29.ª REUNIÃO ANUAL
29th ANNUAL MEETING

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SOCIEDADE PORTUGUESA DE GENÉTICA HUMANA

ABSTRACT BOOK
LIVRO DE RESUMOS

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ISBN: 978-989-35476-0-3
Depósito Legal: 556361/25

Concessão gráfica e impressão: Lousanense, Artes Gráficas

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29.ª REUNIÃO ANUAL
29th ANNUAL MEETING

SOCIEDADE PORTUGUESA
DE GENÉTICA HUMANA

PORTUGUESE SOCIETY
OF HUMAN GENETICS

20-22 NOVEMBER 2025

CONVENTO SÃO FRANCISCO
COIMBRA

Caros Colegas,
Caros Participantes,

É com uma imensa **alegria e emoção** no coração, alicerçadas na firme **razão e sentido de responsabilidade**, que vos damos as calorosas **boas-vindas** à nossa **29ª Reunião Anual da Sociedade Portuguesa de Genética Humana (SPGH)**. Que bom regressar a este lugar tão acolhedor, o Convento de São Francisco, na nossa romântica Coimbra!

Depois de um ano pautado por um trabalho conjunto e dedicado entre a Direção e as diversas comissões, voltamos a reunir-nos para o **maior evento** da Genética Humana em Portugal.

Apresentamos, mais uma vez, um **programa de excelência**, meticulosamente delineado pela Comissão Científica (CC), sob a coordenação da incansável Paula Jorge e a co-coordenação do Sebastião Rodrigues. Este trabalho contou com múltiplos e valiosos contributos da CC e seus colaboradores, da Direção, das Comissões de Bioética, de Políticas Públicas e Educação para a Genética, entre outros.

O nosso muito obrigado a todos!

Pela Direção, estamos particularmente felizes por termos conseguido concretizar aqui dois objetivos a que nos propusemos para 2025: a **aproximação da SPGH às Associações de Doenças Raras** e o **reforço dos laços com os países lusófonos**.

Mantivemos o formato **intenso e enriquecedor** da manhã de quinta-feira **pré-congresso**, aberta e gratuita para todos, com duas sessões educativas em paralelo com os nossos **tradicionais** clubes de Genética Clínica e Dismorfologia e de Citogenética e Genética Molecular. De seguida, teremos uma Sessão Plenária dedicada à assinatura de protocolos e um almoço convívio com as Associações de Doenças Raras, **fortalecendo pontes essenciais**.

O programa formal da Reunião conta com mais de **15 palestrantes** nacionais e internacionais de renome, mesas-redondas estratégicas como o *Building Bridges* com a Sociedade Portuguesa de Hematologia e a sessão sobre Genética Humana nos Países Lusófonos, com foco especial em Cabo Verde. Teremos ainda **21 comunicações orais** e **90 *posters*** selecionados de entre os resumos submetidos. Todos os *posters* serão ativamente discutidos pelos elementos da CC e seus colaboradores.

Trazemos ainda uma **novidade estimulante**: os participantes poderão, com círculos autoadesivos que são fornecidos, assinalar o(s) *poster(s)* da sua **preferência**, promovendo um **diálogo científico ainda mais participativo**!

O nosso **profundo agradecimento** estende-se a todos os que contribuíram de forma decisiva, em particular:

- À **ASIC**, pela coordenação impecável da organização do evento em estreita colaboração com a Direção da SPGH.
- Aos membros da **Comissão Local**, que nos bastidores, garantiram toda a logística e o bom funcionamento da reunião.
- Ao **Convento de São Francisco** e à **Câmara Municipal de Coimbra**, pelo acolhimento.
- Aos **representantes das Associações de Doenças Raras**, por nos honrarem com a sua presença.
- E, claro, o nosso **muito obrigado** a todos os **sponsors**, cujo apoio recorrente e fundamental torna possível a realização da nossa reunião anual.

Esperamos corresponder às vossas expectativas e que todos saiam daqui **enriquecidos** pela **ciência de excelência** e, acima de tudo, pelo **caloroso reencontro de amigos**!

A Direção Efetiva de 2025

Sérgio B Sousa
Janet Pereira
Cláudia Oliveira

Dear Colleagues,
Dear Participants,

It is with **immense joy and profound emotion**—underpinned by firm rationale and a sense of responsibility—that we extend a warm welcome to the **29th Annual Meeting of the Portuguese Society of Human Genetics (SPGH)**. How wonderful it is to return to this most welcoming venue, the Convento de São Francisco, in our romantic Coimbra!

Following a year marked by **dedicated and collaborative work** between the Board and the various committees, we once again gather for the largest event in Human Genetics in Portugal.

We are delighted to present, yet again, an **outstanding programme**, meticulously developed by the Scientific Committee (SC) under the coordination of the tireless Paula Jorge and the co-coordination of Sebastião Rodrigues. This work benefited from multiple, valuable contributions from the SC and its collaborators, the Board, the Bioethics, Public Policy and Genetics Education Committees, among others.

Our deepest thanks to all!

The Board is particularly pleased to have achieved two objectives here that we set for 2025: **bringing the SPGH closer to Rare Disease Associations** and **strengthening ties with Portuguese-speaking countries**.

We have maintained the **intense and enriching format** of the pre-congress Thursday morning, which is open and free to all, featuring two parallel educational sessions alongside our traditional Clinical Genetics and Dysmorphology Club and Cytogenetics and Molecular Genetics Club. Following this, we will hold a Plenary Session dedicated to protocol signings and a networking lunch with the Rare Disease Associations, fostering **essential connections**.

The formal Meeting programme features **over 15 renowned national and international speakers**, strategic round tables such as the **Building Bridges** session with the Portuguese Society of Haematology and the session on Human Genetics in Portuguese-speaking countries, with a special focus on Cabo Verde. We will also have **21 oral presentations and 90 posters** selected from the submitted abstracts. All posters will be **actively discussed** by members of the SC and their collaborators.

We are also introducing an exciting new feature: participants will be able to mark their preferred poster(s) using **self-adhesive circles** provided, promoting an even more **participatory scientific dialogue!**

Our **profound gratitude** extends to all those who contributed decisively, in particular:

- To **ASIC**, for the impeccable coordination of the event organisation, in close collaboration with the SPGH Directorate.
- To the members of the **Local Committee**, who, behind the scenes, ensured all logistics and the smooth running of the meeting.
- To the **Convento de São Francisco** and the **Coimbra City Council**, for their hospitality.
- To the representatives of the **Rare Disease Associations**, for honouring us with their presence.
- And, of course, our sincere thanks to all **sponsors**, whose recurrent and fundamental support makes our annual meeting possible.

We hope to **meet your expectations** and that everyone leaves here enriched by the excellent science and, above all, by the **warm reunion of friends!**

The 2025 SPGH Board

Sérgio B Sousa
Janet Pereira
Cláudia Oliveira

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SCIENTIFIC **PROGRAMME**
PROGRAMA CIENTÍFICO

THURSDAY 20th NOVEMBER

09:00-14:00 ▸ **REGISTRATION**

10:00-12:00 ▸ **PRE-MEETING PARALLEL SESSIONS**

▸ **PROFESSIONAL**

▸ **Session 1:** *Cytogenetics and Molecular Genetics Club* (in Portuguese)
Chairs: **Isabel Carreira**, Faculty of Medicine, University of Coimbra | **Teresa Fidalgo**, Department of Hematology, ULS Coimbra

▸ **Session 2:** *Dysmorphology and Clinical Genetics Club* (in English)
Chairs: **Joana Rosmaninho-Salgado**, Department of Medical Genetics, ULS Coimbra | **Sadaf Farooqi**, School of Clinical Medicine, University of Cambridge, UK

▸ **EDUCATIONAL**

▸ **Workshop 1:**
"Genetic testing interpretation and report – beyond ACMG classification" (in Portuguese)
Júlia Baptista, [1]Molecular Pathology Department, King's College Hospital, Synnovis, London; [2]Faculty of Life Sciences and Medicine, King's College London, UK

▸ **Workshop 2:**
"Gene therapy – from basic to advanced" (in Portuguese)
Luís Pereira de Almeida, **Rui Nobre**, CNC-UC & CIBB & Centro de Excelência em Terapia Genética (GeneT), Coimbra, Portugal

12:10-14:00 ▸ **PRE-CONGRESS PLENARY SESSION**

12:10-12:30 ▸ **CORPORATE SYMPOSIUM I ILC**
Chair: **Cristina Iglésias da Costa**, CEO da ILC- Instrumentos de Laboratório e Científicos, lda
"Redefining Sequencing: From Genomics to 5D Multiomics with AVITI24"
Antonio Fadda, Field Applications Scientist, Element Biosciences

12:30-13:00 ▸ **COOPERATION PROTOCOL SIGNATURE SESSION** (in Portuguese)
Sérgio B. Sousa, President SPGH | **Peter Jordan & Milena Paneque**, CPPEG-SPGH | **Mónica Correia**, Sociedade Portuguesa de Literacia para a Saúde (SPLS) | **Paulo Gonçalves**, União das Associações das Doenças Raras de Portugal (RD-Portugal) | **Rui Barros Silva**, Federação das Doenças Raras de Portugal (FEDRA)

13:00-14:00 ▸ **LUNCH WITH PATIENT ASSOCIATIONS**

14:00-14:15 ▸ **OPENING AND WELCOME**
Sérgio B. Sousa, Janet Pereira, Cláudia Oliveira, Paula Jorge, e representantes da Câmara Municipal de Coimbra, Universidade de Coimbra e Unidade Local de Saúde de Coimbra

14:15-14:50 ▸ **KEYNOTE LECTURE 1**
Chairs: Carla Oliveira | Paula Jorge
"Genome editing methods to interrogate variant function at scale"
Gregory Findlay, The Genome Function Laboratory, The Francis Crick Institute, London, United Kingdom

14:50-15:50 ▸ **INVITED SYMPOSIUM I:** Gene therapy for eye disorders
Chairs: Ana Luísa Carvalho | Rui Nobre

14:50-15:20 ▸ *Invited talk ISI-1: "Gene Therapy for Inherited Retinal Diseases"*
Bart Leroy, Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium

15:20-15:50 ▸ *Invited talk ISI-2: "Gene Therapy in Portugal: Luxturna Outcomes and the Role of IRD-PT"*
João Pedro Marques, Department of Ophthalmology, ULS Coimbra, Coimbra, Portugal

15:50-16:10 ▸ **CORPORATE SYMPOSIUM II QUILABAN**
Chair: **Pedro Pinto**, Quilaban
"CRINGENES: What is different compared to other genomic newborn screening projects?"
Judit García Villoria, Sección Errores Congénitos del Metabolismo-IBC.BGM, Hosp Clínic, Barcelona

16:10-17:30 ▸ **COFFEE-BREAK | POSTER VIEWING AND DISCUSSION**

17:30-18:20 ▸ **SELECTED ORAL COMMUNICATIONS I**
Chairs: Ilda Ribeiro | Fábio J. Ferreira

OC 01 17:30-17:40	João Nogueira	*Dissecting and Predicting the impact of enhancer variants in type 2 diabetes using high-throughput mutagenesis and reporter assays*
OC 02 17:40-17:50	Ana Joana Duarte	*Generation of cellular models for fabry disease: unlocking the potential of IPSCS and gene editing*
OC 03 17:50-18:00	Rafael Graça	*Dissecting non-coding regulation of LDLR and PCSK9 in familial hypercholesterolaemia*
OC 04 18:00-18:10	André Besouro-Duarte	*Differential allelic expression case-control study unveils missing prostate cancer heritability and pleiotropy with breast cancer*
OC 05 18:10-18:20	Frederico Pena	*Establishment of a scalable neuronal reporter model of Machado-Joseph disease based on gene-edited patient cells as a platform for high-throughput drug repurposing*

18:30 ▸ **SPGH GENERAL ASSEMBLY**

FRIDAY 21st NOVEMBER

09:00-10:00 ▸ **SELECTED ORAL COMMUNICATIONS II**
Chairs: Jorge Saraiva | José Ferrão

OC 06 09:00-09:10	Ricardo Amorim	*Clinical trajectories and healthcare burden of hereditary diffuse gastric cancer: prevention versus treatment in european centres*
OC 07 09:10-09:20	Mariana Tomásio Neves	*MUTYH carrier frequency in portugal: navigating the data gap with what we've got*
OC 08 09:20-09:30	Cristina Santos	*Lessons learned from a portuguese inherited retinal disease research cohort*
OC 09 09:30-09:40	Ana Miguel Pinto Capela	*Understanding the genetic basis of hereditary cardiovascular diseases in portugal: a retrospective analysis across two centres*
OC 10 09:40-09:50	Sofia Quental	*RNA analysis as a first-line diagnostic approach for neurofibromatosis type 1: a six-year institutional experience*
OC 11 09:50-10:00	Filipa Oliveira	*Liquid biopsies and (EPI)genomic characterisation of glioblastoma*

10:00-11:00 ▸ **BUILDING BRIDGES:** Sociedade Portuguesa de Hematologia (in Portuguese)
Chairs: Janet Pereira | Celeste Bento
"Advancing Gene Therapy for Red Blood Cell disorders – How Far Are We from an Affordable Cure"
Guests: **Salvador Payan**, Virgen del Rocío University Hospital, Institute of Biomedicine of Seville (IBiS-CSIC) | **Luís Pereira de Almeida**, CNC-UC & CIBB & Centro de Excelência em Terapia Genética (GeneT), Coimbra | **Tabita Magalhães Maia** (Department of Hematology, ULS Coimbra) | **José Carlos Ferreira** (Department of Obstetrics, ULS Santa Maria, Lisboa)

11:00-12:00 ▸ **COFFEE-BREAK | POSTER VIEWING AND DISCUSSION**

12:00-13:00 ▸ **KEYNOTE LECTURE 2**
Chairs: Sérgio B. Sousa | Daniela Oliveira

12:00-12:30 ▸ *"Generative AI meets Genetics: Reasoning with GestaltMatcher"*
Peter Krawitz, University of Bonn; Institute for Genomic Statistics and Bioinformatics, Bonn, NRW, Germany

12:30-12:50 ▸ *GestaltMatcher in practice: Portuguese clinical cases*

12:50-13:10 ▸ **CORPORATE SYMPOSIUM III ROCHE**
Chair: **Catarina Salgado**, Medical Manager, Roche
"The Evolving Role of Liquid Biopsy in NSCLC: Characterizing Resistance Mechanisms"
Manuel Teixeira, Diretor do Serviço de Genética Laboratorial do IPO Porto e Coordenador do Grupo de Oncogenética do CI-IPOP, Porto

13:10-14:00 ▸ **LUNCH BREAK**

14:00-15:00 ▸ **POSTER VIEWING AND DISCUSSION**

15:00-16:00 ▸ **INVITED SYMPOSIUM II:** Pharmacogenomics & Precision Medicine
Pharmacogenomics and Splicing Mechanisms in Health and Disease
Chairs: Sebastião Rodrigues | Marta P. Soares

15:00-15:30 ▸ *Invited talk ISII-1: "Pharmacogenomics clinical implementation in Spanish health system: MedeA pilot project and BioFRAM"*
Adrián LLerena, INUBE Extremadura University Institute for Biosanitary Research, Faculty of Medicine and Health Sciences. Extremadura University Hospital Unit for Pharmacogenomics and Personalized Medicine. Badajoz, Spain

15:30-16:00 ▸ *Invited talk ISII-2: "Splicing tolerance and its limits in disease"*
Maria Carmo-Fonseca, Faculdade de Medicina da Universidade de Lisboa; GIMM - Gulbenkian Institute for Molecular Medicine, Lisbon, Portugal

16:00-16:30 ▸ **COFFEE-BREAK**

16:30-16:50 ▸ **SELECTED ORAL COMMUNICATIONS III**
Chairs: Henriqueta Silva | Peter Jordan

OC 12 16:30-16:40	Liliana Sousa	*Optimizing prevention and care in rare tumour risk syndromes (RTRS): insights from the preventable european consortium*
OC 13 16:40-16:50	Diogo Caetano	*Pharmacogenomic studies in medical genetics: a pilot approach to personalized medicine in practice*

17:00-17:40 ▸ **ROUND TABLE:** Human Genetics in Portuguese-speaking Countries - Cabo Verde (in Portuguese)

17:00-17:15 ▸ *"Molecular Research and Diagnostics in Cape Verde: Insights from Breast Cancer"*
Pâmela Borges, Molecular Biology Laboratory, Agostinho Neto University Hospital, Praia, Cape Verde

17:15-17:40 ▸ Round table with
Raffaella Gozzelino, NOVA Medical School | **Lúcio Lara Santos**, IPO Porto | **Isabel Carreira**, FMUC-UniCV | **Celeste Bento**, ULS Coimbra; ALUA

17:45-18:45 ▸ **INVITED SYMPOSIUM III:** Hematologic Malignancies & Molecular Oncology
Chairs: Sofia Fernandes | Sara Carvalhal

17:45-18:15 ▸ *Invited talk ISIII-1: "Germline predisposition to hematological malignancies"*
Maria Julia Montoro, Vall d'Hebron University Hospitallle - Department of Haematology and Oncology, Barcelona, Spain

18:15-18:45 ▸ *Invited talk ISIII-2: "Diagnostic Experience in Bone Marrow Failure Syndromes and Hematologic Neoplasia Predisposition"*
Margarida Coucelo, Hemato-Oncology Molecular Laboratory, Department of Hematology, ULS Coimbra, Portugal

20:00 ▸ **CONFERENCE DINNER**

SATURDAY 22nd NOVEMBER

08:30-09:30 ▸ **SELECTED ORAL COMMUNICATIONS IV - Clinical Cases**
Chairs: Ana Grangeia | Mário Laço

OC 14 08:30-08:36	Jorge Diogo Da Silva	*Early detection of juvenile myelomonocytic leukemia in CBL-related Noonan-like syndrome through exome sequencing.*
OC 15 08:36-08:42	Diana Macedo Cardoso	*The hidden risk of paracentric inversions: partial 9p trisomy and neurodevelopmental disorder*
OC 16 08:42-08:48	Daniela Oliveira	*Disruption of SPECC1L translation initiation by intragenic deletion: novel pathogenic mechanism in teebi-hypertelorism syndrome*
OC 17 08:48-08:54	Sara Jesus	*Somatic RHOA mosaicism unveiled by deep sequencing: dysmorphology-driven diagnosis*
OC 18 08:54-09:00	Isabel Alonso	*Integrating transcriptomics into the clinical setting for the diagnosis of SH3KBP1 deficiency*
OC 19 09:00-09:06	Pavlina Rayko	*Broadening the phenotypic spectrum of THUMPD1-related syndrome: the oldest reported patient with novel features*
OC 20 09:06-09:12	Eunice Matoso	*Hells-associated episignature enables the diagnosis of ICF-4 syndrome*
OC 21 09:12-09:18	Vera Sousa Marques	*CHARGE syndrome with an additional FBRSL1 variant: interpreting unknown genetic findings*

09:30-10:30 ▸ **BIOETHICS DEBATE:** "Databases and DNA Profiles"
Comissão de Bioética (in Portuguese)
Chairs: Lina Ramos | Mariana Neves | Joaquim Sá
"Forensic DNA Profile Database: Possibilities and Limitations"
Francisco Corte Real, Instituto Nacional de Medicina Legal e Ciências Forenses
"From Biological Samples to Biobanks for Research: Ethical, Legal, and Social Aspects"
Cíntia Águas, Faculdade de Medicina, Universidade de Lisboa

10:30-10:50 ▸ **CORPORATE SYMPOSIUM IV ILLUMINA**
"From Sequence to Diagnosis: Deciphering the Genome"
Chair: Daniela Piazzolla, PhD Senior Manager, Medical Affairs Europe, Illumina
"Illumina Constellation Mapped reads: A new paradigm for Whole Genome Sequencing."
Prof. Massimo Delledonne, PhD Professore di Genetica, Università di Verona

10:50-11:20 ▸ **COFFEE-BREAK**

11:20-11:55 ▸ **KEYNOTE LECTURE 3:** Metabolic Disorders & Adiposity Research
Chairs: Joana Rosmaninho-Salgado | Joana Xavier
"Causes and consequences of obesity: insights from genetics"
Sadaf Farooqi, School of Clinical Medicine, University of Cambridge, UK

11:55-12:05 ▸ **SPGH IN 2026**
Célia Ventura, President SPGH 2026 | Raquel Gouveia Silva, Secretary SPGH 2026, Treasurer 2026-2028 (tba)

12:05-12:30 ▸ **SPGH AWARD LECTURE**
Chairs: Sebastião Rodrigues, Co-chair Scientific Committe | President Elected (tba)

12:30-12:50 ▸ **SPGH AWARDS CEREMONY**
Chair: Paula Jorge, Chair Scientific Committee

12:50-13:00 ▸ **CLOSING SESSION**
Sérgio B. Sousa | Janet Pereira | Cláudia Oliveira

INVITED **SPEAKERS**
PALESTRANTES CONVIDADOS

JULIA BAPTISTA

Dr. Julia Baptista is a clinical scientist and an educator with over 20 years' experience in genetics.

Julia completed her undergraduate degree in Microbial Biology and Genetics at the University of Lisbon (1997-2002) and holds a PhD in Human Genetics from the University of Southampton (2005-2008). She pursued her postdoctoral studies at the University of Bologna in Italy working on identifying diagnoses for patients with neurodevelopmental conditions.

Julia has held a variety of roles across diagnostic services, research and academia. She is currently a Scientific Training Officer at Synnovis and a Visiting Senior Lecturer at King's College London. Julia is involved in a range of activities, providing leadership in education and training for the global healthcare workforce and her main area of expertise is genomic variant interpretation in rare conditions. She is the Deputy Secretary-General for the European Society for Human Genetics and a Section Editor of the European Journal of Human Genetics.

EDUCATIONAL Workshop 1 (in Portuguese)

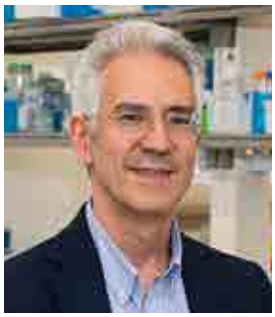
Genetic testing interpretation and report – beyond ACMG classification

Abstract

Technological advancements—particularly the widespread adoption of Next Generation Sequencing (NGS) technologies such as gene panels, exomes, and whole genomes—have significantly reshaped the role of scientists in diagnostic laboratories. Today, scientific responsibilities are increasingly centred on interpreting genomic variants within the context of specific clinical questions. This interpretive process involves classifying variants, assessing their clinical relevance, and determining what information should be included in the final diagnostic report.

The introduction of the ACMG-AMP guidelines in 2015 marked a pivotal change in how laboratories approached variant classification. These guidelines provided a structured framework for evaluating different types of evidence and assigning appropriate weight to each criterion. However, it soon became evident that further refinement was necessary to enhance consistency, accuracy, and adoption. The ClinGen consortium took on the task of implementing and refining the guidelines, offering gene- and condition-specific recommendations as well. Meanwhile, other professional bodies, such as the ACGS in the UK, adapted and rebranded the guidelines, contributing to a fragmented and occasionally conflicting landscape alongside ACMG and ClinGen.

This workshop discusses with examples, that while guidelines are essential to diagnostic practice, they must be supported by scientific judgment, peer collaboration, and multidisciplinary team discussions. Scientists must remain aware of the limitations inherent in any guideline and actively engage in collaborative efforts to ensure that accuracy remains central.



LUÍS PEREIRA
DE ALMEIDA

RUI JORGE
NOBRE

Luís Pereira de Almeida is an Associate Professor at the Faculty of Pharmacy, University of Coimbra. He coordinates the Center for Neuroscience and Cell Biology (CNC), the Center for Innovative Biomedicine and Biotechnology (CIBB), and GeneT – the Portuguese Centre of Excellence in Gene Therapy. Since 1998, he has focused on gene therapy for neurodegenerative diseases, particularly Machado–Joseph disease. Author of numerous publications and leader of over 40 projects, he supervises many PhD students and young researchers. His EU-funded GeneT Teaming Project combines research, affordable vector production and clinical trial facilities to accelerate new gene therapy development.

Rui Jorge Nobre is a Researcher at the Center for Neuroscience and Cell Biology (CNC.UC) and at GeneT-*the Portuguese Centre of Excellence in Gene therapy*, where he coordinates ViraVector, the national infrastructure dedicated to the development and production of viral vectors for biomedical research and advanced therapies. Since 2010, he has been developing innovative gene transfer platforms and therapeutic strategies for genetic diseases, with a particular focus on spinocerebellar ataxias. He is the author of over 80 scientific publications, inventor on several patents, and leads translational research projects in collaboration with academic, clinical, and industry partners.

EDUCATIONAL Workshop 2 (in Portuguese)

Gene therapy – from basic to advanced

Luís Pereira de Almeida & Rui Jorge Nobre,

CNC-UC & Centro de Excelência em Terapia Genética (GeneT), CIB, Universidade de Coimbra, Portugal

Abstract:

Gene therapy has become one of the most promising biomedical approaches for treating inherited disorders. Its progress has followed advances in genetic engineering, better methods to produce viral vectors, and the establishment of research and clinical infrastructures capable of bringing these ideas closer to patients.

In this workshop, we will offer a broad and practical overview of the field, with particular attention to applications in the central nervous system (CNS) and in neurodegenerative diseases with a genetic basis. We will discuss the main therapeutic strategies currently used, from replacing missing or defective genes to selectively reducing the expression of toxic alleles, as well as newer genome editing approaches such as CRISPR/Cas. The choice of delivery system is a central aspect of gene therapy, so we will look at viral vectors, focusing on adeno-associated viruses (AAV), which are already used in approved therapies and are an important platform for targeting the CNS.

The session will also highlight work carried out in Portugal, especially in the context of spinocerebellar ataxias and related neurodegenerative conditions. We will describe the experimental models used, from cell-based systems to animal models, and how these support preclinical evaluation of safety and therapeutic effect.

Another part of the workshop will focus on preclinical studies and translation to the clinic, a process that depends on collaboration among research groups, clinicians, technology platforms, and industry partners. We will share examples of ongoing collaborative efforts and discuss what it takes to move a therapeutic concept toward real-world application.

Finally, we will reflect on the challenges ahead, including the cost and accessibility of therapies, the scalability of vector production, and ethical considerations surrounding genome modification.

By the end of the session, participants should have a clear understanding of the current landscape of gene therapy, the progress already achieved, and the issues that will shape the field in the coming years.

GREG FINDLAY

Dr. Greg Findlay leads the Genome Function Laboratory at the Francis Crick Institute in London. He received his bachelor's degree in biology from Carleton College before completing MD-PhD training at the University of Washington in Seattle. Working with Dr. Jay Shendure, Greg led the development of several new genome editing techniques, including CRISPR-based methods for assaying genetic variants at scale and for tracing cellular lineage. His work pioneering the use of saturation genome editing (SGE) was critical for establishing the impact of high-throughput assays on clinical variant classification. Greg started his independent group at the Crick in 2020, where his lab continues to develop and leverage high-throughput methods to study human genetic variation. The lab has systematically mapped loss-of-function variants in tumour suppressor genes, and more recently, developed new strategies to explore the effects of non-coding variants at scale. The lab's overarching aim is to facilitate precision medicine approaches through an improved understanding of rare genetic variants.

Genome editing methods to interrogate variant function at scale

Abstract:

Our incomplete understanding of how rare human variants contribute to disease phenotypes substantially limits the clinical utility of genetic data. To address the challenge of variant interpretation, the Francis Crick Institute's Genome Function Laboratory led by Dr. Greg Findlay has developed genome editing approaches to interrogate human genetic variants at scale. One approach called saturation genome editing (SGE) has now been used to measure the functional effects of thousands of variants across tumour suppressor genes and rare disease genes, including *BRCA1*, *VHL*, and *RNU4-2*. SGE data reveal diverse genetic mechanisms through which variants exert phenotypic effects and predict with very high accuracy which variants cause disease. Recently, the lab has developed a powerful new platform to perform pooled prime editing, enabling up to 100,000 variants across multiple genes to be screened in a single experiment. The variant effect maps produced by these methods will ultimately reduce uncertainty in genetic testing and advance the application of precision medicine.

BART PETER LEROY

Bart P. Leroy, MD, PhD

Head of the Department of Ophthalmology and Staff Member at the Centre for Medical Genetics, Ghent University Hospital, and Full Professor at Ghent University, Belgium, he is an ophthalmologist and clinical geneticist specialising in, and passionate about, inherited eye disorders.

He trained in Ghent and London (Moorfields Eye Hospital, UCL) and has been a Senior Clinical Investigator for the Research Foundation – Flanders (FWO) since 2010. Bart has contributed extensively to the development of gene therapies for inherited blindness, including as part of the team that brought Luxturna to market in the USA and EU.

Author of over 230 peer-reviewed papers and 15 book chapters, he is an active member of several professional societies, including ISGEDR, ERN-EYE, EURETINA and SGOF. Bart regularly lectures on ophthalmic genetics and visual electrophysiology, with more than 590 presentations at international meetings.

He serves as a reviewer for 27 international journals and multiple funding agencies, and frequently participates in PhD evaluation panels. A dedicated mentor, he has supervised and trained over 30 international fellows and continues to promote awareness of rare retinal disorders worldwide.

Gene Therapy for Inherited Retinal Diseases

Abstract:

We shall discuss the development of and current status of the first and for now only approved gene therapy product voretigene neparvovec (Luxturna®) for *RPE65*-IRD. In addition, an overview of other successful gene augmentation strategies will be discussed. Also, genetic therapy strategies for large genes will be addressed, including technologies such as Anti-sense OligoNucleotides (AONs), dual vector technologies with DNA and RNA-trans-splicing and protein splicing using intein technology.

JOÃO PEDRO TEIXEIRA MARQUES

João Pedro Marques, MD, MSc, PhD, FEBOphth

An ophthalmologist and clinician-scientist at ULS Coimbra, Portugal, he leads the Ophthalmic Genetics Clinic and is also an Invited Assistant Professor at the Faculty of Medicine, University of Coimbra.

He trained in Coimbra and completed advanced clinical fellowships at Moorfields Eye Hospital (UK) and Ghent University Hospital (Belgium). He holds a PhD in Health Sciences from the University of Coimbra (2023).

His research focuses on inherited retinal dystrophies and clinical trials in retinal therapeutics. He has extensive experience as a clinical investigator in numerous retinal and inherited retinal dystrophy trials at ULS Coimbra and the Centre of Clinical Trials (CEC), AIBILI, Coimbra, where he also heads the Clinical Trials Unit at the Unidade de Oftalmologia de Coimbra (UOC).

He is an active member of several professional societies, including EURETINA, ASRS, SPO, GER, and ARVO, and serves as ULS Coimbra's Health Care Provider representative within ERN-EYE, as well as an elected member of the EVICR.net Retinal Dystrophies Expert Committee.

Author of over 90 scientific publications, he has received multiple awards, including the EURETINA YOURS Science Slam (2024) and the M8 Alliance Best PhD Thesis Award (2023). His ongoing work bridges clinical care, translational research, and international collaboration in ophthalmic genetics.

Gene Therapy in Portugal: Luxturna Outcomes and the Role of IRD-PT

Abstract

Voretigene neparvovec (Luxturna) uses an adeno-associated viral vector serotype 2 (AAV2) to deliver a functional *RPE65* gene into retinal cells, restoring enzyme activity and the retinoid visual cycle. Clinical trials demonstrated sustained improvements in functional vision, including improvements in light sensitivity and low luminance mobility testing. Approved by the Food and Drug Administration in 2017 and by the European Medicines Agency in 2018, this innovative gene therapy was first used in Portugal in May 2021. After treating 29 eyes of 16 patients, our center has gathered strong real-world data regarding structural, functional and patient-reported outcomes (PRO) after gene therapy with Luxturna. I will present our results, discuss the challenges and limitations of Luxturna and touch upon the role of our national inherited retinal disease registry – IRD-PT registry (retina.com.pt) – in moving the field of IRD forward in Portugal.

SALVADOR PAYÁN PERNÍA

Dr. Salvador Payán Pernía is a hematologist specializing in red blood cell disorders and iron metabolism diseases, in which he is regarded as a national reference. He works in both laboratory diagnostics and clinical care at Virgen del Rocío University Hospital in Seville, Spain. He is responsible for diagnostic oversight in the Andalusian neonatal screening program for sickle cell disease (SCD) and serves as a member of the Board of Directors of the Spanish Red Cell Group of the Spanish Society of Hematology and Hemotherapy (SEHH), as well as of the Spanish Society of Pediatric Hematology (SEHOP). He is also a member of the scientific committee of the national patient associations for SCD and thalassemia. In addition, he participates as a clinical investigator in national gene therapy development projects for congenital dyserythropoietic anemia, pyruvate kinase deficiency, and Diamond–Blackfan anemia.

BUILDING BRIDGES: Sociedade Portuguesa de Hematologia (in Portuguese)

Advancing Gene Therapy for Red Blood Cell disorders – How Far Are We from an Affordable Cure

Current landscape of gene therapy in hemoglobinopathies

Dr. Salvador Payán-Pernía

Hospital Universitario Virgen del Rocío, Sevilla, Spain

In February 2024, the European Commission approved exagamglogene autotemcel (exa-cel, Casgevy®) for the treatment of individuals aged ≥12 years with severe sickle cell disease (SCD), defined by recurrent vaso-occlusive crises, or with transfusion-dependent thalassemia, who are candidates for hematopoietic stem cell transplantation (HSCT) but lack an HLA-identical sibling donor. Funding for this CRISPR/Cas9-based gene editing therapy has been approved in the United Kingdom and Italy, while in other European countries it remains under evaluation.

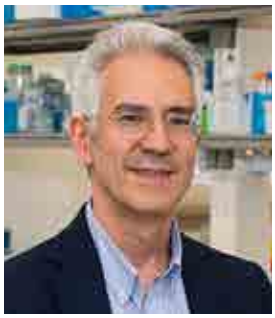
Other gene therapy strategies are under development and are expected to emerge in the coming years. Current research is focusing on *in vivo* gene therapies and and approaches that could overcome the need for myeloablative conditioning with chemotherapy.

The implementation of gene therapy in hemoglobinopathies poses significant challenges. Its high cost, which can exceed one million euros per patient, strains the sustainability of healthcare systems and emphasizes the importance of cost-effectiveness. Economic studies in these diseases are limited, costs vary widely across countries and patients, and the long-term efficacy and safety of the therapy remain uncertain.

The need for specialized centers for treatment administration, referral of patients to these centers, prolonged hospitalizations, and limited manufacturing capacity represent important logistical challenges. Furthermore, advances in HSCT and the availability of alternative pharmacological treatments—such as hydroxyurea in SCD, which is inexpensive and effective in reducing vaso-occlusive crises—necessitate positioning gene therapy within the individualized therapeutic algorithm. Prioritization of some patients over others, or discrepancies between the severity criteria used in clinical trials and those characterizing patients in clinical practice, also pose clinical and ethical challenges.

BUILDING BRIDGES: Sociedade Portuguesa de Hematologia (in Portuguese)

Advancing Gene Therapy for Red Blood Cell disorders – How Far Are We from an Affordable Cure



LUÍS PEREIRA DE ALMEIDA

Luís Pereira de Almeida is an Associate Professor at the Faculty of Pharmacy, University of Coimbra. He coordinates the Center for Neuroscience and Cell Biology (CNC), the Center for Innovative Biomedicine and Biotechnology (CIBB), and GeneT – the Portuguese Centre of Excellence in Gene Therapy. Since 1998, he has focused on gene therapy for neurodegenerative diseases, particularly Machado–Joseph disease. Author of numerous publications and leader of over 40 projects, he supervises many PhD students and young researchers. His EU-funded GeneT Teaming Project combines research, affordable vector production and clinical trial facilities to accelerate new gene therapy development.

TABITA MAGALHÃES MAIA

Dr. Tabita Magalhaes Maia is a hematologist, specialized in the diagnosis and management of inherited red blood cell disorders. Currently serving as the Head of the Red Cell and Iron Metabolism Clinical Unit at the Unidade Local de Saúde de Coimbra (ULS de Coimbra), her clinical and research expertise centers on a wide range of congenital red blood cell disorders, including hemoglobinopathies, enzymopathies, red blood cell membrane and iron metabolism disorders. She manages both pediatric and adult patients, providing diagnostic services and comprehensive and specialized care for rare erythrocyte disorders.

She plays a crucial role in public health initiatives, contributing to the portuguese Newborn Screening pilot program for Sickle Cell Disease. Furthermore, she is the coordinator of a Portuguese reference center for congenital red cell and iron metabolism disorders in the European network EuroBloodNet, helping to standardize care for congenital rare blood cell diseases.

JOSÉ CARLOS P. B. FERREIRA

José Carlos P. B. Ferreira, M.D., Ph.D., is a medical specialist in Gynaecology and Obstetrics and in Medical Genetics, currently holding academic positions as an Assistant Professor in the Department of Gynaecology and Obstetrics at the Faculty of Medicine of Lisbon, Portugal, and Visiting Professor at the Faculty of Medicine of Eduardo Mondlane University, Maputo, Mozambique, and a member of the Scientific Advisory Board of Menarini Labs, Bologna, Italy.

He graduated in Medicine from FMUL in 1986, and completed his specialisation in Gynaecology and Obstetrics in March 1995 at Hospital Garcia de Orta, Almada, Portugal. He obtained certification by the American Board of Medical Specialties in Medical Genetics in 1999, maintaining the certification ever since, and obtained equivalence from the specialist college of the Ordem dos Médicos (Portuguese Medical Association) in 2021. He holds a Ph.D. in Medical Sciences from the University of Toronto, supervised in the Genetics Department of the Hospital for Sick Children, awarded in July 2011.

He has held academic positions as an Assistant Professor at the Albert Einstein College of Medicine and as a Visiting Professor at the Medical University of Warsaw.

He has held professional positions at Hospitais Civis de Lisboa and Hospital Garcia de Orta (Portugal), as a resident, and in the latter also as a specialist in Obstetrics and Medical Genetics, at Montefiore Medical Center, Bronx, NY (as a resident and subsequently as a specialist in Medical Genetics), at Bronx Lebanon Hospital, Bronx, NY, at Jacobi Medical Center, Bronx, NY, as a specialist in Medical Genetics, at Hospital Central de Maputo and Instituto do Coração, Maputo, Mozambique, and at Hospital de Santa Maria, Lisbon, Portugal.

He has published over 50 articles in peer-reviewed journals listed in PubMed with 2754 citations and an h-index of 29, and has been invited to scientific conferences in Europe.

PETER KRAWITZ

Peter Krawitz is a board-certified medical geneticist and Professor of Genomic Bioinformatics at the University of Bonn. He currently serves as Director of the Institute for Genomic Statistics and Bioinformatics and is a member of the Excellence Cluster ImmunoSensation as well as president of the Association for Genome Diagnostics (AGD e.V.), a non-profit entity providing GestaltMatcher Database (GMDB). Peter Krawitz studied medicine at the Technical University of Munich and physics at Ludwig Maximilian University. He completed his residency in human genetics at Charité – Universitätsmedizin Berlin, where he also received his board certification and habilitation in 2016. His scientific training includes research fellowships at the Institute for Systems Biology in Seattle under Prof. Ilya Shmulevich, and at the University of Munich with Prof. Christian Haass. He later joined the Computational Biology group of Prof. Peter Robinson at Charité, where he contributed to foundational work in clinical genomics and phenotype ontology. Prof. Krawitz has been a leading contributor to the development of next-generation phenotyping technologies, including AI-based facial analysis for rare disease diagnostics. His research focuses on next-generation phenotyping, the interpretation of genome sequencing data, particularly in non-coding regions, and on the molecular basis and phenotypic spectrum of GPI anchor biosynthesis deficiencies.

Generative AI meets Genetics: Reasoning with GestaltMatcher

Abstract:

Next-generation phenotyping approaches have demonstrated high accuracy in suggesting diagnoses based on dysmorphic facial features. We propose a novel approach using convolutional neural networks (CNNs) to simultaneously infer Human Phenotype Ontology (HPO) terms and classify disorders based on syndromic faces. By integrating HPO extraction and disorder prediction, our method enables cross-validation of results, improving diagnostic robustness. We aim to enhance both diagnostic support and explainability in AI-driven rare disease classification.

Through GestaltMatcher Database, we set up a large-scale effort to annotate the presence and absence of HPO-terms. As annotation of HPO-terms is a subjective process, we allowed multiple experts to annotate the same images to improve the overall robustness. By simultaneously learning to predict disorder and infer HPO-terms, we can cross-reference inferred terms with disorders to (in) validate predictions. Further, since HPO-terms inherently carry spatial information, we can gain new insights into the predicted disorders.

At the time of writing more than 1000 images, across 10 disorders were annotated with 72 common HPO-terms for the disorders, alongside any term the annotators considered important. We trained several CNNs and compared the performance when predicting HPOs, disorders, and a combination of the two. The latter allowed us to combine the predictions to further improve performance and explainability.

Our proposed approach allows for more and better analyses to be conducted straightforwardly. Further, it inherently improves the explainability of our AI models, improving their usefulness in clinical settings. Additionally, it reduces the subjectiveness of labeling HPO-terms manually and allows clinicians to work more efficiently.

ADRIÁN LLERENA

Adrián LLerena is the Director of INUBE Extremadura Biosanitary Research Institute and the Clinical Research Center in the University Hospital in Badajoz Spain. He is also Professor of Pharmacology and Clinical Pharmacology in the Medical School of the University of Extremadura Spain. He earned his PhD at University of Extremadura and between 1989 to 1993 did his post doc at Karolinska Institute Sweden focusing on Clinical Pharmacogenetics. His teaching experience is of more than 30 years in Spain and of about 9 years in Portugal part time in the University of Beira Interior. He has supervised more than 25 PhD thesis so far. He has also been an invited Professor in different Universities in USA such as University of California at Los Angeles 2004 Mount Sinai Medical School at NY 2005 and Miller Medical School at Miami 2006 and in Latin America such as UNAM in Mexico San Marcos in Peru Chile Brasil etc. He has published more than 240 peer reviewed papers and book chapters. He is also coordinating the RIBEF IberoAmerican network of Pharmacogenetics since 2006. He served in different Scietific societies as IberoAmerican Society of Pharmacogenomics. He has been Principal Investigator in more than 25 national and European research projects. He has also served as reviewer in all journals related to clinical pharmacology and mainly pharmacogenetics and clinical psychopharmacology. Currently is the leader of the IMPaCT WP05-bioFRAM Pharmacogenenetics Implementation and Pharmacogenetics MedeA Clinical Implementation in health System.

Pharmacogenomics clinical implementation in Spanish health system: MedeA pilot project and BioFRAM

MARIA CARMO-FONSECA

Maria Carmo-Fonseca is a Professor at the University of Lisbon Medical School and a Group Leader at the Gulbenkian Institute for Molecular Medicine (GIMM). As a co-founder of the Institute for Molecular Medicine (iMM), a leading biomedical research institute affiliated with the University of Lisbon, she has played a pivotal role in advancing biomedical research in Portugal. Recently, iMM merged with the Gulbenkian Institute of Science, creating GIMM, a unified center of excellence dedicated to advancing research and innovation at the interface of biology and medicine.

From 2011 to 2013, Carmo-Fonseca was a Visiting Professor at Harvard Medical School. She is a distinguished member of several prestigious organizations, including the European Molecular Biology Organization (EMBO), the Portuguese Academy of Sciences, the Portuguese Academy of Medicine, and Academia Europaea. She served as President of the RNA Society (2021-2022), a global organization dedicated to RNA research. She was also an editor for the Journal of Cell Science from 2009 to 2023 and currently serves on the editorial board of the RNA journal. Her research is focused on RNA biology and gene regulation, with a particular interest in understanding RNA splicing in the context of human disease.

Maria Carmo-Fonseca is Professor at the University of Lisbon Medical School and Group Leader at the Gulbenkian Institute for Molecular Medicine (GIMM). She co-founded the Institute of Molecular Medicine (iMM), affiliated with the University of Lisbon, and led the institute as its inaugural Director (2002–2014), followed by her appointment as President. In 2023, iMM merged with the Gulbenkian Institute of Science to form GIMM (https://gimm.pt/), where she now serves on the Board of Trustees.

M. Carmo-Fonseca was also co-founder and advisor for GenoMed, a spin-off company of iMM for molecular diagnosis (2004-2024). She was visiting Professor at Harvard Medical School (2011-2013). She is a member of the European Molecular Biology Organization (since 1994), Academia Europaea (since 2022), Portuguese Academy of Sciences (since 2000) and Portuguese Academy of Medicine (since 1999). She served as President of the RNA Society (2021-2022). She was an editor for the Journal of Cell Science from 2009 to 2023 and currently serves on the editorial board of the RNA journal. She is also a member of the European Research Council (ERC) Advanced Grants Panel (2020-2026).

The vision of her research group is to understand how RNA molecules, with their ability to both encode information and exert catalytic activities, contribute to the regulation of gene expression in health and disease. Her group implemented methodologies that allow tracking single RNA molecules in time and space inside the nucleus of living cells. She did a sabbatical hands-on training at Harvard Medical School, in Tom Kirchhausen's lab, where she developed a strategy that permits direct measurements of intron dynamics in single pre-mRNA molecules in live cells. This approach revealed that splicing can occur much faster than previously thought, opening new avenues to study how kinetic mechanisms contribute to RNA biogenesis. To further investigate the timing of pre-mRNA splicing relative to transcription, she started a collaboration with Nick Proudfoot at the University of Oxford, who had developed a novel strategy for Native Elongation Transcript sequencing (NET-seq). Together they unraveled unanticipated dynamic links between Pol II and the spliceosome. More recently they developed a new approach to characterize nascent RNA, called polymerase intact nascent transcript (POINT) technology. Using this methodology, they mapped nascent RNA 5' ends, established the kinetics of co-transcriptional splicing patterns, and profiled whole transcription units. Prompted by increasing evidence that alterations in pre-mRNA splicing play a role in human disease, Carmo-Fonseca initiated a new research line aiming to investigate how mis-splicing could be targeted for therapeutic applications in heart disease. The group developed a method to produce cardiomyocytes derived from human induced pluripotent cells, which has been patented. Using this model system, they demonstrated that intronic variants identified in patients with inherited cardiomyopathy interfere with splicing and induce an altered cellular phenotype, which can be corrected by splice-switching antisense oligonucleotides.

In recognition to her contribution to Science and Society, Carmo-Fonseca received several honors and awards, namely: Comenda Ordem de Sant'iago de Espada Ilustres Carreira, Portuguese award for literary, scientific, and artistic merit (2001); Gulbenkian Science Award, Fundação Calouste Gulbenkian, Portugal (2007); Prémio Pessoa, Portuguese award in Culture, Art and Sciences (2010); Prémio D. Antónia Ferreira, Portuguese award for Entrepreneurial women (2013); Prémio Aboim Sande Lemos-Identidade Portuguesa, Portuguese award in Art and Sciences (2018); Prémio Universidade de Lisboa for her "exceptional journey as a scientist, with relevant contributions in the field of molecular medicine, and public engagement in promoting science and scientific literacy" (2023).

Splicing tolerance and its limits in disease

Abstract:

Splicing is inherently noisy, with errors occurring occasionally during splice site recognition. Yet, these errors are normally tolerated in healthy individuals. Genetic variation can exacerbate splicing errors, subtly altering the transcriptome. My laboratory is interested in understanding the interplay between stochastic splicing errors and those driven by genetic variants, with a focus on delineating the thresholds that distinguish benign variability from pathogenic consequences in inherited heart disease. By dissecting splicing errors linked to genetic variants that are not associated with disease, I will discuss how cells buffer splicing noise and when this tolerance fails, leading to disease phenotypes. Deciphering these thresholds not only informs the molecular origins of genetic diseases but also sheds light on the evolutionary forces shaping transcriptome robustness in the face of genetic diversity.

PÂMELA CRISTINA
CARVALHO BORGES

Pâmela Cristina Carvalho Borges, PhD

Molecular biologist and biomedical engineer from Cape Verde, Pâmela is currently Principal Investigator of the Cape Verde Molecular Laboratory Implementation Project at Agostinho Neto University Hospital. Her work focuses on establishing breast cancer diagnosis and molecular characterisation in Cape Verde. She holds a PhD from the Instituto Gulbenkian de Ciência, with part of her doctoral research conducted at Rockefeller University, USA, studying telomere biology and genome stability.

A recipient of the 2023 L'Oréal–UNESCO For Women in Science Sub-Saharan Africa Young Talent Award, she has led national initiatives in cancer and infectious disease research, including the coordination of virology laboratories during the COVID-19 pandemic. Pâmela has co-authored several international publications, including in *Nature* and *Nucleic Acids Research*, and is a recognised advocate for scientific capacity building in the PALOP countries.

ROUND TABLE Human Genetics in Portuguese-speaking Countries - Cabo Verde (in Portuguese)

Molecular Research and Diagnostics in Cape Verde: Insights from Breast Cancer and Beyond

Abstract:

Breast cancer is a significant health concern in Cape Verde, but comprehensive data on its presentation, management, and outcomes have historically been limited. This work presents findings from the first large-scale clinical and genetic studies on breast cancer in the country, alongside the implementation of its first molecular diagnostics laboratory and an overview of national efforts in human genetics.

A 24-year retrospective study (2000–2024) at Agostinho Neto University Hospital included 586 patients (98.6% women, 1.4% men). The mean age at diagnosis was 52.1 years (SD 13.6) for women and 70.0 years (SD 16.7) for men. Most cases (39.4%) were diagnosed at stage III. Invasive ductal carcinoma was the predominant histological type. Among 307 patients with immunohistochemistry, 69.4% were luminal, 26.1% triple-negative, and 4.6% HER2-positive. Treatment commonly included surgery (92.8%), chemotherapy (82.1%), and hormone therapy (67.6%); only 33.4% received radiotherapy. Median follow-up was 36.5 months, with a median survival of 137.1 months.

In a parallel hereditary cancer study, 125 patients (selected by age ≤45, TNBC, family history, or male sex) underwent multigene panel testing. Pathogenic mutations were identified in 23.2% of cases. *BRCA2* mutations were most common (51.7%), followed by *RAD51D* (17.9%), *BRCA1* and *RAD51C* (13.8% each), and *BRIP1* and *PMS2* (3.5%). A recurrent *BRCA2* variant (NM_000059.4:c.632-3C>G) found in 12 patients—mostly from Santiago Island—suggests a founder effect.

These efforts were enabled by the creation of Cape Verde's first Molecular Biology Laboratory, now providing in-country genomic testing. A national mapping of research and diagnostic teams in Human Genetics is underway, promoting collaboration between hospitals and universities.

This experience demonstrates the impact of local scientific leadership and molecular innovation in improving cancer care in resource-limited island settings.

Keywords: Cape Verde, Breast Cancer, Hereditary Mutations, Molecular Diagnostics, BRCA, Genomic Medicine, Public Health, Africa

ROUND TABLE Human Genetics in Portuguese-speaking Countries - Cabo Verde (in Portuguese)

RAFFAELLA GOZZELINO

Raffaella Gozzelino, PhD, is Assistant Professor at NOVA Medical School, NOVA University of Lisbon, and founder of *Diáspora Mundi*, a global network promoting knowledge transfer and scientific collaboration across Africa. She leads the Inflammation and Neurodegeneration Laboratory at NOVA and has authored over 35 peer-reviewed papers and multiple books. A dual Italian–Cape Verdean national, she has served as Vice-Chancellor of WASCAL and Rector of the Atlantic Technical University in Cape Verde. Her research focuses on iron metabolism, neuroinflammation and translational medicine. Recognised among the world's top 2% most cited scientists (Stanford–Elsevier, 2024–2025), she is also Co-Chair of DOTCAN and Global Advisor for the ADWIN Foundation. Raffaella has coordinated international projects with WHO, MIT-Africa, and UNESCO, and serves as reviewer for major funding bodies, including Horizon Europe, DFG, and FCT. Her work bridges science, policy, and leadership development across continents.

LÚCIO JOSÉ
DE LARA SANTOS

Lúcio José de Lara Santos, MD, PhD, is senior surgeon, Professor of Surgical Oncology, former director of the Digestive Pathology Unit, currently the coordinator of the Experimental Pathology and Therapeutics Group and member of the IPO's Surgical Oncology Department. He also serves as Adviser of Oncology for ONCOCIR - Education and Care in Oncology, Angola.

Prof. Santos received MD degrees from both Agostinho Neto University, Angola, and Nova University, Lisboa, Portugal. He received his PhD in Surgery and Oncobiology from Porto University, Porto, Portugal. He served a fellowship in General Surgery at the Portuguese Institute of Oncology and received specialized training in esophageal surgery at Leiden Academy and in liver surgery at Hôpital Paul Brousse in Villejuif, France, and The University of Texas MD Anderson Cancer Center in Houston, Texas.

Prof. Santos' research interests include surgical oncology and carcinogenesis, working in Portugal and Angola. As a Consultant Surgical Oncologist and General Surgeon in the Portuguese Institute of Oncology (Digestive Pathology and Breast Cancer Unit), Prof. de Lara Santos conducts research in experimental pathology and oncology, including gastrointestinal, breast cancer, and cancers related to infections. He has extensive experience in organizing oncology facilities such as chemotherapy, radiation therapy, and surgical oncology services, and in standardizing procedures and teaching of general medicine, surgery, and oncology.

Additionally, Prof. de Lara Santos is the author or coauthor of nearly 60 peer-reviewed publications in journals including Nature Communications, Annals of Oncology, BMC Cancer, British Journal of Cancer, and Molecular Oncology.

ISABEL MARQUES
CARREIRA

Isabel M. Carreira is an Emerita Full Professor of the Faculty of Medicine at the University of Coimbra, where she was Deputy Director since 2017 and Director of the Cytogenetics and Genomics Laboratory since 1992. Since 2014, she was the Coordinator of the ISO 9001 quality certification process for all diagnostic laboratories of the Faculties of Medicine and Pharmacy at the University of Coimbra. She was the Course Convenor/Coordinator for several curricular units in the 1st, 2nd, and 3rd cycles at the Faculties of Medicine, Science and Technology, and Psychology and Education Sciences. She is the Coordinator and Promoter of the Master's Degree in Laboratory Clinical Genetics at FMUC, which has been running since 2021/22. She is a Member of the Joint Commission/ Coordinator of the 1st cycle of studies for the Medicine course in the Azores and Cape Verde. She has been the Coordinator of CIMAGO (Centre for Research in Environment, Genetics and Oncobiology) since 2009. She is the Principal Investigator of the Biomarkers in Translational and Clinical Oncology Group at CIBB (Centre for Innovative Biomedicine and Biotechnology). Since its formation, she has been the Co-Chair of the Clinical-Laboratory Geneticists specialty of the European Board of Medical Genetics, of which she was the President in 2021. She was a Founding Member of SPGH and served as its Effective President in 2006 and 2019. She is the Coordinator of the Commission for the Clinical and Laboratory Specialties of Medical Genetics at SPGH. She has been an Effective Member of the National Academy of Medicine of Portugal since 2012.

CELESTE BENTO

Celeste Bento is a Molecular Geneticist with a PhD in Biomedicine. She heads the Laboratory of Red Blood Cells and Iron Metabolism Disorders at the University Hospital of Coimbra, Portugal, and is a Principal Investigator at the "Genes, Populations and Diseases" group at the Research Centre for Anthropology and Health (CIAS), University of Coimbra.

Her expertise focuses on hemoglobinopathies, congenital hemolytic anemias, congenital erythrocytosis and related disorders. She is involved in international initiatives including ERN-EuroBloodNet, RADeep (national coordinator for Portugal), MPN&MPNr-Euronet, the Human Variome Project, INHERENT, and COST-HELIOS.

Celeste is president of the Portuguese Association for Parents and Patients with Hemoglobinopathies (APPDH) and a founding member of ALUA, an alliance of Lusophone healthcare providers addressing sickle cell disease. She has led numerous projects and missions in Portuguese-speaking countries, focusing on capacity-building for healthcare professionals, prevalence studies of HbS, and implementation of institutional measures to support patients and families with SCD.

JULIA MONTORO

Dr. Julia Montoro (MD, PhD) is a hematologist with a special interest in patients with Myelodysplastic Syndromes (MDS) and Hereditary Predisposition Hematological Neoplasms at Vall d'Hebron University Hospital in Barcelona, where she has been working since 2013.

She graduated in Medicine in 2008 and completed her medical training between 2009 and 2013 in the Department of Hematology at Vall d'Hebron University Hospital. During this period, she carried out her Research Proficiency Thesis titled "Causes of Death in Patients with Myelodysplastic Syndrome." She was awarded the Research Promotion Grant from the Spanish Society of Hematology and Hemotherapy (SEHH) for two consecutive years (2014-2015) for the project "Evaluation of the Impact of Autoimmune Disorders in Patients with Myelodysplastic Syndrome."

From August 2016 to December 2017, she undertook a fellowship in the Basic and Translational Research Laboratory at the Herbert Irving Cancer Center of Columbia University in New York, under the supervision of Dr. Azra Raza, an internationally recognized expert in the field of MDS. During this period, she received a grant from the CRIS Foundation to develop the project "Predicting Response to Luspatercept in Myelodysplastic Syndromes: Analysis of Terminal Erythroid Differentiation Using an Ex Vivo System and RNA Sequencing."

She completed her doctoral thesis in September 2019 with the highest distinction, Cum Laude, for her dissertation titled "Improvement of Prognostic Assessment Strategies in Patients with Myelodysplastic Syndromes," which resulted in two first-author publications. Additionally, throughout her career, she has co-authored numerous clinical publications and delivered multiple oral presentations at national and international conferences, both as a co-author and as a lead author.

Dr. Montoro is a member of the Vall d'Hebron Institute of Oncology (VHIO), an internationally renowned cancer research institution. She is also a member of the Spanish Society of Hematology and Hemotherapy (SEHH), the European Hematology Association (EHA), the Spanish Group of Myelodysplastic Syndromes (GESMD), and serves as President of the Catalonia-Balearic Group of Hereditary Predisposition Hematological Neoplasms.

Since January 2021, she has been the coordinator of the Hematological Genetic Counseling Unit at Vall d'Hebron University Hospital, focusing on Myeloid Neoplasms with Hereditary Predisposition and patients with Clonal Hematopoiesis of Indeterminate Potential

Germline predisposition to hematological malignancies

MARGARIDA COUCELO

Head of Hemato-Oncology Laboratory at Unidade Funcional de Hematologia Molecular, Hematology Department, Unidade Local de Saúde de Coimbra, Portugal.

Myeloid neoplasms, Acute Leukemias, Bone Marrow Failure Disorders and Predisposition syndromes to hematologic malignancies.

Specialist in Human Genetics (Ordem dos Biólogos); Clinical Laboratory Geneticist (European Board of Medical Genetics). Master degree in Cellular and Molecular Biology at University of Coimbra and degree in Biotechnological Engineering at Lusófona University from Lisbon.

Member of Coimbra Institute and Research (iCBR) – Group of Environmental Genetics and Oncobiology (CIMAGO), Faculty of Medicine of Coimbra (FMUC); PETHEMA-AML, EWOG-MDS/SAA European Working Group of Myelodysplastic Syndrome (MDS) and Severe Aplastic Anemia (SAA) in children and adolescents

0000-0002-3426-6363

Diagnostic Experience in Bone Marrow Failure Syndromes and Hematologic Neoplasia Predisposition

Abstract

Bone marrow failure syndromes (BMF) are a heterogeneous group of hematological disorders characterized by cytopenia affecting one or more hematopoietic lineages, that can be inherited or acquired, with an increased risk of developing hematologic malignancies and/or solid tumors. The differential diagnosis between these entities is essential for an accurate diagnosis, therapeutic adequacy, selection of donor and conditioning regimens for allogenic hematopoietic stem cell transplant[1,2,3].

The introduction of next-generation sequencing (NGS) has greatly advanced the knowledge of the genetic basis of BMFS, unravelling a marked genetic heterogeneity among patients with similar clinical features. Moreover, revealing that hereditary forms of MDS and AML are more common that previously recognized, occurring in approximately 4-13% of pediatric patients and 5-14% of adults. This advances lead, for the first time in 2016, to the inclusion of a new WHO entity of germline predisposition to hematological neoplasms that was recently updated in 2022[4].

To optimize the diagnosis approach to inherited BMFS and familial predisposition syndromes, in 2019 our laboratory customized a NGS panel encompassing 101 implicated genes (excluding Fanconi anemia genes), that was recently replaced by whole exome sequencing[5].

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FRANCISCO CORTE REAL

Presidente do Instituto Nacional de Medicina Legal e Ciências Forenses.

Professor da Faculdade de Medicina da Universidade de Coimbra.

Médico especialista em Medicina legal.

Foi Presidente da Rede de Serviços Médico-Legais e Forenses de Língua Portuguesa, Presidente do Colégio da Especialidade de Medicina Legal da Ordem dos Médicos, Presidente da Associação Portuguesa de Avaliação do Dano Corporal, Presidente da Sociedade Portuguesa de Genética Humana e Coordenador da Base de Dados de Perfis de ADN.

BIOETHICS DEBATE: "Databases and DNA Profiles" (in Portuguese)

Forensic DNA Profile Database: Possibilities and Limitations

Bases de Dados Forenses de Perfis de ADN: Possibilidades e Limites

Abstract

Acompanhando o avanço científico e tecnológico característico do final do século XX, também a Genética Forense sofreu, nas duas últimas décadas, um desenvolvimento muito significativo. A vulgarização internacional das análises genéticas com fins forenses originou um elevado volume de informação, cuja comparação pode ser pertinente para, por exemplo, se saber se o mesmo indivíduo está implicado em diferentes crimes ou para se identificar o cadáver de uma pessoa desaparecida. Tal facto levou a que muitos países instituíssem Bases de Dados de Perfis de ADN (BDP ADN) a partir de 1995.

Este objetivo foi oficializado na Europa através da Resolução do Conselho de 9 de junho de 1997, relativa ao estabelecimento de bases de dados nacionais de ADN, e da Resolução do Conselho de 25 de junho de 2001, relativa à partilha de perfis de ADN entre os diversos estados membros. A necessidade de partilha de informação entre os diferentes países foi protocolada pelo Acordo de *Prüm* e, posteriormente, pela Decisão do Conselho 2008/615/JAI, em 23 de junho de 2008.

Mais de quinze anos após a publicação da lei que aprovou a criação da BDP ADN e a sua entrada em funcionamento importaria fazer uma análise retrospetiva sobre o processo que levou à publicação da Lei n.º 5/2008, de 12 de fevereiro, bem como descrever a situação atual. Assim, são descritas as iniciativas que originaram a publicação da lei e os aspetos que suscitaram mais controvérsia. São apresentadas as especificidades da BDP ADN, comparando-a, de forma sucinta, com algumas outras bases de dados europeias, descrevendo-se os problemas atualmente existentes. São abordados os pressupostos previstos para a cooperação internacional nesta matéria, tal como os principais desafios que se perspetivam.

CÍNTIA ÁGUAS PEREIRA

Professora Auxiliar Convidada na Faculdade de Medicina da Universidade de Lisboa. Doutorada em Bioética pela Bioética pela Universidade Católica Portuguesa. Licenciada em Direito pela Faculdade de Direito da Universidade de Coimbra. Formadora em Integridade Científica pela Amsterdam University Medical Centre - Projeto Virt2ue.

É Vice-Presidente da Comissão de Ética da Escola Superior de Saúde da Cruz Vermelha Portuguesa – Lisboa, Membro da Comissão de Ética do ABC - Algarve Biomedical Centre e Membro do Grupo de Reflexão Ética da Associação Portuguesa de Cuidados Paliativos. É membro do Conselho de Deontologia da Ordem dos Biólogos. Foi Secretária Executiva do Conselho Nacional de Ética para as Ciências da Vida de 2006 a 2024.

Principais áreas de interesse: Bioética, da génese à prática; Biobancos; Ética e Direitos Humanos; Cuidados Paliativos; Integridade Científica; Ética e Ambiente.

BIOETHICS DEBATE: "Databases and DNA Profiles" (in Portuguese)

From Biological Samples to Biobanks for Research: Ethical, Legal, and Social Aspects

Da amostra biológica aos biobancos para investigação: aspetos éticos, jurídicos e sociais

Um "biobanco" é uma "biblioteca" dinâmica de materiais biológicos (de origem humana ou outra) e informações associadas para múltiplos propósitos, nomeadamente para impulsionar a descoberta científica e, em última análise, melhorar a saúde humana. De pequenas coleções a grandes infraestruturas, os biobancos são hoje infraestruturas imprescindíveis para a investigação científica aplicada à saúde. Podemos encontrá-los em clínicas, hospitais e universidades, entre outras entidades dos setores público, privado ou social. Podem ser orientados para uma patologia ou representativos da população. Integram um ecossistema que envolve participantes, profissionais, investigadores e outros peritos, mas também os financiadores e as instâncias políticas e de regulação, em interação com a sociedade civil.

A crescente organização e profissionalização destas estruturas noutros países, em que os biobancos ganham visibilidade, escala e permanência, contrastam com a situação portuguesa, pois muitos aspetos da constituição e atividade dos biobancos, da sua relação com os participantes e os cidadãos e as condições que os legitimam e organizam continuam, em larga medida, por determinar e regulamentar. Questões relativas ao consentimento, à propriedade de amostras e de dados, ao uso secundário de informação anteriormente colhida para fins de diagnóstico ou terapêuticos, aos benefícios e à comercialização, aos achados incidentais, à proteção da privacidade ou à análise de dados potenciada por poderosos algoritmos, são preocupações centrais dos biobancos que urge acolher e atualizar na realidade portuguesa. Ignorar estas realidades pode criar obstáculos à investigação transnacional competitiva e responsável, erodir a confiança da sociedade e minar o respeito pelas pessoas que, movidas pelo altruísmo, partilham as suas amostras biológicas e dados de saúde altamente sensíveis.

A presente comunicação pretende chamar a atenção para estas questões e fomentar um debate útil sobre a atividade dos biobancos e a forma como intersetam a ética, a ciência e a sociedade.

SADAF FAROOQI

Sadaf Farooqi was born in Wigan, Lancashire, England. She studied Medicine at the University of Birmingham, England where she qualified with Honors in 1993, winning the Queen's Scholarship (Gold Medal). She subsequently trained in Internal Medicine, Diabetes and Endocrinology in Birmingham, Oxford and Cambridge, where she is currently an Honorary Consultant Physician in Diabetes and Endocrinology.

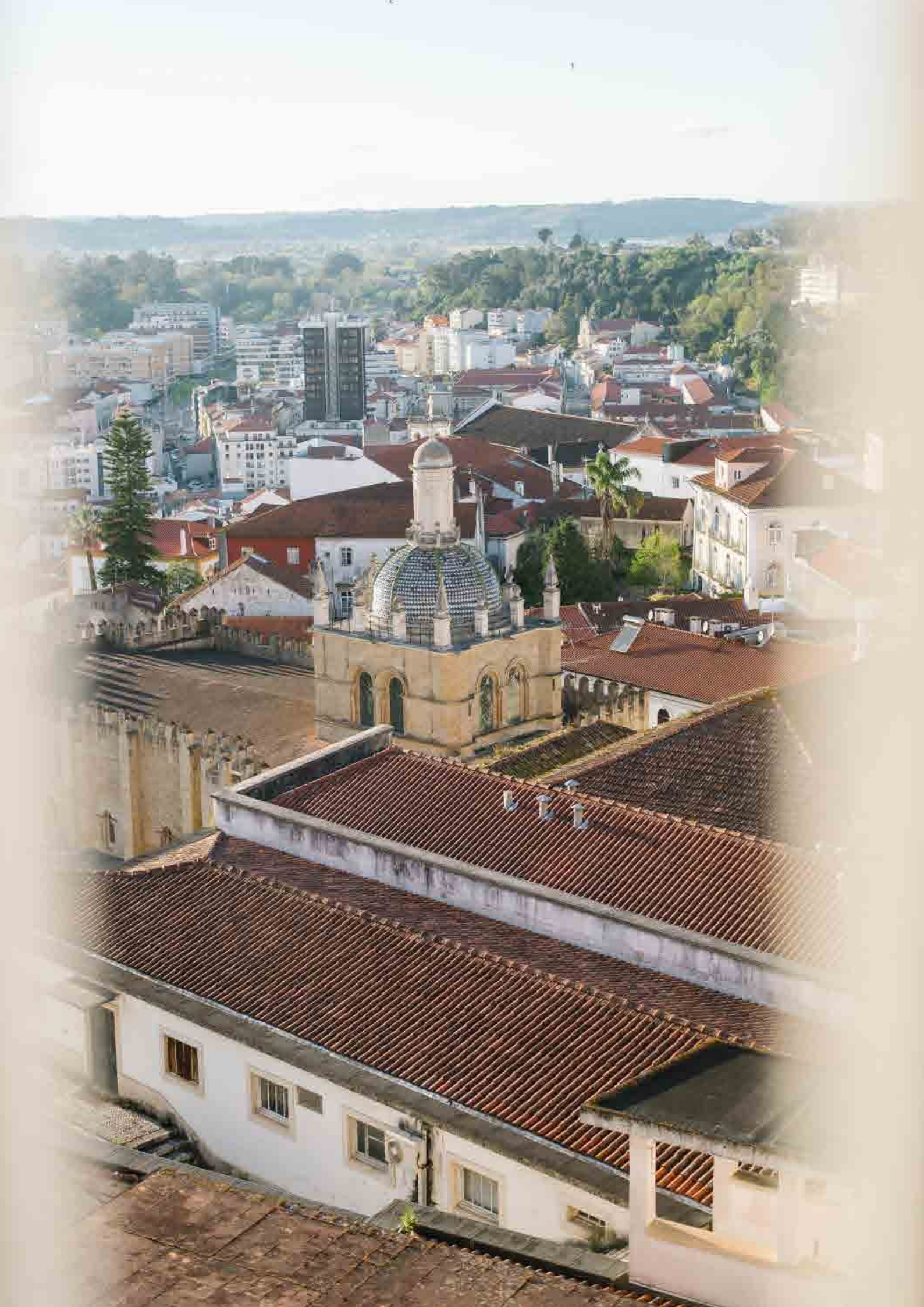
As Professor of Metabolism and Medicine at the University of Cambridge, Sadaf Farooqi is an internationally recognized Clinician Scientist who has made seminal contributions to understanding the genetic and physiological mechanisms that underlie obesity and its complications. Through the discovery and characterization of several genetic disorders disrupting the leptin-melanocortin pathway, her work has fundamentally altered our understanding of how body weight is regulated and led to genetic testing being recommended in clinical guidelines for childhood-onset obesity, globally. In studies of children with congenital leptin deficiency before and after treatment with recombinant leptin, she established that the principal driver of obesity is a failure of the control of appetite, establishing that human eating behavior is strongly regulated by biological factors. Her work on the melanocortin 4 receptor, in which loss of function mutations represent the commonest genetic cause of obesity, has informed the development of targeted therapies for people with multiple subtypes of severe obesity. These treatments are now licensed in many countries and have transformed the lives of children and adults living with these conditions. Her studies have uncovered key neural mechanisms which regulate other instinctive behaviors such as socialization, fear, aggression and maternal care providing mechanistic insights into clinical disorders as diverse as autism, anxiety and postnatal depression.

She has received numerous awards including the American Diabetes Association Outstanding Scientific Achievement Award, Dale Medal (highest honour awarded by the UK Society for Endocrinology), Croonian Medal of the Royal Society (with her mentor Stephen O'Rahilly), Obesity Society Research Achievement Award, Endocrine Society Outstanding Clinical Investigator Award and the Endocrinology Across the Lifecourse Award from the European Societies of Pediatric and Adult Endocrinology. She was elected as a Fellow of the Royal Society in 2021 in recognition of her exceptional contributions to science.

Causes and consequences of obesity: insights from genetics

Abstract

Within an obesogenic environment, there is considerable variation in body weight. These differences in susceptibility to obesity are strongly influenced by genetic factors. Molecular and physiological studies in severely obese patients with inherited mutations have provided insights into the role of the hypothalamic leptin-melanocortin pathway in the regulation of eating behaviour and body weight. Genome wide approaches including whole exome sequencing, are leading to the discovery of new obesity genes. Discovering how genetic variation contributes to weight gain provides a framework for the development of mechanism based therapeutic strategies.



ORAL **COMMUNICATIONS**
COMUNICAÇÕES ORAIS

Thursday 20th November

TIME SLOT	PRESENTING AUTHOR	TITLE
OC 01 17h30-17h40	João Miguel Carvalho Nogueira	*Dissecting and predicting the impact of enhancer variants in type 2 diabetes using high-throughput mutagenesis and reporter assays*
OC 02 17h40-17h50	Ana Joana Duarte	*Generation of cellular models for fabry disease: unlocking the potential of IPSCS and gene editing*
OC 03 17h50-18h00	Rafael Graça	*Dissecting non-coding regulation of ldlr and PCSK9 in familial hypercholesterolaemia*
OC 04 18h00-18h10	André Besouro-Duarte	*Differential allelic expression case-control study unveils missing prostate cancer heritability and pleiotropy with breast cancer*
OC 05 18h10-18h20	Frederico Pena	*Establishment of a scalable neuronal reporter model of Machado-Joseph disease based on gene-edited patient cells as a platform for high-throughput drug repurposing*

Friday 21th November

TIME SLOT	PRESENTING AUTHOR	TITLE
OC 06 09h00-09h10	Ricardo Amorim	*Clinical trajectories and healthcare burden of hereditary diffuse gastric cancer: prevention versus treatment in european centres*
OC 07 09h10-09h20	Mariana Tomásio Neves	*MUTYH carrier frequency in Portugal: navigating the data gap with what we've got*
OC 08 09h20-09h30	Cristina Santos	*Lessons learned from a portuguese inherited retinal disease research cohort*
OC 09 09h30-09h40	Ana Miguel Pinto Capela	*Understanding the genetic basis of hereditary cardiovascular diseases in Portugal: a retrospective analysis across two centres*
OC 10 09h40-09h50	Sofia Quental	*Rna analysis as a first-line diagnostic approach for neurofibromatosis type 1: a six-year institutional experience*
OC 11 09h50-10h00	Filipa Oliveira	*Liquid biopsies and (epi)genomic characterisation of glioblastoma*
OC 12 16h30-16h40	Liliana Sousa	*Optimizing prevention and care in rare tumour risk syndromes (RTRS): insights from the preventable european consortium*
OC 13 16h40-16h50	Diogo Caetano	*Pharmacogenomic studies in medical genetics: a pilot approach to personalized medicine in practice*

Saturday 22nd November - Clinical Cases

TIME SLOT	PRESENTING AUTHOR	TITLE
OC 14 08h30-08h36	Jorge Diogo Da Silva	*Early detection of juvenile myelomonocytic leukemia in CBL-related noonan-like syndrome through exome sequencing.*
OC 15 08h36-08h42	Diana Macedo Cardoso	*The hidden risk of paracentric inversions: partial 9P trisomy and neurodevelopmental disorder*
OC 16 08h42-08h48	Daniela Oliveira	*Disruption of SPECC1L translation initiation by intragenic deletion: novel pathogenic mechanism in teebi-hypertelorism syndrome*
OC 17 08h48-08h54	Sara Jesus	*Somatic RHOA mosaicism unveiled by deep sequencing: dysmorphology-driven diagnosis*
OC 18 08h54-09h00	Isabel Alonso	*Integrating transcriptomics into the clinical setting for the diagnosis of SH3KBP1 deficiency*
OC 19 09h00-09h06	Pavlina Rayko	*Broadening the phenotypic spectrum of thumpd1-related syndrome: the oldest reported patient with novel features*
OC 20 09h06-09h12	Eunice Matoso	*HELLS-associated episignature enables the diagnosis of ICF-4 syndrome*
OC 21 09h12-09h18	Vera Sousa Marques	*Charge syndrome with an additional FBRSL1 variant: interpreting unknown genetic findings*

OC01 DISSECTING AND PREDICTING THE IMPACT OF ENHANCER VARIANTS IN TYPE 2 DIABETES USING HIGH-THROUGHPUT MUTAGENESIS AND REPORTER ASSAYS

J.M. Nogueira[1,2], F.J. Ferreira[1,2], S.M. Borges[1], F. Magalhães[1,2], T. Nogueira[1,2], M. Sousa[1,2], A. Eufrásio[1], J. Bessa[1,2]

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Introduction

Type 2 diabetes (T2D) is a widespread metabolic disorder with severe complications. Many genetic variants associated with T2D risk reside in non-coding cis-regulatory regions, particularly transcriptional enhancers, implicating dysregulated transcription in disease susceptibility. Functional characterization of these enhancers and systematic evaluation of the impact of sequence variants are essential to understand pancreatic gene regulation and to develop predictive models for enhancer dysfunction and disease risk.

Methodology

Candidate pancreatic enhancers were prioritized by intersecting T2D-associated SNPs with enhancer-associated epigenetic signatures of human islets. Their regulatory activity was first tested using massively parallel reporter assays (MPRAs). Functional candidates were further validated by establishing stable endocrine pancreas enhancer-reporter cell lines: single cells were isolated by FACS, genotyped, and clonally expanded. To investigate the impact of sequence variation, we applied two complementary strategies: (i) CRISPR/Cas9-mediated random mutagenesis directly within the integrated enhancer on these lines, and (ii) PCR-based random mutagenesis to generate enhancer variant libraries for MPRA testing. These mutant libraries enabled high-throughput quantification of nucleotide-level effects on enhancer activity.

Results

We validated hundreds of enhancers with significant activity and generated multiple stable enhancer-reporter lines. Random mutagenesis supported the identification of nucleotides and transcription factor motifs essential for pancreatic enhancer function, with certain variants producing reproducible gain- or loss-of-function effects. Integration of these data enabled the development of machine learning models that accurately predict functional outcomes of enhancer mutations.

Discussion

This work establishes a scalable pipeline for functional dissection of non-coding variants in T2D. By linking enhancer sequence variation to regulatory activity, our results not only highlight key elements of pancreatic enhancer grammar but also provide a framework for predicting the impact of genetic variants on gene expression. This approach advances our capacity to interpret the non-coding genome in the context of T2D.

Funding/Financing: La Caixa Foundation grant HR21-01212; FCT COMPETE2030-FEDER grant

Keywords: Type 2 diabetes (T2D), Enhancers, Cis-regulation, Nucleotide Alterations, Machine learning

OC02 GENERATION OF CELLULAR MODELS FOR FABRY DISEASE: UNLOCKING THE POTENTIAL OF IPSCS AND GENE EDITING

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Introduction

Fabry Disease (FD) is a lysosomal storage disorder caused by mutations in the *GLA* gene, resulting in a defective α-GAL A enzyme. This deficiency leads to the accumulation of Gb3 and lyso-Gb3 within lysosomes, resulting in a multisystem disease[1]. Through reprogramming, we obtained induced pluripotent stem cells (iPSCs) derived from fibroblasts of a patient with FD[2] and from a wild-type (WT) control. We used CRISPR/Cas9 to correct the c.860G>A mutation present in the patient's cells[3], as well as to generate a WT *GLA* knockout (KO). The resulting cells were then differentiated into cardiomyocytes, a cell type affected by this disease.

Methods

We reprogrammed the fibroblasts into iPSCs using episomal vectors or Sendai virus. For gene editing, single-guide RNAs (sgRNAs) and Cas9 were nucleofected, and the editing was confirmed by Sanger sequencing. Following colony selection, isogenic cell lines were established. The FD iPSCs, the corrected FD iPSCs, and the WT iPSCs were then differentiated into iPSC-derived cardiomyocytes (iPSC-CMs).

Results

Seven new cell models were generated. Functional studies of the FD iPSCs showed the maintenance of the molecular and biochemical characteristics and a normal karyotype. The KO cell line recapitulated the biological features observed in FD patient cells, with reduced *GLA* expression, lower α-Galactosidase A (α-Gal A) activity (1.5 nmol/h/mg protein), and Gb3 accumulation. The corrected cell line was generated with 75.8% efficiency and 69.6% on-target efficacy. Enzyme activity increased to 579 nmol/h/mg protein (*vs.* 0.78 nmol/h/mg protein in FD iPSCs), accompanied by a marked reduction in Gb3 levels. We successfully generated iPSC-CM lines, which were validated by qRT-PCR and immunofluorescence.

Discussion

Cell modelling is essential for studying the pathophysiology of disease mechanisms. By retaining the characteristics of the original cells, iPSCs are a valuable biological resource for generating specific differentiated cell types affected by the disease, which would otherwise be difficult to access. This study also explored the therapeutic potential of gene editing as a promising approach to altering the course of rare diseases.

References

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3. Guo *et al.*, 2018. Doi: 10.1038/s41598-018-20358-5

Funding: Financial support from FCT/MCTES through the projects UIDB/00211/2020, PTDC/BIM-MEC/4762/2014, and SFRH/BD/118009/2016.

Keywords: Rare diseases; iPSCs; CRISPR/Cas9; Cell models

OC03 DISSECTING NON-CODING REGULATION OF *LDLR* AND *PCSK9* IN FAMILIAL HYPERCHOLESTEROLAEMIA

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Introduction

Familial hypercholesterolaemia (FH) is most often caused by pathogenic variants in *LDLR*, *APOB* and *PCSK9*, yet a substantial proportion of clinically diagnosed patients remain without a molecular explanation. Non-coding regions such as promoters and 5'UTRs are likely contributors but remain poorly characterised. This study set out to systematically investigate the regulatory impact of 100 *LDLR* and *PCSK9* promoter/5'UTR variants.

Methodology

Promoter/5'UTR sequences of *LDLR* (c.-319 to ATG) and *PCSK9* (c.-650 to ATG, mapped by 5'-RACE) were cloned upstream of *Firefly luciferase* in pGL4.10 and modified by site-directed mutagenesis. Variant constructs were transfected into CHO-ldlA7 and Huh7 cells alongside a *Renilla luciferase* control, and reporter activity was quantified under varying cholesterol conditions.

Results

Functional assays revealed a wide spectrum of effects, with both loss- and gain-of-function variants relative to wild-type constructs. These variants are functionally significant, since alterations in *LDLR* and *PCSK9* expression exert opposing effects on circulating LDL-C, thereby influencing FH phenotypes. In *LDLR*, functionally relevant variants clustered within key transcription factor binding motifs (e.g., SREBP, Sp1), while those outside regulatory sites showed minimal effect, underscoring the importance of these elements in gene expression and the value of functional assays for interpreting non-coding variants. Among the variants studied in the *PCSK9* promoter, c.-346G>C and c.-353G>T emerged as particularly relevant due to their opposing effects on transcriptional activity, down regulating and upregulating, respectively. To our knowledge, this study also provides the first experimental definition of the *PCSK9* 5'UTR.

Discussion

These findings reinforce the need to integrate non-coding functional assays into variant classification pipelines, expanding the diagnostic yield and enhancing personalised management of FH. By establishing a scalable framework for functional interrogation, this work advances the systematic characterisation of regulatory variants, underscores their importance for variant classification, and highlights their relevance for improving FH diagnostics and personalised management.

Funding: PerMedFH project funded by La Caixa Foundation in partnership with FCT.

Keywords: Familial hypercholesterolaemia; promoter and 5'UTR variants; functional assays; genetic diagnostics

OC04 DIFFERENTIAL ALLELIC EXPRESSION CASE-CONTROL STUDY UNVEILS MISSING PROSTATE CANCER HERITABILITY AND PLEIOTROPY WITH BREAST CANCER

André Besouro-Duarte[1,2], Letícia P. Leite[1], Nelson Martins[1], Marinella Ghezzo, Ana Teresa-Maia[1,2,3,*] Joana M. Xavier[1,3,*]

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* Joint Last-authors

Prostate cancer (PC) has an estimated genetic heritability of 58%, much of which remains unexplained[1,2]. As most known PC risk loci map to non-coding regions, we hypothesize that unidentified cis-regulatory variants account for part of this missing heritability[3,4]. We propose a case–control approach leveraging differential Allelic Expression (AE) as a quantitative read-out to detect cis-regulatory variants associated with PC risk. This strategy, previously validated in breast cancer (BC) susceptibility studies, provides proof of principle[5]. Finally, we will assess pleiotropy to investigate whether shared cis-regulatory variants contribute to the genetic architecture of both PC and BC.

We analyzed genomic and transcriptomic data from normal prostate of healthy (GTEx, n=116) and PC patients (TCGA, n=51). For TCGA, genotype imputation was performed from array data using the 1000 Genomes Project (phase 3 v5). Genetic ancestry was inferred with ADMIXTURE. RNA-seq data from individuals of European ancestry were aligned to GRCh38 using STAR; variant calling was performed with GATK, restricted to heterozygous sites in matched DNA. AE fractions were computed as the ratio of alternative allele counts to total read counts and compared between groups. Pleiotropy was assessed through a meta-analysis combining our PC results with data previously generated from BC.

Amongst 5 679 tested variants located within 3 180 genes, we identified 133 SNPs in 123 genes with AE levels associated with PC risk modulation ($|\text{effect size}| \geq 0.5$ and q value ≤ 0.05). Meta-analysis with a previous BC study identified 16 pleiotropic variants, representing 22.5% of the PC and 10% of the BC risk-associated SNPs tested in both datasets.

This approach enables the discovery of PC risk variants and target genes by prioritizing cis-regulatory mechanisms. Its robustness is supported by the identification of multiple risk-associated SNPs per gene with consistent effect sizes, as well as of variants and genes previously implicated in cancer susceptibility. The substantial overlap of risk-associated SNPs between PC and BC highlights shared genetic determinants, pointing to common biological pathways underlying disease risk

Keywords: Allelic Expression; Prostate Cancer; Genetic Risk; Pleiotropy

Funding: FCT – 2023.01351.BDANA, DL 57/2016/CP1361/CT0042, PLEIREG 2022.02380.PT CINTESIS UIDB/04255, RISE LA/P/0053/2020, and CCMAR UIDB/04326/2020

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OC05 ESTABLISHMENT OF A SCALABLE NEURONAL REPORTER MODEL OF MACHADO-JOSEPH DISEASE BASED ON GENE-EDITED PATIENT CELLS AS A PLATFORM FOR HIGH-THROUGHPUT DRUG REPURPOSING

Frederico M. Pena[1,2,3,4], Ana R. Fernandes[1,2,3,4], Miguel Lopes[1,2,3,4,6], Jisette Núñez[1,2,3,7], Carlos Matos[7,8,9], Sara Lopes[1,2,3,5], Rosário Faro[1,2,3], Mariana Pires[1,2,3], Ana Pereira[1,2,3], Dina Pereira[1,2,3], Sónia Duarte[1,2,3], Rui Nobre[1,2,3,6], Pedro R. L. Perdigão[1,2,3,10], Luís P. de Almeida[1,2,3,6,7,10]

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Abstract

Introduction

Machado-Joseph disease (MJD) is the most dominant form of autosomal ataxia worldwide. This neurodegenerative disorder is caused by a CAG repeat expansion in the *ATXN3* gene, resulting in a toxic, polyglutamine-expanded Ataxin-3 deubiquitinase. Therapeutics able to reduce mutant Ataxin-3 levels remain a key focus. Drug development is challenged by the lack of disease models that replicate MJD pathophysiology while supporting large-scale applications. Here, we developed a neuronal reporter model for MJD using gene-edited, patient-derived induced pluripotent stem cells (iPSCs) to rapidly monitor endogenous Ataxin-3 levels.

Methodology

We genetically modified MJD iPSCs using CRISPR/Cas9 to integrate a reporter in-frame with mutant *ATXN3*. Gene editing was estimated via *in silico* analysis of Sanger sequencing, and edited clones were validated via PCR amplification and western blot. To recapitulate cellular context, MJD-reporter iPSCs were differentiated into induced neurons (iNeurons) for evaluation of reporter activity and screening of an FDA-approved drug library to identify compounds that modulate Ataxin-3 levels.

Results

We designed CRISPR/Cas9 sequences that edit *ATXN3* with 10-15% efficacy, with 5% of the clones exhibiting bi-allelic reporter integration and correct expression of the Ataxin-3::reporter fusion. MJD-reporter iPSCs were differentiated into homogeneous iNeuron cultures exhibiting neuronal markers within 7 days. Reporter activity connected to endogenous Ataxin-3 was efficiently detected. We next screened 2300 drug candidates and identified, so far, 23 compounds capable of either reducing or enhancing Ataxin-3 levels. Drug candidates will be further validated by assessing MJD hallmarks both in patient-derived neurons and animal models.

Discussion

Overall, we developed a novel MJD neuronal reporter model suitable for large-scale drug screening in a physiological relevant context. We are confident that this work offers a powerful platform for drug repurposing and will help accelerate the identification of promising therapeutic targets for MJD.

Funding: Work funded by the ERDF (COMPETE 2020, POCI); FCT – EXPL/MED-NEU/0936/2021, ViraVector (CENTRO-01-0145-FEDER-022095), CinTech (PRR, Ref PC644865576-00000005; SCARNA (AFM-Téléthon, P25124); ARDAT (IMI2 JU no. 945473), ERDERA ID:101156595, Capacity 2023 ID: 101145599, GeneH ID:101186939, GCure ID: 101186929, and GeneT Teaming Project ID: 101059981, supported by the EU Horizon Europe program.

Conflicts of Interest: None.

Keywords: Machado-joseph Disease; Gene Editing; Neuronal reporter model; High-throughput Screening; Drug Repurposing.

OC06 CLINICAL TRAJECTORIES AND HEALTHCARE BURDEN OF HEREDITARY DIFFUSE GASTRIC CANCER: PREVENTION VERSUS TREATMENT IN EUROPEAN CENTRES

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Introduction

Hereditary diffuse gastric cancer (HDGC), caused by germline variants in CDH1 or CTNNA1, is a rare autosomal dominant syndrome with high risks of gastric and lobular breast cancer. Management includes counselling, surveillance, prophylactic surgery, or cancer treatment. Comparative evidence on outcomes and costs of these care pathways is limited. This study evaluated clinical outcomes and healthcare costs of prevention versus treatment in European HDGC carriers.

Methodology

As part of the Horizon Europe PREVENTABLE project, we compiled a multicentre retrospective cohort (1998–2023) of 426 individuals from 9 centres in 7 European countries. Data were collected via a harmonised IT tool, including interventions, timing, resource use, costs (standardised prices), and survival. Primary outcomes were overall survival and cumulative healthcare cost per patient by care pathway.

Results

Among 246 CDH1 carriers, 203 (82.5%) followed prevention and 43 (17.5%) treatment pathways. In prevention, 56% had prophylactic gastrectomy within 9 months, and 20% of women chose prophylactic mastectomy within 42 months of diagnosis. Despite prevention, 9.4% developed cancer. There were 18 deaths: 16 from diffuse gastric cancer (median age 38.1 years) and 2 from lobular breast cancer (median 68 years). Median follow-up was longer in prevention (43 months) than treatment (32 months). Treatment involved early mortality, intensive systemic therapy, and frequent hospitalizations, leading to higher per-patient costs (€18,360 vs €6,765). Although total prevention costs were higher (€1.37M vs €0.79M), prevention served five times more patients with stable costs, unlike sharp cost spikes in treatment.

Discussion

HDGC prevention resulted in longer survival, predictable care, and lower per-patient costs, while treatment involved early deaths, higher hospital burden, and unsustainable costs. This multicentre evidence supports early genetic diagnosis, structured surveillance, and timely prophylactic surgery as effective and cost-efficient HDGC management strategies.

Funding: Supported by the European Union's Horizon Europe Programme, PREVENTABLE Project (Grant Agreement nº 101095483)

Ethics and Consent: All centres obtained approval from local ethics committees and informed consent from participants, according to national regulations and the PREVENTABLE project framework.

Keywords: Hereditary diffuse gastric cancer; Cancer genetics; Health economics; Prevention; European cohort.

OC07 *MUTYH* CARRIER FREQUENCY IN PORTUGAL: NAVIGATING THE DATA GAP WITH WHAT WE'VE GOT

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Introduction

The lack of a national genetic database requires alternative methods to estimate carrier frequencies. Although clinical whole-exome sequencing (WES) data may have selection biases, they serve as useful proxies for population genetics. This study estimated the carrier frequency of pathogenic/likely pathogenic (P/LP) *MUTYH* variants in Portugal by reanalyzing clinical WES data from a commercial lab. Methodology, developed with the Biostatistics Department, applied strict inclusion criteria and demographic weighting to improve representativeness.

Methods

We analyzed individuals who underwent WES/ WES-based NGS panels over the last 10 years for non-oncological indications, aged <25 years (including prenatal), one individual per family. All *MUTYH* variants were extracted, only P/LP variants were included. Variants were grouped by Portugal's NUTS II regions. Allele frequencies and heterozygous carrier rates were estimated per region assuming Hardy–Weinberg equilibrium, and national prevalence was weighted by 2021 Census data.

Results

We analyzed 1166 qualifying samples. Robust carrier frequency estimates were obtained for Norte (2.98%), Centro (1.7%), and Lisboa (2.85%), covering 84% of the population; other regions lacked sufficient data. A population-weighted average of these three regions yielded a national prevalence of 2.6% (95% CI: 1.65-3.67%). Variant c.1187G>A represented 56.6% of pathogenic alleles (1.4% prevalence), c.536A>G 23.3% (0.6%), and c.1227_1228dup was not detected.

Conclusions

Our estimate exceeds the 1.86% reported in gnomAD for non-Finnish Europeans, but falls below Isidro *et al.*, who reported 2% for c.1187G>A in Portugal. It supports that c.1187G>A and c.536A>G together represent over 80% of pathogenic *MUTYH* alleles in Europeans. Conversely, the absence of c.1227_1228dup validates our clinical impression of its much lower frequency. These findings suggest *MLPA*-based testing, which includes the two most common variants, is a cost-effective first-tier test for individuals with 10–20 adenomas. Our prevalence results support current recommendations against routine reporting of heterozygous *MUTYH* status in WES/NGS panels. Study limitations include sample *bias* and the non-population-based nature of the data, reinforcing the need for a national genomic database to refine carrier estimates and inform public health policies.

OC08 LESSONS LEARNED FROM A PORTUGUESE INHERITED RETINAL DISEASE RESEARCH COHORT

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Introduction

Inherited retinal diseases (IRD) are rare monogenic conditions caused by impairment in retinal or ubiquous molecular pathways. Integration of next-generation sequencing, biomolecular research, and multimodal in vivo imaging has led to rapid advances in the field of ophthalmogenetics.

Methodology

Analysis of clinical and molecular data of patients recruited for the study "Genotipagem de Doentes e Familiares com Distrofias Corio-Retinianas Hereditárias" between 2017 and 2023. Clinical, multimodal evaluation and whole-exome sequencing (WES) were performed as previously described(1).

Results

A total of 339 families were included in this review. 195 probands (57.5%) had a clinical diagnosis of retinitis pigmentosa (RP). A molecular diagnosis was achieved in 83.4% of pedigrees with variants in 81 different genes. A total of 42 novel variants were introduced for the first time in ClinVar. Regarding zygosity, 41.9% of probands had P/LP variantsin homozygosity. Median cumulative autozygome was 67 Mb (for autosomes). The most frequently mutated genes in the cohort were *ABCA4* (n=33), *EYS* (n=22), *USH2A* (n=20), *RPGR* (n=17), and *RHO* (n=9). A deletion of 107.5 kb encompassing exons 13 and 14 of EYS gene had the highest allelic frequency in the cohort. Biallelic variants in genes classically associated with syndromic disease (*INPP5E*, *COQ8B* and *AP5Z1*) were associated with non-syndromic forms of IRD.

Discussion

Analysis of this cohort has shed light on the national and international landscape of inherited retinal diseases over the past ten years. First by providing the first large Portuguese IRD landscape including high prevalence of EYS-related RP. Second by contributing to international databases via novel ClinVar variants. Third, by expanding the phenotypic continuum of COQ8B and AP-5-complex.

Funding: Grants 176097 and 204285 from the Swiss National Science Foundation (CR) and Jürg Tschopp MD-PhD Scholarship (VGP).

Conflicts of Interest: The authors declare no competing interest.

Keywords: inherited retinal diseases; phenotypic expansion; genetic cohort

Ethics and Consent: This study was approved by CES of IOGP and Ethikkommission Nordwest- und Zentralschweiz. Written informed consent was obtained from all individuals or their legal guardians.

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OC09 UNDERSTANDING THE GENETIC BASIS OF HEREDITARY CARDIOVASCULAR DISEASES IN PORTUGAL: A RETROSPECTIVE ANALYSIS ACROSS TWO CENTRES

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Introduction

Hereditary cardiovascular diseases (HCVDs) are a major cause of morbidity and mortality worldwide. Genetic diagnosis can improve disease identification, prognostic evaluation, family screening, and reproductive counselling. However, challenges such as penetrance, variable expressivity, and variant classification complicate clinical management. This study aims to assess the diagnostic yield of genetic testing for HCVDs in a large cohort and to characterize the genes most frequently implicated —a key step toward mapping the mutational landscape in the Portuguese population.

Methodology

A retrospective analysis was conducted on patients from two tertiary centres who underwent genetic testing for HCVDs at a national laboratory between January 2011 and March 2025. Clinical records were reviewed to assess phenotype and genetic testing results. Variants reported before 2015 were reinterpreted according to the ACMG guidelines to ensure accuracy in diagnostic reporting.

Results

A total of 1,259 probands (778 male, 481 female) were included: 930 tested for cardiomyopathies (CM), 291 for arrhythmias, and 38 using extended panels. Pathogenic or likely pathogenic variants were identified in 19.5% of cases, 32.5% were inconclusive (either due to variants of uncertain significance [VUS] or the presence of a pathogenic variant in an autosomal recessive gene), and 48% were negative. The diagnostic yield was highest for CM panels compared to arrhythmia panels, while extended panels yielded more VUS findings. In hypertrophic CM, deleterious variants in *MYH7* and *MYBPC3* were identified in 72% of patients, while *TTN* loss-of-function variants accounted for 50% of cases in dilated CM. Among arrhythmias, genetic testing identified causative variants in 30% of long QT syndrome, but only 5% in Brugada syndrome.

Discussion

This nationwide study reveals a diagnostic yield of nearly 20% for HCVDs, with higher success rates in CM panels. The distribution of disease-specific genetic variants aligns with international findings, highlighting the reliability of genetic testing for certain conditions. Extended genetic panels led to a higher frequency of VUS, underscoring the need for caution in interpretation and continuous reclassification of variants. Importantly, this is the first comprehensive dataset on HCVD genetics in the Portuguese population, providing a foundation for personalized patient management, guiding family screening, as well as advancing our understanding of HCVDs in this population.

Conflicts of interest: None

Key words: Genetic Counselling, Cardiogenetics

OC10 RNA ANALYSIS AS A FIRST-LINE DIAGNOSTIC APPROACH FOR NEUROFIBROMATOSIS TYPE 1: A SIX-YEAR INSTITUTIONAL EXPERIENCE

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Introduction

Neurofibromatosis type 1 (NF1) is caused by inactivating variants in the *NF1* gene. While clinical diagnosis can often be established, molecular confirmation may facilitate the identification of patients in early stages of the disease and is important for appropriate follow-up and genetic counselling. Approximately 30% of pathogenic *NF1* variants impact splicing, and around 10% of these remain undetectable through genomic DNA (gDNA) analysis. This study summarizes six years of institutional experience implementing RNA-based testing as the first-line diagnostic strategy for NF1.

Methodology

Over the last six years, 131 individuals were referred to our centre for RNA-based analysis. Indications for testing included confirmation of a clinical diagnosis of NF1, as well as confirmation or exclusion of the diagnosis in individuals presenting with incomplete clinical features. Full-length *NF1* transcripts (cDNA) were sequenced using eight partially overlapping amplicons. When RNA testing was negative, MLPA was performed to detect large deletions or duplications.

Results

Pathogenic or likely pathogenic variants were identified in 69 of 131 patients (52.7%), confirming the diagnosis. One patient (0.8%) had a variant of uncertain significance (VUS).

Of the 69 positive cases, 39 (56.5%) would have been identified by conventional gDNA analysis. Another 29 patients (42.0%) harboured splice-altering variants, 19 (27.5%) of which involving canonical splice sites or other sequence changes theoretically detectable by gDNA analysis, but requiring RNA testing to confirm splicing impact and pathogenicity. The remaining 10 patients (14.5%) carried deep intronic variants or Alu insertions that would have been missed by exonic gDNA-based methods. In addition, one patient who tested negative by both RNA and MLPA analyses was subsequently found to carry a 5' UTR variant, c.-273A>G.

No variants were identified in the remaining 61 individuals (46.6%), most of whom did not fulfil clinical criteria for NF1, supporting exclusion of the diagnosis.

Discussion

This six-year experience underscores the dual utility of RNA analysis in NF1 diagnostics. In addition to enhancing sensitivity by detecting splicing alterations invisible to conventional gDNA analysis, RNA testing provides evidence of the functional impact of variants already detectable at the DNA level. These findings highlight the importance of integrative molecular strategies for accurate diagnosis and optimized patient care in NF1.

Keywords: NF1; RNA; Splicing

OC11 LIQUID BIOPSIES AND (EPI)GENOMIC CHARACTERISATION OF GLIOBLASTOMA

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Introduction

Glioblastoma (GBM) is the most aggressive and prevalent primary tumour of the central nervous system, characterised by marked heterogeneity, rapid growth, and limited therapeutic options. To address these challenges, we analysed a large multicentric Portuguese cohort to explore the potential of cfDNA as a biomarker in GBM and to characterise the genetic and epigenetic landscape of this tumour.

Methodology

This study included 62 GBM patients from three institutions (Unidade Local de Saúde de Coimbra, Unidade Local de Saúde de Faro and Instituto Português de Ocologia de Coimbra), with the collection of 62 tumour samples and 195 peripheral blood samples at different clinical time points, along with plasma samples from 24 controls. cfDNA was isolated and quantified, and its correlation with clinical parameters was assessed. Tumour molecular characterisation was performed using MS-MLPA to simultaneously assess copy number variation and methylation, and aCGH, to provide a genome-wide overview. Functional enrichment of altered genes was explored through Gene Ontology (GO) terms.

Results

cfDNA analysis demonstrated its ability to reflect tumour burden and monitor treatment response, anticipating radiological progression in some cases, supporting its value as a real-time monitoring tool. Although no robust correlation with overall survival, tumour volume, or progression-free survival was observed, this highlights the impact of inter-patient variability and tumour heterogeneity. MS-MLPA identified amplifications (*CFTR*, *CDK6*, *STK11*, *VHL*) and deletions (*PTEN*, *CREM*, *CDKN2A*, *MGMT*), including cases of double inactivation (*PYCARD*, *PAX5*, *ESR1*, *WT1*, *MGMT*). aCGH confirmed extensive CNVs, with frequent gains on chromosome 7 and losses on chromosome 10, and highlighted 18 genes (*LOC100133469*, *DLX5*, *PEG10*, *DLX6-AS1*, *DLX6*, *TWIST1*, *KLF14*, *CASD1*, *COL1A2*, *SGCE*, *SHH*, *WASHC2A*, *HNRNPH3*, *RUFY2*, *SLC25A16*, *DNA2*, *PBLD,* and *ANTXRL*), many of which have not yet been explored in GBM biology, that are potentially implicated in tumour development and progression. GO term analysis highlighted enrichment of biological processes related to differentiation, cell cycle regulation, apoptosis, DNA repair, and signalling pathways critical for gliomagenesis, providing functional insight into the detected alterations.

Discussion

Altogether, these findings support the clinical potential of cfDNA as a non-invasive biomarker for disease monitoring and emphasise the value of integrating genomic and epigenomic data to capture GBM heterogeneity. Importantly, this study represents one of the largest Portuguese cohorts of GBM patients analysed to date, strengthened by its multicentric design, which increases representativeness and reinforces the relevance of collaborative studies in rare and highly heterogeneous tumours such as glioblastoma.

Funding / Financing: This work was supported by NRC.LPCC/CIMAGO.

Keywords: Glioblastoma; Liquid biopsies; Cell-free DNA; Molecular characterisation; Copy Number Alterations.

OC12 OPTIMIZING PREVENTION AND CARE IN RARE TUMOUR RISK SYNDROMES (RTRS): INSIGHTS FROM THE PREVENTABLE EUROPEAN CONSORTIUM

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Introduction

RTRS are individually rare but collectively impose a substantial clinical, social and economic burden. The PREVENTABLE project aims to assess the clinical, financial and social impact of applying multidisciplinary and specialized care to prevent advanced disease in families suffering from rare tumour risk syndromes (RTRS). Defining patient trajectories, documenting care pathways, assessing prevalence, and evaluating the impact of preventive interventions, while generating educational content, communication guidelines and policy insights is the long term objective.

Methodology

Optimized care pathways were developed by ERN-GENTURIS expert centres in RTRS, incorporating patient settings, clinical trajectories, and procedures for diagnosis, surveillance, prevention, and treatment. These were integrated into an IT platform for real-world data collection. Real world clinical data from 8 RTRS were gathered across 9 European centers. Educational materials for patients and professionals, communication guidelines, and cost-effectiveness models were created to support evidence-based care and policymaking.

Results

A total of 1,108 RTRS families were identified (28% Li-Fraumeni Syndrome (S.), 26% PTEN Hamartoma Tumour S., 16% Peutz-Jeghers S., 9% Hereditary Diffuse Gastric Cancer, 8% Birt-Hogg-Dubbé S., 6% Hereditary Leiomyomatosis and Renal Cell Carcinomas, 6% Familial Malignant Melanoma, and 1% Gastrointestinal Stromal Tumour S.). From these, 1,967 RTRS carriers were offered multidisciplinary care to treat or prevent advanced disease, while 2,026 non-carriers were discharged, reducing unnecessary follow-ups, and alleviating the burden on healthcare systems. Based on the total population of 16.6 million served by the 9 centers, RTRS carrier prevalence was estimated at 1.18:10,000. Multiple pathways of care were documented, capturing both preventive and therapeutic interventions, supporting improved clinical outcomes, survival, and quality of life, while optimizing resource allocation.

Discussion:

This study highlights the importance of a multidisciplinary care model for rare tumour risk syndromes (RTRS), improving outcomes for 1,967 identified carriers while safely discharging 2,026 non-carriers. With a carrier prevalence of 1.18 per 10,000, the approach optimized care, is expected to enhanced quality of life, and reduced strain on healthcare resources.

Funding: Funding from the EU's Horizon Europe Coordination and Support action under Grant Agreement nº 101095483 (PREVENTABLE).

Keywords: *RTRS; Care pathways; Prevention; Education; Cost-effectiveness; Health Policy*

OC13 PHARMACOGENOMIC STUDIES IN MEDICAL GENETICS: A PILOT APPROACH TO PERSONALIZED MEDICINE IN PRACTICE

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Introduction

Pharmacogenetic (PGx)-guided prescription is a well-validated approach to improve efficiency and minimize adverse events of drugs. However, the use of broad pharmacogenomic testing is still mostly unused in clinical practice. We previously established an outpatient consultation to provide broad pharmacogenomic testing and subsequent counselling, within the Medical Genetics service of the Centro Hospitalar Universitário de Santo António. Here we performed a retrospective analysis of the impact of PGx testing.

Methodology

We reviewed all PGx tests, which encompass 25 PGx-actionable genes, for actionable gene-drug pairs (≥ level 2B ClinPGx evidence), and correlated them with the patients' past and present medication. A telephonic quantitative questionnaire (Likert scale) was applied to assess the patient's perception of the utility of the test.

Results

Twenty-six patients underwent PGx testing (median age 28 years) due to a history of adverse reactions, lack of efficacy or for prevention. All participants had at least one actionable variant. Overall, 15 participants (57.7%) would have required therapeutic adjustment for at least one prescribed medication, while 11 participants (42.3%) were classified as high-risk for at least one such drug. Actionable gene-drug pairs were identified for 22 prescriptions (16.1%), involving 19 drugs. All information was provided to prescribing clinicians in a clinical PGx report. Survey responses (n=18) indicated strong agreement with the utility of PGx, and that test results were easy to understand and implement (rate of 89% positive responses in the overall questionnaire). Qualitative assessment revealed that half the respondents highlighted the value of knowing which medications to avoid in the future. Comprehension of the concept/utility of PGx testing positively correlated with the total questionnaire score (ρ=.529, p=.029), albeit clinical impact did not (p=.146), nor did the other demographic, clinical, or PGx variables.

Discussion

this pilot study demonstrates the feasibility and clinical value of PGx testing in a Portuguese hospital. Even in a small cohort, clinically relevant variants for current medication were identified in more than half of the patients. The great value placed by the participants on the prospective benefits of PGx findings, as opposed to their immediate clinical impact, underscores the perceived importance of primary prevention and effective clinician–patient communication. In the future we will assess the clinicians' views of PGx testing.

OC14 EARLY DETECTION OF JUVENILE MYELOMONOCYTIC LEUKEMIA IN *CBL*-RELATED NOONAN-LIKE SYNDROME THROUGH EXOME SEQUENCING

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Introduction

Germline RAS/MAPK pathway gain-of-function variants are associated with well-known monogenic conditions such as Noonan syndrome. These conditions have oncogenic propension, due to the possibility of somatic second hits, therefore increasing the risk of malignancy such as leukemia.

Methodology

Description of a clinical case, laboratorial approach to diagnosis, and further management.

Results

A 2-year-old boy was referred to Clinical Genetics due to persistent hematological alterations. He was assessed in the emergency department at 9 months of age due to acute gastroenteritis. Physical examination revealed hepatosplenomegaly, and the analytical workup was remarkable for normocytic/normochromic anemia, thrombocytopenia and leukocytosis, all of which persistent until the present age. Further analytical assessment revealed neutrophilia, monocytosis, polyclonal B-cell lymphocytosis, and elevated TDN cells. Upon assessment in our consultation, he presented dysmorphic facial features (hypertelorism, downslanting palpebral fissures, and low-set posteriorly rotated ears) and 2-3 bilateral toe syndactyly. Growth and development were normal. Exome sequencing revealed the pathogenic variant c.1111T>C, p.(Tyr371His), in the *CBL* gene, with a VAF of approximately 90%, which was called as homozygous. Inspection of adjacent variants in *CBL* and contiguous genes revealed a similar VAF for all variants, suggesting loss-of-heterozygosity (LOH) of the 11q23 chromosomal region. Sanger sequencing in buccal epithelium DNA revealed the same variant in heterozygosity, indicating the presence of a germline *CBL* variant with LOH in hematopoietic tissue. In combination with clinical criteria, a diagnosis of juvenile myelomonocytic leukemia (JMML) was established, with underlying *CBL*-related Noonan-like syndrome. The patient was referred to haplotyping and is currently waiting for hematopoietic stem cell transplantation (HSCT). Familial studies determined that the germline variant was inherited from the father, which presented with similar dysmorphic features.

Discussion

This case illustrates that accurate detection and interpretation of germline and somatic variants in NGS testing is crucial, as diagnosis of life-threatening conditions can be rapidly anticipated, even when the clinical presentation is atypical, indolent or complex. Reverse phenotyping after exome sequencing therefore allowed the patient to be referred earlier to HSCT, which can improve its long-term prognosis.

OC15 THE HIDDEN RISK OF PARACENTRIC INVERSIONS: PARTIAL 9P TRISOMY AND NEURODEVELOPMENTAL DISORDER

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Introduction

Paracentric inversions (PAI) are chromosomal rearrangements resulting from two breaks within the same chromosome arm, followed by reinsertion in reverse orientation, leading to an inverted sequence for that segment. Despite PAI being frequently regarded as uncommon and clinically "benign", their reproductive consequences remain under-acknowledged; while the presence of an inversion does not necessarily cause an abnormal phenotype, meiotic crossing over in inversion heterozygotes can generate chromosomal imbalances in descendants.

Clinical Case

We present the case of a healthy mother referred to our Medical Genetics consultation due to cancer family history. During evaluation, it was ascertained that the patient had a daughter with intellectual disability who had never been assessed by a geneticist. The daughter was then a 46-year-old woman with developmental delay, behavioural changes, dysarthria, and high-grade myopia, who was presumed to have cerebral hypoxia, although this was never confirmed.

Results

Cytogenetic evaluation revealed that the daughter presented with a chromosome 9 short arm partial trisomy (9p24–9p13 duplication). Fluorescence in situ hybridisation (FISH) confirmed preservation of the subtelomeric region of the same chromosome. Karyotype analysis of the mother identified a PAI involving 9p24–9p13, consistent with the daughter's unbalanced rearrangement and compatible with a meiotic recombination event.

Discussion

The outcome of homologous pairing in PAI carriers depends on meiotic behaviour: a classical crossover within the inversion loop produces an acentric and a dicentric recombinant chromosome, prone to abortion, whereas a U-type exchange results in two monocentric chromosomes, one with a duplication and another with a deletion. Although the literature frequently suggests that PAI are rare and confer negligible reproductive risks, this case illustrates their potential to generate viable unbalanced offspring. It emphasises the critical need for comprehensive genetic counselling in all carriers of such rearrangements, regardless of their presumed rarity or non-pathogenicity.

Conflicts of interest: None

Ethics and Consent: Informed consent was obtained from the patient and family for this clinical case presentation.

Keywords: Paracentric inversion; Partial trisomy; Neurodevelopmental disorder.

OC16 DISRUPTION OF *SPECC1L* TRANSLATION INITIATION BY INTRAGENIC DELETION: NOVEL PATHOGENIC MECHANISM IN TEEBI-HYPERTELORISM SYNDROME

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Abstract

Introduction

SPECC1L encodes a cytoskeletal-associated protein essential for neural crest cell migration and tissue morphogenesis. Pathogenic variants cause variably expressive disorders such as Teebi Hypertelorism Syndrome 1 (TBHS1) and oblique facial clefting. TBHS1 typically presents with hypertelorism, cleft palate, and prominent forehead, often with extracranial malformations and neurodevelopmental phenotypes. Most pathogenic variants underlying the classic TBHS1 phenotype cluster in the CCD2 and CHD domains, disrupting interactions with actin filaments and microtubules. Here, we report the first family with a novel pathogenic mechanism: an intragenic deletion removing the canonical translation start site, disrupting the N-terminal intrinsically disordered region (IDR), and forcing alternative initiation, while sparing the functional domains of SPECC1L.

Methods

We used an *in vitro* overexpression assay in HEK293 cells to assess protein expression and determine the functional impact of the deletion. A literature review was also conducted to delineate *SPECC1L*-related phenotypic variability.

Results

The proband and father presented with craniofacial dysmorphisms consistent with TBHS1 – midface retrusion, hypertelorism, telecanthus and micrognatia - without major congenital anomalies or neurodevelopmental delay. WGS identified a novel heterozygous intragenic deletion of exon 3 in *SPECC1L*, encompassing the canonical start codon. Functional overexpression assays showed translation from downstream alternative start codons, resulting in stable production of truncated isoforms lacking part of the N-terminal IDR.

Discussion

We describe the first intragenic deletion in *SPECC1L*, affecting the canonical translation start site and the poorly characterized N-terminal IDR. The phenotype - limited to craniofacial dysmorphisms without major malformations - is consistent with milder expressivity, similar to cases with variants outside known functional hotspots domains (CCD1 or CCD3). These data highlight a previously unrecognized mechanism of pathogenicity in *SPECC1L* and suggest that the IDR contributes to neural crest development and developmental defects.

Funding: This work was supported by a Friends of Guy's & St Thomas' Hospital Research Grant Ref. 23/03R. D.O. was supported by a Research Mobility Fellowship 2022 - ERN Rare Disease Training and Support Program - European Joint Programme on Rare Diseases (EJP RD), which is funded from the European Union's 2020 research and innovation programme under EJP RD COFUND-EJP N°825575. Y.K. is funded by Guy's & St Thomas' Charity Grant Ref. STR111001. The funders played no role in study design, data collection, analysis, and interpretation of data, or the writing of the paper.

Conflicts of interest: All authors declare no competing interests.

Keywords: Teebi Hypertelorism Syndrome 1; *SPECC1L*; Hypertelorism; Craniofacial dysmorphisms; Alternative translation initiation

Ethics and consent: Written informed consent from participants for the use of clinical data were obtained in accordance with the Declaration of Helsinki. The guardian and adult individual provided written informed consent for presentation of images.

OC17 SOMATIC *RHOA* MOSAICISM UNVEILED BY DEEP SEQUENCING: DYSMORPHOLOGY-DRIVEN DIAGNOSIS

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Introduction

Somatic mosaicism is increasingly recognised as a key contributor to unexplained multisystem disorders. *RHOA* variants, recently implicated in ectodermal dysplasia, remain rarely reported, and their detection often requires dysmorphology-driven suspicion and tissue-specific deep sequencing.

Methodology

A 48-year-old female with congenital hemicorporeal asymmetry, Blaschkoid hypopigmentation, oligodontia, early-onset cataracts with glaucoma, optic atrophy, polyneuropathy, neurosensory hearing loss, scoliosis, cleft palate, and progressive urinary incontinence underwent stepwise evaluation from 2019–2024. Investigations included karyotype, HUMARA assay, targeted NGS panel, CNV analysis of *IKBKG*, whole-exome sequencing, and metabolic screening. Clinical suspicion of *RHOA*-related ectodermal dysplasia prompted skin biopsy and target *RHOA* deep sequencing.

Results

Despite extensive germline studies, no pathogenic variants were identified. Deep sequencing of the skin tissue with a targeted NGS panel revealed a pathogenic *RHOA* variant at 13% allele frequency, compatible with mosaicism, providing a definitive molecular diagnosis consistent with ectodermal dysplasia. This clarified the previously unexplained constellation of multisystem manifestations, guided ongoing multidisciplinary care, and resolved a decades-long diagnostic odyssey.

Discussion

This case illustrates the enduring value of dysmorphology in the genomic era. The constellation of congenital asymmetry, ectodermal anomalies, and neurological features pointed to a mosaic disorder, prompting targeted investigation beyond routine germline testing and leading to tissue-specific deep sequencing, which uncovered pathogenic *RHOA* mosaicism. In an era dominated by high-throughput sequencing, this illustrates how careful clinical observation can remain the crucial first step towards achieving a definitive molecular diagnosis.

Funding/Financing: None.

Conflicts of Interest: SJ, CAS, and JL report no conflicts of interest. SQ reports affiliation with a laboratory that has commercial interests.

Keywords: RHOA, mosaicism, ectodermal dysplasia, deep sequencing, diagnostic odyssey, multisystem disorder.

Ethics and Consents: Written informed consent for genetic testing and case presentation was obtained.

OC18 INTEGRATING TRANSCRIPTOMICS INTO THE CLINICAL SETTING FOR THE DIAGNOSIS OF SH3KBP1 DEFICIENCY

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Abstract

Introduction

Multiomic approaches to genetic diagnosis are becoming an increasing reality in rare disease research. However, testing in the clinical setting is almost exclusively performed with genomic methodologies. With the increasing availability of transcriptomics, it is expected that RNA-directed techniques will be quickly integrated into routine clinical practice, especially for disorders that affect test-accessible tissues, such as immunogenetic conditions.

Methodology

Report of the clinical presentation and genetic diagnostic testing of a single patient.

Results

A 14-year-old boy was referred to for genetic testing due to a suspected inborn error of immunity. Prior medical history was remarkable for learning difficulties, dyslexia and attention-deficit hyperactivity disorder. He presented with recurrent bacterial infection, such as tonsillitis and acute gastroenteritis. Analytical testing revealed IgM deficiency and generalized B-cell lymphopenia, suggesting a likely common variable immunodeficiency (CVID). Exome sequencing detected the presence of the hemizygous c.1005+2dup variant in the *SH3KBP1* gene, classified as of uncertain significance (VUS). The SpliceAI algorithm predicts the variant to cause loss of the splicing donor site in the exon 9/intron 9 border (Δ=0.76). Transcriptomic testing through RNA-Seq of peripheral blood showed the retention of intron 9, which is present in all known *SH3KBP1* transcripts, and presumably causes a frameshift, with no alternatively spliced transcripts detected in this region. This is compatible with the molecular variant and clinical phenotype, as *SH3KBP1* loss-of-function has been described as causal for a humoral inborn error of immunity (OMIM #300310), in which patients show non-severe neurodevelopmental disorders such as in this patient.

Discussion

Despite limited case-based evidence, *SH3KBP1* deficiency (also known as CIN85 deficiency) has been listed as causal for humoral immunodeficiency by the 2024 International Union of Immunological Societies classification. In our case, the impact of the VUS was possible through the employment of a transcriptomic approach, that showed that the splicing change predicted to arise with the DNA variant had in fact an impact in mRNA processing. This case illustrates the importance of transcriptome analysis in the diagnostic workup of rare conditions in the clinical setting.

OC19 BROADENING THE PHENOTYPIC SPECTRUM OF *THUMPD1*-RELATED SYNDROME: THE OLDEST REPORTED PATIENT WITH NOVEL FEATURES

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Introduction

Biallelic pathogenic variants in the *THUMPD1* gene cause a rare genetic condition known as neurodevelopmental disorder with speech delay and ocular abnormalities (NEDSOA). First described in 2022, this syndrome has so far been reported in eight families and is characterized by global developmental delay, speech impairment, moderate to severe intellectual disability, behavioural disturbances, facial dysmorphism, and a spectrum of ophthalmological anomalies. We report a 48-year-old male patient born to consanguineous parents with a novel homozygous *THUMPD1* variant and recurrent fever syndrome.

Methodology

The patient underwent detailed clinical and imagiological evaluation. Clinical exome analysis was performed, and the pathogenicity of variants was assessed according to ACMG guidelines.

Results

The patient exhibited intellectual disability, absent speech, epilepsy, behavioural abnormalities, bilateral cataracts, and hearing impairment. He also had urogenital anomalies (cryptorchidism, hypogonadism, horseshoe kidney), gastrointestinal issues (splenomegaly, hepatic nodules, pancreatic cysts) and type II diabetes. Brain CT showed parasagittal calcifications, ventriculomegaly, and vermis hypoplasia. Since childhood, he has experienced recurrent fever of unknown origin, which raised suspicion of sarcoidosis. Physical examination revealed coarse facial features, frontal balding, spindle-shaped fingers, hypotrichosis, obesity, and hypogonadism. Clinical exome analysis identified a novel homozygous *THUMPD1* variant, NM_017736.5:c.599_604delinsCCAAC p.(Tyr200Serfs*9), classified as likely pathogenic, establishing the diagnosis of NEDSOA. This case is unique in adding urogenital and gastrointestinal abnormalities to the previously recognized neurodevelopmental, ocular, and auditory features.

Discussion

This patient, the oldest reported to date, expands the genotypic and phenotypic spectrum of NEDSOA and underscores the need to screen for urogenital and gastrointestinal abnormalities, features not previously documented. The recurrent fever may represent either an atypical manifestation of this syndrome or a coexisting condition. Further studies are needed to clarify *THUMPD1*'s full clinical impact.

Funding: No funding was received.

Conflict of Interests: No conflicts to declare.

Keywords: THUMPD1, neurodevelopmental disorder, intellectual disability, recurrent fever

Ethics and Consent: Informed consent was obtained from the patient's mother for this case report.

OC20 HELLS-ASSOCIATED EPISIGNATURE ENABLES THE DIAGNOSIS OF ICF-4 SYNDROME

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Introduction

Immunodeficiency, Centromeric instability, and Facial anomalies (ICF) syndrome is a rare autosomal recessive disorder, genetically heterogeneous, characterized by reduced serum immunoglobulin levels, instability of pericentromeric regions of chromosomes 1, 16 and 9 upon mitogen stimulation of T-lymphocytes, and mild facial dysmorphisms. The hallmark of this syndrome is hypomethylation of pericentromeric satellite repeats, with pathogenic variants in *DNMT3B*, *ZBTB24*, *CDCA7* or *HELLS* genes associated with the four subtypes: ICF1, ICF2, ICF3 and ICF4. A subset of patients, however, remains without a defined genetic cause. DNA methylation episignatures have emerged as valuable diagnostic tools with significant clinical utility in rare diseases. This report highlights their role in clarifying variant classification, thereby enabling accurate diagnosis.

Case report

A 15-year-old male patient was referred for conventional cytogenetic analysis due to short stature and hypogonadotropic hypogonadism. A constitutional karyotype of 46,XY was identified, with 16% of metaphases exhibiting multiradiate branching structures in the long arms of chromosomes 1 and 16. These cytogenetic findings raised the suspicion of ICF syndrome. Clinical reevaluation revealed that previously a WES-base NGS panel for immunodeficiency had been done due to hypoimmunoglobulinemia and recurrent respiratory infections, without relevant candidate variants identified. Reanalysis request focusing on *DNMT3B*, *ZBTB24*, *CDCA7*, and *HELLS* genes, identified a homozygous missense variant in *HELLS* (NM_018063.5:c.2062A>G p.Thr688Ala), classified as a variant of uncertain significance. Parents were consanguineous and targeted testing confirmed they were both heterozygous. An Infinium MethylationEPIC array (Illumina) was conducted for the index patient, data interpretation was carried out using the Episign Knowledge Database, and revealed a positive result compatible with an hypomorphic functional impact.

Discussion

The etiological diagnosis of the patient was clarified to ICF-4 Syndrome, consistent with the phenotype, although milder than most cases described. In conclusion, conventional cytogenetic analysis was essential for raising the suspicion of ICF syndrome leading to clinical and molecular revaluation. DNA methylation episignature analysis was important to confirm diagnosis and allow variant reclassification

Conflict of Interest: None declared.

Key words: ICF syndrome; *HELLS*; DNA methylation; episignature

Ethics approval have been obtained from the Ethics Commission of the Faculty of Medicine, University of Coimbra.

OC21 CHARGE SYNDROME WITH AN ADDITIONAL *FBRSL1* VARIANT: INTERPRETING UNKNOWN GENETIC FINDINGS

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Introduction

CHARGE syndrome is a rare genetic disorder, usually caused by pathogenic variants in *CHD7*. While molecular confirmation can be challenging in cases with non-coding variants, methylation profiling has emerged as a powerful diagnostic tool. We report an 8-year-old girl with esophageal and choanal atresia, cardiac defects, iris coloboma, bilateral prelingual hearing loss, cranial nerve anomalies, facial dysmorphisms and neurodevelopment delay.

Methodology

Genetic investigations included aCGH, *CHD7* sequencing/MLPA/cDNA, whole-exome sequencing, methylation profiling (EpiSign), genome sequencing and RNA-based functional studies.

Results

Array CGH, *CHD7* sequencing, and exome sequencing were negative. EpiSign revealed a methylation profile consistent with CHARGE syndrome, thus establishing a diagnosis. Whole-genome sequencing identified the *de novo* heterozygous *CHD7* c.3202-177A>G variant, and cDNA analysis confirmed aberrant splicing with partial intronic retention. Additionally, a *de novo* heterozygous *FBRSL1* frameshift variant (c.724_743dup p.(Leu250Argfs*45)) was detected.

Discussion

The clinical presentation is consistent with the confirmed molecular diagnosis of CHARGE syndrome. This case highlights the utility of epigenomic profiling and functional assays in resolving variant significance and guiding clinical management. However, the craniofacial dysmorphism appears more pronounced than is usually reported within the phenotypic spectrum of this condition. An additional *FBRSL1* variant of uncertain significance was also identified. While recent reports have implicated loss-of-function variants in *FBRSL1* in neurodevelopmental disorders, these cases generally involve the short N-terminal isoforms. In contrast, in the present case, only the long isoform appears to be affected. It therefore remains unclear whether this variant contributes to the phenotype. Although the *FBRSL1* variant is unlikely to account for the complete syndromic presentation associated with *CHD7*, it may nonetheless exert a more subtle influence on the clinical features. This highlights the importance of interpreting emerging gene–disease associations with caution.

Funding / Financing: None declared.

Conflicts of Interest: The authors declare no conflicts of interest.

Keywords: CHARGE syndrome; CHD7; Functional genomics; Rare disease; Epigenomics

Ethics and Consent: Written informed consent was obtained from the patient's guardians.

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P01 TRANSITION BETWEEN GNOMAD V2 AND V4: CONSIDERATIONS FOR VARIANT INTERPRETATION

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Introduction

Population reference datasets are central to variant interpretation, but their use varies across analysis workflows. ClinGen emphasizes frequency-based metrics, while some platforms still rely on heterozygote counts. This distinction matters when moving from gnomAD v2.1.1 to v4.1, where changes in cohort composition and coverage affect allele counts. A non-UK Biobank (non-UKB) subset in v4 could support continued reliance on heterozygote counts, but excluding UKB reduces non-European representation and statistical power. ClinGen recommends using the full dataset, as large biobank cohorts also help mitigate biases and dilute rare disease enrichment.

Methodology

To assess the impact of this transition, we compared gnomAD v2.1.1 to v4.1 across exome and genome data. 3 537 798 ClinVar variants were examined without prior filtering to capture differences in population frequency and heterozygote counts. Outlier detection strategies were applied to evaluate systematic shifts, but the unfiltered dataset served as the foundation for comparisons.

Results and Discussion

Heterozygote counts in v2 and v4 showed no consistent relationship, either with each other or with allele frequencies. Counts for variants of similar frequency often diverged, reflecting cohort composition, sequencing depth, and variant calling. This instability limits their clinical utility and supports prioritizing frequency-based metrics. Coverage differences were also evident, with some clinically relevant loci underrepresented in v4 despite robust presence in v2. Disease-specific datasets are no longer released, as large cohorts cannot exclude all affected individuals, a factor that must be considered in interpreting rare variants.

Conclusion

The transition from gnomAD v2 to v4 requires attention to heterozygote counts, frequency metrics, and coverage differences. ClinGen SVI gene-specific recommendations should take precedence, even if they do not explicitly endorse gnomAD v4. As gnomAD expands in size and diversity, reliance on raw heterozygote counts will become impractical, reinforcing the need for frequency-based approaches to ensure consistent and reproducible variant interpretation.

P02 SYNGAP1 AND THE DEVELOPMENT OF MURINE NEOCORTICAL PROGENITOR CELLS

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Introduction

SYNGAP1 is a major regulator of synaptic plasticity through its interaction with synaptic scaffold proteins and modulation of Ras and Rap GTPase signalling pathways. *SYNGAP1* mutations in humans are often associated with intellectual disability, epilepsy, and autism spectrum disorder. *Syngap1* heterozygous loss-of-function in mice results in impaired LTP, premature maturation of dendritic spines, learning disabilities, and seizures, reflecting the phenotypes of the human SYNGAP1 syndrome. More recently, SYNGAP1 was shown to influence cortical neurogenesis and the proliferation of progenitors in human organoids.

Methodology

In this study we used histological analysis of genetically modified mice carrying heterozygous and homozygous knock-out alleles for *Syngap1 (Syngap1KO)*. We did a comprehensive analysis of progenitor numbers, proliferative capacity and division mode along with quantifications of overall neocortical thickness, and neuronal and glial cell counts at different developmental ages using validated staining methods and EdU labelling.

Results

Here, we show that the absence or haploinsufficiency of *Syngap1* does not influence the properties of neocortical progenitors and their cellular output in mice. Despite careful analysis of the behaviour of neocortical progenitor cells, we did not observe significant alterations in the properties of cortical progenitors and the generation of neocortical cell layers.

Discussion

Our results did not replicate the key findings observed in human organoids. This discrepancy highlights potential species-specific or methodological differences and raises significant questions about the broader relevance of SYNGAP1's role in neurogenesis. The absence of cortical progenitor deficits in *Syngap1* haploinsufficient mice, which exhibit cognitive deficits and seizures, indicates that these phenotypes do not arise from differences in neurogenesis but are more likely due to SYNGAP1's prominent role in the regulation of synapse function.

Funding

R37NS036715 (RLH); R01HG012357 and RF1MH121539 (UM); RS-2025-25403864 (IH) and 2023.11467.PEX doi.org/10.54499/2023.11467.PEX (SB)

Keywords: Syngap1; Neurogenesis; Neurodevelopment

P03 CHRONIC EXPOSURE TO VOLCANIC POLLUTION SHAPES THE ORAL MICROBIOME: AN EXPOSOME STUDY FROM SÃO MIGUEL (AZORES)

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Exposure to outdoor air pollution is a major global health concern, severely affecting respiratory and overall health. Over 10% of the world's population lives near active volcanoes, exposed to volcanic pollutants. On S. Miguel Island (Azores, Portugal), volcanic activity includes fumaroles, CO_2-rich springs, and soil diffuse degassing. The Furnas hotspot emits ~1000 tons of CO_2 daily, with indoor radon reaching up to 510× reference values. A 1991–2001 study reported higher risks of lip, oral cavity, and pharynx cancers in Furnas compared with non-volcanic areas. Since then, the Azores Oncology Centre has been conducting epidemiological studies and launched a screening program for early oral cancer detection (PICCOA).

To assess chronic exposure and oral cancer risk, we conducted a high-throughput exposome study comparing oral microbiomes from regions with opposing volcanic pollution (high-Furnas (FN), low-Ribeira Grande (RG)). Through PICCOA, saliva samples and epidemiologic data were collected from 95 participants (49 in Furnas, 46 in Ribeira Grande) and analysed by next generation 16S rRNA sequencing.

Analysis revealed significant microbiome differences between FN and RG. Alpha diversity was lower in FN (Shannon 5.68, Chao1 267) than in RG (6.04, 331), suggesting microbial loss from exposure to volcanic pollution. *Streptococcus*, *Veillonella*, and *Schaalia* were more abundant in FN, while *Porphyromonas*, *Aggregatibacter*, *Fusobacterium*, and *Treponema* dominated in RG. The genera driving these differences have previously been implicated in oral and head-neck pathologies: *Streptococcus* promotes tumor progression in oral carcinoma, *Veillonella* is an opportunistic pathogen active in dental caries and periodontitis development. *Fusobacterium* is linked to improved survival in head and neck cancer, and toxic to head and neck squamous cell carcinoma cells in co-culture. Functional KEGG pathway analysis revealed regional differences in cysteine and methionine metabolism, selenocompound metabolism, and inositol phosphate metabolism - linked to volcanic elements and heavy metal detoxification - suggesting microbial adaptation to volcanic exposure.

N. Mineiro holds a PhD grant from the Foundation for Science and Technology (FCT; 2023.02660.BDANA).

Keywords: Exposome; Chronic exposure; Volcanic pollution; Oral microbiome; Oral cancer

Ethics approval was granted by the HDES committee. All participants provided informed consent.

P04 DOWNREGULATION OF HEPARAN SULFATE SYNTHESIS VIA mRNA DEGRADATION: AN ANTISENSE OLIGONUCLEOTIDE APPROACH FOR MUCOPOLYSACCHARIDOSIS IIIC

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Introduction

Lysosomal storage disorders (LSDs) are inherited metabolic diseases often associated with severe symptoms, including neurodegeneration. Despite advances in understanding their molecular basis, treatment options remain limited, particularly for neuronopathic LSDs, where the blood–brain barrier restricts the drug efficacy. Mucopolysaccharidosis type III (MPS III) is one example, comprising four subtypes (A–D) that all result from impaired degradation of heparan sulfate (HS). This defect leads to HS accumulation, mainly affecting the central nervous system. Current therapies are scarce, highlighting the need for novel approaches. RNA-based therapies offer promising alternatives, with some already approved for other genetic disorders. Among them, antisense oligonucleotides (ASOs) represent a novel and highly promising class for personalized medicine. These short synthetic molecules bind to RNA by complementarity modulating gene expression. Here, we explore the use of gapmer ASOs as a genetic substrate reduction therapy (gSRT) for MPS IIIC, targeting *XYLT1* to downregulate HS biosynthesis and reduce its storage.

Methodology

Five 2'-MOE-PS gapmer ASOs were designed and tested in patient-derived fibroblasts to evaluate their ability to reduce *XYLT1* mRNA. Their impact on xylosyltransferase I (XT-I) was assessed by western blot, and HS storage levels were measured by LC-MS/MS.

Results

All gapmer ASOs showed high knockdown efficiency at mRNA level but two of them showed best results. Succeeding experiments confirmed these findings, demonstrating a highly reduction in *XYLT1* mRNA (~90%), a decrease in XT-I protein levels and, ultimately, in HS storage (up to 32%)[1].

Discussion

The work demonstrated the proof of concept for the approach in MPS IIIC patient-derived fibroblasts. These findings underscore the transformative potential of gapmer ASOs as a gSRT not only for MPS III but even for other LSDs, highlighting its potential for long-term therapeutic applications.

References

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Funding

This work was financed by national funds through FCT, within the scope of the project ASOS2cureMPSIII-2022.04667.PTDC (https://doi.org/10.54499/2022.04667.PTDC). The authors would like to thank SPDM (2020DGH1834), CECA (UIDB/00211/2020) and AL4AnimalS (LA/P/0059/2020).

Keywords: Mucopolysaccharidosis III (MPS III); RNA therapy; antisense oligonucleotides (ASOs); gapmer ASOs; genetic substrate reduction therapy (gSRT).

P05 GENEST: A NEW BIOINFORMATIC FRAMEWORK TO STREAMLINE MULTIGENE GENETIC TESTS IN THE GENOMICS ERA

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Introduction

Rare diseases (RDs) impose a major burden on patients and families, often linked to a prolonged diagnostic odyssey. Although NGS technologies, together with whole-exome (WES) and whole-genome sequencing (WGS), have transformed molecular diagnostics, each approach retains constraints. Moreover, gene-specific mutational spectrum and genomic regions, such as segmental duplications, may preclude efficient variant detection. Ignoring this interplay can lead to inappropriate test selection, and prolonged time to diagnosis.

Methodology

To address this, we developed GeNesT, a bioinformatics framework designed to characterize the analytical potential of genes. Built on Django and integrated within CGPP's knowledge database GreenBird, it combines a genomic relational database with tools for gene classification, automated virtual multigene panel generation, and quantitative comparison of sequencing strategies through Theoretical Variant Detection Rate (TDVR). Additionally, a dedicated GUI allows intuitive exploration of datasets in the platform.

Results

As proof of principle, it was validated using CGPP's accredited panels for Comprehensive Ataxias (251 genes) and Hereditary Neuropathies (158 genes). The platform effectively visualized gene-specific data, rendering insights into analytical-impacting features such as coverage, mutational spectrum, and genomic contexts. It also successfully replicated the set of analysable regions for each panel, with updated annotations, standardized gene classification and concordant to CGPP's method. TVDR analyses compared short-read (SR) technologies, two WES (classic and extended), and WGS. Both extended WES and WGS covered the full set of PAT/L-PAT variants from ClinVar and HGMD (40,728 in Ataxias; 26,672 in Neuropathies). Additionally, the prior methods achieved similar TVDR, with slight gains over WES classic (~0.7% for Ataxias; ~1.2% for Neuropathies).

Discussion

GeNesT provides a scalable, clinically meaningful framework to bridge genomic complexity and diagnostic decision-making for RDs. The TVDR tool provides quantitative support for the design of multigene panels and offers insights about the pertinence for inconclusive cases reanalysis. Current limitations include dependence on GRCh37, absence of long-reads datasets, and single-sample coverage bias. Future developments will extend functionalities to newer genome builds, sequencing methods and broader coverage datasets.

Conflicts of Interest: No conflict of interest towards this work.

Keywords: WES; WGS; coverage; mutational spectrum; variant detection rate

P06 AN ORGANOID-BASED MULTI-ORGAN-ON-A-CHIP TO MODEL GASTRIC CANCER METASTASIS TROPISM

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Introduction

Gastric cancer (GC) presents clinically into intestinal, diffuse, and mixed subtypes. The intestinal type predominantly metastasizes to the liver, lungs, and bone, whereas the diffuse type invades the peritoneum and lymphatic system. Understanding the histologically driven metastatic pattern is essential to anticipate target organs and improve disease monitoring and treatment. We propose using organ-on-chip technology and human-derived organoids to mimic the complexity of GC metastasis.

We propose using organ-on-chip technology and human-derived organoids to mimic the complexity of GC metastasis.

Methodology

Multiorgan-on-chip (MoC) was designed using CAD and prototyped by xurography. The device will be tested for efficient cell feeding and the ability to sustain multiple cultures with immortalized GC cells and target tissue cell lines. Cell Trace labeling will allow live tracking of dissemination through HUVEC-based artificial vasculature. Metabolism, viability, and cell patterning will be assessed. Phenotype, endothelialization of the main channel, and hydrogel stability will be analyzed by on-chip immunofluorescence.

Results

We designed, generated, and tested a MoC prototype consisting of a single primary tumor site chamber, connected to secondary chambers representing three target tumor sites. The design allows the artificial vasculature to bathe all the interconnected chambers, while a top-down chamber allows efficient feeding of each chamber within the system. A cell tracing procedure tracked primary tumor cells up to 16 days on-chip, which remained alive, enabling the following tumor dissemination. GC organoids and primary tissues mimicking metastatic sites have been generated/obtained to populate the MoC.

Discussion

This MoC prototype will allow establishing robust protocols for cultivating and maintaining GC and metastatic target-organ organoids within the microfluidic environment. This system will also recreate key vascular and mechanical cues, anticipating unique patterns of metastatic spread, and enabling physiologically relevant interactions between primary GC and metastatic sites

Funding

Supported by FCT: 2023.12556.PEX, AICIB and DGS: NCH2024005, Preventable Project: 101095483 and La Caixa: CI24-10293

Keywords: Gastric cancer; Metastasis; Organoids; Organ-on-a-chip;

P07 ALLELE FREQUENCY DISTRIBUTION OF CLINICALLY RELEVANT PHARMACOGENETIC VARIANTS IN GENES WITH CPIC GUIDELINES ACROSS EUROPEAN POPULATIONS: A SCOPING REVIEW

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Introduction

Clinically relevant pharmacogenetic (PGx) variants influence drug metabolism, transport and target interactions, affecting efficacy and risk of adverse reactions. The literature shows variability in drug response across populations with different ancestries, highlighting the importance to study the global distribution of these variants for equitable implementation of personalised medicine (PM).

Methodology

A scoping review, following PRISMA-ScR guidelines, was conducted to map the allele frequency of clinically relevant PGx variants in genes with guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC) across Europe. Only data from individuals without a diagnosed disease were included.

Results

Out of 1709 articles, this review selected 111 published between 1996 and 2024. Our analysis revealed notable regional differences in the frequencies of PGx variants across Europe. *CYP2C9*2* is less prevalent in Eastern Europe, while *CYP2C9*1* shows an increasing gradient from Southeast to Northwest. *CYP2C19*17* is more prevalent in Central and less in Southern Europe, while *CYP2C19*2* and **3* have similar frequencies across Europe. *CYP2D6*9* shows similar prevalence in Mediterranean countries, whereas *CYP2D6*10* exhibits varied distribution, with *CYP2D6*17* being uncommon in Europe. *CYP2D6*41* is slightly more common in Southern Europe. *CYP3A5*3* is highly prevalent, with *CYP3A5*1* frequencies decreasing from South to East of Europe. *DPYD*2A* and **13* are less prevalent in Central and Southern European countries, and *TMPT* variants (**2*, **3A*, **3C* and **4*) have lower prevalence in Europe. Finally, the UK has lower frequencies of *SLCO1B1*37* and **5*, and *UGT1A1*28* than other European countries.

Discussion

PGx variants distribution differs across Europe, reflecting the diverse ancestral backgrounds of European populations. The literature shows that genetic diversity follows a northwest-to-southeast gradient, with geographically adjacent populations typically being genetically closer to one another. To provide adequate PM, healthcare systems must consider these differences. Furthermore, as migration influences the genetic composition of European populations, large-scale studies must be updated to reflect current allele frequencies. These findings emphasise the importance of studying pharmacogene allele frequencies to improve PM across Europe.

Funding

Fundação para a Ciência e a Tecnologia (2024.06461.BDANA) and European Union's Digital Europe Program (Grant agreement #101168231).

Conflicts of Interest

None.

Keywords: Pharmacogenetic variants; European populations; Allele frequency; Clinical Pharmacogenetics Implementation Consortium (CPIC).

P08 UNCOVERING THE MOLECULAR CONSEQUENCES OF LDLR NONSENSE VARIANTS USING FLOW CYTOMETRY

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Introduction

Familial hypercholesterolaemia (FH) is a common monogenic lipid disorder, characterised by LDL-C lifelong elevation and increased risk of premature cardiovascular disease, being caused mainly by pathogenic variants in *LDLR*. Understanding the molecular consequences of these variants is crucial for a accurate diagnosis and personalised FH management. Nonsense variants, resulting from nucleotide substitutions that introduce premature stop codons, typically generate truncated mRNAs subjected to nonsense-mediated decay (NMD), leading to loss-of-function. This study aimed to functionally characterise 5 *LDLR* nonsense variants and evaluate their effects on receptor expression and function and determine if variants towards the C-terminal domain undergo NMD.

Methodology

Nonsense variants (c.898A>T, c.1200C>A, c.1798G>T, c.2488A>T, and c.2546C>A) were generated by site-directed mutagenesis, cloned into pcDNA3 vectors, and transfected into CHO-Δ7 cells, which lack endogenous LDLR expression. Following incubation with fluorescent probes or antibodies, flow cytometry was used to evaluate three parameters: LDLR expression, binding, uptake.

Results

Variants c.898A>T (p.Arg300*) and c.1200C>A (p.Tyr400*) showed markedly reduced activity across all three assays (<10%). In contrast, c.2488A>T (p.Lys830*) and c.2546C>A (p.Ser849*) displayed near-normal activity (>70%) in all assays tested. Variant c.1798G>T (p.Glu600*) displayed an intermediate profile, with reduced activity in expression (47%) and binding (79%), and severely impaired LDL uptake (9%).

Discussion

Traditionally, nonsense variants were assumed to invariably abolish LDLR activity by triggering NMD. Our findings challenge this assumption by showing that variant position is a critical determinant of outcome: while upstream variants lead to complete loss of receptor function, those located near the 3' end may escape NMD and preserve partial or even normal activity. Recognising this variability is crucial not only for accurate FH variant classification, but also for tailoring clinical decision-making, ultimately contributing to more precise diagnostics and truly personalised therapeutic strategies for patients.

P09 SECONDARY ANALYSIS PIPELINE UPGRADES: ASSESSING THE IMPACT ON VARIANT QUALITY METRICS

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Introduction

Variant quality metrics (SNPs and indels) are widely used to assess sequencing data quality and the accuracy of variant calling. Small changes in the version of the secondary analysis pipeline can have a significant impact on variant calls and downstream analysis, potentially leading to the need for confirmation by orthogonal methods. These confirmations, in addition to being costly, are frequently associated with increased turnaround time (TAT).

One of the most commonly used secondary analysis pipelines is DRAGEN. During the upgrade from DRAGEN version 4.0.3 to 4.3.17, significant changes in these metrics were observed. In version 4, DRAGEN introduced machine-learning (ML) algorithms for the recalibration of germline calls by default. Those algorithms have been progressively refined and, with the upgrade to version 4.3.17, these refinements may have led to the observed differences. Furthermore, other enhancements to the variant-calling algorithms, adjustments to filtering parameters, and optimizations in data processing may have resulted in measurable alterations to the quality metrics.

Methodology

To evaluate the impact of these updates, the Base Quality (BQ), Depth (DP), Mapping Quality (MQ), Genotype Quality (GQ), and Allele Frequency (AF) metrics of 9 174 853 variants obtained with both DRAGEN versions were compared. The dataset was also subdivided by variant type and by zygosity, allowing a more detailed analysis of the observed differences.

Results and Discussion

Overall, a slight increase in metrics such as Depth (DP) was identified, while other metrics like Mapping Quality (MQ) suggest a certain bias towards lower values and an opposite bias for higher values.

These results suggest that assessing and validating the impact of upgrades to the secondary analysis pipeline is essential to ensure continuous, reliable results. It is important that laboratories are aware of these changes and consider the need to re-evaluate their pipelines and validation procedures to guarantee the accuracy and reliability of sequencing results.

P10 ISOGENIC CELLS DERIVED-MODELS FOR THE STUDY OF VARIANTS OF UNKNOWN SIGNIFICANCE (VUS) IN A GENETIC RARE DISEASE

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Introduction

It is unavoidable to find a variant of unknown significance in clinical genetic testing nowadays. In the future, with the implementation of genetic newborn screening across more countries, the identification of a disease-causing variant and timely initiation of treatment will be crucial. To assess the impact of a VUS, the availability of a suitable cell model and a robust readout is essential.

Niemann-Pick type C (NPC) is a monogenic disease due to pathogenic variants in *NPC1* gene (in >95 % of the cases) or seldom in *NPC2* gene. It presents considerable clinical variability, overlapping with other diseases, often delaying diagnosis and access to therapy (ies). These are patients with high unmet medical needs, urgently requiring new and quicker diagnostic tools. Additionally, *NPC1* is highly polymorphic with frequent variants of unknown significance (VUS), approximately one third of published variants in ClinVar are classified as VUS. In the cases where these VUS are present in compound heterozygosity, functional studies using patient- derived skin cells are not informative; therefore, improved models are needed to address novel missense variants.

Methodology

In this work, we leveraged an *in vitro* NPC cell model (*NPC1*$^{-/-}$ ARPE-19: arising retinal pigment epithelia cell line) by transducing it with constructs encoding different fluorescently tagged variants of *NPC1*. Those constructs were generated by site-directed mutagenesis using PCR and cloning methods. Later, the constructs were used to produce retroviruses and transduce *NPC1*$^{-/-}$ ARPE-19 cells. The existence of the fluorescent tag at the C-terminal of NPC1 protein is also suitable for high-throughput fluorescence microscopy studies, enabling large-scale studies.

Results

The trafficking and degradation studies on these NPC models were assessed. As expected, the presence of certain missense variants leads to ER retention of the mutated protein, predicting a *NPC1* pathogenic variant.

Discussion

In this study, we use a new *in vitro* model to assess the effect of specific *NPC1* variants on protein trafficking and degradation, distinguishing VUS from pathogenic variants. This work will have a meaningful impact on rare disease patients by characterizing the pathogenicity of novel variants and accelerating diagnosis, thereby reducing the length of the 'diagnostic odyssey'.

Funding

This work received financial support from the National Institute of Health Doutor Ricardo Jorge and FCT (EXPL/BTM-TEC/1477/2021).

P11 TRANSCRIPTION FACTOR-MEDIATED GLIAL REPROGRAMMING AS A PROMISING THERAPEUTIC APPROACH FOR MACHADO-JOSEPH DISEASE

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Introduction

Machado-Joseph Disease (MJD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in the *ATXN3* gene, leading to a toxic gain-of-function of ataxin-3 and progressive neuronal degeneration. Currently, no effective therapies exist.

Methodology

A promising therapeutic strategy involves the direct reprogramming of glial cells into induced neurons (iNeurons), which has shown success in other neurodegenerative conditions but remains largely unexplored in ataxias. We tested five transcription factors (TFs)—Ngn2, Ascl1, Brn2, Dlx2, and Sox11—in wild-type mice using lentiviral (LV) vectors and a Cre-Flex system for astrocyte-specific targeting and lineage tracing via mCherry.

Results

Robust mCherry expression confirmed system efficiency. Ascl1 and Ngn2 delivery increased NeuN and nestin immunoreactivity, indicating successful neuronal induction. Elevated Iba1 and GFAP expression was observed in TF-treated groups (but absent in controls) suggesting specific glial recruitment rather than generalised inflammation.

Ascl1 and Ngn2 were then further tested in symptomatic MJD mice. AAV5 vectors were stereotactically injected into the cerebellar vermis, and AAV presence confirmed via ITR analysis. Behavioural tests showed improved gait, though no significant effects were seen in rotarod or beam walking, likely due to advanced disease stage.

Discussion

These findings support the potential use of Ascl1 and Ngn2 for glia-to-neuron reprogramming in MJD. Future work will compare RNA-based and AAV-mediated delivery to optimise therapeutic strategies.

Funding

This work was supported by the CInTech Technological Hub for Innovation (C644865576-00000005), co-funded by Component C5 of the Portuguese Resilience and Recovery Plan through NextGenerationEU; ERDF (COMPETE 2020, POCI) and FCT (AstroIn2Neurons – 2022.06127.PTDC, DOI: 10.54499/2022.06127.PTDC), ViraVector (CENTRO-01-0145-FEDER-022095); AFM-Téléthon (#29068); ERDERA (ID: 101156595), Capacity 2023 (ID: 101145599), GeneH Excellence Hub (ID: 101186939), GCure Era-Chair (ID: 101186929), and the GeneT Teaming Project (ID: 101059981), all funded by the EU Horizon Europe programme.

Keywords: Machado-Joseph Disease (MJD)/Spinocerebellar ataxia type 3 (SCA3); Transcription factor; induced neurons; glial reprogramming; gene therapy (RNA-based or AAV-mediated delivery)

P12 IDENTIFICATION OF G6PD VARIANTS IN A POPULATION SAMPLE FROM SÃO TOMÉ E PRÍNCIPE

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme defect globally, affecting over 350 million people. A population-based study to assess the frequency of mutations causing G6PD deficiency can be a valuable tool for family and prenatal counseling as well as for defining public health strategies. This study aims to evaluate the prevalence of G6PD mutations associated with hemolytic anemia that are common in African populations, focusing on the G6PD variants A-, Betica, and Santa Maria, among women from São Tomé e Príncipe.

Methodology

The study population includes 113 sub-Saharan women, aged 18 to 49 years (mean 32.83) living in S. Tomé e Príncipe (Central Africa). Genomic DNA was extracted from blood spots and analyzed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for four common G6PD variants: A (c.376A>G), A– (c.376A>G/c.202G>A), Betica (c.376A>G/c.968T>C), and Santa Maria (c.376A>G/c.542A>T), using primers previously described and the endonucleases *Fok*I, *Nla*III, *Msp*I, and *Bsr*FI, respectively.

Results

In the total sample, 33 women were found to be heterozygous for the G6PD deficient allele A– (7 c.376GG/c.202GA and 26 c.376AG/c.202GA), one was homozygous for the G6PD A– variant (c.376GG/c.202AA), and one was heterozygous for G6PD Santa Maria (c.376AG/c.542AT). Of the 226 chromosomes, 35 (15.5% frequency) showed the presence of the G6PD deficient mutation c.376G/c.202A (G6PD A–). The G6PD deficient mutation c.376G/c.542T (G6PD Santa Maria) was found in one chromosome (0.44% frequency). G6PD Betica was not found in the study population. Forty-two additional chromosomes (18.6%) had the *Fok*I restriction site (allele c.376G) that identified the G6PD A variant, and 148 (65.5%) were negative for the *Fok*I restriction site (allele c.376A) representing the G6PD B variant.

Discussion

Our study found that, consistent with data from other West sub-Saharan populations, mutation c.376G/c.202A, that identify the G6PD A– variant, is the most common G6PD deficient allele, occurring in 15.5% of the population. The presence of the G6PD variant Santa Maria may indicate the diverse origins of the São Tomean population. The high frequency of the G6PD deficiency A- variant, which may be linked to malaria resistance in the population, underscores the importance of establishing an effective national screening program, essential to prevent cases of drug-induced anemia, particularly those related to malaria treatment.

P13 RATIONAL DESIGN AND MODIFIED NUCLEOTIDES ENABLE COST-EFFECTIVE PRODUCTION OF THERAPEUTIC CIRCULAR RNA

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Introduction

Circular RNAs (circRNAs) are emerging as a powerful platform for RNA-based therapies, particularly in cancer immunotherapy. However, large-scale production of synthetic circRNAs remains a major challenge due to high manufacturing costs, limiting their clinical translation. Here, we developed a cost-effective, in-house ribozyme-mediated self-circularisation production protocol and optimised circRNA design to enhance translational efficiency and minimise innate immune activation through sequence engineering and nucleotide modifications.

Methodology

CircRNAs were designed through structural sequence variants, codon optimisation and incorporation of chemically modified nucleotides. Capillary electrophoresis was used to confirm RNA quality and circularity, while *in vitro* assays assessed cell viability and circRNA functionality. JMP® software enabled analysis of total and circular RNA yield, circularisation efficiency, and double-stranded RNA (dsRNA) content to identify the optimal *in vitro* transcription (IVT) conditions.

Results

The optimised in-house protocol successfully produced high-quality circRNAs with a mean circularisation efficiency of 87%, and dsRNA levels below 0.5%. Performance was comparable to, or better than, commercial kits, at significantly lower costs. Multivariate analysis identified key synthesis parameters that maximised yield and circularisation efficiency, while minimising dsRNA contaminants. Sequence engineering via structural sequence variants, codon optimisation, and nucleotide modifications differentially impacted RNA performance.

Discussion

In summary, this work demonstrates that our optimised IVT protocol, combined with rational sequence design and nucleotide modifications, enables the cost-effective production of high-quality, functional circRNAs across different cell lines. These results establish a scalable and versatile circRNA platform for next-generation RNA-based vaccines and therapeutics.

Funding

CInTech (C 644865576 00000005), co-funded by Component C 5 Capitalisation and Business Innovation under PRR Plan through the NextGenerationEU Fund; ERDF (Centro 2020 - ViraVector: CENTRO-01-0145-FEDER-022095; COMPETE 2020 & FCT - UID/04539/2025, LA/P/0058/2020); AFM-Téléthon (#29068); and ERDERA (ID: 101156595), Capacity 2023 (ID: 101145599), GeneH (ID: 101186939), GCure (ID: 101186929) and GeneT (ID: 101059981), funded by Horizon Europe (EU).

Conflicts of Interest

The authors declare no conflicts of interest.

Keywords: Circular RNA; *In vitro* transcription; Cost-efficiency; Sequence design; Nucleotide modifications; Translational efficiency; RNA-based therapeutics.

P14 DDX11 SAFEGUARDS CHROMOSOME SEGREGATION: NEW MECHANISTIC INSIGHTS INTO WARSAW BREAKAGE SYNDROME

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Introduction

Warsaw breakage syndrome (WABS) is a rare cohesinopathy caused by biallelic mutations in the DNA helicase DDX11, leading to microcephaly, developmental delay, and the characteristic "railroad" chromosome phenotype. Although defects in sister-chromatid cohesion have long been recognised, the molecular pathway by which pathogenic DDX11 variants drive mitotic failure has remained elusive.

Methodology

Using immortalised patient-derived cells, combined with advanced live-cell imaging and high-resolution chromosome-spread quantitative analyses, we uncovered specific contributions of DDX11 to chromosome segregation fidelity.

Results

WABS-patient cells showed distinct chromosome segregation errors. Importantly, specific DDX11 disease-associated variants are differentially required to sustain a Haspin-dependent phosphorylation gradient at centromeres, essential for the error correction machinery.

Discussion

Disruption of this pathway extends the known functions of DDX11 beyond cohesion establishment and provides novel mechanistic insights into cohesinopathy pathogenesis. Recognising their distinct functions opens the possibility of better understanding the clinical manifestations of WABS.

Funding

Maratona da Saúde, under the Rare disease award, DOI 10.54499/CEECIND/03721/2018/CP1540/CT0003

Keywords: Chromosome segregation; Chromosomes; Rare diseases

P15 PUBLIC GENOMIC COHORT ANALYSES REVEAL BCL6 EXPRESSION AS A PROGNOSTIC MARKER IN LUMINAL A BREAST CANCER

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Introduction

Breast cancer (BC) is the most common malignant neoplasm among women worldwide and remains a leading cause of cancer-related mortality. It is a heterogeneous disease classified into molecular subtypes with distinct prognostic and therapeutic implications. Luminal A is the most prevalent subtype, characterised by high expression of hormone receptors (oestrogen and progesterone), low proliferation rate, and generally favourable prognosis. However, consistent evidence indicates a significant risk of late recurrence and new neoplastic events, posing challenges for clinical follow-up strategies.

Methodology

We analyzed genomic data from the TCGA breast cancer cohort (n = 1247) to assess the prognostic value of the BCL6 gene, a transcriptional regulator previously implicated in tumour progression. Data on BCL6 expression, molecular subtyping (PAM50), and overall survival (OS) were retrieved.

Results

Although BCL6 expression was globally reduced in tumours compared to normal tissue, it was significantly higher in Luminal A tumours than in other subtypes, with a subgroup (44%) maintaining expression levels similar to normal tissue. Importantly, within the Luminal A subtype, higher BCL6 expression was associated with poorer long term survival ($p = 0.041$).

Discussion

These findings support the potential of BCL6 as a stratification biomarker for the risk of long term neoplasm recurrence within Luminal A breast cancer, with possible implications for tailoring the intensity and duration of clinical follow-up.

Conflicts of Interest

The authors declare no conflicts of interest.

Keywords: Breast cancer; Luminal A; BCL6; Prognostic biomarker; TCGA

P16 TRUNCATING *APOB* VARIANTS IMPAIR LDL METABOLISM: FUNCTIONAL EVIDENCE FROM BINDING STUDIES

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Introduction

Familial Hypercholesterolaemia (FH) is a condition caused by pathogenic variants in *LDLR*, *APOB* or *PCSK9* genes, characterised by high levels of LDL cholesterol and premature cardiovascular disease (CVD). APOB variants account for 5–10% of FH cases, most being due to missense variants. Although truncating variants are typically associated with hypocholesterolaemia, some have been reported in clinical FH patients. This study is part of the PerMedFH project and aimed to functionally characterise 4 nonsense *APOB* variants in exon 29 identified in patients with a clinical diagnosis of FH.

Methodology

LDL was isolated through sequential ultracentrifugation from index cases and relatives. Affinity chromatography was used to purify ED-LDLR from HEK293 cells transfected with the pcDNA3.1-EC-LDLR-His plasmid. 96-well plates were coated with the purified ED-LDLR fragments and then incubated with the different *APOB* variants. Antibodies were used for ligand detection, and absorbance was determined at 405 nm.

Results

The variants c.12460G>T/p.(Glu4154*), c.12946C>T/p.(Gln4316*), c.13158del/p.(Glu4387Asnfs7), and c.13576C>T/p.(Gln4526*) showed reduced affinity for the LDL receptor, impairing apoB–LDLR binding and thereby altering LDL metabolism, leading to FH.

Discussion

This work demonstrates that nonsense *APOB* variants are not always associated with hypocholesterolaemia and protective for CVD; variants in exon 29 can escape nonsense-mediated decay and drive FH pathogenesis. By revealing this mechanism for the first time, our findings strengthen the need to include truncating *APOB* variants in exon 29 in diagnostic pipelines and provide a classification according to the patient phenotype and disease (FH). Functional evidence not only validates the clinical diagnosis but also guides risk stratification and supports the development of personalised treatment strategies, ultimately improving outcomes for FH patients.

Funding

PerMedFH project funded by La Caixa Foundation in partnership with FCT.

Keywords: Familial Hypercholesterolemia; *APOB* variants; Nonsense varaints; Functional studies

P17 CLINICAL RELEVANCE OF *POLE* EXONUCLEASE DOMAIN MUTATIONS: INSIGHTS FROM ROUTINE TESTING AUTOMATED QPCR ASSAY

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Introduction

Endometrial cancer is the most frequently diagnosed gynecologic malignancy in high-income countries, with a steadily increasing global incidence. The Cancer Genome Atlas molecular classification stratifies endometrial carcinomas into four prognostically relevant subgroups: (i) POLE-ultramutated, characterized by pathogenic exonuclease domain mutations; (ii) MMR-deficient, associated with mismatch repair protein loss; (iii) p53-abnormal, defined by aberrant p53 expression and extensive copy number alterations; and (iv) No Specific Molecular Profile (NSMP), which lacks the defining alterations. The identification of pathogenic *POLE* mutations in endometrial cancer has significant diagnostic, prognostic, and therapeutic implications. Rapid and accurate detection of these variants is therefore essential in both clinical and translational settings. Next-generation sequencing (NGS) was the primary method employed; however, despite its high sensitivity, NGS is labor-intensive and time-consuming.

This study aimed to implement an automated qPCR test for the detection of clinically relevant *POLE* exonuclease domain mutations.

Methodology

The Idylla™ system is a fully automated, cartridge-based molecular platform that integrates nucleic acid extraction, real-time PCR amplification, and mutation detection in a single-step workflow. The assay targets multiple known oncogenic variants in the *POLE* gene across exons 9, 11, 13, and 14—including hotspot mutations such as P286R, V411L, S297F, and M295R—as well as the *POLD1* exon 12 S478N variant.

Results

Seven samples, previously identified as positive through NGS using the TruSight Hereditary Cancer panel, were utilized as positive controls. Over a 5-month period, 90 endometrial cancer cases were screened using the Idylla™ assay. Of these, 86 (95.6%) were negative for *POLE* mutations, while four cases (4.4%) tested positive. Among the positive cases, three harbored the V411L variant, and one case presented one of the P268R, M295R, and S297F mutations.

Discussion

The Idylla™ POLE-POLD1 Mutation Assay demonstrated high efficiency and ease of use for the detection of clinically actionable POLE mutations in endometrial carcinoma. These results align with what has been reported in the literature. This methodology supports rapid molecular stratification, offering valuable prognostic insight and informing therapeutic decision-making in the management of endometrial cancer.

Keywords: Endometrial cancer, POLE mutations, qPCR test

P18 DECIPHERING THE GENETIC CAUSES OF DIABETIC RETINOPATHY

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Introduction

Diabetic Retinopathy (DR) is the leading cause of vision loss worldwide. With over 530 million diabetic patients requiring regular eye exams, early detection burdens healthcare systems. Understanding the genetic basis of DR is essential to improve risk assessment and clarify disease mechanisms. Most DR-associated variants lie in non-coding regions, particularly transcriptional enhancers, which regulate gene expression. In this work, we aim to identify rare variants in enhancers active in retinal cells and assess their impact on regulatory activity and DR phenotypes using in vitro and in vivo models.

Methodology

We are integrating chromatin accessibility datasets from human retinal cells to define putative enhancers and intersect them with rare variants (RVs) from whole-genome sequencing of 1625 DR patients in the UK Biobank. Functional consequences of RVs will be tested using MPRAs in RPE cells and in hiPSC-derived retinal organoids with reporter constructs to assess enhancer activity across retinal ganglion cells, photoreceptors, and Müller glia. Candidate enhancers disrupted by RVs will be validated in zebrafish CRISPR/Cas9 models under hyperglycemia to assess their contribution to DR phenotypes.

Results

We curated 135 DR-associated genes, linked them to expression profiles across retinal cell types, and mapped chromatin-accessible regions using ATAC-seq data. This yielded catalogs of putative enhancers: 405 in RPE, 2573 in cones, 2556 in rods, 3671 in Müller glia, and 489 in retinal ganglion cells. To enable functional assays, we generated a synthetic oligonucleotide library of enhancer, promoter, and control sequences, and cloned it into a custom reporter system. Initial experiments in RPE cells are underway. This framework establishes functional testing pipelines to investigate how retinal cell type–specific enhancers contribute to DR.

Discussion

This project aims to determine how rare non-coding variants in retinal enhancers contribute to DR development. By identifying disease-relevant enhancers, testing their activity in retinal cells and organoids, and validating key candidates in vivo, we will uncover regulatory mechanisms driving DR susceptibility. The resulting framework will enable prioritization of pathogenic variants and support future prevention and therapy strategies.

Funding

Supported by FCT (2024.05836.BD)

Conflicts of Interest

The authors declare no conflicts of interest

Keywords: Diabetic retinopathy; Enhancers; Cis-regulation; Retinal organoids; Zebrafish

P19 DEVELOPMENT AND VALIDATION OF A SINGLE-CELL RNA-SEQ PIPELINE FOR BREAST-CANCER SENTINEL LYMPH NODES

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Introduction

Early-stage Luminal A breast cancer (BC) generally has a favourable prognosis, but its high prevalence and risk of late metastasis motivate further investigation. Sentinel lymph nodes (SLNs), critical for coordinating the immune response against the tumour, are currently evaluated only for subclinical metastasis using the One-Step Nucleic Acid Amplification (OSNA) assay. We hypothesise that SLNs from Luminal A BC exhibit distinct immune-cell compositions that drive different immune responses, independent of the presence of subclinical metastasis. To investigate this, we aim to predict SLN immune composition from discarded OSNA SLN bulk RNA-seq samples by developing a deep neural network (DNN) deconvolution model. DNN models require large training sets, but bulk BC LN data with known cell type proportions are scarce. To address this, we propose generating large numbers of *in silico* pseudo-bulk samples from well-annotated single-cell RNA-seq (scRNA-seq) profiles.

The present work develops and validates a scRNA-seq pipeline for publicly available BC LN datasets lacking single-cell annotations, providing a resource to support the future pseudo-bulk generation for DNN model training.

Methodology

Publicly available BC LNs scRNA-seq datasets were retrieved from the GEO repository. For datasets lacking annotations, we implemented a pipeline in Seurat (v5.0.1), including clustering and cell-type labelling. Conservative thresholds were applied, assigning a label only when marker expression and cluster separation were unambiguous. To validate this pipeline, we generated artificial samples from a published annotated dataset by randomly selecting a subset of cells and re-analysed them without access to the original labels. Predicted versus true labels were compared at the single-cell level, and confusion matrices, per-class precision, recall, F1 scores and overall accuracy were computed.

Results

Validation showed concordance with published annotations, yielding a reproducible set of annotated cells suitable for future pseudo-bulk generation and DNN training.

Discussion

These preliminary results establish a reproducible resource to enable the future DNN training for predicting SLN immune composition from discarded OSNA bulk RNA-seq samples and to facilitate benchmarking of deconvolution methods.

Keywords: Early-stage breast cancer; Sentinel lymph nodes; scRNA-seq analysis; deconvolution.

Ethics and Consent

Not applicable.

P20 ADDRESSING ANCESTRAL UNDERREPRESENTATION IN ONCOBIOLOGY: ESTABLISHING A SUB-SAHARAN PATIENT-DERIVED CANCER CELL LINE PANEL AND CONDUCTING A HIGH-THROUGHPUT SCREENING OF DRUGS

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Introduction

Cancer is an increasing public health burden, including in sub-Saharan Africa (SSA), where incidence will double in the next 20 years. These rates ask for a better understanding of cancer across SSA regions, which display variable genetic and environmental features when compared to other regions of the globe. This project aims to establish the first PALOP (Portuguese-speaking African countries) patient-derived cancer cell lines.

Methodology

Fresh tumour samples were collected from SSA ancestry patients in biopsies and surgeries at IPO-Porto, H. Santa Maria-Porto and H. Garcia-Orta-Lisboa, totalling 8 cases: 1 salivary gland, 2 breast, 3 prostate and 2 kidney. The conditional reprogramming (CR) was done by co-culturing samples with irradiated mouse fibroblasts (3T3-J2). The cells were monitored by bright-field microscopy, and characterised for autosomal STRs, 1 million SNPs array, karyotype, immunofluorescence (Ki67 and tissue-specific antibodies) and cell growth. A high-throughput screening of drugs (HTS) was performed in one prostate cell line, with Prestwick library (1280 drugs), and then the drug hits were tested in all three prostate cell lines.

Results

The STR DNA profiles were unique, with the absence of the microsatellite instability phenotype. For most of the samples a higher than 75% African ancestry was observed. All cell lines showed a dominant normal karyotype. Ki67 immunofluorescence allowed to confirm proliferation of all cell lines. The HTS validation on the prostate cancer cells identified 15 candidate drugs, namely cardiac glycosides and topoisomerase modulators.

Discussion

We were able to establish the CR technique and new SSA ancestry cell lines, initiating an efficient pipeline for the collection of these tumour samples in Portuguese hospitals. This panel has a potential for new discoveries, as we have demonstrated here through the HTS-validation of repurposed drugs for prostate cancer treatment.

Funding

FCT funded PhD grant (UI/BD/153041/2022) and project (PTDC/BIA-MOL/3986/2021).

Keywords: Population Genetics; Cancer Genetics; Conditional Reprogramming; Patient derived tumour models; In vitro drug test

The study was approved by the Ethics Committees of i3S and all participating hospitals. Written informed consent was obtained from all patients prior to sample collection.

P21 MOLECULAR CHARACTERIZATION OF RECTAL ADENOCARCINOMA: GENOMIC AND EPIGENETIC ALTERATIONS AND THEIR IMPLICATIONS IN DISEASE PROGRESSION

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Introduction

Rectal adenocarcinoma, the most common type of colorectal cancer (CRC), is a heterogeneous disease where genomic instability and epigenetic dysregulation are key factors in tumor development and progression. Despite being a distinct clinical and biological subtype, its molecular basis remains poorly understood, highlighting the importance of characterizing the genetic and epigenetic alterations that drive tumor initiation and growth.

Methodology

A comprehensive characterization of 24 rectal adenocarcinoma samples was performed using complementary techniques, namely conventional cytogenetics, aCGH, and MS-MLPA.

Results

Primary cultures showed frequent numerical alterations, such as trisomy of chromosome 7 and monosomy of Y chromosome. Conventional cytogenetic analysis also revealed complex structural rearrangements, mainly involving chromosome 5, including add(5), der(20)t(5;20), der(5)t(5;17), der(5)t(5;15)(q11.2?;p11.1?), der(15)t(5;15)(q11.2?;p11.1?), and del(5)(p13?). aCGH revealed recurrent gains on chromosomes 7, 8q, 13, and 20, and losses on chromosomes 8p, 17, and 18. Notably, tumor suppressor genes such as *TP53* (17p13.1) and *SMAD4* (18q21.1) are located within these frequently deleted regions. MS-MLPA analysis identified *VHL, PYCARD, GATA5,* and *BRCA1* as often amplified with *GATA5*, *ESR1*, *CDH13* and *WT1* also showing high methylation levels. These results emphasize the coexistence of genomic gains and epigenetic silencing within key regulatory genes.

Discussion

These findings are relevant to colorectal carcinogenesis, as they reflect the chromosomal instability and CpG island methylator phenotype. The integrated genomic and epigenetic information suggests that rectal adenocarcinoma progression results from the interplay between oncogene activation and inactivation of tumor suppressors.

Conclusion

This study provides valuable insights into the genomic and epigenetic patterns of colorectal cancer. This profile could provide information about the alterations underlying disease progression. Nevertheless, it is important to develop large-scale studies with a focus on understanding, analyzing, and validating these preliminary results.

Conflicts of Interest

The authors declare no conflicts of interest.

Keywords: rectal adenocarcinoma; conventional cytogenetic; aCGH; MS-MLPA.

Ethics and Consent

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Commission of Faculty of Medicine of University of Coimbra. Written informed consent was obtained from all patients.

P22 PRELIMINARY INVESTIGATION OF CHROMOSOME INSTABILITY AND DNA REPAIR IN GATA2 DEFICIENCY

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Introduction

Bone marrow failure syndromes (BMFS) are a group of genetic disorders characterized by hematopoietic failure, leading to malignancies such as myelodysplastic neoplasms and acute myeloid leukemia. Inherited BMFS (IBMFS) represent ~30% of pediatric BMFS cases and are a heterogeneous and life-threatening group of diseases caused by pathogenic germline mutations. Hematopoietic stem cell transplantation (HSCT) remains the only potentially curative treatment. However, the prognosis is poorer than for other conditions.

While Fanconi anemia is the most studied IBMFS linked to deficiencies in DNA repair mechanisms, particularly those involving DNA double-strand breaks (DSBs), others, such as GATA2 deficiency, may also impair DNA repair, though the mechanisms remain unclear. This study analyzes chromosome instability (CI) in IBMFS patients and its potential association with HSCT complications.

Methodology

This preliminary work assessed CI using non-treated, DEB-, and bleomycin (BLM)-induced lymphocyte cultures from patients with bone marrow hypoplasia and healthy controls. Statistical analysis compared CI parameters, including the mean breaks per cell (brk/cell).

Results

Case 1 - *GATA2* deficiency. This case showed significantly higher brk/cell in non-treated (0.34) and BLM-induced (1.86) cultures compared to controls. However, there were no differences in DEB-induced cultures. This suggests that *GATA2* deficiency selectively impairs DNA damage pathways.

Discussion

These findings suggest that patients with *GATA2* deficiency may exhibit selective impairment of DNA repair pathways. Previous studies indicate that *GATA2* indirectly regulates DNA repair genes, notably *NPM1A*, through transcriptional regulation. This regulatory network suggests that individuals with GATA2 deficiency may exhibit selective sensitivity to DNA-damaging agents, which could significantly impact the efficacy and safety of HSCT.

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P23 IS ANTISENSE OLIGONUCLEOTIDE-MEDIATED EXON SKIPPING A POTENTIAL THERAPEUTIC APPROACH FOR MUCOLIPIDOSIS II?

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Introduction

Mucolipidosis II (ML II) is a Lysosomal Storage Disorder caused by N-acetylglucosamine-1-phosphotransferase (GlcNAc-PT) deficiency, which impairs lysosomal hydrolases trafficking. Here, we explored an innovative therapeutic strategy based on the use of antisense oligonucleotides (ASOs) to promote targeted skipping of *GNPTAB* exon 19, which harbors c.3503_3504del, the most frequent disease-causing variant. Previously, in ML II patients' fibroblasts, we tested ASOs to induce exon 19 skipping, successfully generating an in-frame mRNA[1]. Now, our aim is to determine if this in-frame transcript leads to increased GlcNAc-PT levels.

Methodology

First, the GlcNAc-PT activity was measured in fibroblasts, but activity levels were similar in ML II and control fibroblasts (treated/non-treated) showing that the assay is not proper to measure endogenous levels. To overcome this, we designed 3 constructs: a WT (full *GNPTAB* cDNA), a del_ex19 (without exon 19) and a mutant (with the mutation c.3503_3504del) that were transfected in HEK293T cells. Then GlcNAc-PT expression was analyzed by Western Blot (WB).

Also, we measured the activity of several hydrolases and evaluated the expression of α-galactosidase A (α-Gal) by WB after ASO treatment.

To further validate this therapy we also generated a novel GlcNAc-PT antibody in rabbits.

Results

Our results showed that HEK293T cells were able to express all the constructs. The WB of both WT and del_ex19 constructs showed bands corresponding to the α/β precursor. However, only the WT construct expressed the β-subunit, suggesting that there is no GlcNAc-PT activity in the absence of exon 19. As expected, in the delTC construct WB no α/β precursor band was detected.

We also observed a slight increase in the activity of various lysosomal hydrolases in ML II fibroblasts after treatment. However, only the α-Gal values were statistically significant, but the WB analysis for this enzyme did not reveal any band in ASO-treated ML II cells.

We also developed a novel antibody for GlcNAc-PT. Preliminary results showed a β-subunit band both in control and patient fibroblasts (unexpected), but in overexpression both WT and del_ex19 constructs presented α/β precursor bands. So, further assays are needed to assess its specificity.

Discussion

Our ASO-based approach effectively promotes exon 19 skipping. However, this strategy, as far as we have been able to prove, is not able to restore any GlcNAc-PT enzymatic activity. Further validation, including co-localization studies are planned to clarify these findings.

Keywords: Antisense Oligonucleotide Therapy; Exon-Skipping; Mucolipidosis II

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P24 CYTOGENOMIC INSIGHTS INTO GLIOBLASTOMA: PREDICTIVE MARKERS OF SENSITIVITY TO 7α-ACETOXY-6β-HYDROXYROYLEANONE (ROY)

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Glioblastoma (GB), the most common and aggressive astrocytic tumor, is characterized by a highly invasive and resistant nature, leading to poor clinical outcomes. The latest WHO classification underscores the importance of cytogenomic profiling in understanding GB biology. Here, we investigate cytogenomic alterations in GB patients and their response to 7α-acetoxy-6β-hydroxyroyleanone - Roy (a natural lead compound isolated by us). Primary cultures were established from tumor tissue of 11 GB patients, alongside 3 established cell lines (U87, A172, H4). Samples were analyzed by karyotyping, array-CGH, and MS-MLPA. Roy's therapeutic potential was evaluated in both 2D and 3D cell models. Across the cohort, cytogenetic analysis revealed complex structural rearrangements, including t(1;12)(p36.3;q13.1), t(4;8)(p16.3;q21.3), and der(22)t(17;22)(q11.2;q13.3), along with frequent chr7 gains and losses of chrs 10, 14, 16, and 22. Diploid/tetraploid subpopulations were also observed. Cell lines exhibited highly unstable karyotypes and extensive CNAs, with some alterations overlapping patient profiles but also displaying additional changes absent in tumors. Array-CGH revealed recurrent CNAs, particularly chr7 gains (*EGFR, CDK6*) and chr10 loss (*PTEN*), common to patients and cell lines. MS-MLPA confirmed frequent deletions of *PTEN* and *CDKN2A*, as well as recurrent methylation of *TP73*, *ESR1*, and *MSH6*. Treatment response to Roy correlated with specific cytogenomic signatures: sensitivity was linked to deletions on chr6 (52–54 Mb) and amplifications on chr11 (2–3 Mb, 31–32 Mb), while resistance was associated with deletions in these regions of chr11. A deletion on chr2 (87–88 Mb) was present in both resistant and intermediate groups, suggesting that it may contribute to, but is insufficient for, resistance, likely acting synergistically with other changes unique to resistant tumors. 3D spheroid assays confirmed Roy's antitumor activity, showing spheroid disintegration and growth inhibition. Overall, this study highlights the genomic heterogeneity of GB and identifies CNAs on chrs 6 and 11 as potential predictive biomarkers of response. These findings support Roy's therapeutic potential; however clinical translation is yet limited due to the small cohort and in vitro nature of the study.

Funding

This work was supported by NRC.LPCC/CIMAGO and by the Foundation for Science and Technology (FCT, Portugal), through the following projects:

UIDB/04539/2020 (DOI: 10.54499/UIDB/04539/2020), UIDP/04539/2020 (DOI: 10.54499/UIDP/04539/2020) and LA/P/0058/2020 (DOI: 10.54499/UIDP/04539/2020) CIBB Strategic Projects.

Conflicts of Interest

The authors declare no conflicts of interest.

Keywords: Glioblastoma; Cancer cell lines; Copy number variation; 7α-acetoxy-6β-hydroxyroyleanone; Antitumor activity; Cytogenetics

Ethics and Consent

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Commission of Unidade Local de Saúde Coimbra (approval no. 483/CES, 02/12/2022). Written informed consent was obtained from all patients.

P25 ULTRA-PORTABLE NANOPORE SEQUENCING WITH ADAPTIVE SAMPLING FOR CLINICAL DIAGNOSTICS: PRELIMINARY EVALUATION USING MONOGENIC DIABETES AS A MODEL

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Introduction

Next-generation sequencing (NGS) is a cornerstone of clinical genetics. Oxford Nanopore sequencing offers distinct advantages, including rapid turnaround, portability, and potential for on-site diagnostics. The ONT MinION enables ultra-portable, real-time genetic analysis outside conventional laboratory settings. Its compact design and flexibility allow deployment in clinical or resource-limited environments, such as low-income regions or emergency situations. Adaptive sampling permits selective enrichment of target regions, supporting in situ genetic diagnostics. Here, we present the implementation of the MinION MK1D with a standard laptop to expand access to genetic testing, particularly for rare diseases, in challenging contexts.

Methodology

A targeted panel of 72 genes implicated or suspected in monogenic diabetes was analysed using Nanopore sequencing with adaptive sampling. Sixteen samples that previously had been Sanger sequencing for GCK, HNF1A, HNF1B and HNF4A genes.were processed. Sequencing performance and data quality were assessed, focusing on coverage depth across target regions and downstream bioinformatic analysis.

Results

All samples yielded sequencing data across the targeted diabetes gene panel. Average sequencing depth was 2–3x, limiting variant-calling reliability. Nevertheless, interpretable results were obtained for all samples, suggesting that while not yet suitable for onsite clinical use, further optimization, including enhanced computational resources, may enable reliable variant detection.

Discussion

Despite current limitations in coverage and analytical confidence, the combination of portability and rapid adaptive sampling sample preparation makes Nanopore sequencing promising. Refinement of panel design, workflow optimisation, and increased computational power are expected to improve coverage, data quality, and variant detection. With further development, this approach may achieve sufficient depth for robust analyses, facilitating decentralised, on-site clinical genetic testing.

Funding

Supported by FCT – Fundação para a Ciência e a Tecnologia, project reference [2023.11834.PEX].

Conflicts of interest

None.

Keywords: Nanopore sequencing; Adaptive Sampling; Clinical genetics; Rare diseases; Diagnostic technologies.

Ethics and consent

The molecular study of monogenic diabetes has been approved by INSA Ethical Committee (NI 136-2023 - 11/04/2023). For all index patients and relatives informed consent was obtained by the referring clinician.

P26 ENGINEERING RNA-TARGETING CRISPR/CAS13 FOR ALLELE-SPECIFIC *ATXN3* KNOCKDOWN FOR TREATMENT OF SPINOCEREBELLAR ATAXIA TYPE 3

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Introduction

Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease, is the most prevalent autosomal dominant ataxia worldwide. SCA3 is caused by an abnormal expansion of the CAG repeat in the *ATXN3* gene, leading to the formation of toxic Ataxin-3 species causing neuronal degeneration. RNA interference sequences for silencing mutant *ATXN3* have been explored for SCA3 treatment; however, these suffer from toxicity and lack of specificity. In this study, we investigated the potential of RNA-guided CRISPR/Cas13d ribonuclease for allele-specific silencing of mutant *ATXN3*.

Methodology

We designed and tested over 120 Cas13d CRISPR RNAs (crRNAs) sequences to direct Cas13d ribonuclease towards single-nucleotide polymorphisms (SNPs) linked to the mutant *ATXN3* allele, or the expanded CAG repeat region. These were assessed in a dual-luciferase reporter assay using HEK293T cells. Several parameters - target position, spacer length, or introduction of sequence mismatches - were analysed to identify optimal Cas13d guides that selectively target mutant *ATXN3*.

Results

Cas13d crRNAs targeting mutant *ATXN3* SNPs attained over 80% silencing of mutant gene expression. Target specificity was primarily affected by the SNP position within the crRNA sequence, with optimal guides exhibiting 40-50% greater selectivity towards the mutant allele. Introduction of additional mismatches further enhanced specificity up to 60%. SNP-targeting crRNAs showed minimal off-target effects on other polyglutamine genes (*ATXN2*, *ATXN7*, *HTT*). While crRNAs targeting the CAG repeat led to over 95% knockdown, their specificity was compromised by silencing non-expanded polyQ genes. We are currently validating the most promising Cas13d sequences both in SCA3 patient-derived neurons and animal models.

Discussion

Our work demonstrates the potential of RNA-guided Cas13d ribonuclease as a therapeutic approach for SCA3. Here, we refined Cas13d sequences for efficient and selective repression of mutant *ATXN3* expression, paving the way for a novel and safe therapeutic route.

Funding

Work funded by the ERDF (COMPETE 2020, POCI); FCT – EXPL/MED-NEU/0936/2021, ViraVector (CENTRO-01-0145-FEDER-022095), CinTech (PRR, Ref PC644865576-00000005; SCARNA (AFM-Téléthon, P25124); ARDAT (IMI2 JU no. 945473), ERDERA ID:101156595, Capacity 2023 ID: 101145599, GeneH ID:101186939, GCure ID: 101186929, and GeneT Teaming Project ID: 101059981, supported by the EU Horizon Europe program.

Conflicts of Interest

None.

Keywords: Spinocerebellar Ataxia Type 3; CRISPR/Cas13d ribonuclease; Allele-specific gene silencing; Single-nucleotide polymorphism; CAG repetitive tract.

P27 COST-EFFECTIVE LINEAR mRNA PRODUCTION THROUGH OPTIMIZED *IN VITRO* TRANSCRIPTION, RATIONAL SEQUENCE ENGINEERING, AND CHEMICALLY MODIFIED NUCLEOTIDES

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Introduction

The development of linear mRNA vaccines for SARS-CoV-2 marked a milestone in medicine. With rapid production, safety, and scalability, mRNA platforms are expanding to cancer and neurological diseases. Scalable, cost-effective in vitro transcription (IVT) is key to optimize yield and quality. This study evaluates three approaches: optimizing IVT conditions, refining DNA templates, and adding modified nucleotides to improve stability, translation, and reduce immunogenicity.

Methodology

We compared three mRNA production methods for mRNAs encoding NanoLuc (879 nts) and NeonGreen (993 nts): two commercial kits (silica- and magnetic bead-based) and a non-commercial method (silica purification). Evaluation included yield (spectrophotometry), integrity (capillary electrophoresis), double-stranded (ds) RNA levels (ELISA), *in vitro* functionality (protein expression in HEK293T and fibroblasts up to 72 h), and cost *per* mg. Using the best method, we tested ten sequence modifications in the regulatory 5'UTR or 3'UTR sequences, and three in the reporter (NanoLuc) coding sequence. For the best sequence, ten chemically modified nucleotides were tested at 25–100% ratios. Functionality and cell viability were assessed *in vitro*.

Results

The in-house IVT method was the most cost-effective, yielding 120–132 µg/IVT with <0.5% dsRNA for most mRNAs, high integrity, and expression comparable to kits, at ~€287/mg (vs. €452 Kit A, €1118 Kit B). Two 5'UTR modifications improved translational efficiency while maintaining integrity up to 72 h. Chemically modified nucleotides gave mixed outcomes, with both gains and losses in yield and functionality.

Discussion

We present a scalable, kit-independent platform for high-quality mRNA synthesis. Combining low-cost IVT protocols, sequence engineering, and rational nucleotide modification, we demonstrate improved yield and functionality, supporting next-generation mRNA therapeutics.

Funding

CInTech (C 644865576 00000005), co funded by Component C 5 Capitalisation and Business Innovation under PRR Plan through the NextGenerationEU Fund; ERDF (Centro2020, ViraVector: CENTRO-01-0145-FEDER-022095; COMPETE 2020 & FCT, UID/04539/2025, LA/P/0058/2020); AFM-Téléthon (#29068); and ERDERA (ID: 101156595), Capacity 2023 (ID: 101145599), GeneH (ID: 101186939), GCure (ID: 101186929) and GeneT (ID: 101059981), funded by Horizon Europe (EU).

Conflicts of Interest

Nothing to declare.

Keywords: RNA-based therapeutics, Linear mRNA, *In Vitro* Transcription, Yield, RNA integrity, dsRNA, Sequence Engineering, Modified Nucleotides, Cost-efficiency, *In vitro* functionality

P28 OPTIMIZED COST-EFFECTIVE PRODUCTION OF SELF-AMPLIFYING RNA FOR RNA THERAPEUTICS: *IN VITRO* TRANSCRIPTION CONDITIONS, SEQUENCE ENGINEERING, AND CHEMICALLY MODIFIED NUCLEOTIDES

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Introduction

Self-amplifying RNA (saRNA) platforms are emerging in next-generation vaccines for their potency at low doses. Scalable in vitro transcription (IVT) is essential to produce high-quality saRNA, but few comparative data exist on workflows balancing yield, quality, functionality, and cost. This study aimed to define a cost-effective, scalable saRNA production strategy and assess how rational sequence design and nucleotide modifications affect RNA quality and function.

Methodology

We compared three IVT methods for saRNA encoding eGFP (8611 nt) and NanoLuc (8500 nt): (1) commercial kit with silica-based purification, (2) non-commercial method with silica-based purification, and (3) commercial kit with bead purification. All were evaluated for yield (spectrophotometry), integrity (capillary electrophoresis), double-strand (ds) RNA levels (ELISA), *in vitro* functionality (protein expression in HEK293T and fibroblasts up to 72 h), and cost *per* mg. The best method was used to generate three NanoLuc saRNA variants with coding sequence modifications and incorporation of ten chemically modified nucleotides at 25–100% ratios in the best-performing sequence, to assess effects on yield, integrity, dsRNA, and functionality.

Results

The non-commercial method yielded140–155 µg/IVT saRNA with <0.5% dsRNA, comparable protein expression to commercial methods, and the lowest cost *per* mg. Specific sequence modifications enhanced translational efficiency without compromising integrity, although benefits were context-dependent. Nucleotide modifications provided mixed results: some improved functionality while others reduced yield or affected saRNA performance.

Discussion

We provide a cost-efficient approach for high-quality saRNA production without proprietary kits. Rational sequence design and nucleotide modifications can modulate performance, but effects are not universally beneficial and need empirical optimization. This work provides a flexible framework for developing next-generation RNA therapeutics.

Funding

CInTech (C 644865576 00000005), co funded by Component C 5 Capitalisation and Business Innovation under PRR Plan through the NextGenerationEU Fund; ERDF (Centro2020, ViraVector: CENTRO-01-0145-FEDER-022095; COMPETE 2020 & FCT, UID/04539/2025, LA/P/0058/2020); AFM-Téléthon (#29068); and ERDERA (ID: 101156595), Capacity 2023 (ID: 101145599), GeneH (ID: 101186939), GCure (ID: 101186929) and GeneT (ID: 101059981), funded by Horizon Europe (EU).

Conflicts of Interest

Nothing to declare.

Keywords: RNA-based therapeutics, Self-amplifying RNA, *In Vitro* Transcription, Yield, RNA integrity, dsRNA, Sequence Engineering, Modified Nucleotides, Cost-efficiency, *In vitro* functionality

P29 ASSESSMENT OF mRNA DELIVERY APPROACHES IN HEK293T CELLS AND HUMAN DERMAL FIBROBLASTS (HDF) WITH LINEAR mRNA, SELF-AMPLIFYING mRNA (saRNA), AND CIRCULAR RNA (cRNA)

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Introduction

mRNA therapies hold transformative potential in vaccines and diseases treatment. Beyond conventional linear mRNA, saRNA and cRNA are emerging as promising therapeutics. However, optimal delivery strategies for different RNA types, particularly in hard-to-transfect primary cells like HDF, remain poorly characterized. This study addresses this gap by comparing transfection across RNA types, cell models, and delivery methods to identify the most effective for each RNA.

Methodology

Linear mRNA (mRNA) (~930nts), saRNA (~8500nts), and cRNA (~1490nts) encoding for luminescent and fluorescent reporters were synthesized by *in vitro* transcription. HEK293T and HDF were transfected with Lipofectamine Messenger Max (LMM; 0.1–0.5µL) and RNA (0.1–0.5µg). Cell viability was assessed using Alamar Blue. For Nucleofection (NF), 0.1–1µg of each RNA type was electroporated into HDFs using the Lonza 4D-Nucleofector. Lipid nanoparticles (LNP) were also tested for mRNA and saRNA. Transfection efficiency was assessed by luciferase assay (20 h), fluorescence microscopy (6-60 h), and flow cytometry (24 h).

Results

The best transfection conditions (LMM volume/RNA amount) in HEK293T cells were: 0.25µL/0.5µg for mRNA, 0.5µL/0.1µg for saRNA, and 0.5µL/0.25µg for cRNA. In HDFs, the best conditions were 0.25µL/0.5µg for mRNA, 0.1µL/0.5µg for saRNA, and 0.1µL/0.1µg for cRNA. Cell viability was above 75%. For mRNA, LMM reached higher transfection (11% at 12 h) than LNPs or NF (<5%), stable for 36 h, while LNPs yielded delayed expression, attaining 17% (0.25 µg) at 54 h, stable for 60 h. For saRNA, NF yielded around 50% at 66h (0.5µg and 0.25µg), stable up to 72h, while NF for cRNA reached 2.4% after 18 hours.

Discussion

This study compares delivery strategies for mRNA, saRNA, and cRNA in HEK and HDF. No single method suited all RNA types: LMM was optimal for mRNA and cRNA, NF for saRNA, and LNPs enabled delayed but prolonged mRNA expression. These findings guide tailoring RNA delivery based on type, intended use, and target cells.

Funding

CInTech (C 644865576 00000005), co funded by Component C 5 Capitalisation and Business Innovation under PRR Plan, the NextGenerationEU Fund; ERDF (Centro 2020 - ViraVector: CENTRO-01-0145-FEDER-022095; COMPETE 2020 & FCT - UID/04539/2025, LA/P/0058/2020); AFM-Téléthon (#29068); and ERDERA (ID: 101156595), Capacity 2023 (ID: 101145599), GeneH (ID: 101186939), GCure (ID: 101186929) and GeneT (ID: 101059981), funded by Horizon Europe (EU).

Conflicts of Interest

The authors declare no conflict of interest.

Keywords: mRNA technology; nucleic acids delivery; RNA-based therapies

POSTERS **CLINICAL CARE / COHORTS**

POSTER Nº	TITLE	PRESENTING AUTHOR	SESSION
P30	*Comparative clinical characterization of the COL4A3 P.(GLY407ARG) variant versus other COL4A3/4 variants in patients with type iv collagen-related glomerulopathies in north-west Portugal*	Diogo Fernandes da Rocha	1
P31	*Optimizing ngs bioinformatic pipeline for PMS2 variant detection: the role of variant allele frequency*	Bárbara Rodrigues	2
P32	*Mlpa analyses as a first-line diagnostic approach in short stature: an eight-year review*	Katerine Torres	1
P33	*Evaluating clinical markers to improve selection of diabetic patients for genetic testing*	Margarida Vaz	2
P34	*Liquid biopsy in oral squamous cell carcinoma: circulating cell-free DNA dynamics and mutational landscape*	Pedro Veiga	1
P35	*Genetic diagnosis of familial hypercholesterolaemia (FH) in Portugal: insights from the portugue FH study*	Beatriz Miranda	2
P36	*Liquid biopsy as a minimally invasive tool for longitudinal genomic surveillance in colorectal cancer*	João Mendes	1
P37	*A new MS-digitalMLPA probe panel for tumour methylation profiling: first evidence from oral cavity carcinomas*	Carolina Mano	2
P38	*Rare rnu4-2 variants in neurodevelopmental disorders: dominant mechanisms prevail in a patient cohort with high consanguinity*	Mariana Ferreira	1
P39	*Genetic insights into primary ovarian insufficiency: towards clinical utility*	Mariana Melo	2
P40	*Impact of incomplete penetrance copy number variants on neurodevelopmental disorders*	Sofia Dória	1
P41	*Implementation and iso 15189 accreditation of a clinical genetic testing for familial hypercholesterolemia*	Tiago Nicolau	2
P42	*MAG-SUDOE: implementing a genetic counseling mentoring program in the southwestern region of europe*	Inês Costa	1
P43	*Molecular characterization of SERPINC1 variants in patients with thrombotic predisposition: a cohort study*	Tânia Costa	2
P44	*Genotyping repeat expansions in neurological diseases by long-read sequencing: a cross-platform study*	Susana Valente	1
P45	*Implementing and validating cytogenetics laboratory workflows for ISO 15189 accreditation and ivdr compliance*	Carlos Medeiros	2
P46	*Reporting incidental and secondary findings – a prospective study*	Lígia Lameiras	1
P47	*Post-market surveillance of whole-exome sequencing under ivdr: one-year experience after technical file completion*	Susana M. Sousa	2
P48	*Insights from a cohort of 44 patients with CDK13-related disorder: incomplete penetrance, variable expresion and genotype-phenotype associations*	Susana Isabel Ferreira	3
P49	*Retrospective analysis of patients who underwent tumor and germline testing for ovarian cancer*	Ariana C Mendes	2
P50	*The mutational spectrum of primary ciliary dyskinesia (PCD) and the diagnostic relevance of non-coding regions: the laboratory perspective*	Patrícia Marques	3
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P52	*Cnvs detected by mlpa in the par1 region in patients with léri-weill dyschondrosteosis and idiopathic short stature*	Nuno Lavoura	3
P53	*Incidental x-chromosome findings during fmr1 trinucleotide repeat screening*	Madalena Garcia	3
P54	*Role investigation of RNU4-2 gene in unsolved neurodevelopmental cases*	Elsa Garcia	3
P55	*Advances in genetic screening for leber's hereditary optic neuropathy (LHON) at laboratory of mitochondrial biomedicine and theranostics (LBIOMIT) - three decades of expertise*	Célia Alcobia Gomes	3
P56	*Genetic study of CFTR: prevalence and mutation spectrum in a large portuguese cohort*	Emília Vieira	3
P57	*A retrospective analysis of MYBPC3 variants: optimized bioinformatics pipeline reveals pathogenic intronic variants in former genotype-negative HCM cases*	Sónia Sousa	3
P58	*Genomic anomalies in men with fertility alterations: a cohort study (2014–2021)*	Érica Rego	3
P59	*Deciphering the genetic modifiers of sickle cell anemia in children: the role of CYB5R3 gene*	Pedro Lopes	3
P60	*Neonatal screening for sickle cell anemia and the impact of genetic counseling for at-risk couples in benguela, angola.*	Roberto Lardoeyt	3
P61	*Cholestasis: genetic analysis of 342 patients over a 10-year period*	Maria José Simões	3

Posters discussion: Session 1 - 20/11 - 16h25-17h25; Session 2 - 21/11 - 11h15-11h55; Session 3 - 21/11 - 14h00-14h55

P30 COMPARATIVE CLINICAL CHARACTERIZATION OF THE COL4A3 P.(GLY407ARG) VARIANT VERSUS OTHER COL4A3/4 VARIANTS IN PATIENTS WITH TYPE IV COLLAGEN-RELATED GLOMERULOPATHIES IN NORTHWEST PORTUGAL

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Introduction

Type IV collagen-related glomerulopathies (Col4G), including the different genetic forms of Alport syndrome (AS), result from (likely) pathogenic variants (PVs) in *COL4A3*, *COL4A4*, or *COL4A5*. Although autosomal dominant AS is generally considered rarer and clinically milder than the other forms, the *COL4A3* c.1219G>C p.(Gly407Arg) variant is frequently identified in heterozygous state in patients presenting with progressive chronic kidney disease (CKD), in northwest Portugal. This study aims to describe the clinical phenotype of these patients.

Methodology

We retrospectively reviewed the clinical records of all patients diagnosed at our center carrying a heterozygous PV in *COL4A3* or *COL4A4*. A total of 176 records were reviewed: 66 patients with *COL4A3* p.(Gly407Arg), 43 with other *COL4A3* PVs, 40 with *COL4A4* PVs, and 19 with variants of uncertain significance (VUS) in any of the genes.

Results

The renal phenotypes of *COL4A3* p.(Gly407Arg) and *COL4A4* PVs are similar and characterized by microscopic hematuria (mH) (~94%), proteinuria (54-64%), CKD stage ≥3 (~29%), and need for renal replacement therapy (RRT) (15-20%). *COL4A3* non-p.(Gly407Arg) PVs exhibited milder renal involvement (mH 68%, proteinuria 38%, CKD ≥3 26%, RRT 15%). Surprisingly, the renal phenotype of the *COL4A3/4* VUS group (mH 65%, proteinuria 27%, CKD ≥3 21%, RRT 10%) did not significantly differ from the latter group. Overall prevalence of hearing loss was ~20%. Survival analyses showed that individuals with the *COL4A3* p.(Gly407Arg) variant consistently progressed to CKD stage 3 over time.

Discussion

Individuals carrying the *COL4A3* p.(Gly407Arg) variant exhibited the lowest renal survival rates and progressed to renal failure more rapidly than carriers of other non-truncating PVs. *COL4A4* PVs carriers were likewise associated with an elevated risk of CKD. These findings emphasize the importance of recognizing Col4G in patients presenting with persistent mH and significant proteinuria or family history of CKD, even in the absence of extra-renal manifestations.

Conflicts of Interest

Nothing to declare.

Keywords: Alport syndrome; Autosomal dominant; Chronic kidney disease, Portuguese ancestry; Type IV collagen-related glomerulopathies.

Ethics and Consent: This study received approval from the Ethics Committee at ULS São João (151/2024).

P31 OPTIMIZING NGS BIOINFORMATIC PIPELINE FOR *PMS2* VARIANT DETECTION: THE ROLE OF VARIANT ALLELE FREQUENCY

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Introduction

Lynch syndrome (LS; OMIM #614337) is an autosomal dominant cancer predisposition syndrome caused by pathogenic variants in mismatch repair (MMR) genes. Among them, *PMS2* encodes a critical subunit of the MutLα complex, which is essential for DNA mismatch repair and preservation of genomic stability. However, molecular analysis of *PMS2* is challenging due to the presence of highly homologous pseudogenes, particularly *PMS2CL*, which interfere with routine next-generation sequencing (NGS) accurate variant interpretation. The aim of this study was to evaluate a bioinformatic approach that can reliably distinguish between variants present in the gene or pseudogene.

Methodology

Twenty cases with *PMS2* heterozygous variants were identified by NGS. All variants were confirmed by long-range PCR. NGS variant allele frequencies were systematically compared with confirmatory Sanger sequencing results.

Results

Of the 20 cases, 12 variants with allele frequencies (VAFs) ≤37% were confirmed be located in the pseudogene *PMS2CL*. Among the remaining 8 cases with VAFs >37%, 5 variants were confirmed in the *PMS2* gene, while 3 were in *PMS2CL*.

Discussion

These findings suggest that variants with VAFs below 35% are reliably assigned to the pseudogene *PMS2CL*, potentially eliminating the need for confirmatory testing. However, additional data are required to refine and validate the proposed VAF cut-off for broader clinical application.

Keywords: Lynch syndrome, *PMS2*, pseudogene, NGS, Long-range PCR

P32 MLPA ANALYSES AS A FIRST-LINE DIAGNOSTIC APPROACH IN SHORT STATURE: AN EIGHT-YEAR REVIEW

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Introduction

Variants in the *SHOX* gene and its regulatory regions, leading to haploinsufficiency, cause a range of disorders associated with short stature (SS), including Langer mesomelic dysplasia, Leri-Weill dyschondrosteosis and idiopathic SS. It has been estimated that 1.1–16.9% of patients with idiopathic SS present *SHOX* haploinsufficiency, of which 80–90% are attributable to copy number variations (CNVs). Consequently, MLPA analysis of *SHOX* and its regulatory regions has become a first-line diagnostic approach in the evaluation of individuals with SS. Here, we report the results of *SHOX* MLPA analyses performed in individuals with SS since its initial use in our department.

Methodology

We performed a retrospective analysis of 267 *SHOX* MLPA reports, consecutively requested between April 2017 and April 2025 at our department, upon referral from clinical genetics or pediatrics.

Results

Over an 8-year period, 267 individuals underwent *SHOX* MLPA testing: 246 for a clinical indication of SS and 21 for family study. A total of 237 results were normal. Among the 30 abnormal results, 22 individuals carried pathogenic or likely pathogenic variants consistent with *SHOX* haploinsufficiency (14 had a clinical indication of SS and 8 identified through family studies), while 8 harbored variants of uncertain clinical significance. For one of the SS patients with abnormal result, the detected probe profile was suggestive of a point variant, leading to subsequent gene sequencing and identification of a pathogenic variant. Overall, 5.24% of index cases were conclusively diagnosed with *SHOX* haploinsufficiency, increasing to 8.24% when family studies are included.

Discussion

This review confirms that a significant proportion of SS cases are associated with *SHOX* CNVs. *SHOX* MLPA is a valuable first-line diagnostic tool in the evaluation of SS and may also suggest the presence of point variants. Genetic diagnosis of these alterations is crucial in children for the timely initiation of hormone therapy.

Keywords: MLPA, short stature, *SHOX*.

P33 EVALUATING CLINICAL MARKERS TO IMPROVE SELECTION OF DIABETIC PATIENTS FOR GENETIC TESTING

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Introduction

MODY diabetes is a genetic condition caused by a single variant in one of 14 genes associated with this disease. Each gene contributes differently to glucose metabolism, so the clinical presentation varies depending on the gene affected. This heterogeneity makes accurate diagnosis challenging, and many cases are misdiagnosed as having type 1 or type 2 diabetes. The "Molecular Study of Monogenic Diabetes" project was established at INSA to identify patients with this condition. This present study compares the phenotype of diabetic patients with and without a pathogenic MODY variant.

Methodology

After molecular diagnosis we analyzed clinical data from patients' questionnaires. Using Rstudio, we performed statistical analyses of age at diagnosis, blood glucose, HbA1c, and C-peptide levels, at the time of diagnosis for index cases with and without a MODY causative variant. Shapiro test and Wilcox test were used to evaluate differences between the two groups.

Results

From all parameters analyzed, the only variable that showed a statistically significant difference was the age at diagnosis, where the MODY group was diagnosed at an early age. The mean results observed for C-peptide levels, for both groups presented values within the non-pathological range.

Discussion

MODY diabetes is a genetic condition difficult to identify because the clinical phenotype is very heterogeneous. We compared four clinical parameters extracted from the questionnaires and concluded that the only variable with statistically significance difference was the age at diagnosis, lower in the MODY group. This finding can be explained by the fact that MODY-GCK is our most prevalent MODY subtype. We conclude that none of these markers is sufficient to correctly identify all cases of MODY diabetes. More biomarkers need to be studied. It is important to have more clinical data from patients to be able to correctly identify possible cases of monogenic diabetes that should always be confirmed. Genetic testing is the only method that can provide a definitive diagnosis.

Conflicts of interest

None to declare.

Keywords: MODY diabetes, clinical markers, genetic testing

Ethics and consent: The molecular study of monogenic diabetes has been approved by INSA Ethical Committee (NI 136-2023 - 11/04/2023). For all index patients and relatives informed consent was obtained by the referring clinician.

P34 LIQUID BIOPSY IN ORAL SQUAMOUS CELL CARCINOMA: CIRCULATING CELL-FREE DNA DYNAMICS AND MUTATIONAL LANDSCAPE

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Introduction

Oral squamous cell carcinoma (OSCC) is often diagnosed at advanced stages with poor outcomes. While tissue biopsy remains the diagnostic gold standard, it fails to capture tumour heterogeneity over time. Liquid biopsy using circulating cell-free DNA (cfDNA) provides a minimally invasive approach for disease monitoring. This study assessed cfDNA dynamics and mutational landscape in OSCC patients to evaluate its utility in treatment monitoring and disease management.

Methodology

Tumour tissue and serial blood and urine samples were collected from 32 OSCC patients recruited at the Maxillofacial Surgery Unit, CHUC, Coimbra, along with samples from non-cancer controls. cfDNA was extracted and quantified from all samples. Five patients were selected and the cfDNA was analysed by next-generation sequencing (NGS) using a targeted gene panel covering 14 cancer-related genes.

Results

At baseline, patients showed slightly higher cfDNA plasma levels (0.372 ± 0.312 ng/µL) compared to controls (0.305 ± 0.088 ng/µL), while urine cfDNA levels showed no consistent differences. Stratification by stage revealed higher cfDNA concentrations in early-stage patients (0.423 ± 0.419 ng/µL in plasma; 0.397 ± 0.191 ng/µL in urine) compared to advanced stages (0.322 ± 0.117 ng/µL in plasma; 0.341 ± 0.209 ng/µL in urine). Longitudinal analysis demonstrated a consistent transient increase in plasma cfDNA immediately after treatment initiation, followed by a decline to near-baseline values. Urine cfDNA levels exhibited less variation over time.

Targeted NGS on the cfDNA of the 5 selected patients revealed 76 variants at baseline: 47.4% exclusive to the tissue, 22.4% found only in the plasma sample and 30.3% shared. *TP53* was the most frequently mutated gene, followed by *KRAS*, *EGFR*, and *APC*. Longitudinal profiles revealed dynamic clonal evolution, with emergence of novel subclones some of them detectable only in plasma or urine.

Discussion

Our findings support cfDNA as a promising biomarker in OSCC. The higher cfDNA concentration in plasma and urine is consistent to what is described in the literature. The transient post-treatment rise in plasma cfDNA likely reflects tumor cell death induced by therapy, with subsequent decline suggesting treatment response. In contrast, urine cfDNA showed less dynamic variation but captured additional variants, showing its complementary role. Together, these findings support the integration of plasma and urine cfDNA analysis for improved detection of tumor heterogeneity.

Keywords: Liquid biopsy; Oral squamous cell carcinoma; Cell-free DNA; Next-generation sequencing

P35 GENETIC DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA (FH) IN PORTUGAL: INSIGHTS FROM THE PORTUGUE FH STUDY

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Familial hypercholesterolaemia (FH) is a genetic disorder characterised by elevated LDL-C levels, which increases the risk of premature cardiovascular disease (pCVD). FH estimated worldwide frequency is 1/300, and in Portugal nearly 33,000 individuals are expected to be affected. To investigate the genetic cause of individuals with a clinical diagnosis of FH, individuals can be referred by their clinicians to the Portuguese FH Study (PFHS) which is free of charge under a research protocol. This work summarises the outcomes of FH genetic testing in the PFHS.

Between 1999 and 2024, 1386 index cases (555 children, 831 adults) fulfilling FH clinical criteria were referred along with 2389 relatives. Lipid profile and genetic testing were performed for all cases. In 2017, a Next Generation Sequencing panel was implemented, covering FH-causative genes (*LDLR*, *APOB*, *PCSK9*) and FH phenocopy genes.

A total of 491 index cases were genetically confirmed with FH. In adults (n=831, aged 18–78), the positive diagnostic rate was 31% (n=260). In this group, likely pathogenic (LP) and pathogenic (P) variants were identified in the *LDLR* gene (91%), *APOB* (7%) and *PCSK9* (2%). Eleven cases of homozygous FH were identified. In 21% of FH-positive adults, pCVD was observed. Moreover, variants of uncertain significance (VUS) were identified in 6% (n=53). In children (n=555, aged 1–17), the positive diagnostic rate was higher (46%, n=231) with 92% of the cases presenting LP/P variants in *LDLR* gene, 6% in *APOB* and 2% in *PCSK9*. Four homozygous FH cases were identified. VUS were detected in 3% (n=17). No CVD was observed in children. C*ascade screening* identified 652 new FH cases, leading to 1143 genetically confirmed individuals, thus showing the relevance of this approach.

Identified FH cases represent only 3.5% of the expected number in Portugal. Functional assessment of VUS remains essential for accurate diagnosis and is ongoing (PerMedFH and FH-EARLY projects). A cost-effective screening chip array is under development (FH-EARLY) to overcome FH underdiagnosis, promoting an early and affordable diagnosis to prevent CVD complications.

Funding

BM was supported (research fellowship) by the PerMedFH project funded by “la Caixa” Foundation and Fundação para a Ciência e Tecnologia under the grant agreement LCF/PR/HP23/52330032.

Key words: Familial hypercholesterolaemia (FH); Genetic testing; Cascade screening

Ethics and Consent: Written informed consent is obtained from all participants before their inclusion in the study, in accordance with the Declaration of Helsinki.

P36 LIQUID BIOPSY AS A MINIMALLY INVASIVE TOOL FOR LONGITUDINAL GENOMIC SURVEILLANCE IN COLORECTAL CANCER

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Funding

UCGenomics a member of Genome[PT] Consortium (POCI-01-0145-FEDER-022184)

Conflict of interest

The authors have no conflicts of interest associated with this publication.

Ethics and Consent

All patients gave written informed consent. The project was approved by the Ethics Committee of the University Hospital Center of Coimbra (ULS Coimbra).

Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed and deadliest cancers worldwide. The diagnosis and monitoring of CRC rely heavily on tissue biopsies. However, this method, considered an invasive procedure, severely limits longitudinal monitoring, highlighting the need for alternative approaches.

In this context, liquid biopsy (LB) has emerged as a promising, minimally invasive tool for monitoring tumor dynamics, allowing for repeated sampling, making it particularly useful for longitudinal disease assessment. With this work, we aim to assess the validity of LB in CRC patients by analyzing the cell-free DNA (cfDNA) in a cohort of patients.

Methodology

In this study, 24 patients diagnosed with stage I to IVA colorectal cancer were enrolled. Plasma samples were collected before therapeutic intervention and three months after surgery or chemotherapy. Cell-free DNA (cfDNA) was analyzed using a next-generation sequencing (NGS) approach with the Oncomine Colon cfDNA Assay, which targets hotspots across 14 genes.

Results

We observed high variability in cfDNA levels across different disease stages, with an average of 4.08 (1.23<CI<6.23, 95%) before treatment, and a trend toward decreased concentrations following treatment, with an average of 2.03 (1.12<CI<2.94, 95%); however, these changes did not reach statistical significance. We found that variant profiles were diverse across treatments. The limits of detection (LoD) ranged from 0.056 to 2.14%, and we found variant allele frequencies (VAF) that ranged from 0.05 (for rare variants) to 59.2% (suspected constitutional variants). We found a hypermutated patient suggestive of Lynch Syndrome, which was later confirmed by pathology. In patients with favorable therapeutic outcomes, variants were either undetectable post-treatment or exhibited reduced VAF. Conversely, patients with disease progression showed both the loss and emergence of new variants. Patients with a higher number of variants and higher VAF showed more advanced tumor stages. The most frequently mutated genes were APC, KRAS, TP53, and PIK3CA. In general, our liquid biopsy results were concordant with clinical findings.

Discussion

This work supports the use of liquid biopsy as a minimally invasive method for molecular monitoring and longitudinal assessment of disease progression in colorectal cancer.

Key Words: Colorectal Cancer, Liquid Biopsy, Cell-Free DNA

P37 A NEW MS-DIGITALMLPA PROBE PANEL FOR TUMOUR METHYLATION PROFILING: FIRST EVIDENCE FROM ORAL CAVITY CARCINOMAS

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Introduction

Oral cancer is often diagnosed at advanced stages, presenting high locoregional recurrence rates and has had limited improvements in survival over recent decades. While distant metastases are uncommon, recurrent disease is frequently detected late, even under intensive surveillance, reducing the chances of curative intervention. Determination of methylation status of tumour suppressor genes would greatly improve molecular characterization of oral cancers. This study aimed to evaluate the potential of a novel technology – methylation-specific digitalMLPA (MS-digitalMLPA), a potentially useful tool for clinical management of oral cancer.

Methodology

10-40 ng of gDNA from fresh frozen tumour tissue of 42 oral cancer patients was analysed using a test version of DM019 Cancer methylation MS-digitalMLPA assay. DM019 detects methylation and copy number in the promoter regions of 53 cancer-associated genes.

Results

MS-digitalMLPA identified several genes with recurrently methylated promoters in this dataset: *CASP8* (*n*=30) on chromosome 2, *HOXA9* (*n*=40) on chromosome 7, *GATA4* (*n*=24) on chromosome 8, *PAX5* (*n*=19) on chromosome 9, and *WT1* (*n*=24) on chromosome 11. Furthermore, multiple copy number gains and losses were detected across several chromosomes. Among the most frequent copy number losses were the 3p, 8p, and 9p arms, with focal deletions at 3p12.3–p14, 3p24.2–p25.3, 8p23.1–p23.3, and 9p21.3. Similarly, the most frequent copy number gains were observed at 5p13.2–p15.33 and 7p15.2–p21.1. These copy number alteration results are consistent with those obtained using the array-CGH technique.

Discussion

This study is the first to evaluate and discuss the clinical applicability of a MS-digitalMLPA assay in (epi)-genetic characterisation of oral cancer. Our results suggest that this MS-digitalMLPA assay may evolve into a useful tool in the clinical management of oral cancer patients owing to its the ease-of use and multiplexing features.

Conflicts of Interest

Lilit Atanesyan are employed by MRC-Holland, manufacturer of commercially available Digital MS-MLPA probemixes. All other authors declare no competing financial interests.

Keywords: Oral cancer; Multi-oncogene panel test; DNA methylation; Copy number gains and losses; MS-digital MLPA

Ethics and Consent: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Commission of Faculty of Medicine of University of Coimbra. Written informed consent was obtained from all patients.

P38 RARE RNU4-2 VARIANTS IN NEURODEVELOPMENTAL DISORDERS: DOMINANT MECHANISMS PREVAIL IN A PATIENT COHORT WITH HIGH CONSANGUINITY

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Introduction

Genetic variants in *RNU4-2*, which encodes U4—a key non-coding small nuclear RNA (snRNA) component of the major spliceosome— have recently been implicated in neurodevelopmental disorders (NDDs). Dominant, de novo variants clustered within an 18-nucleotide region of *RNU4-2* are considered to cause ReNU syndrome (OMIM 620851). Emerging evidence has expanded this spectrum to include novel biallelic variants outside the ReNU hotspot, affecting other functionally important regions of U4, associated with a recessive form of NDD. The main goal was to investigate the role of rare RNU4-2 variants in a large cohort enriched for parental consanguinity, and assess their contribution to NDD/ID.

Methodology

Genome sequencing (GS) data from 22,928 individuals in CENTOGENE Biodatabank were analyzed for rare monoallelic and biallelic variants in RNU4-2 in patients with NDD and intellectual disability (ID).

Results

Among 4,918 individuals with NDD/ID, 48% of whom were reported to have parental consanguinity, 28 patients were identified with eight heterozygous RNU4-2 variants. The most recurrent variant, n.64_65insT, was present in 20 individuals (71%). Additionally, four patients with three different variants, n.125G>A, n.43insT, n.50G>A were found in the homozygous state with compatible phenotype and not established genetic diagnosis.

Discussion

Our findings reinforce *RNU4-2* as a major contributor to autosomal dominant NDD/ID. While recent studies suggest a distinct recessive disorder linked to biallelic *RNU4-2* variants, our data, despite having high percentage of consanguineous individuals, do not support a significant role for recessive inheritance. These results underscore the evolving complexity of *RNU4-2*-associated neurodevelopmental syndromes and highlight the need for further functional and clinical investigation, including exploration of other noncoding genes within the same family.

Conflicts of Interest: All authors are employees of CENTOGENE GmbH

Keywords: RNU4-2, Neurodevelopmental disorders, ReNU syndrome, Non-coding RNA

P39 GENETIC INSIGHTS INTO PRIMARY OVARIAN INSUFFICIENCY: TOWARDS CLINICAL UTILITY

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Introduction

Primary ovarian insufficiency (POI) affects up to 1% of women under 40 years and is a major cause of infertility. Although genetic factors play a key role, the condition remains genetically heterogeneous, with a definitive molecular diagnosis established in only a minority of cases. This diagnostic gap hampers clinical management and targeted interventions. To address it, this study aims to apply advanced *in silico* and integrative genomics approaches to identify and prioritize novel genes or variants involved in POI pathogenesis.

Methodology

Seven POI patients underwent a two-tiered genetic analysis. First, a custom diagnostic panel targeting genes relevant to ovarian biology was applied. Subsequently, whole-exome sequencing (WES) was performed to expand variant detection. EnGenome Expert Variant Interpreter (eVai), which integrates databases such as Franklin, OMIM, ClinVar, and ClinGen, alongside with *in silico* prediction tools, was employed for variant analysis and classification according to ACMG/AMP guidelines.

Results

Both the targeted panel and whole-exome sequencing failed to establish a definitive molecular diagnosis; however, several variants of potential interest were identified. The panel detected two compound heterozygous *HFM1* variants: c.2T>C, predicted to abolish the start codon (start-loss), and c.3122T>C, a missense change located within a functional domain. Additional variants were encountered in the genes *EXO1*, *PCCA*, *NUP107*, *RECQL4* and *COX10*, all classified as VUS or likely pathogenic. Exome-wide analysis confirmed these findings and revealed eleven additional candidates in genes implicated in ovarian biology and DNA repair: *TG*, *EP400*, *LHCGR*, *FANCG*, *NUP205*, *ASH1L*, *REC114*, *GLO1*, *ESR1*, *BMP4*, and *ANAPC1*. Among these, the *TG*: c.4588C>T (nonsense) variant was classified as pathogenic, while all others remained VUS.

Discussion

Although multiple candidate variants were identified, the absence of definitive pathogenic or biallelic findings hindered molecular diagnoses. These results emphasize the critical need for larger cohorts, segregation analyses, and comprehensive functional validation to clarify the role of these candidate variants in POI pathogenesis. We propose a stepwise approach involving both advanced computational predictions and experimental assays to address these gaps.

Funding

This study was supported by national funds: FCT/FSE (Fundação para a Ciência e a Tecnologia) - Project Reference PRT/BD/154979/2023, UMIB (UIDB/00215/2020, and UIDP/00215/ 2020) and ITR (LA/P/0064/2020).

Conflicts of Interest: The authors declare no conflicts of interest.

Keywords: Primary ovarian insufficiency; candidate gene panel; Next-Generation Sequencing; variant classification

P40 IMPACT OF INCOMPLETE PENETRANCE COPY NUMBER VARIANTS ON NEURODEVELOPMENTAL DISORDERS

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Introduction

Neurodevelopmental disorders (NDDs) are highly heterogeneous conditions frequently associated with copy number variants (CNVs). Among them, CNVs with incomplete penetrance represent a major challenge for clinical interpretation, given their unpredictable expressivity and recurrence risks. This study aimed to investigate the role of CNVs with incomplete penetrance located on chromosomes 15, 16, and 22 in patients with NDDs.

Methodology

We conducted a retrospective cross-sectional observational study including 163 patients diagnosed with NDDs and harboring microdeletions or microduplications on chromosomes 15, 16, or 22. CNVs were detected using array-based comparative genomic hybridization (aCGH) or SNP microarray and classified according to ACMG guidelines. Clinical data were retrieved from electronic medical records and integrated with genetic findings to explore genotype–phenotype correlations.

Results

A total of 164 CNVs were identified: 68.3% pathogenic, 14.6% likely pathogenic, and 17.1% variants of uncertain significance. Deletions were more frequent than duplications across all three chromosomes. Inheritance was variable, with 19.5% maternally inherited, 14.0% paternally inherited, 19.5% de novo, and 46.4% undetermined. Clinical outcomes ranged from mild cognitive and behavioral difficulties to severe congenital anomalies and neuropsychiatric disorders, even among individuals carrying identical CNVs.

Discussion

Our findings highlight the complexity of incomplete penetrance CNVs in shaping neurodevelopmental phenotypes, emphasizing their contribution to variability in clinical presentation and the difficulties they pose for genetic counseling. The study reinforces the need for integrating high-resolution genomic testing with comprehensive phenotypic assessment to improve diagnostic accuracy, counseling, and management of affected individuals.

Keywords: Neurodevelopmental disorders; Copy number variants; Incomplete penetrance; Genetic counseling; Microarray

P41 IMPLEMENTATION AND ISO 15189 ACCREDITATION OF A CLINICAL GENETIC TESTING FOR FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction

Familial hypercholesterolemia (FH) is a prevalent genetic disorder characterised by elevated cholesterol from birth, which increases diverse premature cardiovascular complications. Early diagnosis of FH is crucial for managing complications, as it enables timely intervention and improves patient outcomes. Molecular diagnosis plays a crucial role in confirming clinical suspicion, enabling cascade screening of relatives, and guiding preventive strategies. Implementation of this genetic testing requires strict adherence to the ISO 15189 accreditation standard, ensuring the highest level of accuracy and reliability. This work details the implementation and preparation for ISO 15189 accreditation of a clinical genetic test for FH, aiming to bridge the gap between research and clinical diagnostics.

Methodology

This project, conducted at INSA, involved the validation of a Sanger sequencing workflow for the analysis of FH associated genes: *LDLR* and *APOB*. A systematic variant classification protocol was established, adhering to the 2015 ACMG/AMP guidelines and incorporating reviewed and improved specifications from the ClinGen Familial Hypercholesterolemia Variant Curation Expert Panel. A comprehensive analysis and review of all technical documentation was performed to ensure full compliance with the technical requirements of the ISO 15189 standard.

Results

The genetic testing workflow for FH was successfully developed, with its procedures aligned with ISO 15189 requirements. A portfolio of technical documents was created, aligning the entire pre-examination, examination, and post-examination process with ISO 15189.

Discussion

This project established a reliable, accurate, and accreditation ready genetic test for FH. By providing a quality assured tool for accurate diagnosis and preventative management, this diagnosis strengthens the capacity for early FH detection, supporting essential cascade screening programmes and helping cardiovascular disease prevention strategies.

Funding

Not applicable

Conflicts of Interest

None.

Keywords: Familial Hypercholesterolemia; ISO 15189; Genetic Testing; Accreditation; Quality Control

Ethics and Consent: Written informed consent was obtained for all participants before their inclusion in the study. The study protocol and database have been approved by the National Institute of Health Ethics Committee and the National Data Protection Commission.

P42 MAG-SUDOE: IMPLEMENTING A GENETIC COUNSELING MENTORING PROGRAM IN THE SOUTHWESTERN REGION OF EUROPE

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Introduction

Genetic Counselling Supervision (GCS) plays a crucial role in enhancing quality, safety, and effectiveness of genetic services, allowing recognition and management of the emotional impact the practice can have on professionals. However, access to supervision and its practices vary significantly across Europe. The MAG-SUDOE project aims to promote change in health policies in South-Western Europe, through the implementation of a GCS intervention in Portugal, France, and Spain. The objectives are to: explore the feasibility of GCS services; assess the psychological impact of GCS on genetics healthcare professionals; examine effects of GCS on clinical practice; and development of national programs and evidence-based guidelines.

Methodology

The first phase involves mapping studies on GCS provision in each country and a pilot action in Portugal to assess a group supervision experience. Pre- and post-intervention assessments and interviews will collect perceptions of its impact on practice and well-being. Building on this, a one-year intervention will be implemented in the territories to test a cross-country mentoring programme. Its feasibility will be evaluated through psychological and quality metrics.

Results

The consortia has started recruitment through national organizations and associated partners. Preliminary results from mapping studies in France, Spain, and Portugal will be discussed. Heterogeneity of services organization anticipated disparities and confirms lack of GCS routines in the three territories. Evidence from the pilot implementation of GCS with Portuguese professionals will be presented.

Discussion

This project has the potential to address a critical gap in the provision of GCS by producing robust evidence to guide practice and policy in Portugal, Spain, and France. Although differences in healthcare systems and cultural contexts may pose challenges, the findings will help design adaptable strategies that respect national specificities while promoting safe and effective genetic counselling practice and assuring support for professionals.

Funding

Interreg-SUDOE, FEDER, reference S2/4.5/P0159 LG. PhD Fellowship 2023. 00348.BD, FCT

Keywords: genetics healthcare; healthcare policies; genetic counselling supervision; reflexive practice

P43 MOLECULAR CHARACTERIZATION OF *SERPINC1* VARIANTS IN PATIENTS WITH THROMBOTIC PREDISPOSITION: A COHORT STUDY

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Introduction

Antithrombin (AT), encoded by *SERPINC1*, is a key endogenous anticoagulant regulating coagulation. Variants in this gene may lead to AT deficiencies, with variable thrombotic phenotype. Molecular characterization of *SERPINC1* not only confirms AT deficiency in cases identified by functional assays, but also detects variants that may remain undetected by these methods. Therefore, evaluation of patients with a personal or family history of thrombosis should begin with functional studies and be complemented by molecular analysis.

Methodology

The study included 140 unrelated patients with a personal or family history of thrombosis (mean age 45,8 years; 88 F). In addition, 20 family studies were performed. Functional data were collected when available, and molecular testing was carried out using a customized NGS panel. Sanger sequencing was employed for family members and for variant confirmation. All variants were classified according to ACMG guidelines.

Results

In a cohort of 140 probands, 130 had a personal history of thrombosis and 10 had a family history. In 34 cases, 19 distinct *SERPINC1* variants were identified (9 P, 6 LP, and 4 VUS), 7 of which had not been previously reported. In 12 of the 20 family members, the familial variant was confirmed.

Twenty probands exhibited reduced AT activity (mean 53,7%). Eleven individuals showed normal activity (mean 95%), particularly those carrying missense variants - 6 different variants classified as type I, type IIRS, or transient deficiencies. In 3 individuals, AT activity was not measured, although variants of type IIRS or transient deficiency were present. The mean age of the first thrombotic event was 34 years. The spectrum of variants included: missense (68,4%), nonsense (10,5%), large deletions (5,3%), splicing alterations (10,5%), and frameshifts (5,3%). Six variants were classified as type I deficiency, 3 as type II (IIRS, IIPE, IIHBS), 3 as transient, and 7 are not reported.

Discussion

In this cohort, *SERPINC1* variants largely explain the thrombotic phenotype and reduced AT activity. However, pathogenic variants in individuals with normal AT levels suggest undetected functional deficiencies, possibly due to regulatory or structural mechanisms. These findings underscore the need to better understand impact of variant location on protein function, which could enhance genotype–phenotype correlations, risk stratification, and targeted diagnostic and therapeutic approaches.

Conflicts of interest

No conflicts of interest.

Keywords: SERPINC1; Thrombosis; Molecular studies

P44 GENOTYPING REPEAT EXPANSIONS IN NEUROLOGICAL DISEASES BY LONG-READ SEQUENCING: A CROSS-PLATFORM STUDY

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Introduction

Repeat expansions are a significant cause of neurological diseases within their mutational spectra, as seen in a range of clinical entities such as Huntington's disease and various spinocerebellar ataxias. Given the abnormal elongation of short tandem repeats, short-read NGS cannot accurately genotype these repeats, due to limited read lengths, poor read coverage and alignment issues. Long-read sequencing platforms, namely Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT), offer unprecedented capacity to uncover these challenging regions, allowing the simultaneous determination of sizing and sequence composition of the repeat unit.

Methodology

We reanalysed a set of 40 patients previously tested for different repeat expansion diseases. High molecular weight genomic DNA was extracted and sequenced using both PacBio (PureTarget Repeat Expansion Panel v1) and ONT (Adaptive Sampling) platforms. Bioinformatics analysis resorted to the platforms' default workflows for alignment and variant calling, including repeat expansion callers TRGT (PacBio) and Straglr (ONT). We determined the diagnostic detection rates and sensitivity between the two platforms, comparing with the results obtained through conventional methods.

Results

A total of 20 *loci* (*AR*, *ATN1*, *ATXN1*, *ATXN10*, *ATXN2*, *ATXN3*, *ATXN7, ATXN8OS, C9orf72*, *CACNA1A*, *CNBP*, *DMPK*, *FMR1*, *FXN*, *HTT*, *PABPN1*, *PPP2R2B*, *RFC1*, *TBP*, *TCF4*) known to harbour repeat expansions were genotyped using both platforms in our cohort. For ONT and PacBio the diagnostic detection rates were 84.2% and 97.0%, respectively, and the sensitivity rates were 86.1% for ONT and 99.3% for PacBio. The differences can be mainly explained by the higher coverage obtained (682.6x ± 445.8x for PacBio vs. 26.4x ± 10.7x for ONT) and fragment length. ONT missed automated calling of repeat expansions in *C9orf72* and *RFC1 loci.*

Discussion

We present unprecedented data comparing ONT and PacBio in a diagnostic setting. Targeted long-read sequencing allows for routine detection and characterization of repeat expansions underlying neurological diseases. Incorporating these technologies into clinical genomics pipelines holds promise to further accelerate diagnosis. However, differences between the two technologies should be considered, especially for diagnostic purposes, as evidenced in this work.

Conflicts of Interest

No conflict of interest towards this work.

Keywords: Neurological diseases; repeat expansions; diagnostics; Bioinformatics

Ethics and Consent: The i3S Ethics Committee (CECRI) has approved this project (ref. N2.2025).

P45 IMPLEMENTING AND VALIDATING CYTOGENETICS LABORATORY WORKFLOWS FOR ISO 15189 ACCREDITATION AND IVDR COMPLIANCE

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Introduction

Cytogenetic techniques, including karyotyping and fluorescence in situ hybridization (FISH), are fundamental in genetic diagnostics, enabling precise detection of chromosomal abnormalities. Within accredited laboratories, these methods must comply with ISO 15189, which defines quality and competence standards for medical testing. In parallel, the Regulation (EU) 2017/746 (IVDR) establishes enhanced requirements for validation, traceability, and ongoing performance monitoring of in-house diagnostic tests. This study describes the implementation and validation of cytogenetic methods in the context of ISO 15189 accreditation and IVDR compliance, outlining key challenges and emphasizing the importance of sustaining diagnostic quality within a regulated framework.

Methodology

In-house laboratory-developed tests (LDTs) were designed and validated for each technique, in line with IVDR requirements and following a risk-based approach. Validation was performed on stimulated and non-stimulated lymphocyte cultures, lymphocyte direct fixation as well as on fibroblasts. It comprised three components: (i) scientific validation through a literature review; (ii) analytical validation by comparing results across methodologies and assessing culture growth rates; and (iii) clinical validation using external quality controls to confirm the tests' suitability for routine diagnostics.

Results

Scientific validation confirmed that the chosen methodologies are evidence-based and in line with the latest best practices. Karyotyping achieved consistently high success rates, fulfilling all quality criteria: 98% in stimulated lymphocytes, 93% in non-stimulated lymphocytes and 96% in fibroblast cultures. Participation in external quality assessments (EQAs) produced satisfactory results, providing further support for the reliability of both methodologies. For FISH, probe-specific cut-offs were also established (Multiple Myeloma: 5% for translocations, 9% for 17p deletion, 10% for 1p deletion and 4% for 1q amplification). Overall, both techniques demonstrated stable performance, ensuring patient safety and fulfilling the requirements for ISO 15189 accreditation and IVDR compliance.

Conclusion

Incorporating ISO 15189 and IVDR requirements into routine classical cytogenetics enables laboratories to maintain diagnostic quality and patient safety, while preserving the clinical significance of traditional methods in response to changing regulatory demands.

P46 REPORTING INCIDENTAL AND SECONDARY FINDINGS – A PROSPECTIVE STUDY

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Introduction

As genetic testing began to include the simultaneous study of hundreds to thousands of genes, it shifted from confirming a clinical diagnosis to discovering a molecular diagnosis previously unsuspected by clinicians. In addition, many more incidental diagnoses began to be identified. These two facts gave strong relevance to pre-test informed consent, as a guideline for the disclosure of genetic information that is highly relevant to both the health of the patient and that of their family.

A proportion of those incidental results may reveal clinically actionable conditions, and their research is subject to guidelines from the College of Medical Genetics and Genomics (ACMG).

In the absence of symptoms, clear laboratory reporting frameworks are needed to ensure compliance with current legislation and professional guidelines, ensuring the best health promotion.

Methodology

We conducted a prospective study, from August 1 to September 19, 2025, for secondary or incidental findings in reports from whole-exome sequencing (WES), WES-based panels and chromosomal microarray analysis (aCGH). Findings were categorized as secondary or incidental and reported in line with Portuguese Law 12/2005 and the 2025 advice of the of Medical Genetics College Board.

Results

Among 160 exomes analyzed, we identified 7 secondary findings (4.4%) and 4 incidental findings (2.5%).

In 422 WES-based panels, 2 incidental findings (0.5%) were observed, and in 188 aCGH analyses, 1 incidental finding (0.5%) was detected.

In these 14 reports, only in two a secondary or incidental finding were specifically stated, in another two a recommendation for a medical genetics consult was given. In the remaining ten, because adult-onset conditions were detected in pediatric patients, a recommendation for a medical genetics consult for the proband's parents was provided.

The identified pathologies and the laboratory framework will be detailed.

Discussion

The 2025 statement of the Portuguese College of Medical Genetics, aligned with current legislation, emphasizes the importance of structured reporting protocols. Our results demonstrate the need for such frameworks to guarantee genetic counseling in the communication of incidental and secondary genetics diagnoses. This approach ensures that genomic medicine delivers both clinical utility and responsible healthcare, for the patients and their families.

P47 POST-MARKET SURVEILLANCE OF WHOLE-EXOME SEQUENCING UNDER IVDR: ONE-YEAR EXPERIENCE AFTER TECHNICAL FILE COMPLETION

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Introduction

The implementation of Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices (IVDR) has introduced stricter clinical validation and continuous monitoring requirements for both in-house, and commercial genetic diagnostic tests. Post-market surveillance (PMS) is central to ensuring ongoing safety, performance and clinical utility of these tests. While PMS is relatively straightforward for targeted molecular tests, it presents specific challenges when applied to high-throughput technologies such as whole-exome sequencing (WES). The wide analytical scope, evolving interpretation frameworks and reliance on bioinformatics pipelines make performance monitoring and risk management particularly complex for WES.

Methodology

PMS data were collected during the routine diagnostic use of WES over 12 months. Key quality indicators included sequencing coverage, variant detection metrics and the consistency of variant classification. Performance trends were monitored using Levey-Jennings control charts and other statistical process control tools to allow early identification of deviations from established specifications. Additional data sources of PMS included participation in external proficiency testing, user feedback and records of non-conformities, corrective actions, and adverse events. These measures provided a structured framework for evaluating the analytical performance and clinical utility in line with IVDR requirements.

Results

During the monitoring period, WES performance indicators remained stable, with coverage and variant detection metrics consistently within control limits. No critical adverse events occurred. Minor deviations in analytical parameters were identified and resolved through corrective measures. PMS confirmed assay safety and performance robustness while highlighting opportunities to refine monitoring strategies.

Conclusion

This work emphasizes the need set clear, measurable objectives for future PMS cycles to drive continuous improvement, ensure regulatory compliance and enhance clinical utility. Similarly, applying trend analysis to key QC indicators was crucial for enabling preventive action before deviations could affect results, thus preserving the reliability of WES in routine diagnostics.

P48 INSIGHTS FROM A COHORT OF 44 PATIENTS WITH *CDK13*-RELATED DISORDER: INCOMPLETE PENETRANCE, VARIABLE EXPRESION AND GENOTYPE-PHENOTYPE ASSOCIATIONS

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Introduction

Pathogenic variants in *CDK13* gene cause an autosomal dominant disorder, characterized by developmental delay/intellectual disability (DD/ID), impaired verbal language skills, structural cardiac defects, suggestive facial dysmorphisms, behavioral problems, feeding difficulties in infancy and seizures. Genotype-phenotype correlation of reported patients suggests a trend toward milder phenotypes in patients with variants predicted to cause haploinsufficiency, while missense variants affecting the kinase domain appear most likely to be associated with structural malformations and a more severe phenotype.

Methodology

Patients from CENTOGENE Biodatabank with pathogenic and likely pathogenic (P/LP) variants identified in *CDK13* gene via panel/exome/genome/sanger sequencing within a diagnostic setting were analyzed for genotype-phenotype correlation.

Results

44 patients with P/LP variants in *CDK13* gene were identified, 23 patients with missense SNVs, 7*de novo* and 16 of unknow inheritance. In addition, 21 patients harbored loss of function (LoF) SNVs, 4 *de novo*, 13 of unknow inheritance and 4 familial variants.

Discussion

In the cohort of patients with missense variants the phenotypes were rather severe when compared to the cohort of patients with LoF (96% vs 48% with DD/ID; 91% vs 48% with dysmorphisms; 52% vs 24% with cardiac and 17% vs 24% with brain anomalies).

We also identified three families harboring the same previously reported LP frameshift variant, c.484dup(p.Ala162fs). In two families, the variant was detected in both affected index case and apparently unaffected parent; in the third family, it was present in both daughter and father, exhibiting only cardiac anomalies. This variant has been reported multiple times as *de novo* in affected individuals, except for a male patient with hyperphagia and overweight/obesity, with a slight neurological involvement, inherited from mother with reportedly normal development. Additionally, the unpublished variant c.2105dup p.(Asp703Glyfs*2) was also identified in one affected index and in apparently unaffected father.

This data supports the hypothesis that *CDK13* haploinsufficiency may result in a less severe clinical presentation. These findings further suggest the possibility of incomplete penetrance and/or variable expressivity associated with this disorder, crucial factors to consider when interpreting variant pathogenicity and segregation patterns in clinical genetics setting.

Conflicts of Interest

All authors are employees of CENTOGENE GmbH

Keywords (3-5): *CDK13,* incomplete penetrance, variable expression, missense vs LoF SNVs

P49 RETROSPECTIVE ANALYSIS OF PATIENTS WHO UNDERWENT TUMOR AND GERMLINE TESTING FOR OVARIAN CANCER

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Introduction

Ovarian cancer (OC) is the eighth most common cancer in women worldwide and the deadliest gynaecologic malignancy. In Portugal, it accounts for ~3.7% of female cancer deaths. The disease's nonspecific and silent presentation leads to late-stage diagnoses and poor outcomes. Molecular testing, recommended for all epithelial OC, plays a key role in treatment planning, namely in PARP inhibitors use, as well as in risk-reducing strategies, long-term surveillance, and familial risk assessment. Pathogenic or likely pathogenic (P/LP) germline and somatic variants in *BRCA1/2* are found in ~15–20% of cases, with higher prevalence in high-grade serous subtype.

Methods

The study included all women diagnosed in our Medical Genetics Department between 2019 and 2023 who underwent both tumour and germline testing for OC at IPO-Porto. Molecular analysis consisted of NGS of OC susceptibility genes (*ATM, BRCA1, BRCA2, BRIP1, MLH1, MSH2, MSH6, PMS2, PALB2, RAD51C, RAD51D*) in tumour tissue, complemented by *MLPA* for *BRCA1/2* rearrangements and targeted analysis of the Portuguese founder mutation *BRCA2*:c.156_157insAlu in blood sample. This sample was additionally used to confirm the potential germline origin of variants detected in the initial tumour testing by NGS. Clinical, histological, and genetic data were retrieved by retrospective review of medical records. All data were anonymized and analysed using descriptive statistics.

Results

Sixty-five patients were included, 26 (40%) of whom were deceased at data collection. Tumour testing was not possible in 10 cases (insufficient material), and germline testing was performed only in 63 cases. The overall detection rate (DR) for P/LP *BRCA* variants was 17%, comprising a somatic DR of 7% and a germline DR of 11%. Nine patients (14%) were diagnosed with an OC predisposition syndrome due to germline variants in *BRCA1* (6%), *BRCA2* (5%), *PMS2* (2%), and *RAD51C* (2%). Germline variants of uncertain significance (VUS) were identified in three patients (5%; *BRCA1*, *BRCA2*, *ATM*), and the one in *BRCA1* was meanwhile reclassified as LP.

Conclusion

Our centre's experience aligns with previously reported Portuguese cohorts, where P/LP *BRCA* variants are identified in roughly 19–20% of patients (somatic ~6%, germline ~13%). The high mortality reflects late-stage diagnoses and emphasizes the urgent need for earlier genetic testing, preferably at the time of diagnosis, and integration of targeted therapies whenever applicable.

Keywords: ovarian cancer, hereditary ovarian cancer syndrome, genetic testing, risk-reducing strategies, PARP inhibitors

Ethics and Consent

Approved by the Ethics Committee of ULS Coimbra (Appraisal NºOBS.SF.153-2023)

P50 THE MUTATIONAL SPECTRUM OF PRIMARY CILIARY DYSKINESIA (PCD) AND THE DIAGNOSTIC RELEVANCE OF NON-CODING REGIONS: THE LABORATORY PERSPECTIVE

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Introduction

Primary ciliary dyskinesia (PCD) is a rare heterogeneous genetic disease involving the impairment of motile cilia and leading to chronic respiratory disease, laterality defects and subfertility. To date, 54 PCD-associated genes are known, most following an autosomal recessive mode of inheritance. The advances in next-generation sequencing (NGS) and its clinical application through multigene panels based on whole-exome sequencing (WES) allowed to estimate diagnostic yield: 17% to 70%. Nonetheless, a significant number of PCD cases still lack a molecular diagnosis, suggesting the existence of additional causal genes and/or variants in regions not analysed by routine.

Methodology

The aims of this work were to characterize the mutational spectrum of PCD-related genes and evaluate relevance of covering non-coding regions. We reviewed 79 cases tested using WES-based multigene panels for PCD (2018-2025). These included: (1) 56 cases for which coding exons and intronic boundaries were sequenced; and (2) 28 cases where an extended capture-kit was used to include non-coding regions known to harbour disease-relevant variants.

Results

A similar diagnostic rate was obtained in both groups (17.9% for group 1 and 14.3% for group 2). Pathogenic or likely-pathogenic variants (PAT/L-PAT) were identified at 12 different *loci*: *CCNO*, *DNAAF4*, *DNAAF6*, *DNAH11*, *DNAH5*, *DRC1*, *FOXJ1*, *HYDIN*, *PKD1L1*, *RPGR*, *RSPH1* and *TNFRSF13B*. Among the disease-causing variants found, 9 were novel and 10 had been previously described. Additionally, 46.4% and 28.6% of cases (for groups 1 and 2) had variants of unknown clinical significance (VUS), or only one PAT/L-PAT variant in heterozygosity in a gene causing a recessive disease. The scenario of having missed a second recessive allele comprised 28.6% of cases in group 1; but only 3.6% of cases in group 2. These included genes with deep intronic variants associated with PCD such as *CCDC39*, *DNAH11* and *ODAD1*. Moreover, in group 2, capturing deep intronic regions allowed the identification of additional variants (classified as VUS) in *DNAH5* and *DNAH7*.

Discussion

Overall, despite this relatively small cohort, our results stress the importance of (1) expanding analyses beyond exonic regions; and (2) pursuing further molecular studies. In particular, efforts should focus on identifying new PCD-related genes and analysing the expression of variants located beyond canonical exon boundaries or within regulatory regions.

P51 PREVALENCE AND CHARACTERIZATION OF *IKZF1* GENE DELETIONS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: A SNP ARRAY STUDY

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common cancer in children and adolescents. Over the past decades, survival rates in pediatric ALL have exceeded 90%, mainly due to the implementation of risk-adapted treatment strategies. Nevertheless, a detailed understanding of factors influencing relapse risk remains critical to further improving outcomes. Among these, *IKZF1* (IKAROS family zinc finger 1) is a master regulator of lymphocyte differentiation, essential for both T- and B-cell development. *IKZF1* deletions are among the most frequent genetic alterations in childhood ALL, strongly associated with high-risk subtypes such as Philadelphia chromosome–positive (Ph+) ALL, and are used in risk stratification for therapy decisions. They are detected in approximately 15% of pediatric B-cell precursor ALL cases, 60–70% of BCR-ABL1–positive cases, and are rare in T-ALL. Although numerous studies have shown that *IKZF1* alterations confer poor prognosis, their impact under contemporary treatment protocols remains debated. Here, we describe the prevalence and characterization of *IKZF1* deletions in a Portuguese cohort of pediatric ALL patients and explore their implications for risk stratification.

Methodology

221 ALL samples were analyzed by Array Comparative Genomic Hybridization combined with Single Nucleotide Polymorphism array (SNPa). The analysis included the evaluation of ploidy, whole-genome alterations, loss of heterozygosity, and copy number alterations affecting ALL critical genes, including *IKZF1*.

Results

The results of our study revealed a copy number loss of *IKZF1* in 37 cases (17%). The most common alteration was whole chromosome 7 (35%), 7p12.2 region including whole gene (22%) deletion, followed by exon 4-7 deletion in 19% of the cases. *IKZF1* homozygous deletion were not found. On the other hand, it, is relevant that in 78% of the cases in which the *IKZF1* deletion was found, another genetic alteration was present (*ERG, CDKN2A/B, ETV6, PAX5, or RB1*).

Discussion

Careful monitoring of minimal residual disease in affected patients is essential for early detection of relapse, identifying candidates for alternative or intensified therapies. Further studies investigating the prevalence and impact of *IKZF1* deletions, alongside classical cytogenetic and other copy number alterations, are needed to better define its prognostic significance. Incorporating emerging genetic subgroups into risk stratification may enhance therapeutic responses and improve survival outcomes in ALL patients.

P52 CNVs DETECTED BY MLPA IN THE PAR1 REGION IN PATIENTS WITH LÉRI-WEILL DYSCHONDROSTEOSIS AND IDIOPATHIC SHORT STATURE

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The short stature homeobox-containing gene (SHOX, MIM 312865) is mapped to the pseudoautosomal region 1 (PAR1) of both sex chromosomes (Xp22.33 and Yp11.2). *SHOX* is expressed in osteoblasts, bone marrow fibroblasts, and hypertrophic chondrocytes, playing a pivotal role in endochondral ossification.

SHOX haploinsufficiency underlies several well-characterized short stature phenotypes, such as Léri-Weill dyschondrosteosis (LWD, MIM 127300), Turner syndrome, and, in cases of biallelic loss Langer mesomelic dysplasia (LMD, MIM 249700).

Pathogenic CNVs of *SHOX* and point mutations, account for approximately 1.5–15% of idiopathic short stature (ISS, MIM 300582) cases.

The *SHOX* gene comprises 7 exons, giving rise to two main transcripts, whose expression is regulated by cis-regulatory elements located in PAR1.These regulatory sequences include conserved non-coding elements (CNEs) with enhancer or repressor functions, located both upstream and downstream of the *SHOX* coding region. CNVs disrupting these elements may alter gene expression, leading to abnormal skeletal phenotypes.

We analyzed 110 individuals (104 with ISS, 5 with LWD and 1 unaffected parent), using the MLPA (Multiplex Ligation-dependent Probe Amplification) P018-G2 kit, to assess the CNVs affecting *SHOX* gene and its regulatory regions.

In total, 14 CNVs (~12,7%) were identified: 4 duplications (3,6%) and 10 deletions (9,1%), all within PAR1.

We identified deletions in two families with LWD. In one family two daughters inherited a maternal deletion of 37kb on the downstream region (CNE7 to CNE8). The other family, a daughter inherited, from a father with LWD and an unaffected mother, two different deletions: the maternal of 37kb spanning from CNE7 to CNE8 and a paternal with 320kb from CNE-2 to CNE9.

Among the ISS cases 8 CNVs were found, 4 deletions and 4 duplications; three deletions of 75kb in the CNE7 to CNE8 region, and one is spanning almost the entire PAR1. Duplications were heterogeneous, involving the *SHOX* and both upstream and downstream regions.

In our cohort both *SHOX* deletions and duplications were observed. The recurrent 37kb deletion of the CNE7 to CNE8 region highlights it as a critical regulatory hotspot for *SHOX*-related growth conditions, to a great degree in LWD individuals but also in ISS. These results expand the range of *SHOX*-related alterations and reinforce the importance of MLPA, as a cost-efficient and rapid technique, for detecting clinically relevant CNVs in PAR1. For better characterization array-Comparative Genomic Hybridization could be performed.

Keywords: SHOX, Léri-Weill dyschondrosteosis, idiopathic short stature, PAR1, MLPA.

P53 INCIDENTAL X-CHROMOSOME FINDINGS DURING *FMR1* TRINUCLEOTIDE REPEAT SCREENING

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Introduction

Fragile X Syndrome (FXS/FRAXA, MIM#300624) is an X-linked disorder caused by an unstable expansion of the GGC repeats (>200 triplets) in the 5'UTR of *FMR1*, and represents the main cause of inherited intellectual disability. The prevalence of males with a full mutation is about ~1/4000 and it is known that the FMR protein levels and the FXS phenotype are correlated with the degree of methylation of the *FMR1* promoter. Approximately 16-25% of females with a premutated allele (55-200 GGC repeats) are at risk for Fragile X-associated Primary Ovarian Insufficiency (FXPOI, MIM#311360). Older males and females with premutations are at risk for Fragile X Tremor/Ataxia Syndrome (FXTAS, MIM#300623). Besides fragile site A, fragile site E on the X-chromosome is also associated with intellectual disability (FRAXE, MIM#309548). This disorder is mainly a non-syndromic form of X-linked intellectual disability and is the result of silencing of the *AFF2* gene, consequent to an upstream GCC expansion.

Methodology

This is a retrospective study of individuals clinically referred for FXS, FXPOI and FXTAS molecular testing. Molecular testing relied on a homebrew multiplex-PCR, designed for the mutational hotspot regions of the *FMR1* and *AFF2* genes, and a triplet repeat primed PCR (TP-PCR) analysis of the GGC region of the *FMR1* gene. Atypical PCR patterns were studied by amelogenin gender-marker analysis, methylation-sensitive PCR for the *FMR1* gene and/or MLPA. All resulting fragment profiles were analysed following size separation by capillary electrophoresis.

Results and Discussion

Some individuals had normal *FMR1* GGC repeat numbers but atypical X-chromosome patterns. Further investigation of these unexpected results enabled the detection of males with Klinefelter Syndrome (47,XXY) or 48,XXXY Syndrome, and females with Turner Syndrome (45,X) or Triple-X Syndrome (XXX). It is important to emphasize that such incidental molecular findings should always be confirmed using an alternative complementary technique, such as karyotyping and/or array-CGH.

Keywords: *FMR1* gene; X-chromosome anomalies; molecular testing

P54 ROLE INVESTIGATION OF *RNU4-2* GENE IN UNSOLVED NEURODEVELOPMENTAL CASES

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Despite the identification of over 1,400 genes with a confirmed etiological role for intellectual disability (ID), most ID cases (~60%) remain unexplained after genetic testing.

To date, the overwhelming majority of known disease-causing variants are in the ~1.5% of the genome that directly encodes proteins. In contrast, the non-coding genome (the remaining 98.5%) has been relatively unexplored, especially regions far from protein-coding genes.

Some studies identified variants in a 18 base-pair (bp) critical region in the centre of *RNU4-2,* associated with a severe neurodevelopmental phenotype (NDD) and estimated that variants in this region explain ~0.41% of individuals with NDD. These variants map to critical structural elements in the U4/U6 complex. These structural elements are important to correctly position U6 ACAGAGA, to receive the 5' splice-site during initial spliceosome activation, and detail the expression of *RNU4-2* through brain development.

To investigate the potential contribution of *RNU4-2* variants to NDD, we performed Sanger sequencing of this gene, including the 18 bp critical region, in a cohort of ~300 individuals with unexplained NDD. All patients had previously undergone whole-exome sequencing (WES), which did not identify any pathogenic or likely pathogenic variants.

The aim of this study is to compare the frequency of *RNU4-2* variants in our cohort to previously reported data, and to evaluate the potential diagnostic relevance of this non-coding gene in neurodevelopmental phenotypes.

P55 ADVANCES IN GENETIC SCREENING FOR LEBER'S HEREDITARY OPTIC NEUROPATHY (LHON) AT LABORATORY OF MITOCHONDRIAL BIOMEDICINE AND THERANOSTICS (LBIOMIT) - THREE DECADES OF EXPERTISE

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Many patients with optic atrophy, particularly LHON, remain without an identified genetic cause[1,2]. The diagnosis involves screening for mitochondrial DNA (mtDNA) pathogenic sequence variants (PSV): m.3460G>A, m.11778G>A, and m.14484T>C (TOP3)[3].

Over 30 years, 169 cases (212-355 LHON patients estimated in Portugal) have been assigned to our laboratory. The recent GenEye24[3] provides TOP3 screening within 24h. An overview of the approach and results of LHON screening at LBioMiT is presented.

Bigenomic genetic screening was performed by NGS and/or Sanger sequencing and bioinformatics analysis[4]. The GenEye24 relies on real-time PCR with High-Resolution Melting[3].

Genetic Sequencing revealed 14 (11%) and 111 (89%) patients, respectively with and without TOP3 PSV. GenEye24 detected 8 mutant (18%) and 36 wild-type (82%) patients. The distribution of PSVs was 50% m.11778G>A, 22.7% m.14484T>C, 27.3% m.3460G>A. In addition, one case of dominant optic atrophy (c.635_636delAA, *OPA1*) and four patients with other PSVs (mtDNA/ nuclear DNA), in heterozygosity, were found.

The findings highlight that in-depth genetic screening is essential. But there is a place for rapid first-line screening and GenEye24 is an improved alternative for TOP3 screening; the quick response improves the chances of effective treatment and visual function recovery. Moreover, its implementation led to a significant increase in the number of samples screened (44 in 5 years, compared to 125 over 26 years), duplicating the cases screened per year. This increase may be partly attributed to media coverage[3] and the affordability of GenEye24. With 36 news pieces, a total of 1,022,845 people were reached, associated with €90,671.20 in Automatic Advertising Value (AAV). This makes GenEye24 the first line of diagnosis in LHON.

Funding

Portuguese national funds via FCT – Fundação para a Ciência e a Tecnologia, under projects UIDB/04539/2020, UIDP/04539/2020, UID/04539/2025, LA/P/0058/2020, grant 2022.10296.BD; Santhera Pharmaceuticals.

The authors declare no conflict of interest.

1. DOI:10.1016/j.ejpn.2006.11.015; 2. DOI: 10.1093/hmg/ddx273; 3. DOI:10.1016/j.mito.2023.01.006; 4. DOI:10.1016/j.nmd.2017.11.006

Keywords: LHON; mtDNA variant; variant screening; Sanger Sequencing; real-time PCR

P56 GENETIC STUDY OF *CFTR*: PREVALENCE AND MUTATION SPECTRUM IN A LARGE PORTUGUESE COHORT

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Cystic fibrosis (CF; MIM# 219700) is one of the most common life-shortening autosomal recessive disorders in Caucasians, with a frequency of ~1 in 2,000-3,000 live births. In the European population, CF carrier frequency is estimated to be 1 in 20-30 individuals. It is caused by mutations affecting the CF transmembrane conductance regulator (*CFTR*) gene. Over 2000 different variants have been documented to date, the frequency and distribution of which vary significantly among different ethnic groups and geographic regions.

Clinically, CF is classified as Classic Cystic Fibrosis (Classic CF) or CFTR-Related Disorders (CFTR-RD**),** where the classic form is more severe and typically multisystemic.

The aim of this work is to review *CFTR* studies performed in a Portuguese cohort, determining the type and frequency of variants and estimating the prevalence of CF in this population.

A total of 1560 individuals were screened, comprising 221 patients (referred due to symptoms or laboratory tests consistent with cystic fibrosis), 539 asymptomatic individuals (indicated for carrier test due to family history, consanguinity, or suggestive foetal ultrasound findings), and 800 potential gamete donors.

All samples were tested for 69 frequent variants in Europe using a commercial ARMS-based technique. For a more comprehensive *CFTR* study, a subset of cases was also subjected to Sanger sequencing, MLPA and/or cDNA analysis.

In the patients' group, 61 presented at least one pathogenic variant, of which 20 patients presented two mutated alleles. Carrier test was positive in 139 asymptomatic individuals (105 with a classic CF-causing variant) and 35 gamete donor candidates carried a classic CF-causing variant, indicative of a 1 in 22 CF carrier frequency in the population.

A total of 50 different variants were identified; 24 CF-causing, 16 CFTR-RD-causing and 10 involved in both clinical presentations. The most common variant was c.1521_1523del (Phe508del), representing ~34% of mutated alleles. A novel intragenic rearrangement was detected, in which exon 16 was replaced by an intact copy of exon 19, resulting in an aberrant transcript (NM_000492.4: r.2620_2657delins2989_3139).

The results obtained reinforce the relevance of the molecular study of *CFTR***,** not only for confirming the diagnosis but also for identification of carriers, and it has become indispensable in the field of personalised medicine, which depends directly on the identified genotype.

P57 A RETROSPECTIVE ANALYSIS OF *MYBPC3* VARIANTS: OPTIMIZED BIOINFORMATICS PIPELINE REVEALS PATHOGENIC INTRONIC VARIANTS IN FORMER GENOTYPE-NEGATIVE HCM CASES

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Introduction

Genetic variants in the *MYBPC3* gene are among the most common genetic causes of hypertrophic cardiomyopathy (HCM). Recent studies have identified causal intronic variants, introducing cryptic splice-sites located in outside the standard regions of interest of genetic testing. By default, intronic regions outside the canonical splice site (± 8bp) are not analyzed in the daily practice of our diagnostic laboratory. The aim of this work was to conduct a retrospective analysis and optimize the workflow used in routine cardiogenic testing to include the study of important intronic regions.

Methodology

A retrospective study was conducted on a cohort of 658 HCM probands, through the development of a bioinformatics pipeline that allowed us to search for five known intronic *MYBPC3* relevant variants (c.1224-19G>A, c.1224-52G>A, c.1224-80G>A, c.1224-21A>G, and c.906-36G>A).

Results

This analysis allowed the detection of 4 cases with splicing variants (0.6%) that were previously missed using the conventional approach.

Discussion

These results emphasize the importance for laboratories to regularly update their bioinformatics pipelines, incorporating the study of well covered intronic regions, in genes like *MYBPC3*. This strategy can increase the detection of pathogenic/likely pathogenic variants, improving HCM diagnostic yield, without increasing costs or turnaround time for genetic testing.

Conflicts of Interest: Not applicable

Keywords: Intronic, Cardiogenetics, Pipeline

P58 GENOMIC ANOMALIES IN MEN WITH FERTILITY ALTERATIONS: A COHORT STUDY (2014–2021)

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Introduction

According to the 2023 WHO report, infertility affects approximately 17.5% of couples worldwide, 20–30% due to male factors alone. Male infertility is frequently associated with testicular dysfunction or spermatogenesis disorders and a genetic cause is identified in ~15% of cases, mainly chromosomal abnormalities and Y-chromosome microdeletions. Karyotyping is routinely used to detect chromosomal abnormalities such as Klinefelter syndrome (47,XXY), while Multiplex Ligation-dependent Probe Amplification (MLPA) effectively identifies Y-chromosome genomic anomalies in critical regions like AZF (Azoospermia Factor). This study explores a potential genotype-phenotype correlation in men with impaired fertility.

Methodology

This retrospective study included data from 1.108 men with fertility disorders who underwent karyotyping and, in selected cases, MLPA (panel P360-B1, MRC Holland), between 2014 and 2021. Data from all patients including age, clinical indications, karyotype, and MLPA results were collected and statistically analyzed.

Results

Of the 1.108 men, 1.065 (96.1%) had a normal karyotype, while 43 (3.9%) showed chromosomal abnormalities. MLPA was performed in 230/1.108 men (20.8%), revealing 36 (15.7%) Y-chromosome genomic alterations: 25 deletions (69.4%) and 13 duplications (36.1%). AZFc was the most affected region (31 cases; 86.1%), with *CDY2A* and *DAZ2* being the most commonly altered genes (31 [86.1%] and 30 [83.3%] cases, respectively). Chromosomal abnormalities detected by karyotyping were significantly more frequent in azoospermic men compared to those with severe oligoasthenoteratozoospermia ($\chi^2(2) = 16.959$; $p < 0.001$). However, no significant differences were observed in the frequency of Y-chromosome micro alterations among azoospermic, severe oligoasthenoteratozoospermic, or oligoasthenoteratozoospermic (unspecified severity) men ($\chi^2(2) = 3.566$; $p = 0.168$).

Conclusion

These findings highlight the clinical value of combining karyotyping and MLPA in the genetic workup of male infertility, particularly in cases of altered spermatogenesis, contributing to a better understanding of genotype-phenotype correlations.

Conflicts of interest

None

Ethics and Consent: Informed consent was obtained from patients for this presentation.

Keywords: Male infertility; MLPA; karyotype.

P59 DECIPHERING THE GENETIC MODIFIERS OF SICKLE CELL ANEMIA IN CHILDREN: THE ROLE OF *CYB5R3 GENE*

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Introduction

Sickle cell anemia (SCA) is an autosomal recessive disorder caused by the c.20A>T alteration in the beta-globin gene (*HBB),* leading the synthesis of abnormal hemoglobin S (HbS). Under hypoxic conditions, HbS polymerizes within red blood cells (RBCs), causing them to adopt the characteristic sickle shape. This results in a clinically heterogenous disease, characterized by chronic hemolytic anemia, vaso-occlusive crises, and multi-organ damage. The *CYB5R3* gene encodes the enzyme NADH-cytochrome b5 reductase 3, which plays a crucial role in protecting hemoglobin (Hb) from oxidative damage to unfunctional methemoglobin. Patients with SCA may develop methemoglobinemia, particularly under conditions of oxidative stress. This study aimed to evaluate the potential modulatory effects of a *CYB5R3* variant, along with other well-established genetic modifiers within the globin genes, on the phenotypic variability of SCA in pediatric age.

Methodology

Eighty-one children with SCA were followed by pediatricians at two hospitals in the Greater Lisbon area, who characterized their clinical, hematological, and biochemical phenotypes. For this specific study, *CYB5R3*, *HBG2*, *HBA1, and HBA2* genes were genotyped using PCR, Sanger sequencing, and Gap-PCR. Association analyses were performed using SPSS.

Results and Discussion

Co-inheritance of α-thalassemia with SCA was observed in 43.2% of the children and proved to be beneficial, as it was associated with higher RBC counts, milder anemia, and a significant reduction in hemolysis biomarkers (bilirubin and reticulocyte counts). Similarly, elevated fetal Hb levels (HbF ≥10%) were also beneficial, leading to less severe hemolytic anemia. In the *HBG2* gene, the rs7482144_T allele had a frequency of 15% and was associated with higher HbF, reduced hemolytic parameters, lower HbS level, milder anemia, and a lower frequency of clinical comorbidities, except for heart disease. In the *CYB5R3* gene, the rs1800457_G allele showed a very high allelic frequency of 35% and appears to exert a deleterious effect because patients carrying this allele presented with more severe anemia, elevated hemolysis biomarkers, and a greater tendency toward hepatomegaly and cardiac comorbidities.

This study contributes to understanding how genetic modifiers influence SCA severity, complication risk and eventually treatment response. Identifying these factors supports personalized medicine and may help uncover new therapeutic targets.

P60 NEONATAL SCREENING FOR SICKLE CELL ANEMIA AND THE IMPACT OF GENETIC COUNSELING FOR AT-RISK COUPLES IN BENGUELA, ANGOLA

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Introduction

Sickle Cell Anemia (SCA) is a major public health problem due to its high mortality rate. In 2010, an estimated 310,000 infants were born with SCA, the majority in Sub-Saharan Africa (1). The World Health Organization (WHO) recognizes the importance of developing neonatal screening programs and implementing high-impact interventions for this genetic condition, including the identification of at-risk families for optimal genetic counseling in African countries (2-3). At Benguela General Hospital (HGB) in Angola, children are born with SCA and die before a diagnosis is made. There is currently no neonatal screening program for SCA to enable early diagnosis of the disease.

Objective

To identify newborns with hemoglobinopathies and to implement genetic counseling for couples at high genetic risk.

Methodology

A descriptive, cross-sectional, and prospective observational study was conducted, involving neonatal screening of 784 newborns admitted to the maternity ward of HGB. A blood sample was taken from the newborn's heel. The sample was placed on filter paper and processed using isoelectric focusing (IEF) (4). In a second phase, a cascade screening was performed on first-degree relatives of the affected individuals.

Results

784 newborns were studied within the first 5 hours of birth. Of these, 8.2% were genotypically heterozygous (HbAS), 6 (0.76%) were homozygous recessive (HbSS), a lower figure than that obtained in the Luanda screening study (1.5%) (1), and the remaining cases had Hb AA. A Hardy-Weinberg disequilibrium was demonstrated (p=0.001). In a second phase, a cascade screening was performed on 6 families, identifying a total of 5 siblings of the probands with the same disease. These siblings had severe anemia of unknown cause and were unaware of their genetic condition. **Conclusions:** The identification of hemoglobinopathies through the implementation of a neonatal screening program for sickle cell disease was effective and facilitated early clinical management, thus improving the survival rate of diagnosed cases. Genetic counseling for at-risk couples increased awareness about the disease and allowed for the identification of other undiagnosed first-degree relatives. The feasibility of neonatal screening for sickle cell disease, as well as the importance of early implementation of clinical care for affected individuals, has been demonstrated.

Keywords: Sickle cell anemia, neonatal screening, genetic counseling.

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P61 CHOLESTASIS: GENETIC ANALYSIS OF 342 PATIENTS OVER A 10-YEAR PERIOD

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Introduction

Cholestasis is a disease characterized by absent or reduced bile flow, which can progressively lead to liver fibrosis. Genetic cholestatic liver diseases are a heterogeneous group of disorders caused by defects in different genes. During the last decade, whole-exome sequencing was paramount in identifying new genetic causes for cholestatic liver diseases, improving differential diagnosis and its clinical management.

Aim

To characterize the mutational spectrum of genetic cholestatic liver diseases.

Methodology

We reviewed our laboratory database for cholestasis genetic testing from 2016 to 2025, based on a WES-based multigene panel which comprises 150 genes. Only pathogenic or likely-pathogenic variants were considered as diagnostic.

Results

WES-based multigene test for cholestasis was performed in 342 cases.

We established a molecular diagnosis for 40 patients (12%). We identified 24 different variants located at 13 genes: *SERPINA1, ABCB4, JAG1, NOTCH2, PHKA2, HNF1B, COG4, PKHD1, DCDC2, NPHP3, ARG, NR1H4, HSD3B7*. The homozygous Z allele in *SERPINA1* (Alpha-1-antitrypsin deficiency) was found in 12 patients, being the most prevalent variant reported. *ABCB4* (Intrahepatic cholestasis) variants were found in 11 patients (7 previously reported and 1 novel); 4 *JAG1* variants previously reported in the literature and 2 novel in *NOTCH2* were found in a total of 6 patients with Alagille syndrome. The remaining 11 patients carried variants dispersed in the other 9 genes.

115 patients (34%) harboured uncertain significant variants, some of which had the potential for reclassification upon further familial segregation and/or functional studies.

Discussion

The most frequently mutated genes in our cohort were *SERPINA1* (Alpha-1-antitrypsin deficiency), *ABCB4* (Intrahepatic cholestasis), *JAG1* and *NOTCH2* (Alagille syndrome). The other cholestasis-related mutated genes were found occasionally, demonstrating the high genetic heterogeneity of this clinical entity.

Our results demonstrate the utility of WES-based multigene testing in the differential diagnosis of genetic cholestatic liver diseases, with significant implications for clinical management, prognosis and genetic counselling.

Acknowledgements: We thank patients, families and referring clinicians.

Keywords: cholestasis, WES-based multigene panel, molecular diagnosis

POSTERS
CLINICAL CASES

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P62	*BRWD3-linked intellectual disability, macrocephaly and obesity: when x-inactivation shapes the phenotype*	Isabel Serra Nunes	1
P63	*Bilateral congenital cataracts associated with biallelic variants in the CYP51A1 gene*	Catarina Sousa	3
P64	*Familial microdeletion involving SOX4*	Raquel Rodrigues	1
P65	*Suspected parental germline mosaicism in GABBR1-related syndrome: case report of two siblings and literature review*	Inês Beleza	3
P66	*Acute myeloid leukemia: diagnostic and post-transplant challenges*	Luciana Pereira	1
P67	*Overcoming technical challenges: NGS detection of HBA2 deletion in hemoglobinopathies*	Nuno Teixeira	3
P68	*Mosaicism with four distinct cell lines involving chromosome 18: a prenatal diagnostic challenge*	Manuela Mota Freitas	1
P69	*Unreported ZBTB20 in-frame deletion expands the genetic and clinical spectrum of primrose syndrome*	Vera Sousa Marques	3
P70	*Uncovering a numt in COL6A1: diagnostic insights from short-read in a myopathic patient*	Lígia S. Almeida	1
P71	*Dual molecular diagnosis of recessive diseases in a patient with ocular abnormalities: a case report*	Joana Sá	3
P72	*When newborn screening fails: a case of central congenital hypothyroidism*	Joana Da Silva Cardoso	1
P73	*Balanced reciprocal translocation mosaicism: a rare genetic finding in a couple with miscarriage*	Joana Trindade	3
P74	*Connecting the dots: biochemical and genetic insights in a rare LYRM7-linked complex III deficiency*	Maria João Santos	1
P75	*Prenatal diagnosis in a fetus with fetal growth restriction and a 4-copy gain in the 15Q11.2Q13.1 Region*	Laurentino R. Simão	3
P76	*A nipt result lead to the cause of idiopathic short stature in a pregnant women*	Rita Monteiro	1
P77	*Loss of function of PCDH11X gene in the pathogenesis of neurodevelopmental disorders*	Sónia Pedro	3
P78	*The application of long-read whole-genome sequencing for diagnostics: a case report*	Ana Teresa Pais	1
P79	*Promoter variant in F9 causing haemophilia b leyden: a case report*	Ana Francisca Fernandes	3
P80	*The tell-tale heart: the diagnostic value of minor dysmorphic features in cardiogenetics*	Ana Mafalda Gonçalves	1
P81	*Unmasking congenital heart disease in a genetic syndrome: a case report*	Cláudia Nicole Ornelas Rocha	3
P82	*Vexas syndrome – 5 years of diagnosis*	Sara C. Teixeira	1
P83	*Chromosome instability testing for the diagnosis of familial rad51-associated fanconi anemia*	Nádia Neto	3
P84	*Prenatal diagnosis in three cases with 45,X/46,XY mosaicism: the relevance of the contribution of different technologies*	Patrícia Paiva	1
P85	*Disclosing 7Q11.23 Duplication syndrome: clinical insights from two pediatric cases*	Alexandra Mascarenhas	3
P86	*PAX5::AUTS2 fusion in pediatric b-cell acute lymphoblastic leukemia: a relapse-prone genetic subtype*	Ana Margarida Casanova Malta	1
P87	*Conventional cytogenetics as a key tool in the characterisation of a de novo 20P duplication*	Ana Gonçalves; Isaltina Silva	1
P88	*Case report: ngs detection of low-grade trisomy 8 mosaic in MDS*	Anastasiya Hryshkina	1
P89	*Familial rare x/y translocation – a case report*	Marta C. Pinto	1
P90	*Clinical, genetic, and biochemical characterization in patients with porphyria cutanea tarda in São Paulo, Brazil.*	José Franco	1

Posters discussion: Session 1 - 20/11 - 16h25-17h25; Session 2 - 21/11 - 11h15-11h55; Session 3 - 21/11 - 14h00-14h55

P62 BRWD3-LINKED INTELLECTUAL DISABILITY, MACROCEPHALY AND OBESITY: WHEN X-INACTIVATION SHAPES THE PHENOTYPE

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Introduction

BRWD3 is a well-established X-linked intellectual disability gene, typically associated with intellectual disability and macrocephaly in males. More recently, *BRWD3* has also been linked to overgrowth and obesity through its role in promoting activity of DDB1, a key regulator of adipogenesis. We report a rare case of a female patient with Intellectual Developmental Disorder X-linked with Macrocephaly associated with *BRWD3*.

Methodology

We performed a retrospective review of clinical and genetic data from the patient.

Results

A 5-year-old girl was referred to Medical Genetics for class II obesity, global developmental delay, communication disorder and macrocephaly. Family history revealed parental learning difficulties and an older brother with communication disorder. Genetic testing included an array-CGH and obesity gene panel (both negative) followed by exome sequencing, which identified a likely pathogenic *BRWD3* variant, c.1713T>G (p.Tyr571*), in heterozygosity. Segregation analysis confirmed maternal carrier status; the brother's study was negative. X-chromosome inactivation (XCI) analysis in peripheral blood showed a markedly skewed pattern favoring activation of the mutated X-chromosome in the patient (16:84), whereas the mother exhibited a random pattern (32:68).

Discussion

Although monogenic causes of obesity are rare, they should be considered in cases of early-onset severe obesity. X-linked disorders predominantly manifest in males, with females usually unaffected or mildly affected. This case illustrates that skewed XCI can unmask severe phenotypes in females, highlighting the importance of XCI analysis in atypically affected female patients with X-linked conditions.

Funding

Not aplicable.

Conflicts of Interest

Authors declare no conflict of interest.

Keywords: *BRDW3* gene; X-chromosome inactivation; Intellectual Developmental Disorder; Obesity.

Ethics and Consen: Informed written consent was obtained from parents/legal guardians of the patient.

P63 BILATERAL CONGENITAL CATARACTS ASSOCIATED WITH BIALLELIC VARIANTS IN THE *CYP51A1* GENE

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Introduction

Pediatric cataracts are a significant cause of preventable childhood blindness. It can be defined as any opacity of the crystalline lens present at birth or developing during childhood, impairing normal visual development, with an estimated incidence of 1.8 to 3.6 per 10,000 children per year. It is a heterogeneous condition, however, genetic variants are responsible for a significant proportion of cases. Identification of causal variants in pediatric cataracts is essential to support clinical management and treatment planning.

Methodology

We present the case of a 1-year-old girl with bilateral congenital cataracts who was referred for next-generation sequencing (NGS) ophthalmological diseases panel testing. Whole exome sequencing (WES) was performed using Illumina Novaseq 6000. In house bioinformatics pipeline and analysis NGS software for the requested panel were used for variant calling and analysis. The classification and description of the variants were performed according to international recommendations.

Results

The analysis of the targeted NGS panel data identified two heterozygous variants in the *CYP51A1* gene, NM_000786.4:c.270del p.(Leu91*) and NM_000786.4:c.1141C>A p.(Pro381Thr).

Discussion

Here, we report a patient with bilateral congenital cataracts carrying two heterozygous variants in the *CYP51A1* gene, both classified as variants of unknown clinical significance, and neither previously described in the literature. Although *CYP51A1* is a gene currently without an OMIM or ClinGen entity, variants in this gene have been reported, in homozygosity and compound heterozygosity, in patients with cataracts. Given the limited evidence of gene-disease association in the literature, parental testing and the study of other affected family members are essential to confirm the phase of the variants and to confirm their contribution to the disease. This case underlines the importance of clinical and scientific validation of gene panels using multiple sources and the relevance of including genes with limited but emerging evidence of disease association in the literature, ensuring more comprehensive and up-to-date diagnostic approaches.

P64 FAMILIAL MICRODELETION INVOLVING *SOX4*

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Introduction

SOX proteins are transcription factors with a key role in many developmental and pathological processes. *SOX4* inactivating SNVs have been recently associated with Intellectual developmental disorder with speech delay and dysmorphic facies (#618506). It is unclear whether disease arises primarily from haploinsufficiency or a dominant-negative effect. CNV data may help clarify this. However, only 6 patients with deletions encompassing *SOX4* have been reported, and a clear impact on neurodevelopment has yet to be shown. We report 3 new cases with a maternally inherited deletion including *SOX4*, aiming to help delineate the molecular and phenotypic spectrum of these rarely reported cases.

Methods

A 22mo-old boy was referred to Genetics with hypotonia, macrocephaly, brachycephaly facial dysmorphism, clinobrachydactyly of the 5th fingers, and expressive language delay.

His 7mo-old sister had axial hypotonia, facial dysmorphisms and clinodactyly of the 5th fingers.

Their mother had brachyturricephaly, facial dysmorphism, microretrognathia, brachydactyly of the 5th fingers, and mild intellectual disability.

Karyotype and aCGH (180K-CGX-HD) were performed.

Results

Karyotype showed an unbalanced interstitial deletion in 6p22.3 and aCGH revealed a ~5.38Mb microdeletion involving 10 genes, including *SOX4*. Segregation studies revealed that the children's microdeletion was maternally inherited.

Discussion

Among the 10 genes in the deleted interval, *SOX4* is the likely cause of the phenotype: 8 have no known disease association, and *DCDC2* is linked to unrelated autosomic recessive conditions.

Previously reported *SOX4* SNV carriers consistently have hypotonia, speech delay, global developmental delay (DD), variable intellectual disability, a dysmorphic face, and clinodactyly/brachydactyly of the 5th fingers. Patient with CNVs encompassing *SOX4* have been less well characterized, but DD and facial dysmorphism are common. In our family, the girl is too young for a full assessment but, globally, the phenotypes overlap reported cases with SNVs. Our findings support haploinsufficiency as a major pathogenic mechanism, though a dominant-negative effect cannot be excluded.

P65 SUSPECTED PARENTAL GERMLINE MOSAICISM IN *GABBR1*-RELATED SYNDROME: CASE REPORT OF TWO SIBLINGS AND LITERATURE REVIEW

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Introduction

GABBR1 gene encodes for one of the two subunits of the GABA-B receptor, a G-protein-coupled receptor activated by GABA, the main inhibitory neurotransmitter in the central nervous system. Pathogenic variants in *GABBR1* have been associated with an autosomal dominant syndrome characterised by cognitive, motor and/or language impairments, and varying degrees of intellectual disability. To date, four patients carrying *de novo* missense variants in *GABBR1* have been reported in literature[1].

Results

We describe two siblings with intellectual disability referred for genetic evaluation and counselling. The proband (Pt1), a 24-year-old (yo) woman, is the second of three siblings born to healthy, non-consanguineous parents; her older brother (Pt2, 28-yo) is also affected, while her younger sister (22-yo) is unaffected.

Pt1 presented with global developmental delay evolving into intellectual disability, with poor language skills, lack of autonomy, behavioural disturbances characterised by prolonged episodes of mutism and social withdrawal, and a cerebellar venous malformation. She also had reduced hair density and was overweight. Clinical exome analysis did not identify a causative variant.

Pt2 showed intellectual disability, with a broader vocabulary than his sister, and recurrent episodes of severe psychomotor agitation and insomnia. He underwent genetic testing at a later stage, after a newer version of the clinical exome had become available. Exome sequencing identified a rare missense variant in *GABBR1* gene [c.1591G>A, p.(Gly531Ser)], classified as of uncertain significance. Targeted investigation confirmed that Pt1 also carries this variant, which was not detected in her original analysis given that the gene was not included in the previous version of the clinical exome. Parental testing using Sanger sequencing on peripheral blood DNA revealed that neither carried the variant, allowing for its reclassification as likely pathogenic. These findings are also highly suggestive of parental germline mosaicism.

Discussion

This report emphasises the value of systematic re-evaluation of genetic data in unsolved cases and expands the current knowledge of *GABBR1*-related syndrome through the description of two siblings, the oldest patients reported to date. Furthermore, this case highlights the possibility of parental germline mosaicism in apparently *de novo* variants, having significant implications for genetic counselling and recurrence risk assessment.

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Keywords: *GABBR1*; neurodevelopmental disorder; germline mosaicism; *de novo* genetic variants

Ethics and Consent: Informed consent was obtained from the patients' parents for this case report.

P66 ACUTE MYELOID LEUKEMIA: DIAGNOSTIC AND POST-TRANSPLANT CHALLENGES

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Introduction

Genetic analysis is essential for the diagnosis, classification, prognosis, and therapeutic guidance of leukemias. The integration of cytogenetic and molecular techniques is essential to establish accurate diagnoses and clinically meaningful results.

Methodology

We report the case of a 58-year-old woman admitted to IPO Porto with suspected acute promyelocytic leukemia (APL). For diagnostic purposes, a comprehensive genetic evaluation was performed, including karyotyping, fluorescence in *situ* hybridization (FISH), RT-PCR, array analysis with CytoScan XON Assay, and next-generation sequencing (NGS) with TruSight™ RNA Fusion and oncoReveal™ Myeloid panels. She received chemotherapy followed by allogeneic hematopoietic stem cell transplantation from a related male donor.

Results

Bone marrow karyotype revealed the presence of double minutes (dmin). Initial FISH analysis was negative for *PML::RARA, RARA and KMT2A* rearrangements, but it demonstrated *MYC* amplification in 81% of the cells. *MYC* amplification was also detected by array analysis. NGS with an RNA fusion panel did not detect any oncogenic fusions. NGS with a myeloid panel identified a likely oncogenic variant in *U2AF1*. After chemotherapy and allogeneic transplantation, all 20 cells analyzed reflected the male donor karyotype, and in 5 of these cells, a 20q deletion was present, which was also detected by FISH in 23% of the cells.

Discussion

Genetic findings ruled out APL. The *U2AF1* mutation supported the diagnosis of Acute Myeloid Leukemia with myelodysplasia, a subtype associated with poor prognosis [1]. The post-transplant detection of a 20q deletion in donor-derived cells raises the possibility of clonal hematopoiesis or pre-existing donor mosaicism. At present, the clinical significance of this deletion remains uncertain and depends on the broader clinical and hematological context. This case highlights the critical value of comprehensive genetic work-up in guiding diagnosis and therapy.

Keywords: Leukemia; *MYC* amplification; *U2AF*1 mutation

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P67 OVERCOMING TECHNICAL CHALLENGES: NGS DETECTION OF *HBA2* DELETION IN HEMOGLOBINOPATHIES

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Introduction

Hemoglobinopathies are the most prevalent monogenic disorders resulting from mutations in globin genes that affect the various chains of the hemoglobin (Hb) molecule [1]. Conditions in this group include sickle cell anemia (SCA), driven by the abnormal hemoglobin S (HbS), and thalassemias, which result from reduced or absent globin chain production [2]. Diagnosing these disorders with next-generation sequencing (NGS) presents notable challenges, as high sequence homology exists among duplicated genes and pseudogenes, as well as between paralogous globin genes, such as *HBA1*/*HBA2*, *HBB*/*HBD*, and *HBG1*/*HBG2*. Furthermore, repetitive elements complicate accurate detection, including Alu repeats in the alpha cluster and LINE repeats in the beta cluster [3,4].

Methodology

We analyzed genomic DNA from a pregnant couple using an exome-based NGS panel targeting genes associated with hemoglobinopathies. Our sequencing coverage and bioinformatic pipeline enabled the detection of copy-number variants (CNVs) as well.

Results

We present a prenatal case of a couple in which the female element experienced recurrent non-severe vaso-occlusive crises since early childhood, while the male element was healthy. In the pregnant woman, the pathogenic missense variant c.20A>T in the *HBB* gene was detected in homozygosity, consistent with sickle cell anemia (HbSS genotype). Additionally, in the female sample, a large homozygous deletion of approximately 3.8 kb was identified through CNV analysis, involving the *HBA2* gene (and partially the *HBA1 gene*). This was confirmed by MLPA as a 3.7 kb homozygous deletion in the *HBA2 gene*, establishing the ($-\alpha^{3.7}/-\alpha^{3.7}$) genotype, the most frequent in the Mediterranean region and consistent with alpha-thalassemia [5]. This deletion is a modifier of sickle cell disease, which underlies the absence of a severe phenotype in the female element. In the paternal sample, the same pathogenic missense variant in the *HBB* gene was detected in heterozygosity, consistent with sickle cell trait (HbAS genotype).

Conclusion

This case report demonstrates that our NGS approach is robust and sensitive enough to detect not only pathogenic missense variants but also large deletions in globin genes, overcoming technical challenges that typically require targeted long-read sequencing. Importantly, this strategy enabled a comprehensive non-directed prenatal diagnosis of hemoglobinopathies, demonstrating its applicability in clinical reproductive genetics.

P68 MOSAICISM WITH FOUR DISTINCT CELL LINES INVOLVING CHROMOSOME 18: A PRENATAL DIAGNOSTIC CHALLENGE

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Introduction

Chromosomal mosaicism is the presence of two or more cell lines with different chromosome complements which may or not be present in all tissues, in a single individual. The phenotype is influenced by the distribution of abnormal cells in the various tissues, the chromosome involved and the proportion of normal/abnormal lines in different tissues. In invasive PND, detecting mosaicism is a major laboratory challenge that requires additional work, careful cytogenetic analysis and additional cell counts in different cell cultures; in chorionic villus sampling (CVS), it is necessary to exclude mosaicism confined to the placenta.

Clinical Case

A pregnant woman was referred for karyotype in CVS at 14 weeks, due to nuchal translucency (4,1mm) and positive combined screening for trisomy 21/18/13.

Results

18p monosomy was present in all metaphases from two independent cultures, in a mosaic with four unbalanced cell lines:

mos 45,XX,der(15)t(15;18)(p10;p10)[29]/ 46,XX,i(18)(q10)[6]/46,XX,del(18)(p10)[5]/46,XX,r(18)(q10q23)[5]. These findings characterize a chromosome 18 instability and, since they originated from CVS, an amniocentesis was requested, showing an 18p10 deletion in all cells. Parental karyotypes were normal, thus this anomaly is *de novo*.

Discussion

18p Deletion Syndrome (OMIM#146390) is associated with intellectual disability, growth retardation, craniofacial dysmorphism and limb, brain and heart malformation. The fetal phenotype of this syndrome is highly variable and may not be evident during pregnancy. After genetic counseling, parents requested medical termination of pregnancy, and fetal skin karyotype confirmed the 18p deletion. The authors compare the clinical features in this fetus with the previously reported in "18p Deletion Syndrome" and emphasize the importance of collecting new samples of fetal products to confirm/exclude the existence of true mosaics, while highlighting the importance of conventional cytogenetic analysis for both accurate diagnosis and appropriate genetic counselling.

Ethics and Consent

Informed consent was obtained from the mother for this clinical case presentation.

Keywords: mosaicism; 18p deletion; CVS.

P69 UNREPORTED ZBTB20 IN-FRAME DELETION EXPANDS THE GENETIC AND CLINICAL SPECTRUM OF PRIMROSE SYNDROME

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Introduction

Primrose syndrome (OMIM#259050) is a rare autosomal dominant disorder caused by heterozygous variants in *ZBTB20*. It is characterized by intellectual disability, macrocephaly, distinct craniofacial features, and metabolic alterations. We presente a 10-year-old boy with a novel *ZBTB20* variant and compare his clinical and biochemical findings with previously documented cases.

Methodology

Detailed phenotypic evaluation, brain MRI, and metabolic screening were performed. Genetic analysis was carried out by trio whole exome sequencing. Clinical and biochemical features were systematically compared with cases reported in the literature.

Results

The patient had moderate to severe intellectual developmental disorder, total agenesis of the corpus callosum, macrocephaly with brachycephaly and turricephaly, coronal hypospadias, and multiple dysmorphic features. Biochemical investigations revealed normal urinary organic acids and plasma acylcarnitines, with elevated alpha-fetoprotein (AFP) and triglycerides.Trio-exome sequencing identified a *de novo* heterozygous variant c.1810_1812del (OMIM*606025) in *ZBTB20* gene, which has not been previously reported in the literature. The deletion occurs in a functionally constrained region of the gene where most pathogenic variants are known to cluster, which strengthens its candidacy as a disease-causing change. Comparison with published cases shows overlapping features, including intellectual disability, macrocephaly, AFP elevation, and lipid abnormalities, while agenesis of the corpus callosum and hypospadias are less frequently reported.

Discussion

This case contributes to the delineation of the *ZBTB20* phenotype, confirming core clinical and biochemical hallmarks of Primrose syndrome and suggesting additional features (agenesis of the corpus callosum, hypospadias) that may broaden the recognized spectrum. The novel variant described here represents a promising candidate for pathogenic classification, reinforcing the need for clinical–genomic correlation and additional case accumulation.

Funding

No funding was received for this work.

Conflicts of Interest

The authors declare no conflicts of interest.

Keywords: Primrose syndrome; *ZBTB20*; Intellectual disability; Agenesis of corpus callosum; Rare diseases.

Ethics and Consent: Informed consent for genetic testing and publication was obtained from the patient's legal guardians.

P70 UNCOVERING A NUMT IN COL6A1: DIAGNOSTIC INSIGHTS FROM SHORT-READ IN A MYOPATHIC PATIENT

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Introduction

Structural variants (SVs), defined as genomic alterations larger than 50 base pairs, encompass insertions, deletions, duplications, and rearrangements. As a whole they represent a significant source of genetic diversity being implicated in a wide range of diseases and phenotypic traits. However, their detection remains challenging in routine diagnostic settings. We report a patient with a myopathic phenotype in whom a complex structural variant, consisting of a nuclear-mitochondrial DNA segment (NUMT) insertion in the *COL6A1* gene, associated with collagen VI-related myopathy, was identified.

Methodology

Initial genetic analysis was performed using exome sequencing in a diagnostic context. To validate the findings, targeted PCR amplification and Sanger sequencing were employed to characterize the insertion and breakpoint regions.

Results

Short-read sequencing revealed a heterozygous variant in COL6A1, a deletion of nucleotides g.47406554 to g.47406557 (NM_001848.2:c.542_543) that was replaced by a 67-base insertion derived from mitochondrial DNA (MT-RNR2, m.2469–2535), as confirmed by sequence similarity analysis using NCBI BLASTN. The resulting in-frame alteration, classified as variant of uncertain significance, is consistent with autosomal dominant inheritance of collagen VI-related myopathy. Nonetheless, further familial segregation studies are necessary to clarify the inheritance pattern and assess its contribution to the patient's clinical presentation.

Discussion

This case highlights the utility of short-read sequencing as a first-tier approach for detecting structural variants, even complex ones. The identified insertion represents a NUMT, a phenomenon where fragments of mitochondrial DNA integrate into the nuclear genome. The insertion of NUMTs have been implicated as mechanism of disease in several human disorders, such as factor VII deficiency, Pallister-Hall syndrome, mucolipidosis type IV, Usher syndrome type IC, and lissencephaly. Our findings underscore the diagnostic relevance of SVs and the importance of complementary validation techniques in elucidating their clinical significance.

Conflicts of Interest

All authors are employees of CENTOGENE GmbH

Keywords: structural variants, Collagen VI-related myopathy, Nuclear mitochondrial DNA segment (NUMT)

P71 DUAL MOLECULAR DIAGNOSIS OF RECESSIVE DISEASES IN A PATIENT WITH OCULAR ABNORMALITIES: A CASE REPORT

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Introduction

Retinitis pigmentosa (RP) is a group of inherited retinal disorders leading to progressive vision loss, with symptoms such as night blindness, tunnel vision and central vision deterioration. Microspherophakia is characterized by small, spherical lenses, increased lens thickness and high myopia. It can lead to lens dislocation or subluxation and may be associated with ectopia lentis and glaucoma. These diseases highlight the complex interplay between retinal and lens anomalies, with significant implications for the patient's vision and quality of life. We report a patient with multiple ocular abnormalities for whom a dual diagnosis for autosomal recessive (AR) diseases was stablished through whole-exome-sequencing (WES) analysis.

Methodology

44-year-old male presented with nyctalopia, peripheral vision field loss, myopia, increased intraocular pressure, bilateral lens subluxation, phacodonesis and microspherophakia. He was born to apparently non-consanguineous parents, with irrelevant family history. A WES multigene panel focusing on 584 genes associated with ocular disorders was performed.

Results

WES analysis detected a large pathogenic deletion in homozygosity involving the gene *EYS* (c.(2023+1_2024-1)_(2259+1_2260-1)del) that had already been described in patients with RP, and two heterozygous changes in *LTBP2* gene: a likely pathogenic (L-PAT) variant (c.1767_1768dup) and a variant of unknown significance (VUS) (c.578C>A); possibly associated to microspherophakia. Variant segregation analysis demonstrated compound heterozygosity and allowed the reclassification of the VUS to L-PAT, providing a second molecular diagnosis.

Discussion

Disease-causing variants in the *EYS* gene account for ~28% of AR RP cases in Portugal, making it the most frequent genetic aetiology for this disease. Among its mutational spectrum, large deletions are the most common alterations observed. However, the co-occurrence of molecularly characterized RP and isolated microspherophakia remains underreported in the literature, with only a single previously described case lacking a definitive genetic diagnosis. Our case highlights the critical role of WES in diagnosing complex and rare genetic disorders. It enables the identification of multiple pathogenic variants, especially when the clinical phenotype does not pinpoint to a single genetic cause. In patients with overlapping or atypical phenotypes, WES can provide invaluable insights, reducing time to diagnosis and enabling potential management strategies.

Keywords: Dual molecular diagnoses; Retinitis Pigmentosa; Microspherophakia; *EYS*; *LTPB2*

P72 WHEN NEWBORN SCREENING FAILS: A CASE OF CENTRAL CONGENITAL HYPOTHYROIDISM

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Introduction

Congenital Nongoitrous Hypothyroidism type 4 (MIM #275100) is a rare autosomal recessive form of central congenital hypothyroidism (CCH), caused by isolated deficiency of thyroid-stimulating hormone (TSH) due to biallelic pathogenic variants in the *TSHB*, which encodes the TSH β-subunit. If untreated, it result in significant growth retardation and neurodevelopmental delay. CCH may be missed by newborn screening, which relies primarily on elevated TSH levels.

Methodology

We report a 44-day-old infant referred to the Emergency Department with feeding difficulties, macroglossia, perioral cyanosis, holosystolic murmur, umbilical hernia and glabellar hemangioma. The pregnancy, conceived by IVF, was uneventful, and term delivery in the UK occurred by emergency caesarean section. Neonatal course included transient hypoglycemia and mild jaundice, with normal newborn screening results. Clinical, biochemical, cardiac, radiological, and genetic evaluations were performed during hospitalization.

Results

During admission, the infant developed progressive respiratory distress with recurrent desaturation, ultimately necessitating tracheostomy. Cardiac assessment revealed biventricular hypertrophy, aortic valve stenosis, and pericardial effusion. An initial suspicion of metabolic disease was excluded following metabolic studies. Beckwith–Wiedemann syndrome was ruled out. Repeat thyroid function testing demonstrated undetectable TSH and low free T4, consistent with CCH, and levothyroxine replacement was initiated, resulting in marked clinical and biochemical improvement. Trio whole-exome sequencing, identified a homozygous variant in *TSHB*, initially classified as of uncertain significance but later reclassified as likely pathogenic, confirming autosomal recessive congenital hypothyroidism type 4. Cerebral MRI showed normal pituitary morphology, and thyroid ultrasonography was unremarkable.

Discussion

This case highlights a major limitation of current neonatal screening: CCH may go undetected with TSH-based screening. Despite a normal screening result, this infant developed severe hypothyroidism requiring intensive management. Clinicians should maintain a high suspicion for CCH in infants with suggestive clinical features, even when neonatal screening is normal. Early recognition and treatment are essential to prevent irreversible growth and neurodevelopmental consequences.

Conflicts of interest

None.

Keywords: central congenital hypothyroidism; TSHβ, newborn screening

P73 BALANCED RECIPROCAL TRANSLOCATION MOSAICISM: A RARE GENETIC FINDING IN A COUPLE WITH MISCARRIAGE HISTORY

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Introduction

Chromosomal abnormalities are a major cause of infertility and recurrent pregnancy loss. These, can be either numerical (usually involving sex chromosomes) or structural, such as balanced reciprocal or Robertsonian translocations, and can be present in all analysed cells or in a variable proportion of cells (mosaicism).

Balanced Reciprocal Translocation Mosaicism (BRTM) has been rarely reported in literature, mainly observed in individuals with a normal phenotype who experience reproductive failure. Currently, the mechanisms responsible for BRTM and its true incidence in the population remain poorly understood.

Methodology

A young couple was referred to our laboratory due to miscarriages.

Peripheral blood lymphocyte cultures were established for karyotype analysis in GTL banded preparations.

Later, QF-PCR genetic profiling was performed in the male partner.

Results

A normal 46,XX constitution was observed in the female karyotype. In the male partner, mosaicism with two cell lines was detected, with the karyotype described as 46,XY,t(2;3)(p23.3;q23)[7]/46,XY[23].

Quantitative multiplex-PCR revealed a single male profile.

Discussion

Miscarriage history is a common reason for karyotype analysis. In most cases, a normal karyotype is observed. When a structural chromosomal rearrangement is detected, it is almost invariably present in all analysed cells.

Mosaicism for a balanced reciprocal translocation is extremely rare. Usually these individuals have normal phenotypes, as in the present case, and were investigated due to infertility an/or miscarriages.

Chimerism has also been considered as a possible explanation in such cases; however, it can be distinguished from mosaicism based on genotypic differences.

QF-PCR of the male sample revealed a single genetic profile, supporting the interpretation that the observed findings are due to mosaicism rather than chimerism.

It is recognized that BRTM is often underdiagnosed, as standard cytogenetic techniques may not detect low-level mosaicism or cryptic chromosomal rearrangements.

However, detecting BRTM is essential for genetic counselling. If the rearrangement is also present in gonadal tissue, there is a risk of transmitting the rearrangement in an unbalanced form to offspring, potentially resulting in reproductive difficulties and pregnancy loss.

In this case, the couple's conventional karyotype analysis provided valuable information that will support appropriate genetic counselling and reproductive planning.

P74 CONNECTING THE DOTS: BIOCHEMICAL AND GENETIC INSIGHTS IN A RARE LYRM7-LINKED COMPLEX III DEFICIENCY

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Mitochondrial disorders (MD) are heterogeneous and multisystemic. Tissues with higher energy demands are the most affected[1]. Complex III (CIII) is a central structure of the mitochondrial respiratory chain (MRC), involved in several metabolic pathways, but its deficiency is rarely reported.

The advent of NGS allowed identification of more genetic causes, but functional assessment work continues to be an essential tool[1,2].

A blood sample of a patient (4yo,F) with MD suspicion (presenting brittle hair, amaurosis fugax, lower limb hypertonia, moderate mental retardation, delayed speech and language development, short stature and global developmental delay) was sent to our lab. Parents (consanguineous) and her five siblings are healthy. The analysis of mtDNA, WES and MRC complexes' activity were performed.

The patient had CIII deficiency, seen in biochemical analysis. No abnormalities were found in mtDNA. WES bioinformatic analysis, performed with SEQ Platform (Genomize®), indicated the c.73G>A (homozygous, rs587777433) sequence variation in *LYRM7* as the strongest genetic correlation to clinical data[4]. This was considered the probable genetic cause of disease.

LYRM7 is one of the final CIII assembly factors and its anomaly has been reported in cases with CIII deficiency and overlapping symptoms with our case[3,5]. Also, fever has been reported as a possible trigger, as happens in this case.

The MRC study was performed before exome analysis, so it was possible to add this result in SEQ analysis; otherwise, LYRM7 would not be flagged as the most likely cause. This work shows the impact of "conventional" biochemical assay in aiding NGS analysis - a holistic and multidisciplinary approach gets the best possible result and precision, with the most benefit for patients.

Funding: Portuguese national funds via FCT – Fundação para a Ciência e a Tecnologia, under projects UIDB/04539/2020, UIDP/04539/2020, UID/04539/2025, LA/P/0058/2020, grant 2022.10296.BD.

The authors declare no conflict of interest.

1. DOI:10.3390/cells11040637; 2. DOI:10.1002/jimd.12104; 3. DOI:10.1016/j.pediatrneurol.2020.12.010; 4. DOI:10.1002/humu.22441; 5. DOI:10.1016/j.msard.2020.102615

Keywords: Mitochondrial disorders; Complex III deficiency; LYRM7; Next Generation Sequencing

P75 PRENATAL DIAGNOSIS IN A FETUS WITH FETAL GROWTH RESTRICTION AND A 4-COPY GAIN IN THE 15Q11.2Q13.1 REGION

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Fetal growth restriction (FGR) is a common ultrasound finding in pregnancy that can result from maternal, fetal, placental, environmental factors, or their interaction, and its diagnosis is based on ultrasound screening. This finding is associated with a significant increase in perinatal morbidity and mortality. On the other hand, copy number variants (CNV) in the 15q11.2q13.1 region are associated with recurrent microdeletion/microduplication syndromes, in which the phenotype depends on the parental origin of the CNV.

Here we report on a foetus from a healthy 32-year-old woman with early RCF in prenatal screening. A chromosomal microarray analysis (CMA) was requested, revealing a pathogenic gain of 4 copies, with 5.77 Mb, corresponding to the justacentromeric region 15q11.2q13.1. The karyotype was established as mos 47,XX,+mar dn[40]/46,XX[28]. As the outcome of this CNV depended on the parental origin of the affected allele, MS-MLPA was performed, which showed that it was inherited maternally.

The detected CNV is a recurrent known microduplication witch phenotype is dependent on the parental origin of the duplication. When it has maternally origin it has a severe outcome with hypotonia, cognitive deficit, language delay, behavioral disorders, seizures, among others. If the CNV has paternal origin, although some patients might show developmental delays and behavioral disturbances, most cases are rarely symptomatic. In prenatal diagnosis, few cases have been described in the literature, with intrauterine growth restriction being one of the ultrasound abnormalities reported when the abnormality is of maternal origin.

This case reinforces the importance of the combined use of cytogenetic and molecular cytogenetic technologies, such as karyotyping, MS-MLPA, and CMA, which play a key role in identifying the origins and genetic makeup of sSMC, which would contribute significantly to genetic counselling in prenatal diagnosis.

P76 A NIPT RESULT LEAD TO THE CAUSE OF IDIOPATHIC SHORT STATURE IN A PREGNANT WOMEN

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Introduction

An X chromosome alteration was identified in a pregnant woman through non-invasive prenatal testing (NIPT). The woman, 33 years old with short stature, was referred for amniocentesis due to an intermediate risk for trisomy 21 and NIPT that revealed a 17 Mb deletion in the Xp22 region and a 30 Mb duplication at Xq25-q28.

Methodology

Amniotic fluid fibroblasts and peripheral blood lymphocytes were cultured for karyotype analysis. FISH analysis and SNP array CGH were performed to characterise the chromosomal rearrangement.

Results

Amniocytes cytogenetic analysis revealed a female karyotype with a derivative of one X chromosome, resulting from a deletion of the short arm (breakpoint at Xp22.1) and duplication of the long arm inserted on the short arm of the same X chromosome (breakpoint at Xq25). The pregnant's karyotype was found to be the origin of the derivative chromosome. Microarray analysis confirmed the karyotype result and defined the gene content involved in the chromosomal rearrangement.

Discussion

The findings established that both, the pregnant women and the foetus, have the X chromosome with a deletion including *SHOX*, a common cause of idiopathic familial short stature. Foetal sex determination and prenatal diagnosis in male foetus for conventional cytogenetic study was proposed in future pregnancies. Furthermore, exclusion of clinically relevant *SHOX* gene variants in the partner could be considered in the evaluation of Langer mesomelic dysplasia in future couple's offspring.

This case exemplifies an incidental favourable diagnosis establish in the clarification of an abnormal NIPT result.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Keywords: X Chromosome Abnormalities; Prenatal Diagnosis; NIPT; SHOX

P77 LOSS OF FUNCTION OF PCDH11X GENE IN THE PATHOGENESIS OF NEURODEVELOPMENTAL DISORDERS

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Introduction

The *PCDH11X* gene, predominantly expressed in the brain, plays an important role in cell–cell communication, dendritic synaptic plasticity, cerebral lateralization, and verbal ability. Although it is not currently classified as a morbid gene, loss-of-function (LoF) variants in *PCDH11X* have been reported in the literature as having a moderate association whit autism spectrum disorder (ASD). These variants have been described exclusively in males with ASD, and a recent X-chromosome-wide association study highlighted significant associations between intronic and intergenic variants in this *locus* and ASD in males.

We report a 6-year-old male patient presenting a complex neurodevelopmental phenotype, including learning difficulties, mild motor delay, speech sound disorder, selective mutism, and global hypotonia, as well as facial dysmorphisms, such on prominent large ears, midface hypoplasia, and a high forehead.

Methodology

DNA was extracted from blood sample and chromosomal microarray analysis (CMA), was performed using Thermofisher CytoScan HD.

Results

CMA profile identified a 2.80 Mb interstitial deletion at Xq21.31q21.32 (arr[GRCh37] Xq21.31q21.32(90,594,030_93,397,746) x0).

Discussion

The interstitial deletion identified in this patient encompasses three OMIM listed genes, *PABPC5*, *PCDH11X*, and *NAP1L3*, none of which are currently classified as morbid and being associated to disease. However, the patient's phenotype, characterized by neurodevelopmental disorders, aligns with previous reports linking *PCDH11X* alterations to neurodevelopmental impairments.

Although the current level of evidence is considered moderate, this case suggesting a potential role for *PCDH11X* loss of function in the pathogenesis of neurodevelopmental disorders. In particular, the overlap between the clinical features observed and the known functions of *PCDH11X*, especially those related to language and communication, reinforces the hypothesis that this gene, despite not being classified as morbid to date, may contribute causally to such phenotypes when disrupted.

P78 THE APPLICATION OF LONG-READ WHOLE-GENOME SEQUENCING FOR DIAGNOSTICS: A CASE REPORT

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Introduction

Hereditary cerebellar ataxias (HCAs) are a heterogeneous group of neurodegenerative diseases characterized by wide phenotypic and genetic heterogeneity. Core features include cerebellar syndrome, but also other neurological and non-neurological signs. The most prevalent dominantly-inherited HCAs are caused by polyglutamine repeat-expansions (particularly MJD/SCA3 in Portugal), while Friedreich ataxia (FRDA), caused by biallelic GAA expansions in the *FXN* gene, is one of the most frequent recessive forms of HCA.

Methodology

We describe a Portuguese family enrolled in the national population-based survey for HCAs. Three siblings, born to consanguineous parents, presented slowly progressive ataxia in the 4th-5th decade of life, with peripheral neuropathy, and later dysphagia. Brain CT/MRI scans revealed mild to moderate cortico-subcortical cerebellar atrophy in one of the patients. An extensive workup, including study of the most frequent AD and AR ataxias, had been performed abroad. Given the absence of a genetic diagnosis, long-read whole-genome sequencing (LR-WGS) was performed in the index case using PromethION 2 Solo (Oxford Nanopore). Bioinformatics analysis was conducted via EPI2ME (v5.2.3) using the wf-human-variation (v2.6.0) and wf-basecalling (v1.4.5) workflows, with the super-accurate basecaller model (SUP, v5.0.0).

Results

We obtained a total of 22 Mo reads, with an N50 of 7,650bp and a mean read length of 4,970bp. The mean total depth was 36.0×. Variant calling identified > 4 Mo SNVs and 854K indels, with a Ti/Ts ratio of 1.84. Additionally, we detected ~36K structural variants. LR-WGS detected two pathogenic expanded *FXN* alleles (259 and 791 repeats) confirming FRDA. Results were confirmed by conventional method in all patients of this family.

Discussion

This first case entirely performed in our lab, underscores the clinical utility of LR-WGS for rare diseases diagnostics. Unlike conventional methods, LR-Seq allows direct and accurate sizing of expanded alleles and its sequence composition. In addition, enables simultaneous testing of multiple repeat expansion *loci* and a broad mutational spectrum, which is particularly useful for HCA clinical investigation. Thus, LR-WGS can significantly enhance diagnostic accuracy, reduce time to diagnosis, ultimately improving patient care and enable more precise genetic counseling. In FRDA, early and accurate diagnosis is now even more critical given the recent approval of the first disease-modifying treatment (omaveloxolone).

Keywords: LR-WGS; diagnostics; FRDA

P79 PROMOTER VARIANT IN F9 CAUSING HAEMOPHILIA B LEYDEN: A CASE REPORT

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Introduction

Haemophilia B (HB) is a hereditary bleeding disorder asociated with variants in the *F9* gene, located on the X chromosome, leading to reduced/ absent factor IX (FIX) activity and clinical phenotypes ranging from mild to severe. Disease severity depends on the nature and variant location, which may affect protein structure or gene regulation. Variants in the *F9* promoter region are associated with haemophilia B Leyden, a distinctive form characterised by markedly low FIX levels in childhood followed by a progressive increase after puberty.

Methodology

Male patient with clinical and laboratorial diagnosis of severe HB was studied for *F9* variant identification by NGS. Carrier studies were performed on the mother and sister using Sanger sequencing. The variant was classified according to ACMG guidelines.

Results

The patient was diagnosed with severe HB at age 2, showing FIX levels of 1.2% at 6 years, 8% at 17 years, and 7% currently. Recent knee haemorrhages were effectively treated with replacement therapy. There is no history of inhibitor development.

Genetic analysis revealed the variant c.-35G>C in the *F9* promoter region, already described associated with HB Leyden and classified as pathogenic (PS3, PM2, PP5). Family testing showed the mother is a carrier, and the sister is not.

Discussion

This case supports previous reports that variants in the F9 promoter region (c.-50 to c.-18), such as the c.-35G>C variant identified here, cause severe to moderate haemophilia at diagnosis, followed by an age-related increase in FIX levels. This variant is the rarest among those linked to the Leyden phenotype and is associated with more severe presentation and the smallest FIX increase, as observed in this patient.

The severity and typical clinical course of haemophilia B Leyden are explained by the dependence of the F9 gene promoter on ONECUT-type hepatic transcription factors (also known as HNF-6 and related proteins), which are critical during childhood. After puberty, alternative molecular pathways compensate for this deficiency, resulting in the spontaneous improvement of symptoms.

Though less common, recognising these regulatory variants is important due to their impact on F9 expression and potential prognostic and therapeutic relevance. The family study, showing the mother as a carrier, confirms the expected inheritance pattern.

Understanding the natural history of these variants and FIX level dynamics is essential for prognosis and treatment decisions.

Conflicts of interest

The authors declare no conflicts of interest.

Keywords: Haemophilia B Leyden; Genetic diagnosis; *F9* promoter variants; FIX activity;

P80 THE TELL-TALE HEART: THE DIAGNOSTIC VALUE OF MINOR DYSMORPHIC FEATURES IN CARDIOGENETICS

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Introduction

Cardiogenetics is an emerging field focusing on inherited heart disorders. While many conditions primarily affect the heart, some have additional subtle extracardiac features that can be diagnostically informative. We report three illustrative cases where such findings, combined with molecular analysis, were decisive in establishing the diagnosis.

Methodology

Clinical, molecular, and family history data were collected during cardiogenetics consultations. A next-generation sequencing (NGS) cardiomyopathy panel was performed in all index patients, followed by segregation studies in relatives. Phenotypic findings were correlated with genetic variants.

Results

Case 1: A 15-year-old male with recurrent myocarditis also had woolly hair and plantar keratoderma. His 41-year-old mother had a similar phenotype, with a history of 29 episodes of myocarditis. Genetic testing identified a maternally inherited, likely pathogenic *DSP* (NM_004415.4) nonsense variant [c.2917G>T p.(Glu973*)], consistent with arrhythmogenic cardiomyopathy, woolly hair, keratoderma, and tooth agenesis.[1]

Case 2: A 17-month-old boy with hypotonia and epilepsy carried a pathogenic *DSP* (NM_004415.4) nonsense variant [c.1273C>T p.(Arg425*)], inherited from his father. Although the proband's cardiac evaluation was normal, family history revealed myocarditis, woolly hair, and keratoderma in his father and grandfather, consistent with a cardio-cutaneous phenotype.

Case 3: A father and son, both with dilated cardiomyopathy and distal arthrogryposis, are heterozygous for a novel *ACTC1* (NM_005159.5) missense variant [c.1120C>T p.(Arg374Cys)]. This variant, absent from gnomAD and predicted deleterious by REVEL (score 0.948), changes a residue previously linked to combined cardiac and skeletal muscle phenotype. [2]

Conclusions

These cases underscore the potential role of phenotypic assessment in cardiogenetics. Recognising extracardiac manifestations—such as hair, skin, and musculoskeletal anomalies—can indicate specific genetic conditions, guiding targeted testing and enabling earlier diagnosis. The integration of thorough phenotype characterisation with molecular analysis is crucial for accurate variant interpretation, enabling optimal counselling and personalised management.

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Keywords: cardiogenetics; *DSP*; *ACTC1*; cardiomyopathy; arthrogryposis.

P81 UNMASKING CONGENITAL HEART DISEASE IN A GENETIC SYNDROME: A CASE REPORT

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Introduction

Congenital heart disease is the most common congenital anomaly and a major cause of pediatric morbidity and mortality. Often associated with extracardiac anomalies, approximately 30% of cases are linked to an underlying genetic syndrome, highlighting the importance of genetic investigation (PMID: 26413101). Recognition is essential given the implications for prognosis, management and genetic counseling.

Case Description

We report a 10-year-old male, born to non-consanguineous parents, referred at 7 days of life for congenital heart disease, specifically tetralogy of Fallot, and bilateral cleft lip, detected on prenatal ultrasound. Fetal karyotype and microarray were normal. At observation, dysmorphic features were noted, namely hypertelorism, prominent eyes, anteverted nostrils, low-set ears and micrognathia. Additional findings included bilateral syndactyly of the fourth and fifth toes, pectus excavatum, and micropenis. Newborn hearing screening failed otoacoustic emissions and ENT follow-up documented right-sided hearing loss. Ophthalmologic evaluation revealed high myopia associated with mild myopic degeneration, more pronounced in the left eye. Radiographs demonstrated disproportion between the cranial vault and facial skeleton without other dysplastic or metabolic bone abnormalities.

Microarray 750K carried out in 2015 was normal. Clinical exome sequencing carried out in 2017 also failed to identify a genetic diagnosis. It was only identified a heterozygous variant in the *RYR1* gene (NM_000540.3) - c.7373G>A (p.(Arg2458His)), classified as pathogenic and associated with susceptibility to malignant hyperthermia.

Whole-exome TRIO sequencing carried out in 2022 revealed two variants in the *HYAL2* gene in compound heterozygosity: NM_033158.5 - c.217C>T (p.(Gln73*)) and NM_033158.5 - c.421T>C (p.(Trp141Arg)), both classified as variants of uncertain significance. These findings are consistent with the patient's phenotype and dysmorphic features.

Conclusion

Compared with the 17 previously reported cases of HYAL2 deficiency, the present patient shares the commonly described features, including congenital heart disease, craniofacial anomalies, ophthalmologic abnormalities and skeletal defects (MIM # 621063; ORPHA:508476). However, this case is distinguished by the presence of micropenis, a feature not previously documented in the literature. This highlights the phenotypic spectrum of HYAL2 deficiency and emphasizes the importance of genetic evaluation and multidisciplinary care.

P82 VEXAS SYNDROME – 5 YEARS OF DIAGNOSIS

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Introduction

VEXAS syndrome (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic), first described in 2020 (PMID: 33108101) as a novel class of autoinflammatory diseases driven by somatic mutations, is diagnosed genetically by sequencing the *UBA1* gene. Most pathogenic variants are missense that affect methionine at position 41 (PMID: 33108101; 33690815), involved in the transcription of the cytoplasmic isoform of the protein, consequently affecting its cytoplasmic function. Pathogenic variants are typically present at a high variant allele fraction (VAF), although low VAF (<2%) were described in symptomatic patients (PMID 37606963). As it is a gene located on the X chromosome, the majority of affected patients are male, over 50 years of age, whose clinical picture may present inflammatory and hematologic findings. In this study, we review the cases diagnosed by the laboratory over the last 5 years.

Methodology

47 patients with clinically suspected VEXAS whose most common symptoms were: fever, inflammatory polyarthritis, skin lesions, edema, macrocytic anaemia, thrombocytopenia, and myelodysplastic syndrome. A PCR targeting exon 3 (codon 41) of the *UBA1* gene (NM_003334.4; chr.X) was performed, ir order to cover the main pathogenic variants described: c.122T>C p.(Met41Thr); c.121A>G p.(Met41Val); c.121A>C p.(Met41Leu) (PMID 40373178). Library was prepared by tagmentation (QXT, Agilent Technologies) followed by next-generation sequencing (MiSeq, Illumina). Alignment and base identification were performed using the Dragen somatic workflow (Illumina), followed by bioinformatic analysis. The matrices were bone marrow or peripheral blood. VAFs were evaluated.

Results

Genetic testing confirmed the diagnosis of 30% of the patients studied. Of the 47 patients, pathogenic variants were reported in 14: 5 with c.121A>G p.(Met41Val); 2 with c.121A>C p.(Met41Leu); 5 with c.122T>C p.(Met41Thr), and 2 with c.118-1G>C p.?. The median VAF was 57% (range 1-80%), with two cases presented a VAF as low as 1%. 33 cases showed no variants in the analysed regions, all had adequate coverage for the test (minimum of 20 reads per base).

Discussion

Although it is a recently described disease, its prevalence may be higher than initially predicted. The associated symptoms may also make diagnosis difficult. It was not possible to correlate the detected variant/VAF with the symptoms, as they were very common among patients. Sensitive molecular techniques, as NGS are essential to detect low VAF cases (as 1%), which might otherwise remain undiagnosed.

P83 CHROMOSOME INSTABILITY TESTING FOR THE DIAGNOSIS OF FAMILIAL RAD51-ASSOCIATED FANCONI ANEMIA

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Introduction

Fanconi anemia (FA) is a rare, genetically and clinically heterogeneous chromosome instability syndrome (≥22 genes), usually with autosomal recessive inheritance. It is characterized by progressive bone marrow failure, congenital anomalies, and increased cancer risk, but clinical presentation may be mild or nonspecific, hindering early suspicion.

Case Report

We report two brothers, aged 14 and 10 years, with suspected genetic syndrome. The older sibling presented at birth with congenital genitourinary malformations (duplication of the left excretory system, renal asymmetry, hypogenitalism, bilateral cryptorchidism), developmental delay evolving into intellectual disability (ID), craniofacial anomalies, dental anomalies, and bilateral 5th toenail hypoplasia. The younger brother presented only with ID with nonspecific dysmorphic features. Both patients' hemograms were unremarkable.

Results

Karyotype, *FMR1* expansion, and NGS ID panel were negative. SNP-array revealed a heterozygous 129 kb deletion at 15q15.1 overlapping the *RAD51* gene, associated with autosomal dominant FA. Chromosome instability test with diepoxybutane (DEB) showed values within the normal range in both siblings (0.27 and 0.13 breaks/cell, respectively), while higher DEB concentrations presented a typical FA profile with multi-radial figures (1.69 and 2.61 breaks/cell, respectively), establishing the diagnosis of FA subtype R.

Discussion

FA associated with RAD51 has so far only been reported in pediatric patients, all described as de novo variants and, to date, without bone marrow failure. Our findings represent, to our knowledge, the first presumably inherited case of FANCR. The chromosome instability test was decisive for diagnosis: at the standard DEB concentration, the number of breaks per cell remained within the normal range, in contrast to the typical FA profile. However, upon doubling the DEB dose, the cells exhibited a characteristic FA pattern, with increased chromosomal breaks and multi-radial figures. This atypical response highlights the distinct cellular phenotype of FANCR, potentially related to its autosomal dominant inheritance, and emphasizes the importance of adequate analysis of chromosome instability beyond standard conditions.

Funding

UIDB/00215/2020 (DOI: 10.54499/UIDB/00215/2020) ; UIDP/00215/2020 (DOI: 10.54499/UIDP/00215/2020); LA/P/0064/2020 (DOI: 10.54499/LA/P/0064/2020)

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics and Consent

The study was conducted in accordance with the Declaration of Helsinki and approved by the Portuguese data protection authority (CNPD nº 8061/2016). Informed consent for genetic testing was obtained from the legal guardians of the patients.

Keywords: Fanconi anemia; FANCR/RAD51; Chromosome instability; Rare diseases

P84 PRENATAL DIAGNOSIS IN THREE CASES WITH 45,X/46,XY MOSAICISM: THE RELEVENCE OF THE CONTRIBUTION OF DIFFERENT TECHNOLOGIES

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Mosaicism involving the 45,X/46,XY is a rare sex chromosome disorder associated with a broad spectrum of clinical phenotypes, from Turner females to phenotypically normal males with varying degrees of genital ambiguity.

In this study, we report 3 cases with 45,X/46,XY mosaicism in NIPT, karyotyping, array-CGH, Fluorescent in situ hybridization (FISH) and QF-PCR analysis.

In case 1, a 41-year-old pregnant woman, was referred for amniocentesis, due to advanced maternal age. The karyotype identified a mos 46,X,+mar[24]/45,X[16]. To clarify the origin of the marker chromosome, array-CGH was performed and showed a loss of X chromosome, which is compatible with a 45,X cell line. It was not possible to characterize the marker chromosome, probably because it was present in a low level mosaicism. FISH analysis, using CEPY and SRY probes, identified the extra material derived from the Y chromosome contained the centromeric region, but not SRY locus. This alteration was *de novo*, because the karyotype of both parents was normal.

In case 2, a 36-year-old pregnant woman underwent amniocentesis after a positive NIPT that indicated a high risk for monosomy X. The rapid QF-PCR test was carried out and identified a probable mosaicism with a monosomy X cell line and another with Y material. Cytogenetics analysis confirmed the QF-PCR results and identified a mos 45,X[28]/46,XY[2]. Array-CGH was performed and showed a loss of X chromosome, which is compatible with a 45,X cell line, but the Y chromosome was not found.

In case 3, a 29-year-old pregnant woman, with TN>P95, was referred for chorionic villus sampling. The karyotype was 45,X, but the ultrasound examination performed showed a male fetus. To clarify the existence of a possible mosaic, a FISH study was carried out using SRY and CEPY probes, that showed a mosaicism with two cells lines, one with monosomy X and another with a chromosome X and a chromosome Y. After birth, the child was studied and the 45,X/46,XY mosaicism was confirmed in peripheral blood.

Chromosomal mosaicism remains an ever present challenge for diagnosis, especially in prenatal cases. Karyotyping combined with array-CGH, FISH and QF-PCR analysis can significantly improves the detection rate of chromosomal mosaicism, which can be of great value for prenatal diagnosis.

Keywords: 45,X/46,XY mosaicism; prenatal diagnosis; karyotyping; NIPT; array-CGH; FISH; QF-PCR

P85 DISCLOSING 7Q11.23 DUPLICATION SYNDROME: CLINICAL INSIGHTS FROM TWO PEDIATRIC CASES

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Introduction

Duplication of the Williams syndrome critical region on chromosome 7q11.23 is a rare genomic alteration that results in a distinct clinical phenotype, often considered the reciprocal of the Williams-Beuren syndrome deletion. This duplication involves approximately 1.5 to 1.8 Mb and usually encompass genes involved in neurodevelopment, growth regulation, and craniofacial patterning. While the deletion causes the well-characterized features of Williams syndrome, the duplication may present developmental delay, congenital anomalies and variable dysmorphic features.

Case Report

This study reports two unrelated cases of pediatric patients: the first, a male child, approximately one year old at the time of testing, born prematurely at 33 weeks gestation with a birth weight of 1240g due to intrauterine growth restriction that presented profound developmental delay with brain atrophy. The second case is a male child, aged eight years at the time of testing, with global developmental delay.

Results

Array CGH analysis revealed a duplication in the 7q11.23 region, extending approximately 1.4 MB in both cases. In the first case the duplication was inherited from the mother that presented cognitive impairment and depressive-type mental disorder. In the second case there is still information about the origin of the duplication.

Conclusion

The phenotypic variability and incomplete penetrance of 7q11.23 duplication syndrome present challenges in genetic counseling and clinical management. The duplication follows an autosomal dominant pattern of inheritance. Approximately 27% of individuals diagnosed with this syndrome have inherited the duplication from an affected parent. This abstract underlines the importance of further genotype-phenotype correlation studies and careful genetic counseling. Further research is necessary to elucidate the molecular mechanisms underlying this duplication and its contribution to neurodevelopmental and systemic manifestations.

Keywords: Duplication 7q11.23, array-CGH, phenotypic variability

P86 *PAX5::AUTS2* FUSION IN PEDIATRIC B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: A RELAPSE-PRONE GENETIC SUBTYPE

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Introduction

B-cell acute lymphoblastic leukemia (B-ALL) is the most common paediatric malignancy, with prognosis highly dependent on early and accurate genetic characterization. Cytogenetics provides a global overview of chromosomal abnormalities, while next-generation sequencing (NGS) enables precise detection of gene fusions.

Methodology

We present a case of a 5-year old female, demonstrating the complementary value of the karyotype and NGS. Chromosomal banding analysis was performed on bone marrow cultures with and without methotrexate synchronization. Fusion transcript detection was carried out using the TruSight RNA Fusion Panel, with alignment and analysis through the DRAGEN RNA pipeline.

Results

The karyotype at diagnosis showed a triple translocation: 46,XX,t(7;7;9)(p13;p15;p13)[18]/46,XX[2]. NGS identified two fusion transcripts: *PAX5::AUTS2* and *IKZF1::MALSU1*. Follow-up karyotype reveled the persistence of the rearrangement, with clonal evolution with trisomy of the chromosomes 5, 13 and 22, consistent with relapse. Despite haploidentical stem cell transplantation, further relapses occurred, showing persistence of the original translocation and aberrations.

Discussion

This case exemplifies the complementarity between conventional cytogenetics and NGS in the integrated diagnostic workup of B-ALL. A 2025 study describes the *PAX5::AUTS2* fusion as a marker of high-risk disease, frequently associated with relapse and central nervous system (CNS) involvement [1]. However, further cases are needed to better define its clinical impact. In contrast, the co-occurring *IKZF1::MALSU1* fusion represents a novel and uncharacterized finding, potentially expanding the molecular spectrum of B-ALL. Together, these observations not only support the integration of genomic data into classification, but also suggest the emergence of distinct B-ALL subtypes defined by unique fusion profiles.

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P87 CONVENTIONAL CYTOGENETICS AS A KEY TOOL IN THE CHARACTERISATION OF A *DE NOVO* 20P DUPLICATION

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Introduction

Trisomy 20p is a rare chromosomal disorder caused by duplication of all or part of the short arm of chromosome 20 (20p). Reported phenotypes include overall normal growth, mild-to-moderate intellectual disability, speech delay, poor motor coordination and distinctive craniofacial features. Clinical severity correlates with duplication size, with larger duplications being associated with a more severe phenotype. Most cases involve partial trisomy 20p, often due to parental structural rearrangements. Duplication of the entire 20p is rare, and its clinical spectrum remains incompletely defined. We report a *de novo* case, contributing to phenotypic delineation and counselling.

Methodology

A 6-year-old female, born to non-consanguineous parents, was referred for genetic evaluation due to prominent metopic suture with brachycephaly, short palpebral fissures, bilateral 2–3 toe syndactyly, developmental delay, right renal atrophy, and two low-impact fractures. Exome with copy-number variation (CNV) analysis revealed 20p copy number gain, consistent with trisomy of 20p short arm. To further characterise the abnormality, peripheral blood cultures were analysed using G-banded karyotyping, and fluorescence in situ hybridization (FISH) with locus-specific probes was used to confirm the structural rearrangement. Parental karyotypes were also performed.

Results

Patient's karyotype was 46,XX,der(21)t(20;21)(p10;q10)dn. FISH confirmed a derivative chromosome 21 with 20p duplicated material. Both parental karyotypes were normal, supporting a *de novo* origin.

Discussion

This case illustrates a rare *de novo* trisomy 20p, associated with developmental delay, craniofacial dysmorphism, digital anomalies, renal involvement, and bone fragility. The findings highlight the importance of a multimodal cytogenomic approach, combining sequencing techniques, karyotyping, and FISH, for precise characterisation of rare duplications. This work also contributes to refining the phenotypic spectrum of trisomy 20p and provides crucial additional information to support genetic counselling regarding prognosis and reproductive risk.

Conflicts of interest

None

*These authors have contributed in equal parts

Ethics and Consent: Parental informed consent was obtained for this clinical case presentation.

P88 CASE REPORT: NGS DETECTION OF LOW-GRADE TRISOMY 8 MOSAIC IN MDS

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Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by dysplasia and ineffective hematopoiesis within the bone marrow. Clinically they manifest as cytopenia of one or more lineages, resulting in anemia, increased susceptibility to infections or bleeding tendency, and may progress to acute myeloid leukemia (AML). Regular bone marrow evaluations are essential to assess dysplasia, blast percentage, and cytogenetic abnormalities, which guide prognosis and treatment.

We report a 76 year old patient with anemia, rash, constitutional syndrome and erythrocyte sedimentation rate suggestive of inflammation. The patient was referred for karyotyping of bone marrow cultures without stimulation and for an NGS panel performed on a peripheral blood sample.

Methodology

Chromosome analysis was performed on metaphases stained with GTG bands from one or more independent cell cultures performed without stimulation. Whole exome sequencing (WES) with CNV analysis was performed (Illumina Novaseq 6000 ®) and the resulting data was processed and analyzed with an in house bioinformatics pipeline and NGS analysis software.

Results

Analysis of the bone marrow cultures without stimulation reveled 28 metaphases with trisomy 8, while the remaining 4 metaphases showed no chromosomal abnormalities. NGS analysis of the peripheral blood sample also detected trisomy 8 at a low percentage, consistent with a low-grade mosaic. These findings confirm the presence of a clonal population with trisomy 8, detected both by cytogenetics and by molecular analysis.

Discussion

Trisomy 8 is one of the most frequent cytogenetic abnormalities in patients with MDS. In this case, the detection of a low-grade mosaic in the peripheral blood sample by NGS is particularly relevant. Advancements in NGS technology have significantly improved its sensitivity, allowing the detection of low-frequency variants that were previously undetectable. The continuous advances in NGS raise the possibility that, in the future, patients with MDS may preform simple blood tests rather than repeated bone marrow collections, offering a less invasive way to track disease progression.

P89 FAMILIAL RARE X/Y TRANSLOCATION – A CASE REPORT

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Translocations involving X/Y chromosomes are rare with few reports in literature. In most cases breakpoints are in Xp22/Yq11 and rarely occur in other locations. The impact of X/Y translocations in the phenotype is heterogenous and can lead to development, reproductive and endocrinal disorders.

We report a female foetus with a t(X;Y)(q26.3;q11.22) inherited from a healthy mother, referred for prenatal diagnosis because of viral infection. The alteration was first identified in the karyotype, with a derivative X chromosome showing an abnormal terminal region in the long arm. aCGH was performed showing a 17Mb deletion X(q26.3q28) without any gains. C-banding revealed a heterochromatin region on the der(X) chromosome and the suspicion of the presence of Y material was confirmed by MLPA with the probe mix for Y chromosome microdeletions. The Y chromosome material present (Yq11.223q11.23) would be at least 3,6Mb, including the AZFc region; the *SRY* locus (Yp11.31) was absent.

This foetus carries a der(X) from a translocation between the long arms of the sexual chromosomes, with breakpoints in Xq26.3 e Yq11.223. Since the alteration is inherited from a healthy mother a similar phenotype is expected. However, a different pattern of X chromosome inactivation must be considered in these cases.

The Y material on der(x) has no *SRY* gene and the AZF region is partially present (AZFc) and therefore it will not have implications for the development of the female phenotype. At five-month-old the girl had a normal medical exam.

The deleted region on chromosome X (q26.3-qter) partially overlaps the referred in literature "critical region" x(q13-q26) for ensuring a normal ovarian function and, in that way, the girl and her mother are likely to develop symptoms of POF.

Considering the multiple genes deleted on the der(X), some of them with an X-linked recessive inheritance, there is a high risk for a severe phenotype in future male pregnancies, causing miscarriage, embryo lethality or spontaneous abortions.

The multiple aspects that must be considered in the X/Y translocations counselling highlights the need for a thorough characterization of these structural alterations, especially when rare breakpoints are involved.

Keywords: X/Y translocations, prenatal diagnosis, rare breakpoint

*Both authors contributed equally

P90 CLINICAL, GENETIC AND BIOCHEMICAL CHARACTERIZATION OF PATIENTS WITH PORPHYRIA CUTANEA TARDA

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Abstract

Porphyria Cutanea Tarda (PCT) is the most common subtype of porphyria and represents a multifactorial disorder of heme biosynthesis resulting from decreased activity of uroporphyrinogen decarboxylase (UROD). Clinically, PCT manifests with non-inflammatory vesiculobullous lesions that rupture and heal with atrophic scars, predominantly on sun-exposed areas such as the dorsum of the hands, forearms, legs, and feet. To perform an integrated clinical, genetic and biochemical characterization of patients with PCT and their relatives, aiming to improve understanding of disease etiology and identify potential targets for novel therapeutic strategies beyond conventional treatments such as hydroxychloroquine and phlebotomy.

Methodology

Participants clinically defined as PCT patients were recruited from the dermatology outpatient clinics at UNIFESP, USP, and UNICAMP. Laboratory analyses included: (1) genomic DNA extraction and Sanger sequencing of *UROD* gene; (2) RNA extraction and cDNA synthesis; (3) Splicing variant analysis of *UROD* gene mRNA by RT-PCR; (4) Multiplex Ligation-dependent Probe Amplification (MLPA) for large rearrangements; (5) whole exome sequencing (WES) and (6) quantification of UROD enzymatic activity using erythrocyte-derived protein.

Results

To date, 50 patients have been evaluated. Conventional therapy effectively reduced total porphyrin, uroporphyrin, coproporphyrin and heptaporphyrin levels. *UROD* gene sequencing identified clinically relevant variants in 25 of 50 patients: c.952 G>A, c.74G>A, c.185 C>T, c.461 T>C, c. 540 G>A, c. 767 T>G, c.781 T>G, c.912C>A, c. 942 G>A p. Glu314=. The synonymous variant p.Glu314= was the most frequent (10 patients). MLPA analysis identified no large deletions or insertions and no alternative splicing events were detected.

Discussion

The identification and characterization of *UROD* gene revealed seven missense variants and two synonymous variants. WES of the remaining 25 patients is ongoing, and the integration of enzymatic, genetic, and clinical data will refine genotype–phenotype correlations and improve understanding of PCT pathophysiology.

Funding

Financial support from FAPESP (2023/17551-1).

Conflicts of Interest

The authors declare no conflict of interest.

Keywords: Porphyria Cutanea Tarda, *UROD*, Whole Exome Sequencing, Gene Expression. Ethics and Consent: the project followed the UNIFESP Research Ethics Committee (CEP) guidelines, number 0724/2019.

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ISBN: 978-989-35476-0-3
Depósito Legal: 556361/25