20TH INTERNATIONAL SYMPOSIUM OF THE PORTUGUESE SOCIETY FOR METABOLIC DISORDERS

THE CHANGING PARADIGM OF INHERITED METABOLIC DISORDERS IN PORTUGAL

05th April Symposium
06th April Satellite Meeting
2024

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WELCOME ADDRESS

Dear Colleagues and Friends,

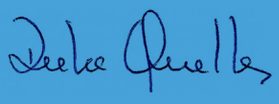
On behalf of the Organizing Committee, it is a great pleasure to welcome you to the 20th International Symposium of the Portuguese Society for Metabolic Disorders (SPDM).

This year´s programme focus on "The Changing Paradigm of Inherited Metabolic Disorders in Portugal", with the scope to explore and review the main advances of Inherited Metabolic Disorders (IMD) diagnosis and treatment in Portugal. The Symposium programme has the privilege to count on the participation of outstanding experts from national IMD reference institutions as well as international worldwide experts who will bring you the most recent scientific advances in the IMD field, including newborn screening, controversies regarding diagnostic techniques and new approaches to IMD treatment such as breastfeeding.

We hope that most of the participants can get together in person, so important for exchange of ideas in an informal environment. The Meliá Ria Hotel offers ideal conference conditions in a modern and pleasant Atmosphere.

This meeting has been made possible by the support of the sponsors, which we acknowledge.

We are delighted to be hosting the 2024 SPDM Annual Symposium, hoping that it will be a success, and very much look forward to welcome all of you in Aveiro.



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Instituto Nacional de Saúde Doutor Ricardo Jorge - Porto, PT

Daniel Gomes – SPDM Board
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Unidade Local de Saúde Santa Maria - MetabERN, Lisboa, PT

SCIENTIFIC COMMITTEE

Anabela Oliveira
Centro de Referência de Doenças Hereditárias do Metabolismo
Unidade Local de Saúde Santa Maria - MetabERN, Lisboa, PT

Arlindo Guimas
Centro de Referência de Doenças Hereditárias do Metabolismo
Unidade Local de Saúde de Santo António - MetabERN, PT

Patrícia Lipari Pinto
Centro de Referência de Doenças Hereditárias do Metabolismo
Unidade Local de Saúde Santa Maria - MetabERN, Lisboa, PT

Rita Loureiro
Centro de Referência de Doenças Hereditárias do Metabolismo
Unidade Local de Saúde Santa Maria - MetabERN, Lisboa, PT

PROGRAMME

SCIENTIFIC PROGRAMME | 5TH APRIL

09:45	Welcome
10:00 - 11:15	**SESSION I - LESSONS FROM NBS** **Chairs:** Hugo Rocha, Anabela Oliveira
10:00	**45 years of Newborn Screening: lessons learned** // Laura Vilarinho, Porto, PT
10:20	**Clinical follow-up of mild diseases – paediatric experience** // Teresa Campos, Porto, PT
10:40	**Clinical follow-up of mild diseases – adult overview** // Arlindo Guimas, Porto, PT
11:00	Discussion
11:15 - 11:45	**COFFEE BREAK AND POSTER VIEW**
11:45 - 13:00	**SESSION II – CURRENT AND FUTURE OF IEM DIAGNOSIS** **Chairs:** Anabela Bandeira, Sara Ferreira
11:45	**Classification of variants in the clinical laboratory: Challenges and solutions** // Belén Pérez, Madrid, SP
12:05	**NGS - implications for clinical diagnosis** // Fabiana Ramos, Coimbra, PT
12:25	**New biochemical markers** // Rafael Artuch, Barcelona, SP
12:45	Discussion
13:00 - 15:00	**LUNCH AND POSTER VIEW**
15:00 - 16:15	**SESSION III – NEW APPROACHES TO IEM TREATMENT** **Chairs:** Ana Paula Leandro, Esmeralda Rodrigues
15:00	**Breastfeeding in IEM** // Júlio Cesar Rocha, Lisboa, PT
15:25	**New options for treatment in CDG** // Mercedes Serrano, Barcelona, SP
15:50	Discussion
16:10 - 16:45	**COFFEE BREAK AND POSTER VIEW**
16:45 - 18:15	**SESSION IV - OVERVIEW OF SPDM PROJECTS** **Chairs:** Esmeralda Martins, Manuela Grazina
16:45	**Improve management of MADD patients – insights and achievements** // Bárbara Henriques, Lisboa, PT
17:00	**Improvement of new amino acid content tables to be used as a tool for the treatment of protein inherited metabolic disorders** // Carla Vasconcelos, Porto, PT
17:15	**A patient reported experience measure (PREM) for quality improvement of pediatric inherited metabolic disorders care** // Teresa Campos, Porto, PT
17:30	**Rare diseases reference centers network knowledge management** // Sandra Mexia, Lisboa, PT
17:45	**Acquired Vitamin B12 Deficiency in Newborns: Positive Impact on Newborn Health through Early Detection** // Patrícia Lipari Pinto, Lisboa, PT
18:00	Discussion
18:15	**AWARDS AND FINAL REMARKS**
18:45	End of the Symposium

SPEAKERS

ARLINDO GUIMAS
OPORTO,
PORTUGAL

Arlindo Guimas, MD

Consultant in Internal Medicine, Unidade Local de Saúde de Santo António (ULSSA) Adult Physician at the National Reference Center of ULSSA.

BÁRBARA HENRIQUES
LISBON, PORTUGAL

Bárbara J. Henriques, PhD

Is Principal Investigator at Biosystems & Integrative Sciences Institute, Faculty of Sciences University of Lisboa (BioISI-FCUL). In 2010 she obtained a Ph.D. in Biochemistry/Structural Biochemistry, at New University of Lisboa (ITQB/UNL). Bárbara's research interests are focused on establishing molecular mechanisms underlying protein misfolding and functional deficiency in the context of rare metabolic disorders, resorting to patient profiling and biochemical and cellular models. This follows international experience obtained during training periods at Aarhus University Hospital (Denmark) and Université Paris Descartes (France), and +18y research experience in rare metabolic diseases. Main scientific contributions translate to establishing the molecular and biochemical basis of therapeutic potential of dietary vitamins, definition of genotype-phenotype relationships, and profiling protein dysfunction in mitochondrial disease. Current research focus is on fatty acid beta oxidation disorders caused by defects on ETF and ETF:QO, and also on leukodystrophies, neurometabolic rare diseases caused by defects in mitochondrial aminoacyl-tRNA synthetases. Bárbara has published +18 articles in international peer-review journals, 4 book chapters, and presented her work in + 50 international meetings. In the last 8Y she raised over 435 kEUR in national competitive calls as PI in 6 research projects, and supervised +20 young researchers, currently 2 PhD and 1 MSc students.

BELÉN PÉREZ
MADRID, SPAIN

Belén Pérez, PhD

PhD in Biological Sciences (1994) from the Autonomous University of Madrid (UAM). She is a Full Professor of the Department of Molecular Biology at the UAM, where she teaches Human Genetics, Genetic Engineering, and Biochemistry. Belen Pérez is a molecular geneticist involved in the biochemical and genetic diagnostics of inherited metabolic diseases. She is the deputy director of the Center for Diagnosis of Molecular Diseases (CEDEM) and the head of a group on CIBER Rare Diseases at the Biomedical Research Institute (IdiPAZ). She has a research line titled "Translational medicine in inborn errors of metabolism and other rare genetic diseases." at the Center of Molecular Biology (CBM-UAM-CSIC)
The activity of her group is aimed at transferring knowledge on rare diseases to the clinical practice to advance in the incorporation of novel and cutting-edge methods for diagnosis, prevention, and therapies through the combination of multi-omic layers (genomic, transcriptomic, epigenomics, and metabolomic) with functional and structural genomics. Finally, the group is interested in developing therapeutic strategies targeted at the mechanism of action of the variants identified in neurometabolic diseases in the era of personalized medicine, specifically in developing pharmacological chaperones, mRNA therapies, or gene editing. She is author of more than 190 papers in high impact factor international journals (ORCID 0000-0002-3190-1958).

FABIANA RAMOS
COIMBRA, PORTUGAL

Fabiana Ramos, MD

Medical Doctor, Faculty of Medicine of the Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
Medical Genetics Residency (Five year program)
Medical Genetics Department, Paediatric Hospital of Coimbra, Portugal.
Consultant in Medical / Clinical Genetics
Medical Genetics Department, Paediatric Hospital of Coimbra, Portugal.
Teaching Assistant of the discipline "Medical Genetics" of the 2nd year of the 6th years Integrated Master in Medicine Faculty of Medicine of the University of Coimbra,Portugal.

JÚLIO CÉSAR ROCHA,
LISBON, PORTUGAL

Júlio César Rocha, PhD

Professor of Nutrition and Metabolism, graduated in Nutritional Sciences (0438N – specialist in Clinical Nutrition), with a post graduate qualification in Clinical Nutrition from the Faculty of Nutrition and Food Sciences – University of Porto (UP) and he has also a PhD in Metabolism, at the Faculty of Medicine, UP.
He has been working in the field of inborn metabolic diseases since 2003. He is Professor of Nutrition and Metabolism at NOVA Medical School teaching in the field of Nutrition and Metabolism to Medicine and Nutrition students. He is also member of the multidisciplinary clinical team at the Reference Centre of Inherited Metabolic Diseases at Unidade Local de Saúde São José, one of the 5 Portuguese Reference Centres for the follow-up of patients with Inherited Metabolic Diseases. He is also a researcher at CINTESIS (Center for Health Technology and Services Research).
He is council member of the SSIEM (Society for the Study of Inborn Errors of Metabolism), Chair of the Dieticians Group of the SSIEM (SSIEM-DG), Chair of the Nutrition Group of the Portuguese Society of Metabolic Disorders (SPDM-GN) and President of the Portuguese Society of Clinical Nutrition and Metabolism (SPNCM).
He is also member of the working group of the European Phenylketonuria Guidelines (EPG 2.0) under the umbrella of the ESPKU.
He is author of more than 75 international, indexed, scientific publications and he has done more than 100 oral presentations/lectures/conferences in more than 13 different countries.

TERESA CAMPOS
OPORTO, PORTUGAL

Teresa Campos, MD

Teresa Almeida Campos, pediatrician, is part of the team of the Reference Center oh Inherited Metabolic Diseases of the Unidade Local de São João. Carried out the Special Cycle of Studies on this scientific field and is currently carrying out doctorate. She is a board member of the Section for Inherited Metabolic Diseases of the Portuguese Society of Pediatrics.

LAURA VILARINHO,
OPORTO, PORTUGAL

Laura Vilarinho, PhD

Degree in Pharmaceutical Sciences by the Faculty of Pharmacy, University of Porto
Ph.D. in 2000 ICBAS University of Porto
Clinical Laboratory Geneticist
Director of Newborn Screening, Metabolism & Genetics Unit, Human Genetics Department, National Institute of Health Doutor Ricardo Jorge (INSA), Porto
PI of I&D Unit of INSA, Porto
Coordinator of Executive Board of National Newborn Screening Program.

PATRÍCIA LIPARI PINTO
LISBON, PORTUGAL

Patrícia Lipari Pinto, MD

Hereditary Metabolic Disease Reference Center, Paediatric Department, Santa Maria's Hospital - Lisbon North University Hospital Center, EPE, Lisbon, Portugal.
Responsible for metabolic diseases consultation at the out-patient clinic for diagnostic orientation and therapeutic management of hospitalised patients with metabolic diseases
Responsible for expert support in metabolic diseases, upon request, to other pediatric units for suspected diagnosis of inborn errors of metabolism.
Member of the Neonatal Intensive Care Emergency team of the Pediatric Department, Santa Maria's Hospital.
Orientation and training of paediatric residents and medical students of the Faculty of Medicine, Lisbon University.
Member of the Portuguese Society of Metabolic Diseases (SPDM).
Member of the Working Group on Lysosomal Storage Diseases of the SPDM.
Member of the Training Unit of the Paediatrics Department of CHULN.
Specialised Training in Medical Paediatrics
Inborn Errors of metabolism (6 months), Neuropediatric (6 months), Genetics (3 months), Paediatric Intensive Care (3 months), Neonatal Intensive Care (3 months), Paediatric Gastroenterology (3 months)
Master's in Medicine At: Lisbon Medical University, Lisbon, Portugal
Master's Degree, with a final course average of 17 out of 20. The final master's work is titled, "Creatine Carrier Deficit - How far do we investigate the cause of a developmental delay?"
Fellowship in Paediatric Metabolic Department at Great Ormond Street Hospital for Children. London, UK.
Fellowship in Pediatric Neurology Department, in Neurometabolic Disorders at Sant Joan de Déu Hospital, with Dr. Àngels García Cazorla, Barcelona-Spain.

CARLA VASCONCELOS
OPORTO, PORTUGAL

Carla Vasconcelos, MD

Nutritionist specializing in Clinical Nutrition at the Nutrition Service of ULS São João and at the Hereditary Center for Metabolic Disorders at ULS São João.

Master's Degree in Paediatric Nutrition at FCNAUP.

RAFAEL ARTUCH
BARCELONA, SPAIN

Rafael Artuch, MD, PhD

Developed his career in Hospital Sant Joan de Déu and its associated research Institute, in Barcelona, Spain. In 1990 he got the degree in Medicine, in 1996 the title of specialist in Clinical Biochemistry and in 1998 the PhD title in Medicine about the biochemical basis of mitochondrial disorders.
His career has been developed in the field of inborn errors of metabolism by studying the biochemical and molecular basis of these disorders, having remarkable experience in biomarker validation for both diagnosis and treatment monitoring and identification of new genes associated with human diseases with 300 indexed in PubMed. One of the main focus in his research is the biomedical aspects of Coenzyme Q10 deficiency.
Dr. Artuch is coordinating a research program inside the scientific network CIBERER (program of metabolic and mitochondrial medicine) and in January 2022, he is the chairman of the Executive Committee of ERNDIM, the largest external quality control scheme program for laboratories working I the diagnosis of inborn errors of metabolism.

SANDRA MEXIA
LISBON, PORTUGAL

Sandra Mexia, MD

Member of the professional order of Nutritionist, 0942N, specialist in Clinical Nutrition.
Degree in Dietetics and Nutrition from the School of Health Technologies of Lisbon.
Master's degree in Epidemiology from the Faculty of Medicine of Lisbon, with the dissertation: "Effectiveness of dietary treatment in MSUD: evaluation of clinical, biochemical and anthropometric parameters".
PhD student in Public Administration, specialization in Health Administration, to perform thesis with the title: "Knowledge Management in Rare Disease Reference Center Networks".
Member of the group of nutritionists of the Portuguese Society of Metabolism.

MERCEDES SERRANO
BARCELONA, SPAIN

Mercedes Serrano, MD, PhD

Pediatrician and Doctor of Medicine from the University of Barcelona.
Neuropediatrician at the Sant Joan de Déu Hospital in Barcelona.
Researcher at CIBERER (Center for Biomedical Research in Rare Diseases).
Associate Professor at the University of Barcelona.

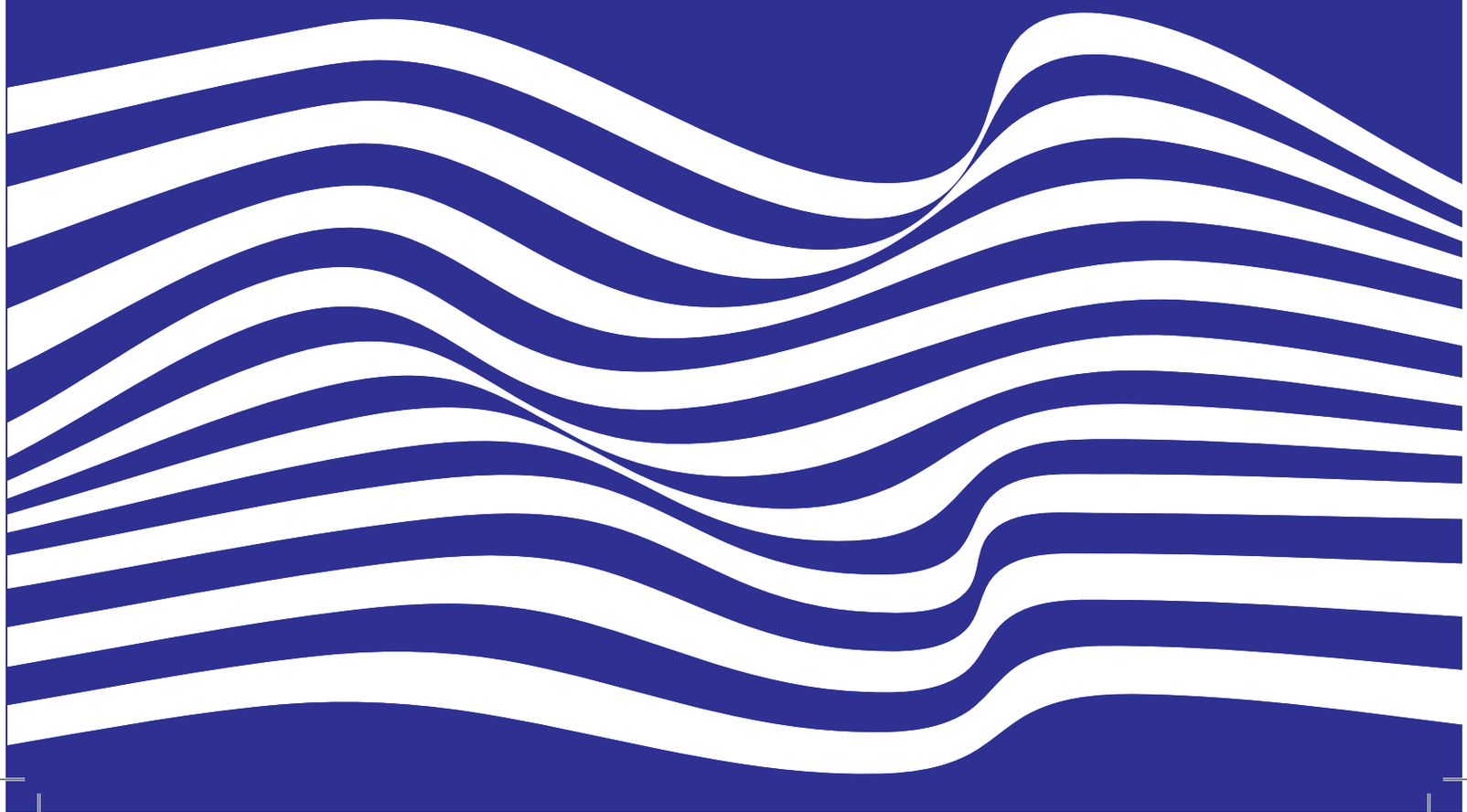
SESSION I

LESSONS FROM NBS

CHAIRPERSONS

HUGO ROCHA,
Newborn Screening, Metabolism and Genetics Unit - Human Genetics Department
Instituto Nacional de Saúde Doutor Ricardo Jorge - Porto, Portugal

ANABELA OLIVEIRA,
Serviço de Medicina Interna, Centro de Referência de Doenças Hereditárias do Metabolismo
Unidade Local de Saúde Santa Maria, Lisboa, Portugal



45 YEARS OF NEWBORN SCREENING: LESSONS LEARNED

Laura Vilarinho

The Portuguese Neonatal Screening Programme (PNSP) began on May 14th of 1979 with the screening for Phenylketonuria (PKU). Over the years, the panel of screened conditions was expanded to the current 28 conditions and allowed the diagnosis of more than 2500 affected neonates. The panel includes 24 Inborn Errors of Metabolism, Congenital Hypothyroidism, Cystic Fibrosis, Sickle Cell Disease and Spinal Muscle Atrophy (ongoing pilot study).

Despite the non-mandatory nature of this programme, its coverage is approximately 100% of neonates born in Portugal, with around 350 samples processed daily in our Unit.

In 2004, was implemented a tandem mass spectrometry (MS/MS) pilot study to test for acylcarnitines and amino acids that allowed the simultaneous screening of multiple conditions in a single sample, increasing the analytic ability of the PNSP. In 2006 a nationwide expanded neonatal screening (NBS) programme for IEM, officially was established.

NBS Programs should be dynamic and adjust themselves to maximize their impact in public health. Since 2004, several adjustments were made to screening criteria of several metabolic disorders including cut-off adjustments, adoption of new screening markers and introduction of second tier testing (2TT) to increase sensitivity and specificity of neonatal screening for disorders with higher number of false positives or false negatives.

CLINICAL FOLLOW-UP OF MILD DISEASES PAEDIATRIC EXPERIENCE

Teresa Campos

The newborn bloodspot screening (NBS) is one of the important public health achievements of the recent medicine, as it allows the precocious identification of some inherited metabolic diseases (IMD) potentially changing their prognosis.

Although management of the classical forms is defined in a certain way for the main screened IMD, there is lack of evidence for the correct guidance of the nonclassic and partial forms of IMD.

Uncertainty can generate practical challenges for health-care providers, patients, laboratory scientists, and program leaders. This can persist for months or years and can lead to very different attitudes, from very lengthy diagnostic odysseys and potentially invasive treatments to more passive monitoring approaches. Balance is needed between prevention of potential risks of no timely intervention and potential impacts on children and families arising from sickness labels.

CLINICAL FOLLOW-UP OF MILD DISEASES ADULT OVERVIEW

Arlindo Guimas

Most of the patients with "mild phenotypes" are identified through newborn screening (NBS) or from family studies from case index.
Since 2006, in Portugal, the extended NBS identified more patients with different hereditary metabolic disorders and consequently more affected family members with "mild phenotypes".

In this presentation, a revision of the patients that could fit in this designation, revealed that this group includes mainly patients with aminoacidopathies (hyperphenylalaninemia and other) and beta oxidation disorders.
In adult life, the management of "mild phenotypes" must be adequate to the potential severity of the disorder and the unknown consequences in the long range of adult life.

An individualized follow up plan should be discussed with the patient, and risk-benefit of every medical intervention must be properly evaluated. In some disorders, the avoidance of triggers/ precipitants could prevent an acute decompensation and should be implemented properly and in proportion to the risk.
Clinicians need to be able to identify some "new phenotypes" that could emerge from the follow up and the impact of the disorder in other comorbidities and aging.

Genetic counseling is crucial to provide the best information for the patient and family and to capacitate them to make informed decisions about fertility.
There is scarce data from "mild phenotype" metabolic disorder through adult life. This data should be gathered and analyzed to provide the clinicians a better evidence about the care of these patients.

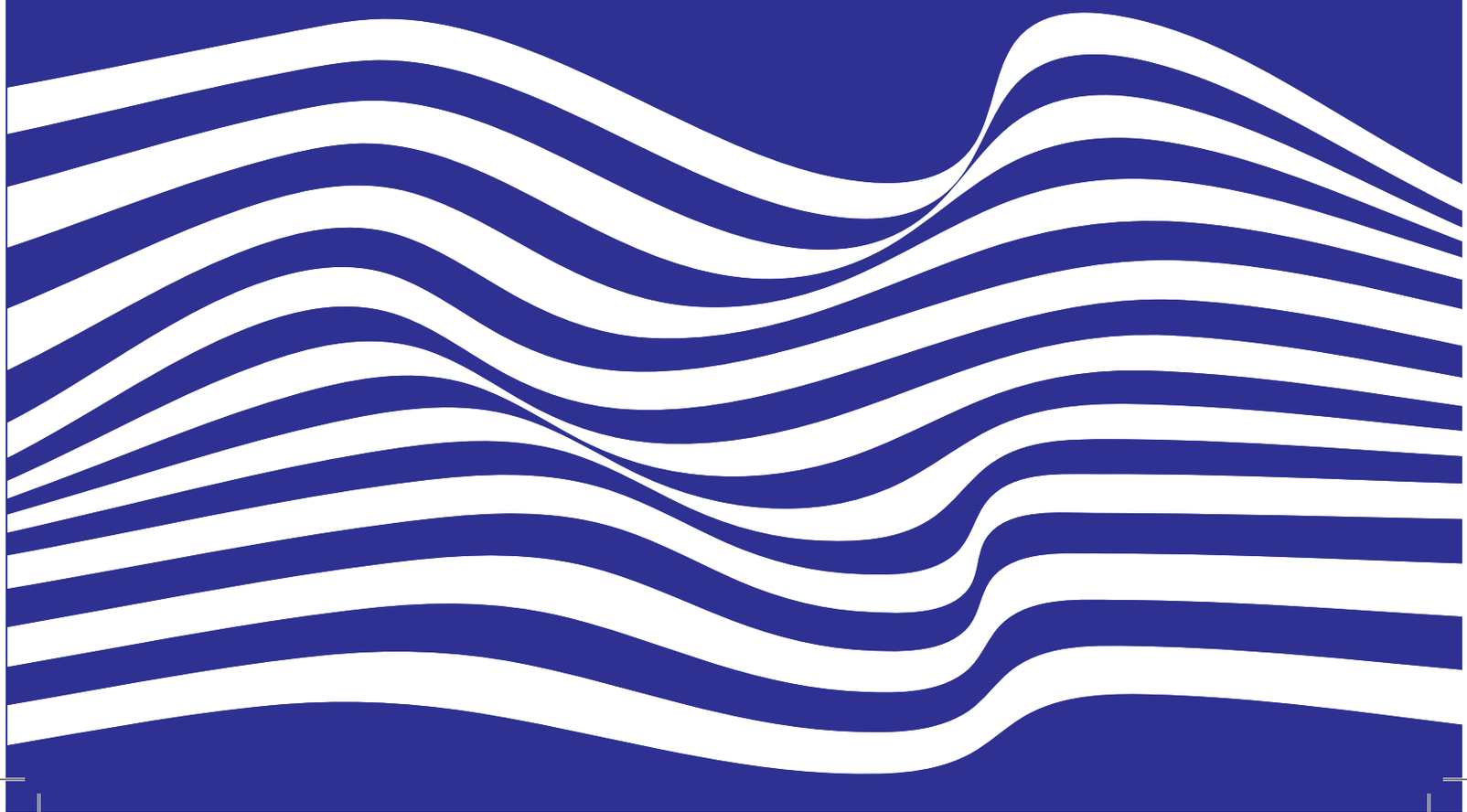
SESSION II

CURRENT AND FUTURE OF IEM DIAGNOSIS

CHAIRPERSONS

ANABELA BANDEIRA,
Centro de Referência de Doenças Hereditárias do Metabolismo
Unidade Local de Saúde de Santo António - MetabERN, Portugal

SARA FERREIRA,
Centro de Referência de Doenças Hereditárias do Metabolismo
Centro Hospitalar Universitário de Coimbra - MetabERN, Portugal



CLASSIFICATION OF VARIANTS IN THE CLINICAL LABORATORY: CHALLENGES AND SOLUTIONS

Belén Pérez

Despite that the diagnosis of inborn errors of metabolism (IEM) have been transformed using the next-generation sequencing technology (NGS), many patients remain unsolved. The reduced diagnosis rate by using whole clinical exome sequencing in the clinical setting as the first line for diagnosing patients with IEM reflects multiple factors, including technical limitations, incomplete understanding of variant pathogenicity, and missing genotype-phenotype association. These factors preclude a diagnosis, increasing the diagnosis odyssey of the patients and families. Short-read and long-read genome sequencing could reduce the diagnosis gap due to the ability to detect variants in non-coding sequences, structural variations, movement of transposable elements, deep intronic mutations, or epigenetics defects. However, the use of these newer technologies is increasing substantially the number of variants of unknown clinical significance (VUS), precluding a diagnosis due to the lack of knowledge of the effect of variants outside the exome sequence. In this work, we present a strategy combining computational tools and functional assay to reclassify the VUS variants into the likely or pathogenic variants to provide an accurate diagnosis and allow the application of personalized therapy and adequate genetic counseling. The integration of ACMG guidelines application by the use of different algorithms in combination with in silico structural analysis, phenotypic data, and functional assay using patient samples reduce substantially the number of VUS.

NGS – IMPLICATIONS FOR CLINICAL DIAGNOSIS

Fabiana Ramos

Inborn errors of metabolism (IEM) are rare disorders, but as a group affect 1/1000 to 1/4000 live-born infants. They have a variable degrees of severity and a broad spectrum of signs and symptoms which overlap with othr conditions, beside a genetic heterogeneity that makes the definitive diagnostic a challenge. The biochemical screening is generally proposed as first-line investigation, mainly when clinical features are suggestive of metabolic disorder and the results drive specific secondary investigations usually based on enzymatic studies or targeted genetic analyses, but this strategy has low detection rate when the clinic is nonspecific, as in situations of isolated intellectual disability. Next Generation Sequencing (NGS) technology allow a simultaneous sequencing of a large amount of DNA sequences and has improved the diagnosis rate in rare diseases, mainly in unspecific or atypical phenotypes and has led to the discovery of new genes and diseases. In IEM due to highly heterogeneity and lack of specific biomarker NGS technology is a powerful tool NGS technology that has been responsible for an increase in the diagnosis rate with faster and more effective diagnoses that benefits the patient. This technology seems to be cost-effective and can be a first-line test in patients with clinical suspicion of this group of disease.

NEW BIOCHEMICAL MARKERS

Rafael Artuch

Neurotransmitter disorders are a group of inherited neurometabolic diseases attributable to disturbances of neurotransmitter metabolism, involving amino acids, cholinergic transmission and biogenic amines (dopamine, serotonin, noradrenaline, and adrenaline). Analysis of amino acids such as γ-aminobutyric acid (GABA), glycine, and glutamate, biogenic amines, and its related cofactors, such as pterins or pyridoxal-phosphate can be done in different biological fluids and are good surrogates for the diagnosis of these conditions. New recently discovered disorders involving synaptic architecture and metabolism, defects in glycine and glutamate specific receptors and transporters, and choline metabolism deficiencies have been published and it will be briefly presented. For these new disorders, no classical biomarkers for diagnosis are available in general, and the diagnosis relays on next generation sequencing techniques. In a clinical point of view, these diseases cause severe neurological affectations, such as movement disorders, early complex encephalopathies epileptic encephalopathies, intellectual disability, behavioral disturbances, and episodes of lethargy. Some neurotransmitter disorders are treatable conditions.

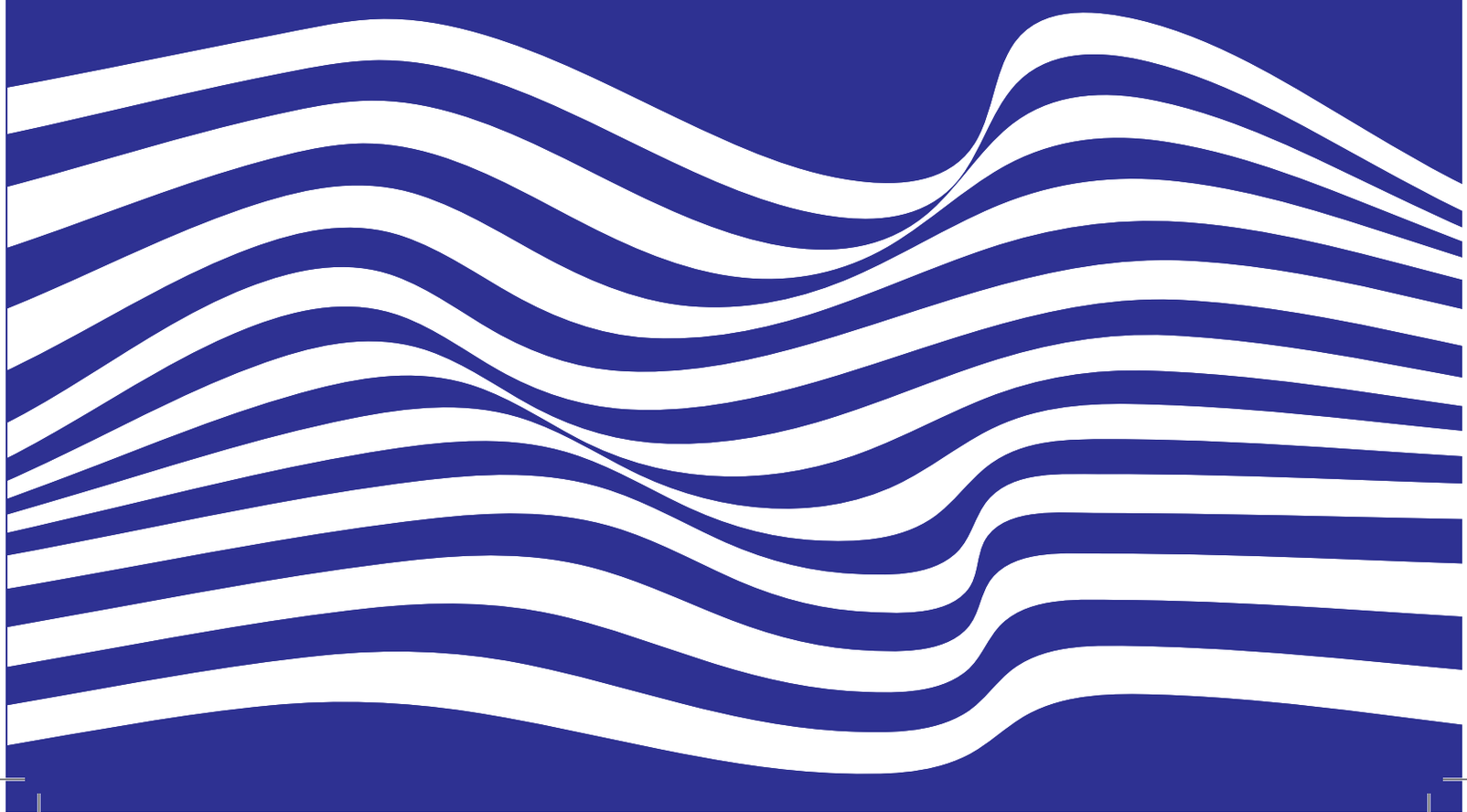
SESSION III

NEW APPROACHES TO IEM TREATMENT

CHAIRPERSONS

ANA PAULA LEANDRO,
Faculdade de Farmácia da Universidade de Lisboa - Lisboa, Portugal

ESMERALDA RODRIGUES,
Centro de Referência de Doenças Hereditárias do Metabolismo -
Centro Hospitalar Universitário São João - MetabERN, Porto, Portugal



BREASTFEEDING IN IEM

Júlio Rocha

It is well recognised the importance of breastfeeding as a strategy providing optimal nutrition for infants whereas the positive health effects on the mother cannot be dismissed. The impact of breastfeeding is not restricted to infant health and growth, but it also modulates development and reduces the risk of several diseases later in life. In line with WHO recommendations, infants should preferably be breastfed until 6 months of age.

Inborn errors of metabolism impose additional challenges since patients with these conditions have special nutritional needs. There are some conditions that result in formal contraindications for breastfeeding, like in patients with classical galactosemia. In other conditions, breastfeeding can for sure be considered, even though it requires strict control of volumes and adequate metabolic control. Although breastfeeding at the breast or from expressed breastmilk is included in management guidelines for some inborn errors of metabolism, there is no universal approach and practices vary widely depending on the type and severity of the metabolic disorder.

Anyway, due to the risk of gut dysbiosis in patients with inborn errors of metabolism, breastfeeding may be even more important for these individuals. There are already spreadsheets constructed to calculate the amounts of protein substitutes and human milk required to cover amino acid, protein, and energy needs in patients with the most important inborn errors of protein metabolism. These tools should be recognised as critically important to increase confidence of multidisciplinary teams, which ultimately may promote breastfeeding practices for these patients.

NEW OPTIONS FOR TREATMENT IN CDG

Mercedes Serrano

Congenital disorders of glycosylation are a growing and heterogeneous group of conditions with different inheritance patterns, prevalences, highly diverse clinical manifestations, and also different therapeutic approaches.

Some conditions can be successfully treated, while in other cases, the approach is symptomatic. If the diagnostic knwoledge is changing and complex, so too are the advances in therapeutics. In this talk, we will review the most recent treatments and their evidence, as well as the possibilities that the future may hold for us.

SESSION V

OVERVIEW OF SPDM PROJECTS

CHAIRPERSONS

ESMERALDA MARTINS,
Centro de Referência de Doenças Hereditárias do Metabolismo
Unidade Local de Saúde de Santo António - MetBERN, Portugal

MANUELA GRAZINA,
Faculdade de Medicina da Universidade de Coimbra - Coimbra, Portugal

IMPROVE MANAGEMENT OF MADD PATIENTS INSIGHTS AND ACHIEVEMENTS

Bárbara Henriques

Multiple Acyl-CoA dehydrogenase Deficiency (MADD, OMIM #231680), a rare disease from the group of inborn error of metabolism (IEM), is an autosomal recessively inherited disorder of fatty acid, amino acid and choline metabolism. MADD results from defects on electron transfer flavoprotein (ETF), and ETF:ubiquinone oxidoreductase (ETF:QO) proteins. These proteins are responsible for transferring electrons from at least 12 dehydrogenases to the respiratory chain, hence mutations on their genes will cause diminished mitochondria β-oxidation and impaired energy production.

In recent years the development of newborn screening programs worldwide resulted in an increased number of MADD patients being identified. Severe forms of disease result in neonatal death ,for milder forms the molecular mechanism that triggers disease symptoms is still unknown, and no effective therapy is established, thus, to make disease prognosis is highly challenging to clinicians.

An enormous gap in the field is the lack of a unifying depository for molecular and clinical data on patients, with the majority of cases found disperse in literature, and many not even reported in international journals with full access to all. In this project we propose to contribute to this societal demand, with tremendous impact in the development of new therapeutic approaches, by organizing a curated database with detailed information on mutations associated to MADD, combining molecular, cellular, and clinical data available in the literature.

In this seminar I will present ongoing experiments on ETF:QO disease variants characterization, and computational analysis of ETF an ETF:QO proteins.

IMPROVEMENT OF NEW AMINO ACID CONTENT TABLES TO BE USED AS A TOOL FOR THE TREATMENT OF PROTEIN INHERITED METABOLIC DISORDERS

Carla Vasconcelos

Determination of the aminocidogram of a vegetal and animal-based group of food and the production of tables with those foods, in what concerns their composition in proteins and amino acids and which can be included in the diet of group of individuals with protein inherited metabolic disorders, in a way of enhancing their quality of life.

The rigorous knowledge of the nutritional composition of the allowed and traditionally more consumed available foods is necessary in a way of a better adjusting the eating plans according to the patient's needs.

The use of a tool which allows to determine the quantity of the toxic amino acid ingested by the patient is considered.

The development of this tool will also allow, in a safe way, the development of appealing and tasty meals and also facilitate on following the nutritional treatment and, consequently, a good metabolic control.

A PATIENT REPORTED EXPERIENCE MEASURE (PREM) FOR QUALITY IMPROVEMENT OF PEDIATRIC INHERITED METABOLIC DISORDERS CARE

Teresa Campos

The concept of patient experience has become central to health system performance measurement and quality improvement. Patients are the most qualified to provide information about what matters to them, and about how they perceive various elements of their interactions with healthcare providers. Patient experience data can derive from qualitative information or quantitative assessment through standardised surveys, namely Patient Reported Experience Measures (PREMs).

Inherited Metabolic Disorders (IMD) are chronic and complex diseases, which lead to patients and their caregivers a high burden due to limiting symptoms and treatments, fear of acute decompensations, and need for frequent use of health services. Thus, and considering the model of patient-centered care, it becomes essential to include in the quality improvement process the considerations of patients regarding their experiences related to healthcare services. For that purpose, this study aims to design and validate a Patient Reported Experience Measure (PREM) for pediatric IMD in Portugal.

RARE DISEASES REFERENCE CENTERS NETWORK KNOWLEDGE MANAGEMENT

Sandra Mexia

This study focuses on the importance of exploring and analyzing the dynamics of the development of scientific activities in the Portuguese Reference Centers (RC) and the relationships that are established between researchers and that allow the creation and transfer of knowledge in a network.

The RCs allow to achieve advantage that can be obtained in the process of network knowledge, as they are organizations where a process of continuous education takes place that facilitates the creation and sharing of knowledge between the elements of each RC and in the national network, process that requires collaboration between the various actors that make up the national network.

Collaborative networks in health are absolutely essential for the creation and sharing of knowledge and allow us to contribute with perspectives that allow us to analyze the dynamics that occur in the network. The knowledge generated in the Networks is thus fundamental for the development of other studies, at various levels, and thus contribute to better health care.

It is intended to contribute to the discussion, among the scientific community, on the importance of knowledge creation in collaborative networks analyzing the process as it occurs and based nationally on the 6 existing CRs in Portugal for the treatment of inherited metabolic disorders and based on methodology in the social network analyze. With the identification of key actors and their role in the creation and transfer of knowledge, studying the type of interaction that occurs between them and identifying the main clusters of researchers and how interactions occur.

The main objective is to study the network of co-authorship and co-citation of, and in scientific articles published in the last six years.

Through the SNA methodology it is intended to build a model that can represent how knowledge creation occurs in national CRs based on scientific articles published since the creation of national CRs in 2016 until December 2022, in a six-year time period, it is intended to evaluate different levels of analysis (interpersonal, organizational and interorganizational) the knowledge in network.

It should be noted that, as expected, hospitals and universities are par excellence local for knowledge creation and allow their transfer and rapid use, which should be taken into account when implementing public policies.

As strengths we highlight the fact that it is a pioneering study in the national network of Reference Centers for the treatment of DHM and that through the results obtained will allow to be the basis for future studies and also to promote the work in the CR network with increased number of publications and consequently the creation and transfer of knowledge in a solid network and based on the prospects of its creation is affirmed as organizations that promote quality knowledge in health care, and with the objective of providing high quality health care and differentiation where knowledge should be added, considered as value.

ACQUIRED VITAMIN B12 DEFICIENCY IN NEWBORNS: POSITIVE IMPACT ON NEWBORN HEALTH THROUGH EARLY DETECTION

Patrícia Lipari Pinto

This study emphasizes the critical need for early diagnosis and treatment of vitamin B12 deficiency in exclusively breastfed infants, particularly those born to mothers with low levels of vitamin B12, to prevent irreversible neurological damage, megaloblastic anemia, and failure to thrive.

Highlighting the significance of early detection of asymptomatic B12 deficiency related to acquired conditions, the research underscores the importance of monitoring serum vitamin B12 levels during pregnancy. It presents demographic, clinical, dietary, and biochemical data, focusing on the evolution of functional biomarkers of vitamin B12 deficiency.

The study enrolled 12 asymptomatic, exclusively breastfed newborns (1-2 months old), identified through expanded newborn screening showing elevated methylmalonic acid and/or total homocysteine levels. Notably, all mothers followed a vegetarian diet, except for three with abnormal B12 absorption, and displayed low or borderline serum B12 and elevated plasma homocysteine levels. Oral vitamin B12 supplementation successfully restored metabolic balance in mothers, while intramuscular hydroxocobalamin injections corrected infants' metabolic abnormalities, leading to healthy outcomes.

POSTERS COMMUNICATIONS

PO 01

UNRAVELING THE GENETIC LANDSCAPE OF HEREDITARY FRUCTOSE INTOLERANCE IN GALICIA

P. Sánchez Pintos; S. Barbosa Gouveia[1,2]; B. Martin López-Pardo[1,2]; M. Vázquez-Mosquera[1,2]; Á. Hermida Ameijeiras[1,2,4]; M. Martínez Olmos[1]; M. Couce[1,2,3,4]

[1] Diagnosis and Treatment Unit of Metabolic Diseases (UDyTEMC). MetabERN. , Clinical University Hospital of Santiago de Compostela, Santiago de Compostela, Spain; [2] IDIS, IDIS-Health Research Institute of Santiago de Compostela, Santiago de Compostela, Spain; [3] Instituto Salud Carlos III, CIBERER, Madrid, Spain; [4] University of Santiago de Compostela, Santiago de Compostela, Spain;

Presenting Author - paula.sanchez.pintos@sergas.es

Main category: Case series

Disease category: Several disease categories

Introduction: Hereditary Fructose Intolerance (HFI) is a rare autosomal recessive disorder resulting from defects in the Aldolase B (ALDOB) gene, leading to impaired fructose metabolism. ALDOB gene encodes the 364-amino acid fructose-1,6-bisphosphate aldolase, a glycolytic enzyme which is involved in the breakdown of fructose mostly found in fruits. This study focuses into the specific genetic characteristics of HFI patients shedding light on the prevalence and clinical implications of endemic variants in the Galician population.

Methods: Targeted sequencing of ALDOB was performed in 12 pediatric and 8 adult patients. SWISS- Model tool was used to predict protein changes providing insights into their function damage. The clinical variables include: type of diagnosis, symptomatology, evolutionary liver function; and assessment of hepatic steatosis.

Results/Case report: Genetic analyses revealed a high prevalence of the c.448G>C and c.360_363delCAAA variants in the ALDOB gene among all the Galician patients diagnosed with HFI, representing 45% (18/40) and 42.5% (17/40,) respectively. Additionally, two different CNVs deletions were identified: exons 2 to 6 in 3 patients and a novel deletion of the complete gene (E1_E9) in compound heterozygosity in one patient. In 2 patients the diagnosis was established by family study prior to the introduction of dietary fructose; and in 18 patients as a result of clinical symptoms (in 7/20 due to diarrhea related with sugar or fruit intake, in 6/20 due to recurrent hypoglycemia accompanied by ketoacidosis episodes in 3; due to hypoactivity episodes related to fruit intake (8/20) or to immunization with vaccine that includes sucrose (1/20) and in 2/20 due to liver dysfunction). Evolutionarily, 12 patients maintain intermittent gastrointestinal manifestations and 13 develop hepatic steatosis and 1 adult mild fibrosis despite good dietary adherence. All maintain normal values of transaminases after diagnosis. When comparing the 2 most frequent variants in homozygosity, the frequency of hypoglycemia and diarrhea at diagnosis (p:0.016) and evolutionarily hepatic steatosis is higher in patients with c.448G>C variant.

Conclusion: The prevalence of c.448G>C variant is high in Galician population, in accordance with previous European studies, although with a lower frequency (45%) than those reported (61 – 67.4%)(1-3). Despite the lack of a clear genotype-phenotype correlation, the homozygous variant c.448G>C seems to have greater hepatic developmental severity and is associated with higher frequency of diarrhea related to fructose consumption ($p<0.05$). Furthermore, molecular modeling revealed that both c.448G>C and c.360_363delCAAA variants were associated with altered enzyme activity, providing mechanistic insight into its pathogenicity. Understanding the functional impact of these variants enhances our comprehension of HFI at a molecular level, facilitating the development of targeted therapeutic intervention. The homogeneity of the identified variants highlights the importance of region-specific genetic studies to tailor diagnostic-approaches effectively.

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PO 02

LONG-TERM STORAGE OF FREEZE-DRIED HUMAN PHENYLALANINE HYDROXYLASE

R. R Lopes; P. R Lino[1]; J. Leandro[1]; M. P Amaro[1]; P. Sousa[2]; J. B Vicente[3]; A. J Almeida[4]; P. Leandro[1]

[1] Metabolism, Genetics and Proteins in Health & Disease Group, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal; [2] Indústria Química e Farmacêutica SA, Sofarimex, Agualva, Portugal; [3] Universidade Nova de Lisboa, Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal; [4] Advanced Technologies for Drug Delivery Group, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal;

Presenting Author - lopes.r.raquel@gmail.com

Main category: Basic science study

Disease category: Intermediary metabolism: energy

Introduction: Freeze-drying is frequently used for biopharmaceutical formulation for its capacity to preserve the structure/function of proteins during storage. To minimize protein misfolding and/or aggregation that may occur during the harsh conditions associated with freeze-drying (chemical/physical stresses), stabilizing compounds are usually included. In this work we studied human phenylalanine hydroxylase (hPAH), the enzyme involved in phenylketonuria. We tested 9 different additives for the hPAH freeze-drying process and followed the activity of the lyophilized protein during 1-year storage at 4 oC.

Methods: hPAH homotetramers were lyophilized (LyoGamma 15) in the absence and presence of additives (5 and 10%; w/v). Enzyme activity, oligomeric profile, thermal stability, and L-Phe binding were determined before, immediately after lyophilization and upon 1-year storage (4oC).

Results/Case report: The tested additives included polyols (glycerol and mannitol), amino acids (glycine and arginine) and carbohydrates (glucose, sucrose, trehalose and melibiose). In the absence of additives lyophilized hPAH tetramers presented only 5% of initial activity. Mannitol and glycerol were unable to maintain hPAH tetramers functional state (0-24% activity) upon freeze-drying, as well as arginine and glycine (7-28% activity) and sucrose (~40% activity). Almost no change in catalytic activity (~100%) was observed for the solutions containing 5% glucose, 5% trehalose and 10% melibiose. Upon 1-year storage of the lyophilized protein, at 4 oC, reconstituted hPAH maintained its activity (≈100%), presented a slightly lower L-Phe activation ratio and an increase in the apparent binding affinities for L-Phe. Importantly no aggregates were observed.

Conclusion: Development of stable freeze-dried proteins requires not only optimization of the freeze- drying process but also identification of the suitable stabilizer agent. In the present work, the tested additives, particularly carbohydrates conferred protection from degradation and loss of the hPAH native state.

The analysis of protein functionality during storage of freeze-dried samples (1-year, at 5 ± 3 oC) allowed us to conclude that the tested additives conferred an excellent protection from degradation and loss of the hPAH native state and biological activity during this long-term storage period.

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PO 03

KRABBE DISEASE: 24-YEAR EXPERIENCE OF A NATIONAL METABOLIC DISEASE CENTER

I. Serra Nunes[1,2]; C. Caseiro[3]; I. Ribeiro[2,3]; S. Figueiroa[4]; C. Garrido[4]; D. Quelhas[2,3]

[1] Serviço de Genética Médica, Clínica de Genética e Patologia, , Centro de Genética Médica Jacinto de Magalhães, Unidade Local de Saúde de Santo António, Porto, Portugal; [2] ICBAS— Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, UMIB— Unidade Multidisciplinar de Investigação Biomédica; ITR—Laboratory for Integrative and Translational Research in Population Health., Porto, Portugal; [3] Laboratório Bioquímica Genética, Serviço de Genética Laboratorial, Clínica de Genética e Patologia,, Centro de Genética Médica Jacinto de Magalhães, Unidade Local de Saúde de Santo António, Porto, Portugal; [4] Serviço de Neuropediatria, Centro Materno-Infantil do Norte, Unidade Local de Saúde de Santo António, Porto, Portugal;

Presenting Author - isabelserranunes@gmail.com

Main category: Case series

Disease category: Complex molecule and organelle metabolism

Introduction: Krabbe disease (KD) is a rare autosomal recessive neurodegenerative disorder. KD has a wide phenotypic spectrum ranging from infantile-onset (before 12 months of age) to late-onset (after 12 months of age and into the seventh decade of life). Krabbe patients, with or without symptoms, have reduced GALC enzyme activity and biallelic GALC pathogenic variants. The authors present a review of KD diagnosed in our National Metabolic Disease Centre during the last 24 years.

Methods: Review of electronic medical and laboratory data of KD patients diagnosed and followed in our centre from 2000 to 2023. Moreover the authors present a phenotypic characterization and literature review.

Results/Case report: ***Case 1:*** Fifty-two-year-old man with progressive spastic paraparesia. Onset in early twenties due to frequent falls. GALC deficiency confirmed KD. GALC sequencing revealed two variants: c.927A>C (p.L309F) and c.1630G>A (p.A544N).

Case 2: Twenty-nine-month-old girl with motor regression. Cerebral MRI showed leukodystrophy. GALC activity was undetectable and two biallelic variants were found in GALC gene: c.884A>T (p.N295I) and c.927A>C (p.L309F). The patient deceased 18 months later.

Case 3: Ten-year-old healthy girl sibling of case 2. Reduced GALC activity and the same genotype confirmed KD. The girl underwent bone marrow transplantation. Five years later is asymptomatic with normal enzyme activity.

Case 4: Five-month-old boy with hypotonia and irritability, showing leukodystrophy in cerebral MRI. GALC deficiency and a 30 kb deletion in homozygoty confirmed KD. Deceased at 15 months.

Conclusion: From the four KD patients here presented, most were later-onset diagnosis. The two siblings distinct clinical phenotype are an example of the lack of genotype-phenotype correlation reported in literature. It is not yet fully understood which factors are responsible for this phenotypic heterogeneity, but environmental insults or variant effects, such as alternative splicing are some of the hypothesis. KD treatment options are limited to supportive mesures in symptomatic patients and bone marrow transplantation disease-modifying therapy in asymptomatic patients.

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PO 04

UNDERSTANDING THE ROLE OF ACYLATIONS ON FATTY ACID OXIDATION ENZYMES

B. Henriques[1]; R. Domingos[1]; J. Ribeiro[1]

[1] Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal;

Presenting Author - bjhenriques@fc.ul.pt

Main category: Basic science study

Disease category: Several disease categories

Introduction: Metabolic regulation encompasses a complex interplay of genomic, proteomic, and metabolomic adjustments within cells. Non-enzymatic post-translational modifications (PTMs) known as acylations, such as succinylation and glutarylation, have emerged as important regulators in mitochondrial enzymes [1]. The extent of acylations is closely associated with the accumulation of metabolites such as succinyl-CoA and glutaryl-CoA that occur under certain conditions such as fasting, caloric restriction or in several metabolic disorders, creating an unique scenario for anomalous protein acylation [2].

Methods: Exploring this concept which suggests that enzymes involved in pathways that handle these metabolites are more prone to protein acylation, we combine biochemical and biophysical techniques to address acylations impact on structure and function of the Medium Chain Acyl-dehydrogenase (MCAD) enzyme.

Results/Case report: It has been recently shown that the enzyme glutaryl-CoA dehydrogenase (GCDH), which participates in amino acid catabolism, is prone to high levels of glutarylation due to an increase in glutaryl-CoA production stimulated by lysine catabolism, and this modification diminishes enzyme activity [3]. Moreover, it was demonstrated that this acylation was regulated by sirtuin5 (Sirt5). We have also showed that ETF (electron transfer flavoprotein) is prone to succinylation, and the modification abolish protein activity. Therefore, it is important to address if this also affect other dehydrogenases involved in fatty acid oxidation, such as MCAD.

Conclusion: Our results indicate that purified MCAD is easily succinylated and glutarylated in the test tube. While glutarylation did not impact enzyme activity, succinylation increased it; with both not showing major differences in protein structure or stability. Further, Sirt5 incubation reverts succinylation and brings function to native levels.

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PO 05

INKT CELLS IN FABRY DISEASE PATIENTS: FROM BENCH TO BEDSIDE

I. Mondragão-Rodrigues[1,2]; M. Miranda[3]; C. Meireles[4]; J. Oliveira[5,6]; M. Macedo[1,7]

[1] Cell Activation and Gene Expression Group, Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal; [2] Doctoral Program of Biomedical Sciences, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Porto, Portugal; [3] Immunobiology Group, Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal; [4] Translational Cytometry Facility, Instituto de Biologia Molecular e Celular (IBMC) and Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal; [5] Nephrology & Infectious Diseases R&D group, Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal; [6] Service of Medical Genetics, São João University Hospital Centre (CHUSJ), Porto, Portugal; [7] Medical Sciences Department, University of Aveiro, Aveiro, Portugal;

Presenting Author - imrodrigues@i3s.up.pt

Main category: Translational science study

Disease category: Lipid metabolism and transport

Introduction: Fabry disease (FD) is a rare X-linked hereditary lysosomal storage disease caused by deficient activity of the lysosomal enzyme α-galactosidase (α-Gal), resulting from pathogenic variants in its gene (GLA). The clinical phenotype ranges from a classical, more severe early-onset form with multi-organ involvement, to a milder later-onset form, predominantly affecting the heart. Invariant Natural Killer T (iNKT) cells are lipid specific CD1d reactive T lymphocytes with a restricted TCR repertoire. These cells play important roles in cancer, auto-immunity and infection.

Methods: This work aims to study in detail the iNKT cell population in a diverse group of FD patients. Blood samples from FD and control (CO) subjects were characterized through flow cytometry equipped with spectral technology (Cytek Aurora).

Results/Case report: It has been reported that in α-Gal knockout mice, iNKT and CD4+ iNKT cells are decreased (1). In addition to that, it was shown by our group, that late-onset FD have a blood frequency of iNKT cells similar to healthy subjects and, similarly to mice, a decreased percentage of CD4+ iNKT cells (2). The preliminary results of this work show that FD have a significant increase in iNKT CD4-CD8α- cells' frequency (CO:21%±14; FD:42%±14), a tendency for a decrease in iNKT CD4+ cells (CO:36%±24; FD:22%±18) and no alteration in iNKT CD8α+ cells (CO:31%±22; FD:32%±17). Both in FD and CO, the iNKT CD8α+ are seen to be αα homodimer (CO:70%±16; FD:92%±12) in contrast to CD3+CD1d-cells CD8α+ that are majorly αβ heterodimer (CO:92%±6; FD:93%±5). Interestingly, FD patients iNKT CD8α+ cells have a higher percentage of αα when compared to CO.

Conclusion: Currently, we are recruiting additional patients to allow us to transpose and correlate alterations in the immunological system with the biochemical and clinical aspects of the patients.

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PO 06

INVARIANT NATURAL KILLER T CELLS IN A-GALACTOSIDASE KNOCKOUT MICE

I. Mondragão-Rodrigues[1,2]; L. Matias[1,3]; C. Meireles[4]; M. Macedo[1,5]

[1] Cell Activation and Gene Expression Group, Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal; [2] Doctoral Program of Biomedical Sciences, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Porto, Portugal; [3] Chemistry Department, University of Aveiro, University of Aveiro, Aveiro, Portugal; [4] Translational Cytometry Facility, Instituto de Biologia Molecular e Celular (IBMC) and Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal; [5] Medical Sciences Department, University of Aveiro, Aveiro, Portugal;

Presenting Author - imrodrigues@i3s.up.pt

Main category: Basic science study

Disease category: Lipid metabolism and transport

Introduction: Fabry Disease (FD) is a rare genetic lysosomal storage disease caused by mutations in the GLA gene, that encodes the enzyme α-Galactosidase (α-Gal) responsible for catalysing globotriaosylceramide (Gb3). In young α-Gal knockout (KO) mice there is accumulation of Gb3 and Invariant natural killer T (iNKT) cells are reduced in spleen, thymus and liver. iNKT cells are lipid reactive, CD1d-restricted T cells, expressing semi-invariant TCR. This work aims to characterise in- depth iNKT cells in α-Gal KO mice, in the already mentioned organs and extend this analyse to lymph nodes (LN) and blood.

Methods: Herein, α-Gal KO mice and control wild type (WT) littermates with B6.129 background aged between 10-12 months were used. Blood, inguinal LN, spleen, liver and thymus were retrieved, processed, stained and the cells characterize through flow cytometry equipped with spectral technology (Cytek Aurora).

Results/Case report: When comparing α-Gal KO and WT mice, there is a tendency for lower iNKT cell frequency in α-Gal KO mice in all organs except thymus. We also found decreased CD4+ iNKT cells in Fabry mice's liver and spleen compared to WT mice. This tendency for lower CD4+ iNKT cells was also seen in the thymus and blood of α-Gal KO. In mice, iNKT cells are characterized as either CD4+ or negative for both CD4 and CD8 (Double negative, DN), but typically do not express CD8 (1,2). Our results in aged mice show a surprising population of CD8+ iNKT cells in the peripheral organs of all animals analysed regardless of the mice's genotype (WT / α-Gal KO) (LN: 48%±14/ 35%±15; blood: 79%±24/ 84%±4; spleen: 8%±5/ 18%±10; liver: 51%±16/ 65%±14; thymus: 2%±2/ 2%±1). Furthermore, these CD8+ iNKT cells were found to be predominantly CD8αβ with a variable TCR Vβ chain.

Conclusion: Ongoing experiments are being carried out to further understand the biology of mice CD8+ iNKT cells and explore differences between α-Gal KO and WT mice.

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PO 07

MONOALLELIC ETFDH VARIANTS ASSOCIATED WITH VARIABLE ACYLCARNITINE PROFILES IN A FAMILY

M. Lopes Pereira[1]; D. Quelhas[2,3,4]; I. Ribeiro[2,3]; C. Azevedo Soares[1,3,4,5,6,7]; R. Sousa Martins

[1] Serviço de Genética Médica, Centro de Genética Médica Dr. Jacinto Magalhães, Unidade Local de Saúde de Santo António, Porto, Portugal; [2] Unidade de Bioquímica Genética, Centro de Genética Médica Dr. Jacinto Magalhães, Unidade Local de Saúde de Santo António, Porto, Portugal; [3] Unit for Multidisciplinary Research in Biomedicine, Instituto de Ciências Biomédicas Abel Salazar/Universidade do Porto, Porto, Portugal; [4] Centro de Referência de Doenças Hereditárias do Metabolismo, Unidade Local de Saúde de Santo António, Porto, Portugal;[5] Departamento de Microscopia, Instituto de Ciências Biomédicas Abel Salazar/Universidade do Porto, Porto, Portugal; [6] Departamento de Ciências Médicas, Universidade de Aveiro, Aveiro, Portugal; [7] i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [8] Serviço de Medicina Interna, Unidade Local de Saúde de Santo António, Porto, Portugal;

Presenting Author - u15468@chporto.min-saude.pt

Main category: Case report

Disease category: Lipid metabolism and transport

Introduction: Biallelic deleterious variants in ETFDH are causative of multiple acyl-CoA dehydrogenase deficiency (MADD) an inborn error of mitochondrial fatty acids beta-oxidation. MADD is included in the expanded newborn screening with certain acylcarnitine species being a biomarker. Monoallelic deleterious variants are associated with MADD´s carrier status, with some carriers presenting normal to near normal acylcarnitines. Here we report a family of patients with a monoallelic pathogenic ETFDH variant, with an acylcarnitines profile similar to MADD patients' profile.

Results/Case report: After the initial diagnosis of MADD in a female patient, we identified the frameshift pathogenic variant c.1648_1649del p.Leu550Valfs*4 in ETFDH in heterozygosity in her mother. At the time of her daughter's diagnosis, mother's acylcarnitines profile was similar to MADD patients (increased C6, C8, C10, and C12) that normalized with riboflavin supplementation, except for C8. Furthermore, the mother´s paternal half-sister, also heterozygous for the familial variant, exhibited an acylcarnitine profile suggestive of MADD. The patient's maternal grandfather, who was also heterozygous for the familial variant, succumbed to massive rhabdomyolysis and hepatic failure during a COVID-19 infection at the age of 66. The clinical presentation was disproportionately severe for a COVID-19 infection, raising suspicion about the contribution of the familial variant.

Conclusion: This case underscores the complexity of interpreting the clinical significance of the heterozygous state for MADD contributing to growing suspicion of clinical relevance. Further studies are needed to elucidate appropriate recommendations for managing heterozygous.

PO 08

ARTIFICIAL INTELLIGENCE-ASSISTED MISSENSE VARIANT RECLASSIFICATION IN A PATIENT WITH BONE FRAGILITY

D. Nogueira[1,6;] A. Bandeira[2,3]; C. Azevedo Soares[1,3,4,5,6,7]

[1] Serviço de Genética Médica , Centro de Genética Médica Dr. Jacinto Magalhães, Unidade Local de Saúde Sto António, Porto , Portugal; [2] Serviço de Pediatria, Centro Materno-Infantil do Norte, Unidade Local de Saúde Sto António, Porto, Portugal; [3] Centro de Referência de Doenças Hereditárias do Metabolismo, Unidade Local de Saúde Sto António, Porto, Portugal; [4] Unit for Multidisciplinary Research in Biomedicine, Universidade do Porto/Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal; [5] Departamento de Ciências Médicas, Universidade de Aveiro , Aveiro, Portugal; [6] Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal; [7] i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;

Presenting Author - diogonogueira00@gmail.com

Main category: Case report

Disease category: Cofactor and mineral metabolism

Introduction: Artificial intelligence (AI)-based algorithms are increasingly being used to reclassify genetic variants and integrate them into comprehensive classification systems. AlphaFold is an AI protein structure prediction tool that serves as the primary source for missense classification algorithms AlphaMissense and AlphScore. We applied the AlphaMissense to a variant of unknown significance (VUS) in COL1A2 identified in a patient with an atypical presentation of osteogenesis imperfecta.

Methods: A COL1A2 variant was classified using American College of Medical Genetics and Genomics 2015 criteria. AlphaMissense predictions were applied to this variant of unknown significance identified in our patient.

Results/Case report: A ten year old male patient presented bone fragility with fractures in the spine with shortening of the vertebrae and grayish sclerae. Multigene panel identified a heterozygous variant, c.757G>T p.(Gly253Cys), in COL1A2, classified as VUS. His mother with bone fragility also had the same variant. COL1A2 is associated with osteogenesis imperfecta (OI), but given the atypical predominance of vertebral bone fragility, not usual observed in OI, a final diagnosis for this family was not clear.

AlphaMissense classified the familial variant as deleterious (moderate) with a high score: 0.977, raising the suspicion of a deleterious effect of the missense variant. AlphScore predictions were not availale for this protein.

Conclusion: AI algorithms like AlphaFold-driven missense variant predictors hold promise for elucidating genetic disorders in clinical practice. While challenges like validation and optimization remain, AI has the potential to revolutionize patient care by enabling faster, more accurate diagnoses and, potentially, more personalized treatment.

PO 09

NEURODEGENERATIVE GENETIC DISEASES: THE BEGINNING OF A NEW PUZZLING ODYSSEY

M. Rodrigues[1,2,3,4]; O. Amaral[5,6]; S. David[1]

[1] UID, Departamento de Genética Humana, UID-Genética Humana das Doenças Infeciosas, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa., Lisboa, Portugal; [2] Patologia Clínica – Lab. Bioq. Genética/Endocrinologia Especial, Hosp. D. Estefânia, CHULC, Lisboa., Lisboa, Portugal; [3]BioISI— Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Portugal., Lisboa, Portugal; [4]Faculdade de Ciências e Tecnologia de Lisboa, FCUL _ Departamento de Química e Bioquímica, Lisboa., Lisboa, Portugal; [5] Departamento de Genética Humana, UID - Doenças Lisossomais de Sobrecarga, Instituto Nacional de Saúde Doutor Ricardo Jorge, Porto, Lisboa, Portugal; [6]Centro de Estudos de Ciência Animal (CECA, ICECA), Universidade do Porto, Portugal., Lisboa, Portugal;

Presenting Author - m.rosario.rodrigues@insa.min-saude.pt

Main category: Basic science study

Disease category: Intermediary metabolism: nutrients

Introduction: Gaucher disease (GD) is the most frequent lysosomal storage disease in Portugal, and it is caused by a deficiency in lysosomal glucocerebrosidase. The biallelic variants of the glucocerebrosidase gene (GBA1; 1q21), which codifies lysosomal β-glucosidase (Gcase) and is responsible for the glucosylceramide (GLcCer) degradation, is the most common cause of the disease. GD is generally categorized into three major forms: non-neuropathic type 1; early onset and rapid neuropathic degeneration type 2; and late onset and slower course type 3.

Methods: Although the majority of Portuguese patients present non-neuropathic form (t1), some patients develop atypical symptoms, namely the appearance of neuropathic findings in patients originally diagnosed as type 1. One of the most common neurological associations in type1 patients is Parkinson's disease

Results/Case report: Patients with GD type 1 and carriers of GBA1 mutations are at risk for the development of parkinsonian manifestations. However, the recent recognition of the association of GD with the development of Parkinsonism, defies this classification. These atypical cases demonstrate that there`s a continuum in the phenotype of GD patients and a mixture of clinical characteristics that can lead to the so-called diagnostic odyssey. We believe that the primary reason for the development of GBA1 associated PD is endoplasmic reticulum misfolding of GCase and the activation of the Unfold Protein Response (UPR), changes in the plasmatic membrane and the endosome secretion, all of which lead to degeneration and inflammation of the dopaminergic cells, associated with lysosomal- autophagic dysfunction and eventual death.

Conclusion: The transport of lysosomal proteins is made through vesicles, requiring the elucidation in biomarkers, and the mechanisms. In the current investigation, we hypothesise that these atypical phenotypes may disclose the association of genetic or biochemical variants that may allow a more precise diagnosis. The first objective of the present doctoral work is to establish collaboration with medical reference centers to investigate clinical cases for the existence of genetic and biochemical markers and to raise awareness to this issue. The corresponding workflow will be presented and discussed.

PO 10

FUNCTIONAL AND STRUCTURAL RESCUE OF THE PREVALENT PATHOGENIC P.K329E MCAD VARIANT WITH HIT MOLECULES IDENTIFIED BY VIRTUAL SCREENING

C. A. Madeira[1]; C. A. Bonito[2]; F. V. Ventura[1]; R. J. Ferreira[3]; J. B. Vicente[4]; P. P. Leandro[1]

[1] Pharmaceutical Sciences and Medicines, Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; [2]Chemistry and Biochemistry, LAQV@REQUIMTE, Faculty of Sciences, Universidade do Porto, Porto, Portugal; [3]Computational Chemistry, Red Glead Discovery, Lund, Sweden; [4]Biological Chemistry, Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal

Presenting Author - catarinalexmad@gmail.com

Main category: Basic science study

Disease category: Lipid metabolism and transport

Introduction: Medium chain acyl-CoA dehydrogenase (MCAD) deficiency is caused by ACADM mutations, the most common being c.985A>G (p.K329E) [1]. Upon MCA dehydrogenation, electrons are transferred from the FAD cofactor to the electron transferring flavoprotein (ETF), thus feeding the respiratory chain. MCAD/ETF interact through MCAD α-helices C and D and ETFβ recognition loop (RL) [2].

ETFβ-RL derived peptides, previously shown to stabilize p.K329E, were used to develop a new fingerprinting function to score a virtual screening campaign and five small molecules (SMol) with structural similarity were found.

Methods: The effect of the molecules on recombinant wild-type and p.K329E MCAD activity and stability was characterized by studying the thermal stability, proteolysis susceptibility, enzymatic activity using artificial (phenazine methosulfate) or natural ETF electron carriers and catalytic activity recovery.

Results/Case report: From the studied SMol, SMol4 presented the most promising results regarding p.K329E MCAD structural stability, increasing its melting temperature (Tm) by 2.15 oC, without significantly interfering with the wild-type Tm. SMol4 also increased p.K329E resistance to limited proteolysis by at least ~45%. Four of the tested SMol increased the p.K304E MCAD catalytic activity after 10-min incubation at 37 °C (SMol2, SMol3 and SMol5) and 42 °C (SMol3, SMol4 and SMol5). The natural electron acceptor ETF was used to characterize the effect of SMol on the p.K329E variant function and to confirm that they do not interfere with the MCAD/ETF interaction. The presence of SMol1 and SMol2 prompted an increase in enzymatic activity when compared with the artificial electron acceptor PMS (52% and 55%, respectively).

Conclusion: Following the previous results on the ability of peptides derived from ETFβ-RL to rescue p.K329E stability and function, small molecules are advantageous and can overcome the constraints of peptides as therapeutic agents. The identified small molecules showed the potential to protect p.K329E stability and activity. Additional studies on the MCAD/SMol interaction mechanisms needs to be performed, as well as their effect on the cellular context, to confirm these molecules as hit compounds for the development of the first pharmacological therapy for MCAD deficiency.

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PO 11

ENHANCING DIAGNOSTIC ALGORITHMS FOR GM2 GANGLIOSIDOSES THROUGH BIOCHEMICAL ANALYSIS

A. Mendonça[1,2]; C. Caseiro[1]; I. Ribeiro[1,2]; E. Pinto[1]; S. Rocha[1]; H. Ribeiro[1]; C. Ferreira[1]; E. Silva[1]; F. Laranjeira[1,2]; L. Lacerda[1,2]; I. Carrilho [3], A. Bandeira [3,4], D. Quelhas[1,2]

[1] Biochemical Genetics Laboratory, Laboratory Genetics Service, Centro de Genética Médica Jacinto Magalhães (CGMJM), Genetics and Pathology Department, Unidade Local de Saúde de Santo António (ULSSA), [2]Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, [3] Serviço de Neuropediatria, Centro Materno-Infantil do Norte, ULSSA, [4] Unidade de Doenças Hereditárias do Metabolismo do Serviço de Pediatria, Centro Materno- Infantil do Norte, ULSSA.

Presenting Author - abreuana2007@gmail.com

Main category: Case report

Disease category: Lipid metabolism and transport

Introduction: GM2 gangliosidoses are lysosomal disorders due to GM2-ganglioside accumulation caused by hexosaminidases (HEX) or GM2 activator protein deficiency. Three GM2 have been described: a)Tay-Sachs disease (TSD) - includes B variant (absent Hexosaminidade A (HEXA) activity) and TSD B1 variant (HEXA inactive for sulphated substrate) b)Sandhoff disease (SD) (HEXA and B deficiency) and c)AB variant (HEXA and B normal, but defective GM2 activator protein). Central nervous system dysfunction is the hallmark of GM2 patients (1) (2). Biochemical studies are crucial to distinguish these three Gangliosidosis.

Methods: Five GM2 patients diagnosis required total HEX and HEXA activity in plasma and leukocytes,using 4-methylumbelliferyl-N-acetylglucosaminide (MUG) as substrate. Specific HEXA activity used sulphated MUG (MUGS) as substrate. HEXA, HEXB and GM2A genes sequencing complemented the biochemical findings.

Results/Case report: ***Pt 1:*** 7 months old boy, cherry red spot, pyramidal symptoms. Reduced HEXA, total HEX activity and abnormal electrophoresis. Homozygous pathogenic variant in HEXB, confirmed SD.
Pt 2 and 3: Two siblings, 11 and 4 years girls, with neurodegenerative phenotype. Both with reduced HEXA activity, normal total HEX, but abnormal electrophoresis. The two siblings have the same pathogenic variants in HEXA, confirming TSD.
Pt 4: 7 years old boy, with psychomotor regression. Low HEXA activity with MUGS, normal total HEX activity, and abnormal electrophoresis with MUGS. Homozygous pathogenic variant in HEXA, confirmed GM2 gangliosidosis B1 variant.
Pt 5: 2 years old girl, epilepsy, cherry-red spot, psychomotor regression. Total hexosaminidases and hexosaminidase A were normal as well as electrophoresis. Presence of a homozygous pathogenic variant in GM2A, confirmed GM2 gangliosidosis AB variant.

Conclusion: This cohort exhibits the three GM2 gangliosidoses reported in literature. Cerebellar atrophy, cherry-red spot and developmental regression are criteria to considering GM2 laboratorial diagnosis. Currently, no curative treatment is available; therefore, genetic counselling to provide prenatal diagnosis/preimplantation options is of extreme relevance for affected families. Innovative therapies are necessary and several clinical trials, namely enzymatic replacement therapy, pharmacological chaperone, hematopoietic stem cells transplantation and gene therapy (3) are currently underway.

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PO 12

EXAMINING HOW ENZYME REPLACEMENT THERAPY AFFECTS THE INVARIANT NATURAL KILLER T CELLS IN INDIVIDUALS WITH ACID SPHINGOMYELINASE DISEASE

C. Andrade[1,5]; M. Cardoso[3]; A. Guimas[4]; I. Mondragão-Rodrigues[5]; P. Chaves[3]; T. Fonseca[5]; F. Macedo[2,5]

[1] Chemistry, Universidade de Aveiro, Aveiro, Portugal; [2] Medical Science, Universidade de Aveiro, Aveiro, Portugal; [3] Inherited Metabolic Disorders Reference Centre, CHUSJ, Porto, Portugal; [4] Internal Medicine Service, CHUSA, Porto, Portugal; [5] Cell Activation and Gene Expression, i3S, Porto, Portugal;

Presenting Author - catarinaandradejp@hotmail.com

Main category: Translational science study

Disease category: Lipid metabolism and transport

Introduction: Acid sphingomyelinase deficiency (ASMD) is a rare lysosomal disease caused by SMPD1 gene mutations, leading to sphingomyelin accumulation. Sphingomyelin restrains the activation of invariant Natural Killer T cells (iNKT), crucial for immune regulation. Consequently, upon this accumulation in ASMD mice and patients, there is a decrease in iNKT cell frequency [1], which in mice can be prevented by Enzyme Replacement Therapy (ERT). ERT is currently available for the visceral form of ASMD.

Methods: This study recruited two male Portuguese ASMD patients undergoing ERT with an escalating dosage that reached the full dose in week 14. A male patient with 67 years, asplenic, with diffuse interstitial lung disease with an important lung compromise and a male patients with 52 years with splenomegaly.

Results/Case report: Both patients reached the full ERT dose at 3 months of treatment. One of the patients interrupted ERT after reaching the full dose, the 52 years old patient continued ERT and was analyzed 3 months after the maximum ERT dosage. At that time we were able to observe normalization in platelet counts and improvement in biomarkers, namely Lyso SM-509. The immunological analyses show no significant change in iNKT cells frequency or phenotype, in both patients.

Conclusion: Our current results indicate that no effects on iNKT cells are seen until maximum ERT dosage is reached, hinting at a possibly still inadequate time of enzyme to allow changes in iNKT cells, or that disease progression is already too advanced for reversal of iNKT cell defect with ERT. Importantly the patient maintained under ERT show improvement of the metabolic parameters. While the presented results span only for 6 months post-ERT initiation, we plan to extend this research for over a year, while emphasizing the need for a larger recruitment of patients to enrich our analysis further.

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PO 13

CLINICAL VARIABILITY IN A FAMILY WITH SIALURIA

C. Gómez Vieites[1]; P. Sánchez Pintos[1]; B. Martín López-Pardo[1]; S. Barbosa Gouveia[1]; M. Couce Pico[1]

[1] Pediatrics, Hospital Clínico Universitario Santiago de Compostela, Santiago de Compostela, Spain

Presenting Author - carmen.gomez.vieites@sergas.es

Main category: Case series

Disease category: Intermediary metabolism: others

Introduction: Sialuria is an ultra-rare autosomic dominant disease due to variants in GNE gene, that encode the bifunctional enzyme UDP-N-acetylglucosamine 2-epimerase/N-Acetylmannosamine Kinase. That produces failures in allosteric negative feedback, which initiates the biosynthesis of sialic acid, causing cytoplasmic accumulation and increased excretion in urine. There are <20 cases described, all characterized by slightly infiltrative facies, prolonged neonatal jaundice, hepatomegaly, repeated respiratory infections, developmental delay and neurological symptoms.

Methods: We describe three sisters with identical genotype and different clinical expressiveness.

Results/Case report: Very preterm twin studied for elevated sialic acid (SA) in urine (6374µmol/mmol create; normal:20.5-224.5) in NBS. She showed epicantus, hypertelorism, wide nose with low-set ear, mild truncal hypotonia. Hepatomegaly with diffuse increase echogenicity; multiple periventricular white matter signal alterations were observed. Genetic study revealed heterozygous pathogenic variant in GNE gene (c.G797A). Family study found twin sister and firstborn diagnosed with sialuria, sharing similar features and elevated SA urinary excretion with the same genotype. Mother had slightly increased SA excretion but molecular study was negative in blood, saliva and urine. The twins had neonatal jaundice and later developed macrocephaly and global developmental delay. The older sister displayed mild symptoms and mild neurological involvement. Hepatomegaly and slight transaminase elevation persisted in all.

Conclusion: Our case series confirms the favorable evolution of developmental delay in these patients and demonstrates in a novel way the genotype-phenotype variability in this pathology. The maternal biochemical alteration suggests a germline mosaicism that could not be confirmed.

PO 14

USE OF CIPAGLUCOSIDASE ALFA IN INFANTILE-ONSET POMPE DISEASE: A CASE REPORT

C. Oliveira[1]; S. Dias[1]; P. Janeiro[2]; F. Cosme Silva[1]; S. Nobre[1]; A. Gaspar[2]; J. Cruz[1]

[1] Pharmacy Services, ULS Santa Maria, Lisbon, Portugal; [2]Department of Paediatrics, Metabolic Diseases Unit, ULS Santa Maria, Lisbon,Portugal;

Presenting Author - catarina.mb.oliveira@gmail.com

Main category: Case report

Disease category: Several disease categories

Introduction: Pompe disease is an IEM caused by a deficiency of the enzyme acid alpha-glucosidase, leading to glycogen accumulation. Infantile-onset Pompe disease (IOPD) is a severe form characterized by hypertrophic cardiomyopathy (HCM), hypotonia and respiratory failure. Recently, a new form of ERT with increased cellular uptake, cipaglucosidase alfa has been developed, in combination with miglustat, which increases its half-life and distribution. There are no published studies on the use of this ERT in patients with IOPD.

Methods: We describe the case of a 7-year-old girl, with a severe form of IOPD, diagnosed at 3 months old, presented with HCM, tetraparesia with scarce spontaneous movements, poor facial expression, preserved cognitive and social capacity, and invasive ventilation via tracheostomy.

Results/Case report: At 6 months, alglucosidase was started and escalated to 40mg/kg every 2 weeks, with marked improvement in HCM but no gain in motor function. At the age of 7 y, cipaglucosidase alfa with miglustat was requested with the expectation of stabilising the motor and respiratory symptoms. Before the first administration, a paediatric pharmaceutical consultation was held, with the parents and nurse team, to assist with administration of the enzyme, and the preparation and administration of the extemporaneous oral miglustat solution. Cipaglucosidase alfa (30 mg/kg, every 2 weeks) and miglustat (dose adjusted to age and weight) have been administered for 16 months. Currently, the patient shows voluntary oscillatory upper limb movements, which were previously absent and greater respiratory autonomy. There was a single episode of local allergic reaction with mild edema, resolved with antihistamines.

Conclusion: This new form of ERT has achieved improvements in motor and respiratory function, compared with the baseline. So far, it has proven to be well-tolerated. The pharmacist team has been crucial in guaranteeing good manufacturing practices, supporting the parents and other professionals, ensuring good compliance and reporting and managing adverse reactions. We hope that, in the future, more patients with no therapeutic alternative, can benefit from this ERT.

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PO 15

MUCOLIPIDOSIS: CLINICAL PRESENTATIONS AND GENETIC INSIGHTS

B. Martín López-Pardo[1]; P. Sánchez Pintos[1]; S. Barbosa Gouveia[1]; E. Monteagudo Vilavedra[1]; M. Couce Pico[1]

[1] Unit of Diagnosis and Treatment of Congenital Metabolic Diseases, University Clinical Hospital of Santiago, Santiago de Compostela, Spain

Presenting Author - beatriz.martin.lopezpardo@sergas.es

Main category: Case series

Disease category: Complex molecule and organelle metabolism

Introduction: Mutations in GNPTAB gene can result in mucolipidosis (ML) type II or IIIα/β, depending on GlcNAc-1-phosphotransferase enzyme activity. GNPTAB encodes crucial α and β subunits for lysosomal enzyme synthesis, underlying autosomal recessive lysosomal storage diseases. ML IIα/β exhibits severe symptoms due to complete enzyme absence, often detected prenatally or neonatally. ML IIIα/β presents with reduced enzyme activity, resulting in milder symptoms and delayed onset (1,2). Moreover, mutations in GNPTG are associated with ML IIIγ (3), though clinically indistinguishable from other ML III forms.

Methods: We report on three cases of ML, covering ML II α/β and ML IIIγ. Clinical data were collected from patient records, including medical history, physical examination findings and laboratory results. Genetic analysis was conducted through WES to identify pathogenic variants in GNPTAB and GNPTG genes.

Results/Case report: Infant with IUGR, transient neonatal hyperparathyroidism, trigonocephaly, dysphagia, psychomotor and growth delay. WES identified a frameshift variant homozygous in GNPTAB gene (3503_3504delTC(p.Leu1168GLNfs*5)), absence of GNPT functional activity confirming ML IIα/β. Girl with family history of abortion due to fetal dysplasia, IUGR, showed craneofacial dysmorphisms, skeletal abnormalities and hypertrophic cardiomyopathy. Identification of compound heterozygous variants in GNPTAB gene: c.738del,p.(Lys246Asnfs*21) and g.102,132,628-102,143del compatible with ML II-III, pending functional analysis of the variants though clinically suggestive of ML IIα/β. Teenage girl with short stature, bilateral carpal tunnel syndrome, plantar fibromatosis and bone dysplasia. Maternal uniparental isodisomy with the variant NM_032520.4:c.238_243del (p.80_81del) in the GNPTG gene, consistent with ML IIIγ.

Conclusion: Mucolipidosis type II and III are severe lysosomal storage diseases with a wide clinical spectrum associating skeletal dysplasia and multisystem involvement, with ML II representing the greatest phenotypic severity and ML III (α/β or γ) a milder form. The lack of early bone series in ML type II has prevented the search for early characteristic radiological signs such as femoral overlay, changes resembling rickets, and astragalocalcaneal stippling, present in the first year of life and disappearing later. Transient neonatal hyperparathyroidism is a common presentation form in ML type II α/ β.

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PO 16

CRISPR/CAS9 AND ZEBRAFISH AS TOOLS TO STUDY RARE GENETIC DISEASES: A NEW ANIMAL MODEL FOR MUCOLIPIDOSIS II

M. Moutinho[1,2,3,4]; M. Coutinho[2,3,4]; S. Alves[2,3,4]; L. Moreira[2,3,4]

[1] Biology Department, Faculty of Science of University of Porto, Porto, Portugal; [2] Inherited Metabolic Diseases in Humans and Animals, CECA-ICETA - Centro de Estudos de Ciência Animal, Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto, Porto, Portugal; [3] Department of Human Genetics , National Health Institute Dr Ricardo Jorge, Porto, Portugal; [4] N/A, Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Porto, Portugal;

Presenting Author - m.teixeira.moutinho@insa.min-saude.pt

Main category: Other

Disease category: Complex molecule and organelle metabolism

Introduction: Mucolipidosis type II (MLII) is a Lysosomal Storage Disorder caused by pathogenic variants in GNPTAB (the most frequent, c.3503_3504.delTC) (1, 2). Currently, there is no treatment for MLII. For its development, new in vivo models that recapitulate the disease phenotype are needed. Zebrafish is a cheaper, easier to maintain and genetically engineer vertebrate alternative to mice (3). Hence, we are using CRISPR/Cas9 to create a zebrafish MLII knock-in (KI) containing the gnptab mutation orthologous to the most frequent in humans, suited for preclinical testing of new therapies directed to it.

Methods: Bioinformatic tools were used to find the orthologous mutation to c.3503_3504.delTC in zebrafish, and design guide RNAs (gRNA) and donor DNA template for KI. CRISPR/Cas9 components were microinjected at 1-cell stage embryos. HRM and Sanger Sequencing are used to genotype and confirm the editions.

Results/Case report: We determined the corresponding orthologous mutation to c.3503_3504.delTC in the zebrafish as a TG deletion in exon 19, that, in theory, will generate a truncated protein as in MLII. A first experiment showed that out of 12 surviving microinjected larvae, 11 had frameshift mutations in gnptab, 8 of which with an edition percentage higher than 80%, showing that microinjection was effective, and the gRNA has high frameshift efficiency. However, no KI events occurred. Homology Directed Repair (HDR), the cellular mechanism that originates KIs, is much less efficient than non- homologous end joining (NHEJ), responsible for the frameshift mutations that originate knock-outs (KO), so the number of embryos to be injected and analyzed needs to be substantially increased. Currently, we are analyzing the results of a following experiment with more than 100 microinjected embryos.

Conclusion: We have optimized the microinjection procedure for CRISPR/Cas9 gnptab and the gRNA used is very efficient. Results confirm that HDR is a much less efficient mechanism than NHEJ, so the KIoptimization is ongoing, namely by increasing the number of injected embryos.

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PO 17

INVESTIGATING CELLULAR TARGETS, POTENTIAL BIOMARKERS, AND DISEASE-CAUSING VARIANTS TO UNDERSTAND THE COMPLEXITY OF NIEMANN-PICK TYPE C DISORDER

A. Assunção Sandiares[1,2,3,4]; M. Gonçalves[1,2,3,4]; H. David[5]; M. Cardoso[6]; J. Ferrão[7]; L. Vieira[7]; I. Ribeiro[8,9]; D. Quelhas[8,9]; S. Alves[1,2,3]; M. Encarnação[1,2,3]

[1] Human Genetics Department, Research and Development Unit,, National Institute of Health Ricardo Jorge, Porto, Portugal, Porto, Portugal; [2] Center for the Study of Animal Science, CECA-ICETA, University of Porto, Porto, Portugal; [3] Associate Laboratory for Animal and Veterinary Sciences , AL4AnimalS, Porto, Portugal; [4] Biology Department, University of Aveiro, Aveiro, Portugal; [5] i4HB, UCIBIO-REQUIMTE, Faculty of Pharmacy, University of Porto, Porto, Portugal; [6] Reference Center for Inherited Metabolic Disorders, University Hospital Centre S. João, Porto, Portugal; [7] Technology and Innovation Unit, Human Genetics Department, National Institute of Health Ricardo Jorge, Lisbon, Portugal; [8]Unidade de Bioquímica Genética, Centro de Genética Médica Dr. Jacinto Magalhães, Centro Hospitalar e Universitário do Porto (CHUP, Porto, Portugal; [9] Unit for Multidisciplinary Research in Biomedicine, ICBAS-UP, Porto, Porto, Portugal;

Presenting Author - ana.sandiares@insa.min-saude.pt 969358295

Main category: Other

Disease category: Lipid metabolism and transport

Introduction: Niemann-Pick type C (NPC) is a monogenic autosomal recessive disease caused by pathogenic variants in NPC1 or NPC2 genes, leading to lipid accumulation. Disease-causing variants and other genetic variants can alter the phenotypic outcome. microRNAs (miRNAs) can help comprehend the complexity of NPC by examining their role on gene regulation and potential biomarker applications(1). Next-generation sequencing (NGS) approaches will be useful to answer these questions. This study aims to identify differentially expressed miRNAs in NPC patients and sequence Clinical-Exome in two atypical NPC cases.

Methods: NGS analyses, namely Clinical Exome and RNA-Seq were conducted. The RNA-Seq allowed to study the miRNA profile and the most prevalent hits were validated in patient´s serum using qRT-PCR studies. Sanger sequencing and Western blot were performed to study the variants impact on mRNA level and protein.

Results/Case report: In this research, two miRNAs were identified as differently expressed and are now being validated in patient's blood serum. The Clinical Exome Sequencing investigation of the two patients with atypical NPC clinical manifestation resulted in the discovery of a variant of uncertain significance (VUS) in the NPC1 gene (in one allele) and two VUS in a kinase-encoding gene (in compound heterozygosity). In the other patient, two distinct variants in the NPC1 gene were discovered; one of them has already been reported in the literature as pathogenic, while the other is a VUS. Further research into the interactions between the variants reported in the two genes is now planned.

Conclusion: In conclusion, due to the severity and complexity of this disease it is essential to identify novel and more effective biomarkers to decrease the diagnosis timeline, especially for late-onset forms. It is also necessary to study other contributors besides disease-causing variants in NPC1 or NPC2 genes to discover novel disease players.

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PO 18

COBALAMIN C DEFECT: THE EXPERIENCE OF A REFERENCE CENTRE (RC) FOR METABOLIC DISORDERS

J. Magalhães[1]; R. Sousa Martins [1,2]; S. Rocha [1,2]; R. Ribeiro[1,2]; E. Martins[2,3]; A. Guimas[1,2]

[1] Medicine, Unidade Local de Saúde de Santo António, Porto, Portugal; [2] Reference Centre for Hereditary Disorders of Metabolism, Unidade Local de Saúde de Santo António, Porto, Portugal; [3] Pediatrics, Unidade Local de Saúde de Santo António, Porto, Portugal;

Presenting Author - jnuno.magalhaes@gmail.com

Main category: Case series

Disease category: Intermediary metabolism: nutrients

Introduction: Cobalamin (Cbl) related disorders comprehend various diseases, from common scenarios like acquired/nutritional Cbl deficiencies to rarer entities linked to inborn errors of Cbl absorption, trafficking and intracellular metabolism, including Cbl-dependent remethylation pathways(1). Cobalamin C (cblC,OMIM: 277400) defect is the frequentest disorder(2) of intracellular B12 metabolism, yet globally rare with low incidence(3), being caused by MMACHC gene mutations, inherited in an autosomal recessive manner. Our aim is to describe a group of cblC patients followed in a RC for Metabolic Disorders.

Methods: A review from our Outpatient Clinic for Inborn Errors of Metabolism was conducted to identify adult patients with cblC. Their characteristics were then retrospectively analysed, including demographics, clinical presentation, current phenotype, genetic study, and treatment approach.

Results/Case report: 6 adult patients identified with male predominance (2:1). Genetic testing confirmed 5 cblC cases; 1 patient is awaiting definitive results. Median age: 38.2 years. Diagnosis in infancy prevailed (5), presenting as refractory epilepsy/developmental delay. Despite early neurological symptoms, 1 patient was diagnosed only as an adult, already reliant on external assistance. Additional manifestations observed: skeletal (marfanoid habitus: 2; kyphoscoliosis: 1), and ocular (optic neuropathy: 1; severe myopia: 2); there was no relevant cardiovascular, respiratory, or renal involvement. Treatment: all patients in combination strategy with parenteral OHCbl (daily dose: 1- 2,1mg), and oral betaine (daily dose: 42.5-200mg/kg). Latest follow-up analysis: homocysteine (Hcy) levels were under the threshold of 100 µmol/L in 5 cases (only one < 50); the remaining patient had a measurement of 113 µmol/L.

Conclusion: cblC is a rare disease of intracellular Cbl metabolism with typical childhood presentation (90%)2, as observed in our sample. Neurological involvement was present in all cases, though the broad phenotype may eventually lead to diagnosis delay. All received proper OHCbl/betaine doses, yet 1 patient had slightly elevated Hcy on follow-up, suggesting need for titration. The clinical impact of good metabolic control on the disease's natural evolution is unknown. Given the rarity of the disease and the consequent lack of robust evidence, regular reports from RCs are crucial for further guidance.

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PO 19

OPTIMIZATION OF THE ASSAY CONDITIONS FOR PREPARATION OF HUMAN PHENYLALANINE HYDROXYLASE ENZYMOSOMES

Gama M. [1,2], Lopes R. R. [1], Corvo M.L. [2], Leandro P. [1]

[1] Metabolism, Genetics and Proteins in Health and Disease Group, Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa; [2] Drug Delivery and Immunoengineering Group, Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa.

Presenting Author - fc52709@alunos.fc.ul.pt

Main category: Basic science study

Disease category: Intermediary metabolism: others

Introduction: Novel pharmacological therapies are still needed for the treatment of the full spectrum of Phenylketonuria (phenylalanine hydroxylase (PAH) deficiency). A promising therapeutic approach is enzyme reposition therapy using PAH enzymosomes. We previously optimized PAH modification with N-succinimidyl S-acetylthioacetate (SATA) that will allow conjugation with maleimide present in prepared liposomes. Having optimized the modification step, we further optimize the conjugation step and herein we focused on the physico-chemical and functional characterization of the obtained PAH- enzymosomes.

Methods: Purified PAH tetramers were obtained using the protocol in use in our group. PAH was modified using the previously optimized molar ratio of PAH:SATA (1:8). Modified SATA-PAH was deactelylated with hydroxylamine (for -SH group exposure) and then conjugated with a maleimide-PEG- PE liposome preparation

Results/Case report: A good yield of recovered modified SATA-PAH as well as a good thioacetylation degree (fluorescamine assay) was obtained. Conjugation conditions were tested using different buffers and PAH:liposomes ratios. The obtained PAH enzymosomes were characterized regarding protein and phospholipid content. Mean particle size, Zeta potential and enzyme activity were monitored during time. Preliminary results indicate that the PAH protein is very sensitive to the buffer used for liposome conjugation as well as to the ratio of protein:lipids present in the formulation. The PAH-enzymosomes stored at 4 oC, for 3 weeks, retained the capacity to hydroxylate L-phenylalanine generating L-tyrosine, when compared to the "naked" enzyme under the same conditions. Furthermore, PAH enzymosomes maintained their physico-chemical characteristics.

Conclusion: Herein we provide evidence that although PAH is considered a marginally stable protein its functional properties are retained upon several steps of chemical modifications as long as the assay conditions are precisely controlled. Importantly, when compared to the "naked" PAH, maintenance of protein activity was observed indicating that association to liposomes probably protects the protein from misfolding and/or aggregation. Although further optimization steps are necessary this work could bring new avenues for the treatment of Inborn Metabolic Disorders using enzymosomes.

PO 20

INDUCED PLURIPOTENT STEM CELL LINES AS TOOLS TO UNDERSTAND AND TREAT MPSIII

M. Barbosa Almeida[1,2,3,4]; S. Carvalho[1,3,4]; J. Santos[1,3,4,5]; L. Moreira[1,3,4]; M. Moutinho[1,3,4,5]; A. Duarte[1,3,4,6]; P. Gaspar[1,3,4,7]; M. Coutinho[1,3,4]; S. Alves[1,3,4]

[1] Research and Development Unit, Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge, Porto, Portugal; [2]Department of Medical Sciences, University of Aveiro, Aveiro, Portugal; [3] Center for the Study of Animal Science , CECA-ICETA, Instituto de Ciências, Tecnologias e Agroambiente, University of Porto, Porto, Portugal; [4] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS) AL4AnimalS, Faculty of Veterinarian Medicine, University of Lisbon, Lisboa, Portugal; [5] Biology Department, Faculty of Sciences, University of Porto, Porto, Portugal; [6] ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal; [7] Newborn Screening, Metabolism and Genetics Unit; Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge, Porto, Portugal;

Presenting Author - matilde.almeida@insa.min-saude.pt

Main category: Other

Disease category: Complex molecule and organelle metabolism

Introduction: Mucopolyssacharidosis type III (MPSIII A-D)are caused by inherited defects in one of the enzymes involved in the breakdown of glycosaminoglycan heparan sulfate(HS).Patients exhibit severe and progressive neurodegeneration, with no effective treatment available(1). Patient-derived Induced pluripotent stem cells(iPSC) are a great tool for pathophysiological studies and for drugs screenings, as they retain the donors' genetic background while presenting a high proliferative capacity and differentiation potential(2). Here we address the establishment/characterization of 2 novel MPSIII iPSC lines.

Methods: For disease modeling, the Epi5TM Episomal iPSC Reprogramming Kit was used in MPS IIIC and IIID fibroblasts. Enzymatic assays, Sanger Sequencing and episomal clearance assays were carried out. Karyotype and HS content assessment are ongoing.

Results/Case report: Around 24 and 20 days after transfection, for MPS IIIC and MPS IIID, respectively, the first iPSC colonies were observed. The absence of the episomal vectors was confirmed for both cell lines, as well as their disease-related genotypes. Enzymatic assays revealed either absent (MPS IIIC) or residual (MPS IIID) activity for the corresponding enzymes.

Conclusion: Both cell lines exhibited MPS III-linked genotype and phenotype. However, extra analyses must be conducted. Further perspectives include the differentiation of these cells into neurons for subsequent drug testing.

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PO 21

TYROSINEMIA TYPE III: CLINICAL, NEUROCOGNITIVE AND BEHAVIOURAL FEATURES OF TWO SIBLINGS FOLLOWED-UP IN A PORTUGUESE REFERENCE CENTRE FOR INHERITED METABOLIC DISORDERS

C. Carmona[1,2,3,4]; M. Almeida[1,2,3,4]; V. Magalhães[2,4,6]; S. Pinto[2]; L. Vilarinho[5]; A. Cunha[2]; A.Bandeira[2]; J. Correia[2]; R. Martins[2]; S. Rocha[2]; R. Ribeiro[2]; A. Guimas[2]; E. Martins[2,3,4]

[1] Centro de Genética Médica Jacinto Magalhães, Centro Hospitalar Universitário de Santo António, Porto, Portugal; [2] Centro de Genética Médica Jacinto Magalhães, Centro de Referência Para as Doenças Hereditárias do Metabolismo, Porto, Portugal; [3] Universidade do Porto, Unidade Multidisciplinar de Investigação em Biomedicina, Porto, Portugal; [4] Universidade do Porto, Laboratório para a Investigação Integrativa e translacional em Saúde Populacional, Porto, Portugal; [5] INSA, Instituto Nacional de Saúde Ricardo Jorge, Porto, Portugal; [6] Universidade do Porto, Instituto de Saúde Pública, Porto, Portugal;

Presenting Author - carla.carmona@chporto.min.saude.pt

Main category: Case report

Disease category: Intermediary metabolism: nutrients

Introduction: Tyrosinemia type III (TYRSN3) is an autosomal recessive disorder caused by the deficiency of 4-hydroxyphenylpyruvate dioxygenase (4-HPD) and is the rarest form of tyrosinemia. The full clinical spectrum of this disorder remains variable and unclear. However, neurological involvement, including intellectual impairment and attention deficit disorder with hyperactivity (ADHD) have been described. We report the case of two siblings diagnosed with TYRSN3 at different ages. We aimed to characterize them on their clinic features, neurocognitive development and adaptive functioning.

Methods: We studied two siblings, diagnosed at different ages, following a protein-restrictive diet supplemented with tyrosine-free amino acid mixtures since diagnosis. We present their clinical features evaluation, metabolic control, neurocognitive profile, school outcome and adaptive behaviour.

Results/Case report: We found in both the same mutation in homozygosity [p.A33T(c.97G>A)]. Physical examination and growth were normal [weight and height in the 50 - 95th percentile] as well neurological examination [normal EEG and MRI] and metabolic control [Tyr values < 300 µmol/l]. At age of ten, we found global intellectual values (IQs) slightly below the mean of healthy population reference norm, with a similar psychometric profile [IQ=68 in the late diagnosed; IQ=81 in early diagnosed]. Both were diagnosed with ADHD initiating treatment with methylphenidate with a positive response. Their last neurocognitive evaluation at age of 14 and 22 years showed a slight increase in IQ [IQ=85 in both siblings]. They attend normal school with an adapted curriculum and adjustments to the assessment process [8th and 12th school year]. They also had a normal level of adaptive functioning.

Conclusion: The follow-up of patients with TYRSN3 by a multidisciplinary team monitoring frequently their clinical features, metabolic control and neurocognitive potential, enabled a clinical intervention and an appropriate psycho-educational support which seems to be reflected in a better long-term metabolic control, allowing these patients to cope with the demands of living with a chronic illness and thus adapt successfully to different life contexts.

PO 22

UNVEILING THE SIRTUIN 4-DEPENDENT ROLE IN HYPERAMMONEMIA AND MITOCHONDRIAL TARGETS: OPTIMIZATION OF AN EXPRESSION SYSTEM FOR PRODUCTION OF RECOMBINANT SIRT4

R. Simões[1]; M. Silva[1]; P. Leandro[1]

[1] Metabolism, Genetics and Proteins in Health & Disease, Research Institute for Medicines- iMed.ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal;

Presenting Author - ricardo-moura@live.com.pt

Main category: Basic science study

Disease category: Intermediary metabolism: others

Introduction: Sirtuins are a family of NAD-dependent enzymes in the regulation of vital metabolic pathways in human (1). The importance of mitochondrial sirtuin 4 (SIRT4) has emerged recently (2,3). This work aims to implement a prokaryotic expression system and to establish the best experimental conditions to obtain SIRT4 in high yields and purity grade to further characterize its enzymatic activities. Glutamate dehydrogenase and Pyruvate dehydrogenase complex are known deacetylation targets of SIRT4. How their interactions are affected during hyperammonemia need further elucidation (4).

Methods: The mature SIRT4 cDNA, cloned into the pET-29a expression vector, was synthesized (GeneCust). Several expression conditions were tested using: i) Immobilized metal affinity chromatography to purification ii) SDS-PAGE and ImageJ densitometric analysis to estimate the purity grade of expressed SIRT4.

Results/Case report: The mature SIRT4 was expressed in fusion with a N-terminal hexahistidyl tag (6xHis), in Escherichia coli BL21 (DE3) strain, with the expected molecular mass (≈34 kDa). Different expression conditions were tested, namely: culture media Luria Bertani (LB) and Terrific Broth (TB); 0.5 and 1 mM IPTG; 27°C and 37°C; 4h and 17h; Tris-HCl and Phosphate lysis buffer; and 1% TritonX-100 (TX). For IMAC purification an imidazole gradient (10-250 mM) was used. A significant expression of SIRT4 was obtained using LB medium, 1 mM IPTG, and 27 °C incubation. Production of SIRT4 during 4h and 17h resulted on a 18% and 9% yield, respectively (250mM imidazole). SDS-PAGE analysis of pellet fractions showed a high content of SIRT4. Following inclusion of TX in lysis buffer an increment in the levels of soluble SIRT4 was obtained.

Conclusion: Advances on the optimization of the expression conditions to obtain soluble SIRT4 were achieved. However, results highlighted the challenges associated with SIRT4 purification, notably its tendency to aggregate due to low solubility. This attribute was evidenced by the intense band observed in the pellet fraction of SDS-PAGE gels and the increase in the yield of soluble protein when a detergent (TritonX-100) was added to the lysis buffer. Overall, results provide significant steps to obtain recombinant SIRT4 to be further investigated, unveiling its biochemical and structural properties.

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PO 23

ADVANCEMENTS IN NON-INVASIVE DIAGNOSIS OF LYSOSOMAL STORAGE DISORDERS

P. Gaspar[1]; R. Neiva[1]; L. Silva[1]; L. Vilarinho[1]

[1] Unidade de Rastreio Neonatal, Metabolismo e Genética; Departamento de Genética, Instituto Nacional de Saúde Doutor Ricardo Jorge, Porto, Portugal;

Presenting Author - paulo.gaspar@insa.min-saude.pt

Main category: Translational science study

Disease category: Complex molecule and organelle metabolism

Introduction: Lysosomal storage disorders are a group of rare diseases that affect approximately 1 in 4,000 live births in Portugal (1). They are characterized by multisystemic involvement and a wide range of phenotypical presentations, often overlapping with other diseases. As a result, many patients experience a lengthy process of seeking a correct diagnosis, known as the "diagnostic odyssey". To address this challenge, a new tandem mass spectrometry method has been developed for the urinary identification of both oligosaccharidoses and mucopolysaccharidoses (MPS).

Methods: Urine specimen underwent LC-MS/MS analysis for non-reducing end (NRE) glycosaminoglycans and oligosaccharides residues biomarkers, according to recent publications (2,3).

Results/Case report: In this study, we conducted an analysis of 150 urine samples consisting of controls, MPSs, Oligosaccharidoses, and suspicious samples. In our analysis, we were able to accurately identify specific signatures for almost all MPSs in the urine samples. Furthermore, we were able to differentiate between different subtypes of MPSIII. Additionally, we observed that these biomarkers can also reflect the efficacy and impact of the administered therapy. We also investigated the oligosaccharidoses and successfully linked oligosaccharide residues to their respective disorders.

Conclusion: This method is suitable for a rapid and easy identification of MPSs and oligosaccharidosis in urine. It is very specific and sensitive, allowing also the discrimination of other LSDs. In the near future we intend to analyze such biological markers in dried blood spot.

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PO 24

EXPANDING THE CLINICAL SPECTRUM AND DISEASE BURDEN OF LEIGH SYNDROME SPECTRUM

C. Fraga [1]; C. Correia[1]; M. Coelho[1]; J. Correia[1]; A. Bandeira[1]; E. Martins[1]

[1] Pediatria, Centro Hospitalar Universitário do Porto - ULSSA - CMIN Reference Center for Metabolic Diseases, Porto, Portugal

Presenting Author - carolinamoraesfraga@gmail.com

Main category: Case series

Disease category: Intermediary metabolism: energy

Introduction: Leigh Syndrome (LS) is the most frequent neurodegenerative mitochondrial disease in the pediatric population (1:40.000). Leigh Syndrome Spectrum, a more recent concept, includes both Leigh syndrome with its diagnostic criteria (clinical, neuroradiological, biochemical and molecular) and the Leigh like-syndrome (incomplete criteria). LS has a wide clinical spectrum with a cumulative disease burden over time.

Methods: Retrospective review of pediatric patients diagnosed with LS (complete criteria) in a Tertiary Pediatric Center since 2000, evaluating clinical presentation and natural history.

Results/Case report: 7 patients were diagnosed with LS (four female), median age at diagnosis: 3.5 years (range: 5 months-9 years). Presentation included horizontal nystagmus (1), intermittent ataxic episodes triggered by infections (2), respiratory acidosis triggered by sedation to perform brain magnetic resonance imaging (MRI) (2) or by infection (1) and apneic episodes (1). Hypotonia and/or global developmental delay were previously described in all. There was no reported consanguinity. All MRI had characteristic symmetrical multiple bilateral lesions (T2W hypersignal) predominantly in basal ganglia, suggesting Leigh Syndrome. All patients had molecular confirmation; three have the most frequently associated mutation (m.8993T>G). The two patients who were diagnosed in the first year of life, died shortly after, at the ages of 14 months (m.8993T>G mutation) and 20 months (NDUFS1 gene mutation).

Conclusion: LS has a vast clinical and genetic heterogeneity, with characteristic cerebral lesions associated with cognitive and motor deterioration. Our sample, however, also highlights the systemic nature of LS, with cardiac and endocrine involvement, beyond the neurological. Also, visual and hearing impairment should also be systematically screened. Currently, there are no curative options for LS. Disease progression with feeding difficulties (subsequent aspiration risk) and multifactorial motor limitations will require specific support, apart from dietary management and cofactors administration.

PO 25

QUALITY OF LIFE IN PHENYLKETONURIA - IMPACT OF DISEASE AND TREATMENT ON PAEDIATRIC PATIENTS AND FAMILIES AT ULS COIMBRA

J. Almeida[1]; J. Gante[1]; S. Ferreira[1,2]; F. Ferreira[1]; L. Diogo[1,2]

[1] Centro de Referência de Doenças Hereditárias do Metabolismo - MetabERN, Unidade Local de Saúde de Coimbra, Coimbra, Portugal;
[2]Faculdade de Medicina, Universidade de Coimbra, Coimbra, Portugal;

Presenting Author - joanaalmeida@ulscoimbra.min-saude.pt 918446339

Main category: Basic science study

Disease category: Intermediary metabolism: nutrients

Introduction: Phenylketonuria (PKU) is a rare genetic neurometabolic disorder. In our Country, where it is diagnosed through newborn screening since 1979, its incidence is about 1:10000. The accumulation of phenylalanine in the tissues leads to chronic brain dysfunction, which can be prevented by a lifelong phenylalanine-restrictive diet. The strict and demanding diet and the need for close follow-up from an early age can be expected to affect the health-related quality of life (HRQoL) of patients and their families. We aimed to assess HRQoL in paediatric patients and their families followed at our Centre.

Methods: HRQoL was assessed using a generic measure, the Pediatric Quality of Life Inventory TM (PedsQLTM) Version 4.0 – Portuguese: Parent Report for Toddlers (PRT)-ages 2-4 years(y); Parent Report for Young Children (PRYC)-ages 5-7y; Child Self-Report (CSR)-ages 8-12y; Teens Self-Report (TSR)-ages 13-18y.

Results/Case report: Fifteen PKU patients (8 female-53%) were included (mean age=9,9±5y): 2-12y (n=11); 13-18y (n=4). Four PRT; 5 PRYC, 3 CSR and 4 TSR have been filled. In total score, the mean of the group revealed QoL of 84,3 and a Physical Health Summary Score of 85,6. Psychosocial Health Summary Score had the lowest score (78,5). In PedsQL, the group's result was: Social Functioning=89,2, Physical Functioning=85,6, School Functioning=81,3 and Emotional Functioning=81. In the mean results of PRT items, no concerns were identified. In the other Parent and Child Reports of the PedsQL, the results obtained raise concern (<75) in the following items: Lifting something heavy; Doing chores around the house; Low energy level; Feeling afraid or scared; Feeling angry; Worrying about what will happen to him or her; Paying attention in class; Forgetting things; Missing school to go to the doctor or hospital.

Conclusion: Untreated PKU is associated with intellectual disability and other neurological manifestations. Treatment from the first days of life can prevent most brain damage, but it is demanding in terms of compliance and social and financial costs. HRQoL is currently a major focus and specific questionnaires for PKU have being developed. Overall, our patients showed excellent HRQoL. Most domains were reported to be little or not affected. Worse QoL was found in psychosocial health. Particular care should be paid to emotional health and school performance, especially attention problems, in PKU patients.

Acknowledgements: To patients and their families, and to all clinical professionals involved in their diagnosis, treatment and monitoring.

PO 26

NUTRITIONAL COMPOSITION OF SPECIAL LOW PROTEIN FOODS REIMBURSED IN PORTUGAL FOR PATIENTS WITH INBORN ERRORS OF METABOLISM

M. Corredeira[1]; M. Ferreira de Almeida[2,3,4,5]; S. Pinto[2,3]; V. Magalhães[2,3,4,6]

[1]Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Porto, Portugal; [2]Centro de Genética Médica Doutor Jacinto de Magalhães (CGMJM), Centro Hospitalar Universitário de Santo António, Unidade Local de Saúde de Santo António, Porto, Portugal; [3]Centro de Referência na área de Doenças Hereditárias do Metabolismo, Centro Hospitalar Universitário de Santo António, Unidade Local de Saúde de Santo António, Porto, Portugal; [4]Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Porto, Portugal; [5]Unidade Multidisciplinar de Investigação Biomédica (UMIB), Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto,Portugal; [6] Unidade de investigação epidemiológica - EPIUnit, Instituto de Saúde Pública da Universidade do Porto (ISPUP), Porto,Portugal;

Presenting Author - margaridacorredeira@gmail.com

Main category: Basic science study

Disease category: Intermediary metabolism: nutrients

Introduction: Inborn errors of metabolism (IEM), mainly of amino acids, require a treatment with a natural protein-restricted diet using special low protein foods (SLPF) supplemented with protein substitutes. In Portugal, the Dispatch 14319/2005 full reimburse SLPF upon prescription in legally defined treatment centres (TC). CGMJM, also a TC, coordinates the national SLPF acquisition and delivery. Although SLPF meet the low protein requisite, more detailed data on nutritional composition is needed due each IEM particularities. We aimed to analyse the nutritional composition of SLPF available in Portugal.

Methods: Data on the nutritional composition of each SLPF (labels and suppliers technical sheets) was collected. A descriptive analysis of the nutritional composition per 100g was carried out for energy, protein, total fat, saturated fatty acids (SFA), carbohydrates (CHO), sugars, fibre and salt.

Results/Case report: Seventy-four SLPF (substitutes of natural foods) from 12 food groups are available: rice&pasta-16; cookies-15; cakes-9; breakfast cereals-4; chocolates-5; flour-4; bread-7; baby cereals-4; meat-2; egg-2; milk-4 and cheese-2. The median SLPF energy/100g was 365kcal, with chocolates and cookies as the highest contributors. The median protein content was 0.4g, with cheese, chocolates and meat substitutes having the highest values. Median fat and SFA were higher in chocolates (33.4 and 21.0g, respectively) followed by cheese and cookies. All products had a CHO content higher than 50g, except meat, cheese and milk substitutes. Breakfast cereals, baby cereals and cakes had the highest median sugar levels (91.0, 87.0 and 84.0g, respectively). Excepting eggs and meat (median 47.1 and 18.9g, respectively), all products had a low fibre content. Bread had the highest median salt content (1.4g).

Conclusion: Due to the need for a reduced protein content, SLPF have a high fat and CHO content. SFA, sugars and salt have also been shown to be high. Therefore, instead of considering these foods to be freely consumed, it is necessary to take into account the specific nutrient composition of each SLPF when deciding on the quantity to prescribe, according to treatment particularity and patient's individual needs, and encourage the industry to reformulate some less balanced products.

PO 27

GLUTARIC ACIDURIA TYPE 1 - AN ULTRA-RARE GENETIC DISEASE WITH A WIDE CLINICAL SPECTRUM

S. Ferreira[1,2]; S. Freitas[2]; S. Moreira[1]; N. Baptista[1]; R. Sousa[3]; S. Carvalho[3]; L. Diogo[1,2]; M. Carmo Macário[1]

[1] Centro de Referência de Doenças Hereditárias do Metabolismo - MetabERN, Unidade Local de Saúde de Coimbra, Coimbra, Portugal; [2]Faculdade de Medicina, Universidade de Coimbra, Coimbra, Portugal; [3]Serviço de Neuroradiologia, Unidade Local de Saúde de Coimbra, Coimbra, Portugal;

Presenting Author - saralopesferreira@ulscoimbra.min-saude.pt

Main category: Basic science study

Disease category: Intermediary metabolism: nutrients

Introduction: Glutaric aciduria type1 (GA1) is a rare neurometabolic disorder of lysine, hydroxylysine and tryptophan catabolism, due to biallelic pathogenic variants in the GCDH gene. Glutaryl-CoA dehydrogenase deficiency leads to accumulation of glutaric, 3-hydroxyglutaric and glutaconic acids and glutarylcarnitine in tissues. The clinical spectrum is wide, ranging from early presentation of hypotonia/macrocephaly and acute encephalopathic crises to asymptomatic cases. Brain MRI often shows suggestive changes (widening of the sylvian fissures). Treatment is based on a lysine-restricted diet and carnitine.

Methods: A descriptive analysis based on anonymized data collected from the clinical files of 8 patients (Pts) (1 male) followed at our Centre in the last 30 years (1992-2023) was performed.

Results/Case report: Newborn screening (NBS) led to diagnosis of 4 Pts: 2 newborns (NB) with high glutarylcarnitine and 2 mothers, 24 and 26 years-old (y), due to hypocarnitinemia in their NB; the remaining 4 Pts were diagnosed at 9-11months of age (m) based on symptoms. Six Pts had clinical manifestations (mostly macrocephaly and hypotonia) presenting from prenatal to 15m. Their diagnosis was done at a median age of 11m (3m-26y). GDCH gene study confirmed diagnosis in all. Brain image of 7 showed typical abnormalities in all. All Pts had carnitine supplementation and children had a lysine-restricted diet. One of the Pts diagnosed as NB is asymptomatic at 6y; the other, 2y, has mild hypotonia and relative macrocephaly. Three Pts evolved with generalized dystonia. One of these died at 4y, after an acute decompensation. The current age of the survivors is 22m-41y (median 27y); 3 adults lead independent lifes.

Conclusion: The clinical variability seen in this cohort reflects the phenotypic spectrum of AG1. The positive impact of the inclusion of AG1 in the national NBS programme was observed, allowing the detection of 2 NB and 2 adult Pts with clinical manifestations dating back to infancy. Early diagnosis and treatment are of paramount importance to improve the quality of life of patients and to allow for timely genetic counselling. Multicentre studies of this ultra-rare and probably under-diagnosed disease are warranted to establish the natural history of the disease and to optimise therapeutic interventions.

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Acknowledgements: To patients and their families, and to all laboratory and clinical professionals involved in their diagnosis, treatment and monitoring.

PO 28

FOLLOW-UP OF ADULT PATIENTS WITH INBORN ERRORS OF METABOLISM DURING THE YEAR OF 2023 AT THE REFERENCE CENTRE FOR HEREDITARY METABOLIC DISORDERS OF THE UNIDADE LOCAL DE SAÚDE SANTA MARIA, LISBOA

A. Belo[2]; D. Gomes[1]; P. Almeida Nunes[2]; A. Oliveira[1]

[1] Medicine Service, Reference Centre for Metabolic Disorders at the Unidade Local de Saúde de Santa Maria, Lisbon, Portugal; [2]Dietary and Nutrition Service, Reference Centre for Metabolic Disorders at the Unidade Local de Saúde de Santa Maria, Lisbon, Portugal;

Presenting Author - anabelabelosantos@gmail.com

Main category: Other

Disease category: Several disease categories

Introduction: Inborn Errors of Metabolism (IEM) are a heterogeneous group of disorders that may be inherited or may occur as the result of spontaneous mutations. These diseases involve failure of the metabolic pathways involved in either the break-down or storage of carbohydrates, fatty acids, and proteins. Although any given inborn error of metabolism is very rare, taken as a group, inborn errors of metabolism occur in 1 in 2500 births.. (1)A large percentage of known IEM require specific and essential nutritional adjustments.

Methods: At the Reference Centre for Hereditary Metabolic Disorders at the ULS Santa Maria, there are two units, the Pediatrics and the Adults, both include a Dietitians team. The aim is to present the case series of the Adult Nutrition patients evaluated at the outpatient clinic, for the year 2023.

Results/Case report: In addition to face-to-face Nutrition appointments, several contacts are made with patients via telephone or email. In 2023, 123 patients were evaluated, for a total of 290 appointments, of which 174 were in-person and 116 were non-face-to-face. The total number of face-to-face appointments was divided into the following pathologies: Phenylketonuria: 85; Maple Syrup Urine Disease: 11; Homocystinuria: 7; Tyrosinemia: 5; Propionic aciduria: 1; Methylmalonic aciduria: 2; OTC deficit: 2; Citrullinemia: 4; Argininosuccinic aciduria: 3; MCADD: 2; VLCADD: 3; Galactosemia: 7; Glycogenoses: 9; Other 33. The total number of non-face-to-face appointments was divided as follows: Phenylketonuria: 61; Maple Syrup Urine Disease: 22; Homocystinuria: 12; Tyrosinemia: 8; Propionic aciduria: 3; Methylmalonic aciduria: 1; Citrullinemia: 5; VLCADD: 1; Galactosemia: 1; Others: 2.

Conclusion: As previously mentioned, nutritional support is essential for most of these diseases, and it is necessary to maintain regular contact with patients, for dietary adjustments according to Phemeasurements, or assessing dietary compliance, e.g., often in non-face-to-face appointments.

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PO 29

EYE-HEAD MOVEMENTS ANALYSIS IN ADULTS WITH GLUCOSE TRANSPORTER TYPE 1 DEFICIENCY SYNDROME (GLUT1-DS)

J. Alves[1]; J. Durães[1,2]; R. Machado[1]; F. Ramos[2,3]; S. Ferreira[2,4]; A. Morgadinho[1]; L. Diogo[2,4]; J.Lemos[1]; M. Macário[1,2]

[1] Neurology Department, ULS Coimbra, Coimbra, Portugal; [2]Inherited Metabolic Disease Reference Center/Metabern, ULS Coimbra, Coimbra, Portugal; [3]Genetics Department, ULS Coimbra, Coimbra, Portugal; [4]Centre for Child Development – Pediatrics Department, ULS Coimbra, Coimbra, Portugal;

Presenting Author - josemiguel.s.alves@gmail.com

Main category: Case series

Disease category: Intermediary metabolism: energy

Introduction: GLUT1-DS is a rare disorder that impairs brain function due to a defective GLUT1 protein, which is crucial for transporting glucose to the brain (1). Symptoms can vary greatly and may evolve over time (2). Early signs in children with GLUT1-DS often include paroxysmal eye–head movements, which are said to decrease over time (3). However, research on eye–head movements findings in adult GLUT1-DS patients is lacking. Our aim was to characterize eye-head movement abnormalities in the latter group.

Methods: Eye-head movements recording was performed on adult patients with genetically confirmed GLUT1-DS followed at the Unidade Local de Saúde de Coimbra.

Results/Case report: We included 7 patients (42.9% male) from five different families, with a mean age of 33.4 years (95% CI 20.2 - 46.7), mean age of symptom onset of 6.8 years (95% CI 1.7 - 11.8), and mean disease duration of 23.9 years (95% CI 10.4 - 37.3). 3 patients were on ketogenic diet. On eye- head movements analysis, there was head tremor (28.6%), head myoclonus (14.3%), mild to moderate ocular apraxia (42.9%), anticipatory saccades during pursuit (42.9%), sometimes more pronounced during vertical pursuit (28.6%), hypometric saccades (42.9%), torsional (28.6%) and downbeat (14.3%) spontaneous nystagmus, gaze evoked nystagmus (28.6%) and decomposed pursuit (14.3%). None showed paroxysmal eye–head movements.

Conclusion: While paroxysmal eye–head movements seem to be absent in adult GLUT1-DS population, we were able to show that mild to moderate eye-head movements abnormalities remain through adulthood. Particularly the presence of ocular apraxia together with head tremor/myoclonus should raise suspicion for GLUT1-DS, in the proper clinical context.

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PO 30

MEDIUM-CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY FROM THE NEWBORN SCREENING LAB TO THE CLINIC

S. Ferreira[1,2]; J. Neves[2]; Í. Oliveira[1]; F. Martins[1]; L. Vilarinho[3]; L. Diogo[1,2]; A. Oliveira[1,2]

[1] Centro de Referência de Doenças Hereditárias do Metabolismo - MetabERN, Unidade Local de Saúde de Coimbra, Coimbra, Portugal; [2]Faculdade de Medicina, Universidade de Coimbra, Coimbra, Portugal; [3]Unidade de Rastreio Neonatal Metabolismo e Genética, Instituto Nacional de Saúde Dr Ricardo Jorge, Porto, Portugal;

Presenting Author - saralopesferreira@ulscoimbra.min-saude.pt

Main category: Basic science study

Disease category: Intermediary metabolism: nutrients

Introduction: Medium-chain acyl-coA dehydrogenase deficiency (MCADD) is the most common fatty acid b-oxidation disorder and the inherited metabolic disease most frequently detected in expanded newborn screening (NBS) in Portugal (1:8830). It impairs fatty acid derived energy and ketogenesis, leading to long fasting hypoglycemia, metabolic acidosis and coma when hepatic glycogen stores become depleted. The aim of this study was to describe diagnostic, clinical features, treatment and disease progression of MCADD patients younger than 18 years old followed in our Centre (January 2004 - December 2022).

Methods: We performed descriptive analysis of clinical, newborn screening, biochemical and genetic data. Correlation between medium-chain acylcarnitine levels at diagnosis and three genotype groups (c.985A>G homozygosity/ compound heterozygosity/ others) was sought. IBM SPSS Statistics version 29.0 was used.

Results/Case report: The study cohort included 29 patients (15males) from 23 families. They were diagnosed by NBS. Participants ranged in age from 11 months to 16 years (mean 7.3; median 6). Most (21; 72%) were of Gypsy ethnicity. Octanoylcarnitine (C8), the primary biomarker of the disease, was significantly elevated in all. The c.985A>G variant (accounting for 86% of the alleles) was homozygous in 21 patients (72%). No significant differences in NBS acylcarnitines were found between the genotype groups. Treatment and outcome were analysed in 28 patients: all were compliant with fast avoidance; 23 (82%) had L-carnitine supplementation, according to plasma levels; six (21%) had one or more hospitalisations to prevent/treat metabolic decompensation (>=3 months of age); none had arrhythmia and/or cardiomyopathy caused by MCADD; 14 (50%) had some neurodevelopmental impairment (one had a del.15q11.2 syndrome).

Conclusion: This cohort has a high prevalence of severe pathogenic genetic variants, especially in homozygosity. With early diagnosis and treatment, most children did not develop clinically significant disease. Although carnitine deficiency is a common finding in MCADD, carnitine supplementation remains controversial. Sociocultural and genetic factors may explain the high prevalence of neurodevelopmental impairment observed in this cohort. Further studies of larger cohorts are needed to understand the impact of MCADD on developing brain beyond the lack of glucose during metabolic crisis.

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Acknowledgements: To patients and families and all professionals in the laboratory and clinic that were involved in their diagnosis, treatment and monitoring.

PO 31

ANTHROPOMETRIC CHARACTERIZATION OF THE MSUD POPULATION FOLLOWED AT REFERENCE CENTRE FOR HEREDITARY METABOLIC DISORDERS OF UNIDADE LOCAL DE SAÚDE SANTA MARIA, LISBOA

R. Santos Loureiro[1]; A. Belo[1]; J. Palhotas[1]; P. Almeida Nunes[1]; P. Janeiro[3]; P. Lipari[3]; R. Jotta[3]; D.Gomes[2]; A. Oliveira[2]; A. Gaspar[3]

[1] Dietary and Nutrition Service, Reference Centre for Metabolic Disorders at the Unidade Local de Saúde de Santa Maria, Lisbon, Portugal; [2] Medicine Service, Reference Centre for Metabolic Disorders at the Unidade Local de Saúde de Santa Maria, Lisbon, Portugal; [3] Pediatric Service, Reference Centre for Metabolic Disorders at the Unidade Local de Saúde de Santa Maria, Lisbon, Portugal;

Presenting Author - anabelabelosantos@gmail.com

Main category: Other

Disease category: Intermediary metabolism: nutrients

Introduction: Maple Syrup Urine Disease (MSUD) is an inborn metabolic disorder caused by branched-chain α-ketoacid dehydrogenase deficiency resulting in the accumulation of the branched chain amino acids (BCAA) leucine, isoleucine and valine. Treatment consists of diet with natural protein restriction, supplemented with BCAA-free mixture and, if the patient is responsive, supplement with enzyme co- factor thiamine. Some of the goals of nutrition therapy are to maintain plasma BCAA within targeted treatment ranges and ensure an adequate nutritional status, normal growth, development and health maintenance.

Methods: All patients are regularly evaluate by the dietitians team, in the outpatient clinic, and data presented here reflects the last evaluation. Weight and length were obtained by standard techniques and were expressed as age- and sex-specific z-scores or classified accordingly with body mass index (BMI)

Results/Case report: In our center we follow 17 patients with MSDU, 9 (53%) children (<18 years old) and 8 (47%) adults (18 years old). Regarding the cohort of 8 children individuals, all were diagnosed in by neonatal screening, except one that had a late diagnose. The mean current age of this population was 8.22 years old. They have an average weight of 31.04kg and an average height of 1,40m, (mean BMI of 16kg/m2). All of these children, presented a normal nutritional status, except for the late diagnosed individual, which had a severe wasting status (with a weight for height z-score: –5,043). Regarding the adult population, 9 of whom had a late diagnosis, the results are: mean age, weight, height and BMI of 24.37 years old, 60.13kg, 1.58m and 24.09kg/m2, respectively. 5 patients of our adult population presented a normal range classification for BMI, 2 of them are overweight and 1 has obesity class I.

Conclusion: The results reinforce the importance of neonatal screening, with malnutrition being observed more commonly in late diagnosed patients Regular nutritional assessments, with appropriate adjustments of micro and macronutrients, is essential for these patients, under highly restricted protein diets in order to achieve a better outcome in quality of life with less metabolic decompensations, and hospital admissions. Nevertheless, dietitians must be aware of these long-term restricted diets, and prevent malnourishment in MSUD patients.

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PO 32

CLASSIC HOMOCYSTINURIA – EARLY DIAGNOSIS SURELY MAKES A DIFFERENCE

Í. Rocha e Oliveira[1]; S. Moreira[1]; H. Rocha[2]; J. Almeida[1]; L. Diogo[1]; S. Ferreira[1]

[1] Centro de Referência de Doenças Hereditárias do Metabolismo , ULS Coimbra – MetabERN, Coimbra, Portugal; [2] Unidade de Rastreio Neonatal Metabolismo e Genética, Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto, Portugal;

Presenting Author - irisrocha7@gmail.com

Main category: Case series

Disease category: Intermediary metabolism: nutrients

Introduction: Classic Homocystinuria (CH) is an autosomal recessive inherited disorder of metabolism caused by cystathionine β-synthase (CBS) deficiency. It has a wide clinical spectrum, with eye (myopia/ectopia lentis), skeletal system (marfanoid habitus, osteoporosis, scoliosis), vascular system (thromboembolism) and central nervous system (developmental delay, seizures, psychiatric problems) involvement. In recent years, it can be diagnosed through neonatal screening program (NSP). Treatment aims to lower plasma methionine (Met) and total homocysteine (tHcy) levels and prevent/reduce manifestations.

Methods: Retrospective descriptive study of the patients with classic homocystinuria followed in our center in the last 40 years. Comparative analysis of clinical evolution of two family related patients, one diagnosed after clinical presentation and other through NSP.

Results/Case report: Six patients were followed in our center (three males). Two were referred through NSP. The others were diagnosed at a median age of 8 yo (8-19) with intellectual deficit and ectopia lentis (3), marfanoid habitus (2) and osteoporosis (3). Plasma Met and tHcy levels were in the same magnitude: Met median levels-600 (68-708); tHcy median levels-188 (75-345). Plasma CBS activity was undetectable in 4/4. Pathogenic variants were found in 5/5. All were non-responsive to pyridoxine and treated with Met-restricted diet and betaine. Two patients (current age: 35y, 18y) had a common ancestor and shared the c.1566delG variant: the first in homozygosity, the later in compound heterozygoty. The first presented at 8 y with intellectual deficit (global IQ-59), ectopia lentis, marfanoide habitus, osteoporosis and scoliosis. The later, diagnosed in neonatal period, remained asymptomatic (global IQ- 104)

Conclusion: CH is an ultra-rare, treatable genetic disease that can be detected through NSP, at least in most severe forms. Early treatment can prevent clinical manifestations, allowing a fruitful life, as shown by the different evolution of our two patients. This is in accordance to other studies that support the importance of NSP in CH long term outcome improvement. (3) In our Country, CH can be detected in NSP when Met levels are high, using tHcy as a second-tier. This is most important since early treatment improves prognosis. On the other hand, early diagnosis allows adequate genetic counseling.

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Acknowledgements: To patients and their families, and to all laboratory and clinical professionals involved in their diagnosis, treatment, and monitoring.

PO 33

COBALAMIN C DEFICIENCY - SAME GENOTYPE, DIFFERENT PROGNOSIS

R. Diogo[1,2]; C. Álvares[2]; S. Ferreira[1,2]; J. Almeida[1]; C. Nogueira[3]; L. Diogo[1,2]; A. Oliveira[1]

[1]Centro de Referência de Doenças Hereditárias do Metabolismo - MetabERN, Unidade Local de Saúde de Coimbra, Coimbra, Portugal; [2]Faculdade de Medicina, Universidade de Coimbra, Coimbra, Portugal; [3]Unidade de Rastreio Neonatal Metabolismo e Genética, Instituto Nacional de Saúde Dr Ricardo Jorge, Porto, Portugal;

Presenting Author - ruialdiogo@gmail.com

Disease category: Cofactor and mineral metabolism

Introduction: Cobalamin C deficiency (CblCD) is a rare disease caused by biallelic pathogenic variants in the MMACHC gene. The cellular inability to convert vitaminB12 to the coenzymes adenosylcobalamin and methylcobalamin leads to deficiency of methylmalonyl CoA mutase and methionine synthase with accumulation of propionylcarnitine, methylmalonic acid (MMA) and homocysteine (Hcy) and low methionine (Met). The resulting multisystem dysfunction includes the nervous system and the eye. Hydroxocobalamin and betaine are the mainstays of treatment. The prognosis is poor, especially in early-onset phenotypes.

Methods: Descriptive analysis of clinical presentation, diagnosis (biochemical findings and variants in the MMACHC gene), treatment and disease progression of 5 patients (Pts) with the diagnosis of CblCD, less than 18 years old, followed at our Hospital Center between 2002 and 2021 (20 years).

Results/Case report: CblCD was diagnosed in 5 Pts (3 males), 4 through newborn screening (NBS). The pathogenic variant c.271dupA in MMACHC gene was detected in homozygosity in all Pts. The first manifestation of the disease was hypotonia in the first weeks of life in all Pts. All progressed with retinopathy and global development delay and 3 with non-thrombotic cardiovascular signs. Two Pts died: 23 months (presented in first day of life with metabolic acidosis and coma) and 7 years (type 1 diabetes mellitus diagnosed at 2). No correlation was found between MMA, Hcy or Met levels and age at onset or severity of the clinical manifestations. In spite of early treatment, MMA and Hcy remained elevated. Although the disease has progressed in all, the 3 surviving Pts are stable. The youngest has significant intellectual disability. The others attend regular school with visual aids and good performance.

Conclusion: The c.271dupA variant is associated with significant metabolic impairment and early clinical presentation, as occurred in our cohort. All Pts were treated with parental high doses of hydroxocobalamin and oral betaine, among other less consensual therapeutic interventions. Nevertheless, MMA and Hcy remained elevated. Although NBS allowed early diagnosis and treatment in 80% of Pts, prognosis was poor with high mortality (40%) and intellectual disability/ visual deficit in surviving pts. Despite the small cohort, this is the experience of our Centre, in collaboration with the E-HOD registry.

Acknowledgements: To patients and their families, and to all laboratory and clinical professionals involved in their diagnosis, treatment and monitoring.

PO 34

PHENOTYPIC HETEROGENEITY AND NATURAL HISTORY OF EARS2 DEFICIENCY – REPORT ON 2 CASES AND FOLLOW-UP DATA ON ONE OF THE FIRST DESCRIBED CASES

M. Paiva Coelho[1]; G. Pelayo[1]; C. Nogueira[2]; J. Correia[1]; A. Bandeira[1]; L. Vilarinho[2]; E.Martins[1]

[1] Reference Center for Inherited Metabolic Disorders, Unidade Local de Saúde de Santo António, Porto, Portugal; [2] Metabolism and Genetics Unit, Human Genetics Department, National Institute of Health Dr. Ricardo Jorge, Porto, Portugal;

Presenting Author - margarida.pcoelho@gmail.com

Main category: Basic science study

Disease category: Intermediary metabolism: nutrients

Introduction: Glutamine-associated mitochondrial synthetase aminoacyl-tRNA deficiency is caused by disturbed mitochondrial protein translation due to biallelic variants on the nuclear encoded EARS2, first described in 2012. With only ~50 patients published worldwide, it appears to have a very defined phenotype (neonatal or childhood-onset of leukoencephalopathy with thalamus and brainstem involvement and hyperlactacidemia) but scarce data is available on natural history of these patients, ranging from clinical and radiological improvement to early death.

Methods: We present follow-up data on one of the first published patients and 2 clinical reports of patients with biallelic variants on EARS2, making a total of 5 patients in Portugal.

Results/Case report: ***A.*** Male, present at the third week of life with feeding difficulties and failure to thrive and corpus callosum agenesy. He died at 2 months of age with refractory metabolic acidosis and hyperlactacidemia.

B. Male, first published (Steenweg et al. , 2012) as thepatomegaly, feeding difficulties, motor delay, progressive spasticity and severe white matter involvement, basal ganglia and thin corpus callosum. Follow up showed a severe but static phenotype with spasticity, complete dependency of others, remaining in palliative care at the age of 14.

C. Female, with hypotonia noted since the first's months of life. Brain MRI at 15 months showed extensive leukoencephalopathy with basal ganglia lesions and lactate peak. After a long period without deterioration and progressive acquisitions, she showed slow progressive ataxia without regression, despite neuroradiological progression.

Conclusion: These cases are well representative of the phenotype spectrum of the disease: i) neonatal presentation with lactic acidemia, corpus callosum agenesia and early death to ii) early onset with severe static phenotype to iii) early onset with mild phenotype with initial improvement but later slow progressive neurological symptoms. Excluding those with neonatal presentation, neurological outcome does not correlate with MRI nor the age of presentation. A possible reversible phenotype in EARS2 should be carefully elicited since despite clinical improvement, disease can later progress.

PO 35

CYP46A1 EXPRESSION IN NPC1 CELLS MAY AMELIORATE AUTOPHAGIC DYSFUNCTION BY FACILITATING AUTOPHAGOSOME-LYSOSOME FUSION

I. Caria[1]; S. Nunes[1]; M. João Nunes[1,2]; A. Neves Carvalho[1,2]; J. Reis[1]; J. Mateus[3,4]; R. Mendes Almeida[1]; M. Castro-Caldas[1,5]; M. João Gama[1,2]; C. M. P. Rodrigues[1,2]; S. Xapelli[3,4]; M. José Diógenes[3,4]; N. Cartier[6]; F. Chali[6]; F. Piguet[6]; E. Rodrigues[1,2]

[1] iMed.ULisboa, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; [2] Departamento das Ciências Farmacêuticas e do Medicamento, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; [3] Instituto de Farmacologia e Neurociências, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; [4] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; [5] Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Almada, Portugal; [6] NeuroGenCell, INSERM U1127, Paris Brain Institute (ICM), Sorbonne University, CNRS, APHP, University Hospital Pitié Salpêtrière, Paris, France;

Presenting Author - inescaria13.8a.evs@gmail.com

Main category: Basic science study

Disease category: Lipid metabolism and transport

Introduction: Niemann-Pick type C1 disease (NPC1D) is an inherited lysosomal storage disorder, arising from mutations in NPC protein, which results in the accumulation of unesterified cholesterol and glycosphingolipids in late endosomes(LE)/lysosomes (1). Autophagy dysfunction has also been reported (2). However, how alterations in cholesterol homeostasis may compromise autophagy needs further studies. Recently, we showed that CYP46A1 ectopic expression in cellular models of NPC and in Npc1tm(I1061T) mice corrected cholesterol metabolism and promoted its redistribution from within the LE/lysosomes (3).

Methods: To study how CYP46A1 ameliorates pathological features in NPC1D, we used NPC1-KO Hela cell line overexpressing CYP46A1 by adenoviral or lentiviral-mediated expression. Plus, we did colocalization studies in Npc1tm(I1061T) mice that received an injection of CYP46A1-coding adeno- associated vector.

Results/Case report: We started by confirming CYP46A1 effect in correcting LC3II/I levels. Accordingly, NPC1-KO HeLa cells transduced with CYP46A1-encoding vector significantly decreased LC3II/I ratio in comparison with cells transduced with the control vector. Conversely, p62 levels remained elevated, suggesting that CYP46A1 expression may only partially ameliorate the autophagy block. To evaluate in which step of autophagy flux CYP46A1 may intervene, we immunostainned Rab7 (LE), LC3 (autophagosomes) and LAMP2 (lysosomes) in NPC-KO cells expressing either empty or CMV-CYP46A1- Flag vectors. CYP46A1-expression seemed to increase the number of LAMP2-positive vesicles and the percentage of LC3-puncta overlapping LAMP2-puncta. We did not observe differences over the percentage of LC3 that colocalized with Rab7-positive puncta. Double immunostaining of LAMP1 and LC3 was also done in Npc1tm(I1061T) mice.

Conclusion: Our results so far suggest that CYP46A1 may have a beneficial role in ameliorating autophagy dysfunction. Whereas CYP46A1-expression does not appears to have an effect on initial stages of autophagy, like amphisome formation, we were still able to observe an increased number of lysosomal vesicles and increased colocalization between LC3 and LAMP2-positive pixels. Thus, we propose that CYP46A1-expression may partially revert the autophagy block by directly promoting autophagosome-lysosome fusion.

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TANGIER DISEASE: THE IMPORTANCE OF A SYSTEMATIC APPROACH TO A SUSPECTED METABOLIC DISEASE

Â. Fonseca[1]; J. Durães[2,3]; M. Santos[3,4]; J. Rosmaninho-Salgado[3,4]; M. Macário[2,3]

[1] Neurology, Hospital Pedro Hispano, ULS-Matosinhos, Portugal, Matosinhos, Portugal; [2] Department of Neurology, Hospitais da Universidade de Coimbra – ULS Coimbra, Coimbra, Portugal; [3] Reference Centre for Inborn Errors of Metabolism, Centro Hospitalar e Universitário de Coimbra, ULS-Coimbra, Coimbra, Portugal; [4] Department of Genetics, Centro Hospitalar e Universitário de Coimbra, ULS- Coimbra, Coimbra, Portugal;

Presenting Author - angelofonseca.med@gmail.com

Main category: Case report

Disease category: Lipid metabolism and transport

Introduction: Tangier disease is an extremely rare disorder of lipid metabolism characterized by impaired cellular cholesterol efflux and subsequent accumulation of cholesterol esters in various tissues. Clinically, this disease manifests with hepatosplenomegaly, corneal opacity, peripheral neuropathy, tonsillar and skin xanthomas.

Methods: Case Report presentation.

Results/Case report: A 70-year-old man with thrombocytopenia since he was 40 years old, chronic liver disease, hepatosplenomegaly, repetitive skin ulcers and amygdalotomy at age of 6 was sent to neurometabolic diseases clinic for suspected peripheral neuropathy in the context of an inborn error of metabolism. His family history was positive for thrombocytopenia in his sister and aunt. Cognitive and neurological examination and EMG studies were normal. Laboratory analysis revealed low total cholesterol, HDL cholesterol and Apolipoprotein A1. Liver biopsy showed reticuloendothelial system involvement, hemosiderosis and cirrhosis, and bone marrow biopsy revealed foamy macrophages aggregates. Due to a high level of suspicion, a NGS panel for inborn errors of metabolism was performed and revealed a truncanting homozygous variant in the ABCA1 gene, highly suggestive of Tangier disease (MIM:#205400; ORPHA:31150).

Conclusion: Low total cholesterol in the setting of a characteristic pattern of accumulation disorders should raise the suspicion of Tangier disease, with autosomal recessive inheritance. The diagnosis of a rare disorder of lipid metabolism with a predominant presentation in adulthood and a late diagnosis emphasizes the importance of genetic studies in identifying rare metabolic disorders in adults.

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LARGE VESSEL VASOSPASM IN PRIMARY MITOCHONDRIAL DISORDERS: EPIPHENOMENON OR A CAUSAL RELATIONSHIP?

Â. Fonseca[1]; M. Seco[1]; S. Moreira[1]; C. Duque[1]

[1] Department of Neurology, Hospital Pedro Hispano, ULS-Matosinhos, Matosinhos, Portugal

Presenting Author - angelofonseca.med@gmail.com

Main category: Case report

Disease category: Intermediary metabolism: energy

Introduction: Primary mitochondrial disorders (PMD) encompass a large spectrum of clinical syndromes, ranging from encephalopathy and stroke-like episodes (as seen in MELAS) to deafness and diabetes, cardiomyopathy, seizures, and migraine. However, the heterogeneity within these disorders often pose challenges in their diagnosis and management. So far, there is a large amount of data supporting the involvement of small vessels in mitochondrial disease and their dysfunction in stroke- like episodes. However, no evidence has been reported about large vessel disease such as extracranial carotid artery vasospasm

Methods: Case Report.

Results/Case report: A 41-year-old Chinese woman, with history of antithrombin III deficiency, cardiomyopathy and a brother who died of heart disease, was admitted for episodes of right periorbital pain and left hemiparesis. CT angiography during one such episode showed right internal carotid artery (ICA) stenosis. Later, she experienced left periorbital pain and transient monocular blindness. Left ICA stenosis was documented, without the contralateral stenosis, suggesting vasospasm. Corticotherapy was initiated, along with ongoing vasodilators and anticoagulation. A POLG mutation and mtDNA deletions in muscle were found.

Conclusion: POLG mutation with mtDNA deletions suggest an underlying PMD posing the hypothesis of causality for the event. Although no large artery vasospasm has been previously described, mitochondrial dysfunction is known to cause disruption on cellular energy metabolism and impair endothelial function with nitric oxide depletion which collectively may lead to dysregulated vascular tone and vasoconstriction such as those observed in the presented case. However, further research is needed to understand the underlying mechanisms and optimize management strategies.

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