. The placenta is a complex and vital temporary organ that plays a crucial role in fetal growth and development. As the interface between the mother and the fetus, any disruption in placental function can have profound implications for both maternal and fetal health. RNF122 is a gene expressed in several tissues including the placenta and is associated with key cellular processes such as cell viability, necrosis, and apoptosis. It also functions as a ubiquitin ligase, mediating ubiquitination, involved in protein signalling and degradation.

Methodology

Forty-nine human placental samples from second trimester pregnancy losses (PL) were collected, twenty-nine being classified as controls (known PL causes, e.g. infections, cervical insufficiency and umbilical cord prolapse) and twenty were classified as idiopathic (iPL). Transcriptome analysis was performed using RNA-Seq, on 11 placental samples (6 controls and 5 iPL). Differential gene expression analysis was considered statistically significant with a false discovery rate value (FDR) below 0.05. The results were confirmed by Real-Time qPCR (n=29 controls and n=20 iPL). Additionally, proteins were extracted from10 placentas and western blot for protein quantification is currently in progress.

Results

The RNF122 was the only differentially expressed gene (DEG) identified in transcriptome analysis, comparing controls and iPL placentas from the second trimester of pregnancy. We observed upregulation of RNF122 transcripts in the iPLgroup. Although transcriptional heterogeneity was observed in RNA-Seq, upregulation in iPL was consistently observed in a larger cohort analysed by qPCR. Western blot analysis is ongoing to assess protein levels and DNA methylation at the promoter and enhancer regions of RNF122 is also being conducted.

Discussion

These results suggest that RNF122 may play a role in placental development and function, being a potential molecular biomarker for pregnancy complications. However, further research is needed to elucidate the specific functions of RNF122 in placental biology.

TWO CASE STUDIES IN PRENATAL GENETIC DIAGNOSIS: THE IMPACT OF GENE-DISEASE RELATIONSHIP EVALUATION

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PRENATAL

Introduction

Prenatal testing (PNT) involving genes with limited or unclear gene-disease relationships (GDR) necessitates careful evaluation by group of experts, considering evidence, ethical implications, and potential consequences for fetuses and families. CENTOGENE has established a GDR protocol, based in ClinGen framework for clinical validity of gene-disease associations, that can be used to include or reject genes with limited or unclear GDR in PNT context. Here we described 2 cases of prenatal testing performed for genes KCTD3, for which no OMIM entity is described, and SLC20A2, with limited association with autosomal recessive idiopathic basal ganglia calcification-1.

Methodology

Upon prenatal genetic testing request, variant classification and family history are evaluated before test acceptance. CENTOGENE GDR protocol is often applied when unclear GDR is suspected. Prenatal testing proceeds if all the criteria are met.

Results

We demonstrate two examples of the applicability of CENTOGENE GDR protocol. In the first case, a KCTD3 variant, NM_016121.3:c.166C>T p.(Arg56*) was previously identified in homozygous state in the family. PNT was requested based on positive family history of epileptic encephalopathy and hydrocephalus. After evaluation, KCTD3 reached a strong GDR for autosomal recessive KCTD3-related developmental delay and seizure disorder. PNT was performed, and the variant was detected in homozygous state. In the second case, a SLC20A2 variant, NM_006749.4:c.613+1G>A was previously identified in homozygous state in the family. PNT was requested based on positive family history of global developmental delay, Moyamoya phenomenon and seizures. After evaluation, SLC20A2 reached a moderate GDR for autosomal recessive SLC20A2-related disorder, being also accepted. For this case the variant was detected in homozygous state in the fetus.

Discussion

These cases demonstrate the importance of careful assessment of GDR data, along with the use of genetic data repositories. The implementation of a GDR protocol is of an utmost importance for an accurate interpretation of the genetic findings and may help to close the knowledge gap in genetic research and diagnostics.

Conflict of interest

All authors are employees of CENTOGENE GmbH

FMR1 BEYOND POI: A NOVEL MARKER FOR PREDICTING IVF SUCCESS IN INFERTILE FEMALES

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Introduction

The FMR1 gene premutation (55-200 CGG repeats) has been well-established as a monogenic cause of fragile X-associated primary ovarian insufficiency (FXPOI, #311360). Females with primary ovarian insufficiency (POI) often seek in vitro fertilization (IVF) as a treatment option. However, the global success rate of IVF remains low, even for individuals without POI.To investigate whether FMR1 allelic complexity can serve as a predictor of ovarian reserve function and IVF success in infertile females, we employed the allelic score, a robust measure incorporating total CGG repeat length and AGG interspersion pattern, using the formula developed by our group.

Methodology

This study included 121 infertile females undergoing intracytoplasmic sperm injection (ICSI). FMR1 allelic complexity was assessed following conventional PCR and triplet-primed PCR to determine total CGG repeat length and AGG interspersion pattern, respectively. FMR1 allelic complexity was calculated using the formula previously described by our group. Ovarian reserve markers and IVF outcomes were extracted from clinical records.

Results

Samples were categorized into two distinct groups based on FMR1 allelic complexity. No significant differences in ovarian reserve markers or IVF outcomes were observed between the groups. However, in the dissimilar group, a significant negative correlation was found between the allelic score of allele 1 and both the number of injected metaphase II and two pronuclei (2PN) oocytes. Notably, when the allelic score of allele 1 exceeded 150, fewer 2PN oocytes were observed.

Discussion

Our findings demonstrate a correlation between the allelic score of allele 1 and the number of 2PN oocytes in females within the dissimilar group. Given that the number of 2PN oocytes is a marker of successful fertilization, our results suggest that FMR1 allelic complexity may be a potential predictor of IVF success. These findings highlight the importance of elucidating the role of FMR1 allelic complexity in predicting IVF outcomes and ultimately to development of more effective IVF treatments.

BABIES BORN AFTER PREIMPLANTATION GENETIC TESTING – A MILESTONE IN PGT FOR PAF DISEASE

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Introduction

Preimplantation Genetic Testing (PGT) has emerged as an indispensable tool for preventing the transmission of hereditary diseases, such as transthyretin-related hereditary amyloidosis (OMIM 105210) (PAF). This comprehensive 25y retrospective analysis included a total of 236 couples referred for PGT-PAF, of which 222 underwent at least one ovarian stimulation cycle, culminating in a total of 476 cycles.

Material & Methods

From the total 476 cycles, 69 (14.5%) cycles did not result in embryo biopsy. From the remaining 407 cycles, 2,027 embryos were biopsied. The mean maternal age was 32.6 years. From 1999 to 2019, biopsies were performed on D3 (blastomeres). From 2019 onwards, D5 biopsy (trophectoderm cells) was gradually introduced and became the standard practice. A total of 1730 embryos were biopsied on D3, and 297 were biopsied on D5.

Results

The genetic testing showed that 880 embryos were pathogenic variant free (N), while 998 carried the risk allele for the pathogenic variant (M) and 140 without result (92 due to amplification failure and 48 inconclusive). Of the 880 N embryos, 738 did not undergo ploidy testing, and 142 were further tested for PGT-A, with 59 being aneuploid and 83 euploid. A notable deviation from the expected was observed when the variant was maternally inherited (331N:422M), with a ratio of approximately 1:1.28, while the paternal origin followed an expected 1:1 ratio (549N:576M) ($p<0.044$). A total of 307 embryo transfers (ET) were performed, including 230 fresh and 77 frozen. Among these, 113 resulted in confirmed biochemical pregnancies, leading to 94 ultrasound confirmed and 82 term pregnancies. Of these pregnancies, 65 were singletons, 16 were twins, and one was a triplet, with 5 ongoing singletons pregnancies. A total of 100 babies were born (46 males and 54 females).

Conclusion

PGT prevents the transmission of pathogenic variants to offspring, offering families the opportunity to have children free from the risk of hereditary diseases. This technology serves as a powerful tool in modern reproductive medicine, granting affected families the prospect of a future unburdened by genetic conditions.

POSTERS

SESSION III

TP53 VARIANT INTERPRETATION: CHALLENGES AND IMPLICATIONS FOR PATIENT MANAGEMENT

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Introduction

Germline TP53 variants are traditionally linked to Li-Fraumeni syndrome (LFS) and are typically diagnosed through family history and specific cancers. Nowadays, the use of widespread cancer panels has revealed TP53 variant carriers who do not meet classic criteria. Despite established guidelines for TP53 variant classification, some variants, such as c.845G>A p.(Arg282Gln), remain challenging to interpret. This study aimed to analyze this specific variant by assessing its frequency within our cohort, examining family phenotypes, and reviewing existing classification data.

Patients and methods

Review of all patients who underwent TP53 gene testing and tested positive for the c.845G>A variant. Data collected included clinical characteristics such as gender, personal and family cancer history, and tumor features. Additionally, we reviewed available data and functional studies regarding the c.845G>A variant.

Results

From 4352 index patients tested for TP53 variants, 7 (0.16%) harbored the c.845G>A variant. Preliminary analysis of clinical data shows variability in phenotypes compared to classical TP53 phenotypes. Although data review found conflicting results, in a new functional assay, the mutant protein demonstrated a loss of transcriptional activity on key target genes in both yeast and lymphoblastoid cell lines.

Discussion

Should this variant be classified as likely pathogenic based on functional data? Such classification would significantly impact surveillance strategies, risk reduction measures and family planning. Current TP53 surveillance guidelines already place pressure on the national healthcare system and raise concerns about the psychological challenges for patients.

Although its association with classical LFS is unclear, the potential for this variant to act as a low-penetrance allele raises concerns about cancer risk. A comprehensive understanding of modifier factors and enhanced collaboration among centers are essential for refining patient care.

DRAFTING THE GENOMIC PROFILE OF ORAL CANCER, AIMING TOWARDS PERSONALIZED TREATMENT AND FOLLOW-UP

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CANCER GENETICS

Introduction

Oral cancer (OC) is the most common among head and neck cancers, and frequently has an aggressive behavior, with relapses and metastasis. This study aimed to characterize the whole genome of OC samples, identifying key altered chromosomes and genes to better understand the disease's molecular mechanisms. We also explored the potential of using liquid biopsies in the follow-up of these patients.

Methodology

Genomic characterization of 32 oral cancer tissue samples was conducted using aCGH. Additionally, cfDNA from 174 plasma samples was quantified at multiple time points during treatment. Principal Component Analysis (PCA) was used to reduce copy number variation data dimensionality, retaining components explaining at least 70% of the variance. These components were used in a logistic regression model to predict disease stage, as well as in a k-means cluster analysis, which identified two clusters. The association between these clusters and disease stages was evaluated using Fisher's exact test. Additionally, linear mixed models were applied to cfDNA data, and the variation slopes of cfDNA over time were computed and related to disease stage, assessing the differences through the Kruskal-Wallis test.

Results

We observed a uniform pattern of chromosomal deletions across patients, with a higher incidence in stage IV. Amplifications, while more heterogeneous, showed significant changes in chromosomes 3, 5, and 8, varying by disease stage. Principal component analysis of cytogenetic band variations extracted five principal components, which were then used in a logistic regression to differentiate between stage groups. The model was statistically significant (p=0.019) with an accuracy of 64.5%, which improved to 77.4% (p=0.002) when patient age was included. Amplification data revealed two distinct clusters (silhouette = 0.694), which were associated with disease stage (p=0.023). Analysis of cell-free DNA in blood showed a trend toward differences (p=0.066) in the rate of change across stages.

Discussion

Our findings demonstrate the feasibility of monitoring OC patients using cfDNA. Combining liquid biopsy analysis with tissue genomic characterization offers a more comprehensive tumor profile and improved patient stratification.

LOCALLY ADVANCED RECTAL CANCER GENOMIC PROFILE – PRELIMINARY RESULTS

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Introduction

The current locally advanced rectal cancer (LARC) treatment guidelines involve the use of neoadjuvant therapy, which has shown variable and unpredictable tumor response. There is an urgent need to identify biomarkers to define tailored therapeutic strategies. The main objective of this study was to characterize the (cyto)genomic profile of LARC using Array Comparative Genomic Hybridization (aCGH) and karyotyping.

Methodology

Tumor tissue samples collected from biopsies of 21 rectal cancer patients were subjected to DNA extraction, followed by analysis using aCGH. Primary cell cultures were also established from tumor samples for karyotyping analysis. Additionally, the WiDr cell line, derived from colorectal adenocarcinoma and obtained from ATCC, was used.

Results

aCGH revealed gains and losses across the majority of chromosomes, with losses frequently observed in chromosomes 5, 8, 10, 12, and Y, and gains in chromosomes 3, 13, 14, 20, and X. Several important genes and signaling pathways related to carcinogenesis are mapped to these regions. The WiDr cell line exhibited a triploid set of chromosomes, with an average of 70 chromosomes per metaphase. In addition to numerical changes, this cell line also displayed a wide variety of structural alterations and complex rearrangements, such as del(4)(q32.1), der(5)t(5;13)(p15.3;q12), der(13;13)(q10;q10), and del(X)(p11.3). Aneuploidies of whole chromosomes and unbalanced structural rearrangements were the most common cytogenetic alterations observed in primary rectal tumor cell cultures.

Conclusions

Multiple chromosomal alterations have been detected in LARC. Further investigations are required to establish a clinical correlation with the response to neoadjuvant therapy and identify predictive biomarkers.

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IMPORTANCE OF COMBINED METHODOLOGIES IN DIAGNOSING HEMATOPOIETIC AND LYMPHOID TUMORS

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CANCER GENETICS

Tumors of hematopoietic and lymphoid tissues represent a diverse group of hematologic malignancies, including myeloproliferative neoplasms and B-cell neoplasms, like chronic lymphocytic leukemia and multiple myeloma. The differential diagnosis of these conditions relies on a combination of morphological assessment, immunophenotyping, and genetic analysis, aiming for accurate diagnosis, risk stratification, and effective treatment selection. Genetic diagnostics encompass molecular biology and cytogenetic techniques. Both conventional cytogenetics and advanced methods like fluorescence in situ hybridization (FISH) and microarrays play crucial roles. Each method provides unique insights into the tumor's characteristics, essential for differentiating among various hematologic malignancies. But are all these tools necessary to obtain a differential diagnosis? In addition, can the type of sample and the use of cultures influence the results?

In order to answer these questions, karyotyping in peripheral blood (D1) and bone marrow blood (D2 and D3), high-definition microarray, and the genetic test FISH were carried out in three male patients – D1, D2, and D3 – with suspected chronic lymphocytic leukemia, multiple myeloma, and myeloproliferative neoplasm, respectively.

For the three individuals, karyotypes of normal constitution 46,XY[30] were observed. However, the results of FISH and microarray showed the presence of chromosomal gains and losses, and in the case of D2, the presence of the FGFR3/IGH fusion gene was also observed by FISH. The discrepancy in results is partly due to differences in the resolution of the techniques, but also to the type of sample used and possibly the infeasibility of the abnormal clone in culture, leading to a natural selection of the normal clone.

This work aims to discuss the importance of the combined performance of several methodologies, as well as the achievement of different results in the direct biological sample and after cell culture, and how these results can influence the patient's diagnosis, leading to a more accurate result that may contribute to the prognosis of the pathology as well as to therapeutic guidance.

KMT2A GENE REARRANGEMENTS IN LEUKEMIA: A STEPWISE STRATEGY FOR GENETIC CHARACTERIZATION

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Introduction

Genetic characterization of leukemias is essential for understanding the disease biology, prognosis, and treatment selection. A strategic combination of molecular and cytogenetic techniques allows identification of genetic alterations and enables accurate classification of leukemia subtypes, while optimizing time and costs.

Methodology

We report two cases: a 17-year-old male with suspected T-cell acute lymphoblastic leukemia and a 68-year-old female with chronic myelomonocytic leukemia, possibly progressing to an acute phase. Bone marrow samples from both underwent genetic analyses: karyotyping, fluorescence in situ hybridization (FISH) with targeted probes and next-generation sequencing (NGS) using TruSight RNA Fusion panel.

Results

In the first case, the karyotype revealed a trisomy 7 with other alterations. FISH detected a loss of 3'KMT2A probe, while NGS identified a KMT2A::TNRC18 fusion transcript. In the second case, a t(5;11)(q32;q23) translocation was detected in the karyotype, consistent with a KMT2A rearrangement. FISH showed a deletion of 3'KMT2A probe, and NGS revealed a KMT2A::MATR3 fusion transcript.

Discussion

Karyotyping revealed chromosomal abnormalities but lacked specificity for full genetic characterization. FISH suggested KMT2A involvement but could not differentiate between deletions and translocations. Due to the broad range of potential KMT2A fusion partners, FISH analysis using break apart probes is insufficient for a complete characterization. NGS allowed the detection of specific fusion transcripts in both cases, providing crucial genetic insights.

A tiered approach is ideal. Karyotyping is an affordable first step, offering an overview of chromosomal abnormalities. FISH is useful for detecting specific rearrangements, although limited by being a targeted analysis. NGS should be performed in cases where karyotyping and FISH analysis are insufficient for a complete characterization. This strategy balances cost with the need for comprehensive genetic characterization, aiding diagnosis, prognosis, and therapeutic decisions.

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CLINICAL CASE REPORT: EMERGENCE OF T(7;11)(P15;P15) IN A CASE OF CHRONIC MYELOID LEUKEMIA

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CANCER GENETICS

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm defined by the presence of BCR-ABL oncogene as the result of the reciprocal translocation t(9;22)(q34;q11.2). It is manageable with therapy, but when treatment fails, the disease may be progressing into a blast crisis.

We present a case of a female patient with an initial diagnosis at 2022 of de novo CML and the presence of t(9;22), under therapy and monitorization.

This year we received a new bone marrow sample after no response to therapy for karyotype to screen for possible acquisition of additional chromosomal alterations.

Method

Cytogenetic analysis was performed on metaphases stained with GTG bands and FISH analysis with the Vysis probe for t(9;22) BCR/ABL rearrangement.

RT-PCR was carried out to identify transcripts p210, p190 and p230 and BCR/ABL quantification (p210) was performed by real-time quantitative PCR (RQ-PCR) for the p210 transcript.

Results

The initial sample was tested by both conventional karyotype and FISH analysis as well as RT-PCR, at 2022. All the techniques confirmed a de novo CML case.

Follow-up proceed every 3-4 months, by BCR/ABL quantification (p210), without achieving a Major Molecular Response (<0.1% IS BCR/ABL).

Due to the lack of response to therapy, the new bone marrow sample was received by our laboratory to perform conventional karyotyping and BCR/ABL fusion gene sequencing, which was not possible to be performed.

Karyotype, however, revealed a second chromosomal alteration involving chromosomes 7 and 11, t(7;11)(p15;p15), in addition to the previous t(9;22)(q34;q11.2): 46,XX,t(7;11)(p15;p15), t(9;22)(q34;q11.2)[20]

This translocation is rare and mainly observed in AML or CML in blast crisis [Aurelia M. Meloni-Ehrig, t(7;11)(p15;p15) NUP98/HOXA13, Atlas Genet Cytogenet Oncol Haematol. 2016-12-01].

Discussion

In this patient, the absence of response to therapy likely resulted from this clonal evolution. Karyotype analysis is still an essential part of a patient's workup in particular moments of the disease, as it allows to guide further testing, narrowing the molecular targets, and, therefore, achieve a better orientation of treatment for this patient.

FUNCTIONAL RNA STUDY: AN USEFUL TOOL FOR DIAGNOSIS OF IDIOPATHIC PULMONARY FIBROSIS – A CASE REPORT

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Introduction

Idiopathic Pulmonary Fibrosis (IPF; OMIM #616373) is a rare, irreversible, and progressive disease of the lungs, associated with older age, smoking habits and other environmental exposures. Common genetic variants and rare mutations contribute to its etiology.

RTEL1 gene is a DNA helicase which plays a crucial role in DNA replication, genome stability, DNA repair and telomere maintenance. Heterozygous variants in this gene are associated with IPF and are inherited in an autosomal dominant pattern.

In this study, we present a case report from a patient who showed clinical signs characteristic of IPF.

Methods

Female 48-year-old with personal and familial history of IPF, whose mother died with IPF. Whole-Exome Sequencing (WES) was performed and bioinformatic analysis was focused on our laboratory 16 genes panel, associated with IPF.

Results and Discussion

WES study revealed the presence of a heterozygous silent variant, in exon 1 RTEL1: c.102G>A (p. Gln34=). The variant was classified as unknown clinical significance variant (VUS), according to ACMG guidelines. This silent variant was predicted to affect the splicing of exon 1. Functional RNA study was performed.

The variant found in genomic DNA, was not detected in the RNA sample. The absence of this variant RNA allowed us to prove the monoallelic expression of RTEL1 gene, confirming that this variant is responsible for the exon 1 skipping.

This study allowed us to reclassify this variant as likely pathogenic, confirming the IPF diagnosis.

Functional RNA studies are an useful tool to aid in the classification of variants that may affect splicing, specially in cases in which segregation studies aren't available.

SKIN SPOTS AND GENETIC MOSAICISM: THE POTENTIAL OF ORAL MUCOSAL SNP ARRAY

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

Skin spots are frequently associated with mosaic genetic alterations, hence the importance of a detailed clinical assessment of the patient and genetic testing (PMID: 32972601).

Here we present a clinical case in which a genetic testing allowed the detection of a mosaic genetic alteration in a girl with neuropsychomotor developmental delay and skin hypochromic spots.

Methodology

The woman's peripheral blood sample was analysed using the SNP array technique, Affymetrix Cytoscan 750K, and the genomic hybridization results were analyzed using the ChAS 4.2 Software.

Results

The SNP array revealed a female genomic profile with:

- a mosaic interstitial loss (between 1 and 2 copies) on 13q14.2q34, of 66,334 Mb and involving 163 protein coding genes;
- and an heterozygous terminal loss at 13q34, of 916 Kb and involving the protein coding genes TMCO3, TFDP1, ATP4B, GRK1, TMEM255B, GAS6, RASA3, CDC16, UPF3A, CHAMP1.

This result is compatible with the presence of two cell lines: one cell line corresponding to around 80% of the cells and with loss of the terminal region 13q34qter and the other cell line, corresponding to around 20% of the cells, with the loss of the 13q14.2qter region, which may correspond to the presence of mosaicism of structural rearrangements of chromosome 13, namely a ring chromosome 13.

Discussion

The results obtained revealed the existence of a mosaic which may correspond to chromosomal rearrangement(s) of chromosome 13.

The proportion of the mosaic can vary between different tissues, resulting in different phenotypes. Therefore, in cases where patients exhibit skin spots and no genetic alterations are found in peripheral blood samples, it would be interesting to consider performing SNP array analysis on the buccal mucosa when deemed relevant, as obtaining an epithelial sample by scraping the oral mucosa is significantly less invasive for patients than performing skin biopsies, which is particularly important considering that most patients are children.

WHOLE EXOME SEQUENCING (WES) IN NEONATAL HEALTH: EARLY DIAGNOSIS AND PRECISION CARE

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Introduction

Exome sequencing is a powerful tool for detecting rare or hereditary genetic diseases in newborns. Since many health problems in the neonatal period have genetic etiology, with manifestation on the first days of life, such as metabolic diseases, rare syndromes, or immune disorders, exome analysis can assist in early diagnosis, which is crucial for timely treatment, prognosis and counseling.

Material/Methods

Blood sample was collected from a 4-week-old male newborn with hypocalcemia and hypomagnesemia. Genomic DNA was extracted using a standard protocol. WES technique was performed on the NextSeq 550 (Illumina) using Twist Comprehensive Exome (Twist Bioscience). Bioinformatic analysis was focused on a hypoparathyroidism panel of genes and patient clinical phenotype.

Results/Discussion

Our laboratory targeted hypoparathyroidism panel and phenotype-based analysis was completed after 5 working days of the sample collection and identified one heterozygous variant in FAM111A gene: c.1706G>A (p.Arg569His) (rs587777011). According to ACMG guidelines we classified this variant as Likely Pathogenic. The clinical information provided allowed us to conclude that this variant was compatible with autosomal dominant Kenny-Caffey Syndrome, 6 (OMIM#127000).

Our laboratory rapid-WES is an effective and time-saving tool and can be used as a first-choice diagnostic option in critically ill newborns.

A FAMILY DIAGNOSIS AFTER A 6 KB SNP ARRAY DELETION

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

In clinical practice, it is rare for an untargeted SNP array study to establish a diagnosis when variants smaller than several tens or hundreds of kilobases are identified. Here we present the case of a 6 Kb deletion.

Methodology

Pregnant woman with a history of learning difficulties, a posterior fossa arachnoid cyst similar to Dandy-Walker malformation, epilepsy until ten years old, asymmetrical pseudobicuspid aortic valve and bilateral inguinal hernia corrected by surgery. An Affymetrix Cytoscan 750K SNP array was performed, followed by MLPA analysis (MRC Holland) for FLNA gene. Then MLPA was conducted in the sample from the patient's mother who has epilepsy and periventricular heterotopias.

Results

The SNP array identified a heterozygous interstitial loss of 6 Kb in Xq28, involving exons 35 to 48 of the FLNA gene, a loss which is at the resolution limit of the technique. To confirm this loss, an MLPA analysis was carried out, which confirmed the loss and revealed a exon 4 duplication.

Discussion

The FLNA gene is associated with periventricular heterotopia 1 [MIM 300049] among other diseases. The SNP array did not cover exons 26 to 34, so it was undetermined if other exons were involved. MLPA of this gene (exons 4, 11, 22, 25, 29, 38) confirmed the deletion of exon 38 and revealed a heterozygous duplication of exon 4.

This loss is classified as probably pathogenic, and also probably corresponds to the aetiological diagnosis of the phenotype in both patients. Genetic counselling was recommended; there is a 50% recurrence risk for this X-linked dominant disease in the future newborn female.

The availability of detailed clinical information and the analyst's critical thinking enabled the identification of an under-resolution variant in a SNP array study that was subsequently confirmed as being relevant in defining the risk of recurrence.

AP4E1 AS A DIFFERENTIAL DIAGNOSIS OF L1CAM IN A NEONATAL CONTEXT – A WES BASED APPROACH

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Introduction

L1CAM gene is associated with the most frequent form of congenital hydrocephalus, often with spastic paraparesis, adducted thumbs and stenosis of Sylvius' aqueduct. Although we are more than a decade into the NGS era, this study is still frequently requested in perinatal contexts in males due to the apparent specificity of the phenotype. In our clinical case the phenotype suggested pathology associated with L1CAM, but when this study was negative the subsequent WES identified an aetiological diagnosis whose phenotype may overlap with that of the classically known pathology.

Methodology

Newborn with noncommunicating hydrocephalus, aqueductal stenosis, thin corpus callosum, short thumb, hitchhiker thumb and generalised hypotonia. Complete studies of L1CAM gene (SNVs and CNVs). Then whole exome sequencing performed at Illumina Novaseq 6000, data processed with in-house pipeline, analysis by NGS software.

Results

Negative results for the L1CAM gene. Both c.1658G>A p.(Trp553*) and c.3214_3215del p.(Leu1072Alafs*10) variants were detected, in heterozygosity, in the AP4E1 gene (chr.15). These variants are expected to introduce a premature stop codon, being predicted a truncated protein and/or its loss of expression due to mRNA decay.

Discussion

Before segregation studies, one variant was classified as probably pathogenic and the other as of undetermined clinical significance. The phenotype of the pathology associated with the AP4E1 gene, spastic paraplegia 51 [MIM 613744], can overlap with that of the L1CAM gene, as in the clinical case [PMID: 25552650] with hydrocephalus, stenosis of the aqueduct of Sylvius, hypotonia and psychomotor development delay. In order to establish the aetiological diagnosis, it will be necessary to clarify through family segregation studies that the variants are in trans.

Even in the presence of apparent phenotypic homogeneity, an approach using high-throughput sequencing, through whole exome sequencing, covering all protein-coding regions, could be a useful addition, if not a substitution, to a single gene approach.

STEP BY STEP: A NAT10 VARIANT CASE REPORT

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

Heterotaxy with left isomerism is a congenital condition marked by abnormal organ arrangement, including cardiac defects, polysplenia, venous irregularities, and external dysmorphisms. It can be linked to ciliopathies, genetic disorders impacting cilia crucial for cell signaling and movement in development. NAT10 gene variants have been associated with heterotaxy in patients with ciliary aplasia.

Case Presentation

We present a case of a 3-year-old girl diagnosed with left atrial isomerism. Prenatal assessments indicated a complete atrioventricular septal defect, atrioventricular congenital block, persistence of the left superior vena cava and left abdominal isomerism, all confirmed after birth. She also has situs inversus and an abnormal venous return, with both the superior and inferior vena cava draining into the coronary sinus, while the pulmonary veins drained into the right atrium.

Her first surgery, performed on day two of life, involved pacemaker implantation, that later migrated into the abdominal cavity, making programming difficult. At age two, she underwent a second surgery to correct the drainage of the left pulmonary veins and the atrioventricular defect, along with a new pacemaker implantation.

Besides cardiovascular issues, she presents dysmorphic features, including epicanthus, bilateral inguinal hernia, scoliosis and short stature. She was diagnosed with obstructive sleep apnea syndrome, requiring non-invasive ventilation in the first two years, which has been discontinued last year.

Amniocentesis during pregnancy showed no pathogenic variants in chromosomal studies or ciliopathy panel. After birth, genetic consultation led to trio exome sequencing, identifying two variants in the NAT10 gene (c.1588A>G and c.1760G>A) of unknown significance. Segregation study revealed that the variants were present in trans.

Discussion

There's a previously reported case of a patient with a homozygous NAT10 variant in a patient with heterotaxy and ciliary aplasia.

Conclusion

Although the role of NAT10 gene's still uncertain in the pathophysiology of cardiac related diseases, there are studies that already demonstrate its involvement in cardiovascular system.

UNUSUAL COMPLEX REARRANGEMENT IN A HEALTHY WOMAN

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Introduction

Structural chromosomal rearrangements occur during meiosis or mitosis, commonly involving two chromosomes, with the possibility of more chromosomes being involved in complex anomalies. Translocations in an apparently balanced state are relatively frequent, with an estimated of 1/560-1/1100 carrier individuals in the overall population. These rearrangements present as cytogenetic anomalies without abnormal phenotypic features yet associated with reproductive risks (increased risk of infertility, spontaneous miscarriage, and/or chromosomal imbalance in the embryo, with possible foetal malformations and eventual childbirth).

Methodology

We report a 20-year-old woman referred to Medical Genetics for preconception counselling due to a complex chromosomal rearrangement, which was first karyotyped at patient's own prenatal amniocentesis and later confirmed at birth using peripheral blood. The previously described chromosomal rearrangement was cytogenetically confirmed and better characterised during her revaluation as an adult. The patient did not present any associated phenotypic features or medical issues.

Results

The structural chromosomal anomaly comprised of an apparently balanced complex rearrangement between four autosomes (chromosomes 3, 4, 8 and 13), described as 46,XX,t(3;8;4;13)(3pter 3q13.3::8q21.2 8qter;8pter 8q12::3q23 qter;13qter 13q21.2::4p15.2 4qter;13pter 13q21.2::3q13.3 3q23::8q12 8q21.2::4p15.2 4pter). In other words, this karyotype revealed the presence of multiple rearrangements consisting of four derivative chromosomes, encompassing translocations between chromosomes 3 and 8, and chromosomes 4 and 13, and a derivative chromosome 13 with segments of the other chromosomes.

Discussion

Previous attempts of medically assisted reproduction with preimplantation genetic diagnosis have demonstrated low success rates of chromosomally balanced embryo implantation in couples with complex rearrangements (estimated at 1/50 embryos). Thus, our patient expectably presents with higher reproductive risks regarding the observed four-chromosome complex rearrangement, in comparison to the associated increased risk in a two-chromosome structural anomaly.

THE URGENCY FOR A CHANGE IN GENETICS HEALTHCARE PROVISION: VIEWS FROM PORTUGUESE MEDICAL GENETICISTS

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DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

Introduction

In the last decades, genetics has experienced significant technological advancements worldwide. Medical genetics specialty exists in Portugal since 1998. However, the increasing demand for genetic consultations is coupled with severe limitations of the few existing genetic services. This study aimed to promote sharing and discussion among genetic medical professionals, to outline concrete actions to address gaps in clinical practice.

Methodology

A qualitative methodology for this exploratory, descriptive study by conducting three online focus groups. The focus groups were conducted with 19 specialists in medical genetics. The data were analyzed using the thematic analysis method to extract the main themes from the discussions.

Results

Four conceptual themes emerged: (i) framing Portuguese genetic services in light of the European context; (ii) improvement of medical genetics education and population literacy; (iii) transforming of medical genetics services; and (iv) operationalizing the change. The results demonstrated that increasing training resources and strengthening multiprofessional teams by hiring more genetic professionals, such as clinical geneticists, molecular geneticists, and other genetic specialists, is crucial to enhancing the responsiveness of genetic services. Integrating medical genetics into all specialties and primary care, as well as updating the national network of medical genetics, are critical points for increasing equity and enabling healthcare to be provided more fairly. Including other medical genetics professionals such as genetic counsellors, nurses and psychologists also plays a significant role in providing comprehensive and quality care.

Conclusion

Urgent action is imperative to bring about a transformation in Genetics healthcare services. A new care model with a multidisciplinary, multiprofessional and collaborative approach within healthcare teams is necessary in Portugal. The findings are compiled as recommendations to support the profession moving forward that can be applied to other healthcare contexts worldwide.

NEED FOR INTEGRATION OF GENETIC COUNSELORS IN THE PORTUGUESE HEALTHCARE: THEIR ADDED VALUE FROM THE MEDICAL GENETICISTS' PERSPECTIVE

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Introduction

This work explores the potential role and benefits of integrating genetic counselors (GCs) into the Portuguese healthcare system. Genetic counseling has evolved into a specialized discipline globally, playing an increasingly significant role in personalized medicine and healthcare systems. Despite its expansion and integration into public health systems in various countries, Portugal has yet to fully incorporate this profession. The study addresses the increasing demand for genetic services and how GCs could enhance the quality and efficiency of care, specifically from the perspective of Portuguese medical geneticists.

Methodology

A qualitative methodology was employed, using two focus groups composed of medical geneticists from various public genetic services in Portugal. This approach allowed for an in-depth exploration of the participants' views on the integration of GCs. The discussions were transcribed and analyzed thematically to identify key patterns and insights.

Results

The research identified perceived advantages of incorporating GCs in areas like workload reduction, professional complementarity, and better patient care. However, it also highlighted several barriers, such as limited training capacity and the lack of official recognition for GCs within the national healthcare framework. Medical geneticists were generally in favor of incorporating GCs into multidisciplinary teams, but there was a consensus that systemic changes, including policy and legislation, would be necessary to support such integration.

Discussion

The study sheds light on the significant potential of GCs to improve genetic healthcare services, targeting further investigation into the practical implementation and long-term outcomes of incorporating GCs into Portugal's healthcare system.

The authors argue that integrating GCs in Portugal could significantly enhance personalized genetic services and empower individuals to make informed decisions about their health. However, the study emphasizes the urgency of the systemic changes, including policy adjustments and sustained investment in education and training programs, are essential to make this integration a reality.

PERICENTRIC HETEROCHROMATIC INVERSION OF CHROMOSOME 1: BENIGN VARIATION OR FERTILITY IMPACT?

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REPRODUCTIVE GENETICS

Introduction

Chromosome inversion inv(1)(p13q21) is classified as a benign heteromorphism, without functional or phenotypic effects; its impact on reproductive disorders e.g. infertility/recurrent miscarriages are subjects of debate. Heteromorphic variants may interfere with chromosome pairing, synapsis, and recombination during meiosis, leading to unbalanced gametes. Also, pericentric inv(1) has been linked to azoospermia, potentially impairing spermatogenesis and disrupting critical regions essential for reproductive function. Still, the exact mechanism by which pericentric heterochromatic inversion affects human infertility is unclear, and the relationship between specific inv(1) breakpoints and clinical outcome needs clarification.

Methodology

We report two clinical cases referred for conventional and molecular cytogenetic studies:

1 - male presenting with azoospermia;

2 - female presenting with an abortion and failure to induce ovulation in the context of medically assisted reproduction.

In both cases, conventional GTL-banding karyotyping was performed on metaphases obtained from peripheral blood according to standard procedures. In case 1, multiplex ligation-dependent probe amplification (MLPA) was applied according to the SALSA MLPA MRC-HOLLAND® protocol for detection of Y chromosome microdeletions.

Results

Case 1 revealed a 46,XY,inv(1)(p13q21) karyotype and normal amplification for the Y chromosome by MLPA. Case 2 revealed a 46,XX,inv(1)(p13q21) karyotype; the partner's karyotype was normal, 46,XY.

Discussion

This study describes two clinical cases of pericentric heterochromatic inversion on chromosome 1 and investigates the link between chromosomal heteromorphisms and human infertility. It analyses the effects of the inversion's breakpoints position and compares these findings with previously published cases. Additionally, potential mechanisms impacting male and female infertility are discussed. The authors emphasize the critical role of conventional cytogenetic analysis in the study of chromosomal heteromorphic regions and highlight the need for improved characterization of these regions to fully understand their clinical value.

FRAGILE SITE 16Q22 IN A MAN AND WOMAN FACING FERTILITY CHALLENGES: A CYTOGENETIC APPROACH

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Introduction

Fragile sites are specific chromosomic regions that are prone to breakage and may manifest as gaps, constrictions, or breaks in the chromosome structure. They can be classified as common or rare based on their frequency in the population, and their chemical induction during cell culture. Nevertheless, there are spontaneous breaks that may occur without any induction, eg. FRA16B, located at 16q22. Typically inherited, this region contains an expanded 33-bp AT-rich minisatellite repeat, and is the most prevalent fragile site, occurring in ±5% of the European population. Although fra(16)(q22) is usually benign, some publications have linked it to infertility, miscarriages, and severe oligozoospermia.

Methodology

We report two cases: a 39 yo male diagnosed with oligoasthenoteratozoospermia and a 40 yo female with recurrent miscarriages, both referred to our Cytogenetics Laboratory. Following standard protocols, chromosome analysis was performed using GTL banding on metaphases from peripheral blood cultures and MLPA panel P360 (MRC Holland) was done in the male patient.

Results

The male´s karyotype revealed, in 14 of 54 examined cells, multiple abnormalities at fra(16)(q22), including chromatid breaks, translocations, 16q22-qter deletions, and radial/triradial configurations. MLPA analysis confirmed normal amplification of all analysed Y chromosome regions. The female's karyotype revealed, in 14 of 30 examined cells, anomalies also involving fra(16)(q22), such as chromatid breaks, 16q22-qter deletions/duplication, and the presence of a 16q22-qter acentric fragment.

Discussion

The authors describe two individuals with infertility phenotypes exhibiting spontaneous 16q22 fragile sites. Studies indicate that infertility cannot be fully linked to the presence of fra(16)(q22), however, it may possibly contribute to a higher occurrence of structural abnormalities leading to unbalanced gametes. Further investigations are required for complete understanding of the molecular mechanisms associated with this fragile site and its impact on infertility. Cytogenetics is still crucial in the diagnosis of fra(16)(q22), improving genetic counselling of these patients.

A RARE CASE OF FULL TRISOMY 22 SURVIVING TO LATE GESTATION

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Introduction

Trisomy 22 is the second most common autosomal trisomy found in miscarriages. In contrast, is rarely observed in 2nd or 3rd trimester, or in live-born infants, especially non-mosaic cases. Clinical features typically include intrauterine growth restriction (IUGR), cardiac and craniofacial malformations (PMIDs: 16239659,38540405). We report a case of full trisomy 22 in a pregnancy submitted to medical termination of pregnancy (TOP) at 20 weeks.

Methodology

Array Comparative Genomic Hybridization (aCGH) (Agilent,4×180 K) was performed on chorionic villi sample (CVS) (13 weeks). Karyotype was done on amniotic fluid sample (AFS) and on fetal tissues (calcaneus, skeletal muscle, liver, myocardium and spleen). Parents' karyotypes were also performed. Fluorescence in situ Hybridization (FISH) using the TUPLE 1 probe was carried out on AFS.

Results

The aCGH revealed a trisomy 22 in a male fetus. To rule out the possibility of confined placental mosaicism, karyotype was performed on AFS, showing an unbalanced Robertsonian translocation involving chromosomes 13 and 22. Parental Karyotyping indicated maternal inheritance, being the result: 46,XY,der(13;22)(q10;q10),+22 mat. First trimester ultrasound showed increased neck translucency, absent nasal bones and subcutaneous edema. In the 2nd trimester was identified flattened facial profile, microcephaly, partial agenesis of the corpus callosum and severe tetralogy of Fallot. FISH on AFS further confirmed trisomy 22. Following TOP, karyotype was performed on different tissue samples, to exclude tissue mosaicism. Trisomy 22 was confirmed in all analyzed tissues.

Discussion

Our study presents a rare case of an alive-fetus with full trisomy 22 persisting beyond the 1st trimester. Karyotype, performed in different tissues, allowed to prove a non-mosaic trisomy present in the fetus. Given the phenotype variability and the few cases reported in the literature with full trisomy 22, ultrasonographic findings provide valuable clues for the genotype-phenotype correlation and provides the opportunity to evaluate the most common features associated to this condition. Among others, IUGR with severe cardiac anomalies (our fetus showed severe tetralogy of Fallot), facial and dysmorphic ears were described in the literature associated with mosaic or full-trisomy 22 (PMID: 38540405). A detailed autopsy will be crucial for a comprehensive anatomy assessment, helping in the management of this rare condition.

NON-INVASIVE PRENATAL TESTING FOR THE DETECTION OF LOW-EXPRESSIVITY MOSAIC TRISOMY 21

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Introduction

non-invasive prenatal testing (NIPT) has become a staple in prenatal screening of common aneuploidies, typically outperforming conventional screening methods in terms of sensitivity and specificity. However, performance of NIPT in the detection of mosaic forms of such aneuploidies, which correspond to approximately 5% of cases, is still unclear.

Methodology: we describe a clinical case where paired-end sequencing-based NIPT was performed, followed by invasive prenatal testing for conventional karyotype and SNP-array of amniocytes.

Results

a healthy couple (34 year old female, 39 year old male) opted for NIPT at 10 weeks gestational age. With an 18% fetal fraction, the log likelihood ratio (LLR) was 66.55 for trisomy 21 (T21), and 73.41 for mosaic T21 (positive if > 2.5), concluding that there was a high risk for T21. Conventional karyotype on cultured amniocytes revealed mosaic T21, with 3% of trisomic cells (47,XX,+21[4]/46,XX[126]). The first and second trimester ultrasounds, as well as a fetal echocardiogram, were unremarkable. Repeat amniocentesis was performed at 21 weeks. A direct amniocyte QF-PCR revealed a 1:1 chromosome 21 pattern, while a SNP-array detected 12% T21 (arr(21)×3 [0.12]). Conventional karyotype on cultured amniocytes revealed 5% trisomic cells (47,XX,+21[3]/46,XX[61]). The pregnancy was terminated, and the fetal exam identified dysmorphic features, including macroglossia and low-set ears, without major organ anomalies.

Discussion

this case shows that NIPT technologies may also detect low expressivity mosaic forms of common aneuploidies, which would likely be missed in conventional screening methods, further pointing towards near-perfect sensitivity. While the phenotype of mosaic trisomy 21 can vary between asymptomatic to complete Down syndrome, it is unpredictable as it depends on the expressivity of the mosaic in the different tissues. This poses huge challenges for genetic counselling and pregnancy management.

PRENATAL RARE BALANCED COMPLEX CHROMOSOMAL REARRANGEMENT IN A NEWBORN WITH APPARENTLY NORMAL PHENOTYPE

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Introduction

Constitutional structural abnormal rearrangements (CCRs) involving at least three breakpoints on two or more chromosomes are dubbed complex chromosome rearrangements. CCRs are rare cytogenetically abnormalities, balanced or unbalanced, often arising as de novo events. Carriers of apparently balanced CCR can be phenotypically normal or manifest clinical abnormalities, due to loss or gain of genetic material, gene disruption in the breakpoints or gene positional effects due to the genome reorganization. Phenotypic abnormalities increase with the number of CCR associated breakpoints. Most of balanced rearrangements occur in females, and about half of them are inherited.

Methodology

A 37 years old pregnant woman was referred for amniocentesis at 22W + 4D gestation due to cervical insufficiency.

Aneuploidy screening and fetal karyotype were requested. Later, fetal microarray test and parental karyotypes were performed.

Results

Quantitative multiplex-PCR revealed absence of aneuploidies.

Fetal karyotype was described as:

46,XX,der(2)(2pter 2q23::4q33 4qter),der(4)(4pter 4q23::14q24.3 14qter),der(14)(14pter 14q24.3::4q23 4q33::2q23 2qter).

Fetal microarray study and maternal karyotype were normal (paternal sample not yet available).

Discussion

The karyotype analysis identified a CCR involving chromosomes 2, 4 and 14. CCRs are rarely observed and often are associated with mental retardation and congenital abnormalities (usually unbalanced forms), recurrent spontaneous abortions and infertility (usually balanced forms). The child was born premature (~26 weeks), without phenotypic abnormalities.

Microarray in the prenatal sample excluded small imbalances, compatible with the otherwise normal phenotype of the baby. However, careful follow-up of the child´s development is paramount. Conventional karyotype in this case contributed with valuable information for future counselling, where implications of this rearrangement for the child and offspring should be addressed, namely the higher risk for pregnancy loss and abnormal outcomes. Parental karyotypes are fundamental for familial guidance and recurrence risks.

THROMBOEMBOLISM CORRELATED WITH PROTEIN S DEFICIENCY – USEFULNESS OF MOLECULAR STUDY

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Introduction

Autosomal dominant protein S deficiency (PSd) is one of the major genetic risk factors in Venous Thrombosis. The prevalence of PSd resulting from variants in PROS1 is low and probably underdiagnosed. Diagnosis can be difficult since: functional tests are poorly responsive to mild deficiencies; some variants can occur with normal plasma levels; and different conditions can temporarily lower protein S levels. PROS1 molecular study improves the diagnostic yield of PSd, allowing genotype/phenotype correlation and identification of patients at risk.

Methodology

We have studied PROS1 in patients with (i) PSd deficiency or boarderline plasma levels or (ii) normal plasma levels but severe thrombosis history. The study included 268 subjetcs (208F,60M;2-92Y) with Pulmonary Embolism (3%), Venous Thrombosis (51.5%), Arterial Thrombosis (6%), obstetric complications (20.9%); 18.7% had a family history of thrombosis. Functional studies: Free and Total PS plasma levels, confirmed in 2 different samples. Molecular studies: PCR, Sanger sequencing and MLPA.

Results

Of the 268 subjects, 206(77%) had PSd and 62(23%) had normal PS levels. 27 different variants in PROS1 were identified in 39.9%(n=107), twelve not yet reported. These variants included: missense/nonsense (77.8%), small deletions (3.7%) and duplications (7.4%), large deletions (7.4%) and regulatory (3.7%). PROS1 p.Val191Cysfs*6, p.Cys226*, p.Ala307Cysfs*22, p.Arg451*, p.Glu465*, two large deletions were found in severe PSd. Two compound heterozygosities were found in three cases with severe PSd. The PS Heerlen variant (p.Ser501Pro) was found in 39% of subjects and correlated with a milder phenotype. Within the individuals with variants, 13.1% had normal PS levels.

Discussion

The type and location of the variant correlated with phenotypic severity. PROS1 molecular study allowed the identification of variants in all severe and moderate deficiencies, but the success in milder deficiencies was lower. Also, we found variants in patients with normal levels. Due to its added value, a molecular study should be considered to identify patients with history of thrombosis and “normal/borderline” levels of this protein.

UNLOCKING THE SECRETS OF DEPRESSION: HOW EXCISE-INDUCED MYOKINES AND GENETICS SHAPE BRAIN HEALTH

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MULTIFATORIAL DISORDERS

Introduction

Depression affects over 300 million people and is the leading cause of global disability. Characterized by persistent sadness, guilt, suicidal thoughts, and fatigue, it disrupts daily life and relationships. While antidepressants and therapy are common treatments, physical exercise has emerged as a powerful non-pharmacological option.

Research in rodent models highlights the potential of voluntary running to reduce depression-like behaviors. Exercise stimulates physiological responses across tissues, particularly skeletal muscle, which releases neurotrophic myokines like cathepsin B and Metrnl. A 2016 study found that exercise increases circulating cathepsin B, promoting neurogenesis in the hippocampus, a region crucial for mood regulation.

Methodology

In this study, two female C57BL/6 mice were anesthetized, and the gastrocnemius muscle was excised for analysis. The muscle was homogenized in RIPA buffer and centrifuged. The supernatant was stored at -80 °C for future analysis. Protein concentration was quantified using the Pierce™ BCA Protein Assay kit to ensure accurate measurements.

For the Western blot, proteins were denatured by boiling in SDS buffer, transferred to PVDF membranes, and incubated with primary and secondary antibodies. Visualization was performed with ECL reagent.

Results

Our findings confirmed the presence of the myokines Metrnl and cathepsin B in the resting gastrocnemius muscle. The intensity of the protein bands detected directly correlated with the amount of protein loaded onto the gel.

Discussion

The discovery of myokines like cathepsin B and Metrnl in mouse skeletal muscle provides a promising basis for future research on how exercise might enhance their role in depression treatment. These myokines regulate inflammation and metabolism in both mice and humans. Since exercise alleviates depression-like symptoms in mice, this paves the way for further exploration in human studies, particularly as the same genes are involved. Genetic variations may play a critical role in individual responses to exercise, influencing both susceptibility to depression and the effectiveness of exercise-based interventions.

THE ANGIOTENSIN-CONVERTING ENZYME INSERTION / DELETION POLYMORPHISM IN THE PORTUGUESE POPULATION AND THE RISK OF COVID-19

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Introduction

COVID-19 is a new complex multisystem disease caused by SARS-CoV-2. The renin-angiotensin-aldosterone system (RAAS) plays an important role in the pathogenesis of COVID -19. Angiotensin-converting enzyme-1 (ACE1) and angiotensin-converting enzyme-2 (ACE2) play a vital role in maintaining RAAS homeostasis. A recent meta-analysis showed that the D allele of the ACE1 gene was clearly related to an increased risk of COVID-19 severity. Our aim was to contribute to the knowledge of the frequency of alleles I (alternative allele) and D (ancestral allele) in Portuguese populations.

Methods

A case-control study was carried out using a candidate gene association study (CGAS) approach. Participants were recruited from a convenience sample representative of the general Portuguese population and a sample of COVID-19 patients diagnosed at the HFF. The stratification of the clinical severity of SARS-CoV-2 /COVID-19 infection was in accordance to the Norma Nº013/2022 of 28.11.2022 of the Direção Geral de Saúde (DGS). The ACE1 gene polymorphism was investigated in all the samples by PCR, visualized in agarose gel electrophoresis. The study were authorized by the INSA and HFF Health Ethics Committee and carried out in accordance with the 1975 Declaration of Helsinki.

Results

From the demographic and clinical characterization, the phenotypes of the cases and control groups for the study were defined. PCR optimization was carried out and like other authors we confirmed that in heterozygous samples the D allele is preferentially amplified, implicating the use o an insertion specific PCR whenever the DD genotype was observed.

Conclusion

The ACE1 gene contains an intronic deletion and insertion (D/I) polymorphism, which has been associated with severe forms of COVID-19. The D/I of ACE1 (rs4646994) is among the most common human polymorphisms of the ACE1 gene and is possibly responsible for variable levels of the ACE1 protein. The DD genotype results in the highest plasma level of ACE1. As different populations can show variations in the prevalence of certain genetic variants, an important additional piece of information for interpreting the results of genotype-phenotype association studies is to know the frequency of the variant in question in the population of the ecogeographic region of the study.

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JG's participation was in the context of her Bachelor's degree in Biology from the Instituto Superior de Agronomia of the Universidade de Lisboa.

DF's participation was in the context of her Master's degree in Applied Microbiology from the Faculdade de Ciências da Universidade de Lisboa.

INFLUENCE OF GENETIC VARIANTS IN GENES RELATED WITH BLOOD CELL ADHESION TO VASCULAR ENDOTHELIUM ON SICKLE CELL ANEMIA SEVERITY AND RISK OF MALARIA

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ORGAN-SPECIFIC GENETICS: BLOOD

Introduction

Sickle cell anemia (SCA) is a genetic disease caused by the c.20A>T mutation in the HBB gene, which origins the abnormal hemoglobin S and sickle-shaped erythrocytes. The disease is characterized by chronic hemolytic anemia and inflammation, vaso-occlusive crisis, and recurrent infections. It is particularly prevalent in the African continent where malaria is also a public health issue. This study aimed to investigate if the genetic variability of genes related with blood cell adhesion to vascular endothelium, such as CD36 and ICAM-1, can affect SCA manifestations in angolan pediatric patients, as well as their risk of getting malaria.

Methodology

The study enrolled 65 patients with SCA, between 3 and 12 years old, living in Luanda or Caxito, Angola. Their clinical, biochemical and hematological phenotypes were gathered in follow-up appointments with the pediatrician. Eleven polymorphic regions were genotyped in CD36 and ICAM-1 genes using PCR, nested-PCR, Sanger sequencing, and fragment analysis by capillary electrophoresis. SPSS was used for statistical data analysis.

Results and Discussion

Concerning the CD36 gene, the genotype/phenotype association study revealed that the rs3211891_C allele is negatively associated with the mean hemoglobin concentration (p=0.013). SCA patients with genotypes containing the C allele have a significantly lower hemoglobin concentration than the others, indicating a more severe anemia. In the ICAM-1 gene, genotypes containing the rs5491_T allele are associated with a significantly lower number of erythrocytes (p=0.044) and higher LDH levels (p=0.020) than wild-type genotypes. This suggests that it worsens anemia and hemolysis rates. Conversely, genotypes containing the rs5496_A allele are associated with a higher hemoglobin concentration (p=0.009) and erythrocyte count (p=0.036), as well as with a lower percentage of reticulocytes (p=0.044) than others, indicating an improvement in the severity of SCA, namely in anemia level and hemolysis rate. Regarding malaria, SCA patients with genotypes containing the ICAM-1 rs5494_T allele show a 5.63-fold higher risk of malaria compared to those with wild-type genotypes (OR=5.63, 95%CI 1.07-29.73, p=0.028).

Conclusion

This study provided a valuable contribution to the understanding of the genetic modifiers of vascular cell adhesion and its influence on SCA pediatric phenotypic variability and risk of contracting malaria, in Angola.

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PHENOTYPIC VARIABILITY AND CARDIOVASCULAR RISK IN HEREDITARY AORTOPATHIES: GENETIC AND CLINICAL FINDINGS FROM A CARDIOGENETICS COHORT

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Introduction

Hereditary connective tissue disorders (HCTDs) involve various genetic conditions affecting the cardiovascular (CV), ocular and skeletal systems. Aortopathies are common in HCTDs and can lead to life-threatening complications; those can be classified as syndromic (Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS), Ehlers-Danlos syndrome (EDS) type IV) or nonsyndromic (familial thoracic aortic aneurysm and dissection (TAAD)).

Methods

Retrospective medical review of patients with molecularly confirmed aortopathies or variants with potential clinical relevance, followed in our Cardiogenetics Clinic from 2015-2024.

Results

We evaluated 55 patients (54% male, n=30); 26 adults (43-years-old± 14) and 29 pediatrics (9-yo ± 5), 71% index cases. Overall, 96% had disease related signs and/or manifestations. CV phenotype (61%) included aortic dilation (53%), aortic dissection (10%), mitral valve prolapse (20%) and bicuspid aortic valve (3%). Endovascular repair was performed in 19% of cases. Extracardiovascular signs and manifestations included skeletal (72%) and ocular (56%) anomalies. Among patients without CV manifestations, 62% were pediatric; 1 case began follow-up at 14yo after parent's sudden death.

Molecular analysis revealed variants related to MFS (74%); LDS (11%); EDS (7%); TADD (4%); Lujan-Fryns (1%) and Combined Osteogenesis imperfecta and EDS type 2 (1%); 2 patients had dual diagnoses. Variant classification (n=39) within this group of genes (FBN1, SMAD3, SMAD2, TGFBR2, COL5A1, COL3A1, COL1A2, LOX, ACTA2, MED12) revealed 51% likely pathogenic variants and 41% pathogenic variants; 3 cases (8%) classified as VUS were also considered ("hot-VUS").

Conclusions

This study highlights genetic heterogeneity, variable expressivity and phenotypic overlap within HCTDs, particularly concerning CV involvement. MFS was the most common condition in this cohort. In this group, 39% of patients did not present CV findings, suggesting that molecular variants alone may not predict CV outcomes, suggesting the need for additional biomarkers to better stratify risk.

Multidisciplinary management is essential for early optimal care, ensuring genetic counselling and family risk assessment.

A CANDIDATE GENE STUDY ON THE ROLE OF MTHFR, IRF6, PAX7, AND TP63 SNPS IN SUSCEPTIBILITY TO NON-SYNDROMIC OROFACIAL CLEFTS IN A PORTUGUESE POPULATION

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MULTIFTORIAL DISORDERS

Background

Non-syndromic orofacial cleft (NSOC) is among the most prevalent birth defects, with an incidence of approximately 1/1000 live births worldwide, varying according to the population and type of defect. The aetiology of NSOC remains elusive, being considered a multifactorial trait, dependent on a complex architecture of multiple genetic variants and environmental factors. This study aimed to verify the role of four single-nucleotide polymorphisms (SNPs) in susceptibility to NSOC in Portuguese patients, using a candidate gene approach.

Methods

Study sample comprised 254 individuals, 120 cases, and 134 controls. All non-consanguineous individuals and the control group had no family history of orofacial cleft. 64.2% of included patients had unilateral cleft lip and palate, 21.7% had bilateral cleft lip and palate, 5.83% had cleft lip only and 8.33% had cleft palate only. SNPs in the MTHFR (rs1801133), IRF6 (rs642961), PAX7 (rs742071), and TP63 (rs9332461) genes were studied using TaqMan probes. Statistical analysis was performed with the Chi-square test and odd ratios with a 95% confidence interval. The adjusted p-value was calculated using the Benjamini-Hochberg false discovery method.

Results

Hardy-Weinberg equilibrium was confirmed for the four SNPs. There were no statistically significant differences in allele frequencies between controls and cases. A statistically significant association was only highlighted for TP63 gene for the genotypic (p=0.04) and over-dominant model, (p=0.016; OR 1.897; CI 95% [1.144-3.147]), revealing a protective effect for heterozygotes.

Conclusion

No evidence of association of the evaluated SNPs with NSOC was found in this study. However, the results suggest a heterozygotes advantage for rs9332461 in TP63 gene.

HEREDITARY THROMBOCYTOSIS: A FAMILIAL CASE WITH TRIPLE-NEGATIVE SCREENING FOR SPORADIC ESSENTIAL THROMBOCYTHEMIA MUTATIONS

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Introduction

Hereditary Thrombocythemia (HT) is a rare inherited disorder presenting with clinical features similar of Essential Thrombocythemia (ET), a clonal acquired disorder with inherent risk for malignant transformation. Although most ET patients have driver mutations in JAK2 (V617F), CALR and MPL genes, about 12% of ET cases are triple-negative. Rare cases of HT associated to germline MPL variants, not related with canonical MPL variants, have been described. Here we present the study of a family with a history of thrombocytosis without any of the canonical MPL mutations associated to sporadic ET.

Methodology

The study focused on two siblings (9 and 15 years old), attending paediatric haematology clinic, due to persistent thrombocytosis (≥450 x109/L). The older had a history of mucosal haemorrhage, mild splenomegaly; the bone marrow biopsy presenting dysmorphic megakaryocytes and mild hypercellularity, suggestive of a myeloproliferative neoplasm (NMP). Standard NPM study was done by Polymerase Chain Reaction (PCR) and Sanger Sequencing (SS) targeting JAK2 V617F, CALR exon 9 and MPL exon 10. NGS with the Oncomine Myeloid Panel (Thermo Fisher Scientific) was performed to look for non-canonical MPL variants. DNA samples were extracted from hair follicles to access the germline nature by SS. The siblings were referred for further clinical genetic counselling. DNA from buccal swabs (patients's parents) and hair follicle (from a third brother) was used to segregate the variant in the family.

Results

Standard NMP screening of the two affected siblings revealed triple-negative results; further NGS assay revealed a known HT-related pathogenic MPL variant – NM_005373.3:c.317C>T p.(Pro106Leu) in exon 3 with a 99% variant allele frequency (VAF). Hair follicle analysis confirmed homozygosity in both siblings. Both asymptomatic parents and third brother were heterozygous for the familial variant.

Discussion

The identification of the MPL p.(Pro106Leu) variant confirms its role in HT in this family. HT follows an autosomal dominant inheritance pattern, with a gene dosage effect, associated with moderate-to-severe thrombocytosis in homozygosity and absent or mild disease in heterozygosity. Differentiating HT from sporadic ET is critical, as HT does not carry the same risk of malignant transformation. Extended screening for non-canonical MPL variants in triple-negative thrombocytosis ensures an accurate diagnosis and adequate familial follow-up.

Declaration of interests: the authors declare no competing financial interests.

INVESTIGATING NEW GENETIC CAUSES OF HEREDITARY SPASTIC PARAPLEGIA IN PORTUGUESE FAMILIES

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NEUROLOGICAL DISEASES

Introduction

Hereditary spastic paraplegias (HSPs) are a complex group of neurodegenerative disorders, mainly characterized by progressive spasticity and weakness in the lower limbs. Advances in molecular genetics allowed the identification of more than 80 spastic paraplegia (SPG) loci/genes. Our group has conducted a population-based survey in Portugal, giving us a unique HSPs cohort to study. After testing for HSPs genes, several families (65/183 families, 36%) still missed a genetic diagnosis. Following up on this work, we aim to identify new genetic causes in these undiagnosed families.

Methodology

In this study, we performed whole-exome sequencing (WES) using an Illumina platform in two to three patients from each of 20 selected families. Bioinformatic analyses were performed on RD-Connect Genome-Phenome Analysis Platform (GPAP) to filtrate and prioritize genes/variants.

Results

We have discovered relevant candidate variants segregating in 9 out of the 20 families selected for this study. In four of these families, the variants were found in genes (KCNA2, TUBB2A, PYCR2, TOR1A) previously associated with other neurological conditions, potentially expanding the clinical spectrum of these disorders. In one family, we identified a novel variant in AMFR, the gene responsible for SPG89. Four families presented new candidate causal genes with functions in lipid and mitochondria metabolism. The variants identified in these genes were classified as variants of uncertain significance; thus, functional experiments are required to validate their pathogenicity and impact on protein function.

Discussion

With this work, we expect to increase the diagnostic yield in our Portuguese cohort of HSPs, while finding new causal variants and genes. Ultimately, our results can open new perspectives on the molecular mechanisms underlying HSPs, contributing to the identification of therapeutic targets.

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THE ROLE OF KINESINS IN HEREDITARY CEREBELLAR ATAXIA AND SPASTIC PARAPLEGIA: INSIGHTS FROM PORTUGUESE FAMILIES

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Introduction

Hereditary cerebellar ataxia (HCA) and hereditary spastic paraplegia (HSP) are rare neurodegenerative disorders with overlapping phenotypes and genetic causes. Among causal genes, variants in kinesin (KIF)-coding genes (KIF1A/SPG30; KIF1C/SPG58/SPAX2; KIF5A/SPG10) significantly contribute to both disorders. In this study, we performed molecular and clinical characterization of HCA and HSP families with causal variants in KIF genes.

Methodology

We analysed 250 families with HCA and 100 families with HSP, identified during the Portuguese population-based survey; and 49 families with HSP, from the CHUdSA. Clinical and family history were previously collected by the same neurologists. Variants were identified by gene panel or exome sequencing, using Illumina platforms.

Results

Overall, KIF genes were responsible for HCA or HSP in 7 families (7/399, 1.75%). In the HCA cohort, one family presented a heterozygous de novo variant in KIF1A (1/250, 0.4%), while two families each had a novel homozygous variant in KIF1C (2/250, 0.8%). In the HSP cohort, we identified a novel, heterozygous, de novo variant in KIF1A in one family (1/149, 0.67%), along with four different heterozygous variants in KIF5A within five families (5/149, 3.35%) - two novel and two previously reported. Patients with KIF1A variants (both in the protein motor domain) exhibited neuropathy; however, the HCA patient showed an extensor plantar response without spasticity, while the HSP patient had a complex phenotype, including intellectual disability. Both KIF1C variants were associated with spastic ataxia; one patient had brainstem atrophy. KIF5A variants were associated with pure or complex HSP. Interestingly, members of one family, all carrying the same KIF5A variant, showed different phenotypes: either neuropathy or HSP.

Discussion

Consistent with other studies, KIF5A variants were the most frequent and KIF1A de novo variants were found in both HCA and HSP. Remarkably, two novel KIF1C variants were identified only in HCA; KIF1C variants are a very rare cause for both disorders.

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RACGAP1 AS A POTENTIAL GENETIC MODIFIER OF AGE OF ONSET IN ATTRV30M AMYLOIDOSIS

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NEUROLOGICAL DISEASES

Introduction

Transthyretin (ATTRv) amyloidosis is a life-threatening genetic disease caused by variants in the TTR gene, that lead to the buildup of amyloid fibrils in various organs. The most common pathogenic variant is Val30Met (ATTRV30M) and affected individuals exhibit considerable variation in symptoms, disease severity, and age of onset (AO). Recent studies from Liz MA. and collaborators (data not published) showed that RAC1 has a determinant role in neurodegeneration in a mouse model of ATTRv amyloidosis. In this study, our aim was to identify genetic variants of RAC1 and its Guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) that might be associated with ATTRV30M amyloidosis AO variability.

Methods

DNA samples were collected from 175 individuals with ATTRV30M amyloidosis (92 early-onset (AO<40yrs) and 83 late-onset cases (AO>50yrs)). Genotyping was performed using Precision Medicine Diversity Array and data concerning RAC1 and its GAPs and GEFs were extracted and analyzed. We found that RACGAP1 (RAC1 GAP) SNP rs615382 was significantly associated with late disease onset. Functional studies were performed and for this purpose plasmids with the reference (C) and alternative (A) alleles of rs615382 were transfected into HEK293T and SH-SY5Y cell lines, followed by dual-luciferase reporter gene assays to assess the SNP's influence on RACGAP1 expression.

Results

RACGAP1 SNP rs615382 showed a mean increase of 20 years in heterozygous individuals and 34 years in homozygous carriers in disease onset. The A-allele, associated with late-onset ATTRV30M, significantly increased RACGAP1 expression by approximately 35% and 20% in SH-SY5Y and HEK293T cells, respectively.

Discussion

The herein presented results confirmed that the alternative A genotype increases RACGAP1 expression, potentially leading to greater RAC1 inactivation and reduced neurodegeneration.

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