HYBRID EVENT

17TH INTERNATIONAL SYMPOSIUM OF THE PORTUGUESE SOCIETY FOR METABOLIC DISORDERS

SEPTEMBER, 8 - 10 | HOTEL LUX - FÁTIMA, PORTUGAL

2021

LINKING METABOLIC SIGNATURE TO IEM TREATMENT

organizing by

SOCIEDADE PORTUGUESA DE DOENÇAS METABÓLICAS

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WELCOME ADDRESS

Dear Colleagues,

On behalf of the Organizing Committee, I have the great pleasure to invite you all to join us for the 17th International Symposium of the Portuguese Society for Metabolic Disorders (SPDM), on the 9th and 10th of September 2021.

Due to the limitations imposed by the SARS-CoV-2 pandemic, the SPDM 2021 Organising Committee will host a hybrid event, being the physical venue held at the Lux Fátima Park Hotel, in the city of Fátima. An online platform will broadcast the event and allow the participation of all those that cannot be physically present.

The motto of this year SPDM Symposium is **"Linking metabolic signature to IEM treatment".** We have invited key world experts to bring us the most up to date research in the field and help to create a fruitful scientific discussion environment. To further stimulate discussion and participation, this year program includes sessions of "oral communication to the table", alongside with another dedicated to general oral communications. We encourage all those working or with interest in the field of inborn errors of metabolism to participate and share their research/work, as oral or poster communications.

We are delighted to be hosting the 2021 SPDM Annual Symposium and very much look forward to welcome all of you in the city of Fátima.

Hugo Rocha
2021 Symposium Chairperson

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Hugo Rocha – *SPDM Board*
Instituto Nacional de Saúde Doutor Ricardo Jorge, Porto

PROGRAM

SCIENTIFIC PROGRAM

SEPTEMBER, 8TH

17:45 | 18:30	SPDM Nutrition Group Meeting
19:00 | 19:45	Welcome drink

SEPTEMBER, 9TH

08:30	Registration opening
09:15	Symposium Opening
09:30 | 10:30	**Session I - IEM's mapping**
09:30 | 10:00	MetabERN - where are we now? *Maurizio Scarpa, IT*
10:00 | 10:30	Broadening the concept of IMD: Novel disorders and international classification *Carlos Ferreira, USA*
10:30 | 11:00	Coffee break
11:00 | 13:00	**Session II - Newborn Screening for IEM**
11:00 | 11:30	Newborn Screening in Europe and initiatives tp develop programmes in low - and middle-income countries *Jim Bonham, UK*
11:30 | 12:00	New tests and future directions in the NBS for IEM *Giancarlo LaMarca, IT*
12:00 | 12:30	Treatment follow-up of screened disorders *Paula Garcia, PT*
12:30 | 13:00	Oral Communications to the table
13:00 | 14:00	Lunch / Poster view
14:00 | 15:50	**Session III - From CbL to Hcy**
14:00 | 14:30	Remethylation disorders: from cobalamin absorption, intracellular pathways to MTHFR deficiency *Matthias Baumgartner, CH*
14:30 | 15:00	Inherited disorders of transsulfuration: clinical presentation, diagnosis, and recent advances in therapy? *Viktor Kozich, CZ*
15:00 | 15:30	The transsulfuration pathway at the crossroads between homocysteine and hydrogen sulfide metabolism: (dys)regulation and (patho)physiological implications *João Vicente, PT*
15:30 | 15:50	Oral communications to the table
15:50 | 16:20	Coffee break
16:20 | 17:20	**Session IV - IEM Nutritional and Pharmacological treatment**
16:20 | 16:50	Current and future approaches in PKU treatment *Cary Harding, USA*
16:50 | 17:20	Micronutrients – role in IEM treatment *Júlio César Rocha, PT*
17:20 | 18:00	**Session V - General Oral communications**
18:00 | 19:30	Thematic Meeting - PKU Breaking the Dogma - (Programme page 11)
20:30 | 21:45	Symposium dinner

SEPTEMBER, 10TH

09:00 | 10:30	**Session VI - The acetyl-CoA cross**
09:00 | 09:30	Pyruvate dehydrogenase complex deficiency in Portugal: the clinical, metabolic, and mutational landscapes and its peculiar characteristics *Hana Pavlu-Pereira, PT*
09:30 | 10:00	Ketotic glycogen storage diseases (GSD): current knowledge, management, and monitoring *Ulrike Steuerwald, DE*
10:00 | 10:30	How to find your way through metabolic disorder in patients with rhabdomyolysis *Pascal Laforêt, FR*
10:30 | 11:00	Coffe break
11:00 | 13:00	**Session VII - Lysosomal Disorders**
11:00 | 11:30	A new face in the diagnosis and monitoring of lisosomal disorders *Johannes Aerts, NL*
11:30 | 12:00	Gene therapy for lysosomal storage disorders: advances, challenges and perspectives. *Maria José Castro, ES*
12:00 | 12:30	Immunomodulation and ERTs *Simon Jones, UK*
12:30 | 13:00	Oral Communications to the table
13:00 | 14:00	Lunch / Poster view
14:00 | 15:30	**Session VIII - The ammonia roundabout**
14:00 | 14:30	New insights into the pathophysiology and molecular basis of UCD's *Johannes Haberle, CH*
14:30 | 15:00	Potential role of gene therapy for OTC deficiency *Alvaro Hermida, ES*
15:00 | 15:30	A novel small molecule approach for the treatment of propionic and methylmalonic acidemias *Marshall Summar, USA*
15:30 | 16:00	Coffee break
16:00 | 17:30	**Session IX - Down Town Mitochondria**
16:00 | 16:30	Mitochondrial plasticity - role in muscle pathology *Rita Ferreira, PT*
16:30 | 17:00	Renal involvemente in Mitochondrial Disorders *Margarida Coelho, PT*
17:00 | 17:30	The diagnosis of mitochondrial disease using multi-omic methodologies *Charlotte Alston, UK*
17:30 | 17:45	**Bolsa SPDM Dr Aguinaldo Cabral 2019** *Sandra Alves, PT*
17:45	**Awards / Closing remarks**

09TH SEPTEMBER 2021

ORAL COMMUNICATIONS

12h30 – 13h00 | Session II – **Newborn Screening for IEM**

OC 01	When the newborn screening prevents ketoacidosis, *J Tenete, Vila Nova de Gaia, PT*
OC 02	Trifunctional protein deficiency – case report of neonatal onset, *M Rodrigues, Porto, PT*
OC 03	Carnitine uptake deficiency in asymptomatic patients – the importance of 5' untranslated region (UTR) of SLC22A5, *H Santos, Vila Nova de Gaia, PT*

15h30 - 15h50 | Session III – **From CbL to Hcy**

OC 04	A Case Series of Paediatric Classical Homocystinuria – nutritional status, dietary intake and metabolic profile, *RS Loureiro, Lisboa, PT*
OC 05	New predictives for low vitamin B12 in newborns, *P Lipari Pinto, Lisboa, PT*

17h20 | 18h00 | Session V – **General Oral Communications**

OC 09	Long-term, sustained efficacy and safety results from a phase 1/2 clinical trial of an AAV8-mediated liver-directed gene therapy in adults with glycogen storage disease type Ia, *Maria-Luz C Pico, Santiago de Compostela, SP*
OC 10	AAV8 Gene Therapy as a Potential Treatment in Adults with Late-Onset Ornithine Transcarbamylase (OTC) Deficiency: Updated Results From a Phase 1/2 Clinical Trial, *Maria-Luz C Pico, Santiago de Compostela, SP*
OC 11	Genotypic and phenotypic features of the mothers of children with mtDNA-associated Leigh syndrome, *CA Soares, Porto, PT*
OC 12	New causes of persistent or recurrent 3-methylglutaconic aciduria – expanding the differential diagnosis of secondary 3-MGA-uria, *F Freitas, Porto, PT*

10TH SEPTEMBER 2021

ORAL COMMUNICATIONS

12h30 - 13h00 | Session VII – **Lysosomal Disorders**

OC 06	Targeted RNA-based therapies for Mucopolysaccharidosis, *JI Santos, Porto, PT*
OC 07	Functional characterization of five novel mutations found in patients with suspicion of Fabry disease, *P Varela-Calais, São Paulo, BR*
OC 08	NIEMANN PICK Type C – 26 years of diagnosis in a Portuguese Reference Center, *AM Capela, Porto, PT*

INVITATION

9TH SEPTEMBER 2021

18:00 - 19:30 (GMT)

PKU
BREAKING THE DOGMA

THEMATIC MEETING

JOIN US!

Organized and sponsored by:

BIOMARIN

Phenylketonuria (PKU) is a rare, inherited, chronic and life-long condition.
Advances in the treatment of this disease led to different perspectives on its management, explored in this meeting.

AGENDA (GMT)

18:00 - 18:05	**Welcome**
18:05 - 18:20	**Kuvan in Infants** | *María Bueno Delgado*
18:20 - 18:35	**The problem with self-awareness in PKU** | *Cary O. Harding*
18:35 - 18:50	**New road map for nutritional management** | *Júlio César Rocha*
18:50 - 19:20	**Open Discussion** Moderator: Júlio César Rocha *Álvaro Hermida Ameijeiras, María Bueno Delgado*
19:20 - 19:25	**Patient Experience**
19:25 - 19:30	**Conclusions**

SPEAKERS

MARÍA BUENO DELGADO
Paediatrician. Hospital Universitario Virgen del Rocío.
Sevilla, Spain

CARY O. HARDING
Clinical Geneticist. Oregon Health & Science University.
Portland, OR, USA

JÚLIO CÉSAR ROCHA
Nutritionist. Centro Hospitalar Universitário de Lisboa Central.
Lisbon, Portugal

ÁLVARO HERMIDA AMEIJEIRAS
Internist. Hospital Clínico Universitario de Santiago de Compostela.
Santiago de Compostela, Spain

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EU-MPRL-PKU-00040 – August 2021

SPEAKERS

SESSION I
IEM'S MAPPING

MAURIZIO SCARPA
ITALY

MAURIZIO SCARPA, MD PhD, paediatrician, is the Director of the Regional Coordinating Centre for Rare Diseases at the University Hospital of Udine, Italy. He is Professor of Paediatrics at the Dept. for the Woman and Child Health, University of Padova, Italy, and the Co-Founder of the Brains For Brain Foundation, together with Prof. David Begley, Kings College of London, London, UK. Prof. Scarpa earned his medical degree, doctorate and residency in Paediatrics at the University of Padova in Italy. He completed a postdoctoral fellowship in molecular biology and gene expression at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, and in genetics and gene therapy at the Howard Hughes Medical Institute, Institute for Molecular Genetics, at Baylor College of Medicine in Houston, Texas, USA. He has been the Director of the PhD Course on Genetics and Biochemistry at the Dept. for the Woman and Child Health at the University of Padova, Italy. He served as Vice-Dean for the International Affairs at the University of Padova and Director of the International Affairs Office at the Faculty of Medicine of the University of Padova, Italy.He is the Coordinator of the European DGSANTE Project, INNERMED.
Prof. Scarpa has extensive expertise as a basic scientist in genetics and biotechnology, as well as a clinician in the diagnosis and treatment of paediatric rare disorders; neurometabolic diseases in particular. Together with dr. Christina Lampe he founded the Center for Rare Diseases at the Helios Dr. Horst Schmidt Kliniken in Wiesbaden, Germany. He is especially interested in developing innovative health approaches for the diagnosis and the treatment of metabolic inherited diseases; to this aim he is also collaborating with major biotech companies as an external independent expert. Prof. Scarpa´s teaching and educational interests aim, among other things, is for the development of a MD/PhD European Programme on Inherited Metabolic Diseases. Prof. Scarpa is the Coordinator of the European Reference Network for Hereditary Metabolic Diseases, MetabERN, formed by 78 healthcare providers in 23 EU countries and treating about 60,000 patients with metabolic diseases and he is the Past-Chair of the ERN Coordinators Working Group. Prof. Scarpa is an author of approximately 140 international peer reviewed clinical and scientific papers, book chapters and reviews.

CARLOS FERREIRA
WASHINGTON DC, USA

CARLOS R. FERREIRA is board-certified in Internal Medicine, Medical Genetics, and Clinical Biochemical Genetics, and specializes in genetic disorders of bone development and inborn errors of metabolism. He led a team of researchers that elucidated the molecular defect underlying Saul-Wilson syndrome, a rare disorder of vesicular trafficking leading to skeletal dysplasia. He contributed to the finding of disturbed tryptophan catabolism as a new etiology of Catel-Manzke syndrome, and to finding the cause of a rare hematologic disorder, combined alpha-delta platelet storage pool deficiency. He was part of several collaborations that led to the discovery of novel genetic disorders, including a primary CoQ10 biosynthetic defect, a genetic form of renal Fanconi syndrome due to GATM aggregates, a rare neuromuscular defect (Carey-Fineman-Ziter syndrome), a novel neurodegenerative condition (TRAK1 deficiency), and a skeletal dysplasia in the dysosteosclerosis-Pyle disease spectrum. Dr. Ferreira participates as faculty in the annual North American Metabolic Academy (NAMA) course. He has authored more than 90 indexed publications, one of which recently received the Emmanuel Shapira Award (bestowed to the best paper published in Molecular Genetics and Metabolism).

SESSION II
NEWBORN SCREENING FOR IEM

JIM BONHAM
UK

JIM BONHAM is currently the National Laboratory Lead for the Newborn Screening Blood Spot Programme in the UK on behalf of Public Health England. He is also President of the International Society for Neonatal Screening with more than 500 members in 40 countries. In 2012 he led a study to introduce additional inherited metabolic disorders into the national newborn screening programme in the UK. Four of these were incorporated as part of the programme in England and Wales from 2015. He has interests in the organisation, quality and effectiveness of newborn screening and how this might be optimised and extended to benefit patients and families in the UK and Europe and more recently as part of the Global Taskforce on newborn screening, to low and middle income countries. Prof Bonham has played an active role in several national and international professional organisations including the SSIEM, Royal College of Pathologists, UKNSLN, MetBioNet, NEQAS and UKAS. He helped found the British inherited Metabolic Disease Group and the International EQA provider for Inherited Metabolic Disorders – ERNDIM. His efforts in these areas were generously recognised by the award of an MBE in 2020.

GIANCARLO LAMARCA
FLORENCE, ITALY

GIANCARLO LAMARCA Assistant Professor of Pharmacology, granted by the University of Florence, since November 1st 2007. Head of Newborn Screening, Clinical Chemistry and Pharmacology Lab, Meyer Children's Hospital, Florence, Italy, since 2011. Associate Professor of Clinical Biochemistry and Clinical Molecular Biology, granted by the University of Florence, since November 1st 2015. Qualified as full Professor of Clinical Biochemistry and Clinical Molecular Biology, granted by the University of Florence, since November 1st 2016. He is (2018-22) the President of the Italian Society for the Study of Inherited Metabolic Diseases and Newborn Screening.
Prof la Marca received several awards in his career, such as:
- Paul Harris Prize 2012, for research in pediatric preventive medicine
- "Excellence of the Year" prize, 2013, Lions Club, Scandicci, Florence"
- International Society for Neonatal Screening: ISNS Dussault Medal 2014 to "honour a researcher who has made a significant contribution to neonatal or other population-based screening which is recognized as such".
- Voa Voa Award 2015: "For personal and professional commitment made in the pediatric research and application of concrete scientific solutions for the benefit of society as a whole"

PAULA GARCIA
COIMBRA, PORTUGAL

PAULA GARCIA is a Paediatrician with experience in inherited metabolic diseases.
Workplace: Reference Center for Inherited Metabolic Diseases of Centro Hospitalar e Universitário de Coimbra, Portugal. MetabERN partner.
Other: President of Inherited Metabolic Diseases section of Portuguese Paediatrics Society and member of the executive commission of Newborn Screening National Program

SESSION III
FROM CBL TO HCY

MATTHIAS BAUMGARTNER
ZURICH, SWITZERLAND

MATTHIAS R. BAUMGARTNER studied medicine at the University of Basel and graduated in 1992. He then completed the postgraduate course in experimental medicine and biology at the University of Zurich and the Biozentrum of the University of Basel. After completing his training as a pediatrician at the University Children's Hospital of Basel (UKBB) and at the Hôpital Necker - Enfants Malades in Paris, Prof. Baumgartner went to the USA in 1999, where he worked as a Postdoctoral and Clinical Fellow at the Mc Kusick-Nathans Institute of Genetic Medicine of the Johns Hopkins University, Baltimore. In 2001, he returned to Switzerland as Senior Physician for Metabolic Diseases, first at the UKBB, and from 2003 at the University Children's Hospital Zurich. In 2008, he was appointed Professor of Metabolic Diseases at the University of Zurich. Prof. Baumgartner heads the Department of Metabolic Diseases, is Medical Director of Newborn Screening Switzerland, Director of the Research Center for the Child since 2017, and Director Research & Teaching and Member of the Executive Board at the Children's Hospital Zurich since 2020. He is an internationally known metabolic paediatrician and scientist with a main research interest in disorders of intracellular cobalamin metabolism including the homocystinurias and methylmalonic acidurias; he is a steering committee member of the European networks and registries for Homocystinurias and remethylation disorders (E-HOD, www.e-hod.org) and Intoxication type Metabolic Diseases (E-IMD, www.e-imd.org) and an editor of the Journal of Inherited Metabolic Disease.

VIKTOR KOZICH
PRAGUE, CZECH REPUBLIC

VIKTOR KOŽICH, M.D., Ph.D. is a Professor of Medical Genetics at the Department of Pediatrics and Inherited Metabolic Disorders, General University Hospital in Prague and Charles University-First Faculty of Medicine, Prague, Czech Republic. Viktor Kožich graduated from the School of General Medicine, Charles University in Prague in 1985. Since his graduation he has been working in the Institute of Inherited Metabolic Diseases and he specialized in clinical biochemistry and medical genetics, in 2012 he became the full Professor of Medical Genetics. His main interests are genetic, biochemical, clinical, epidemiological and ethical aspects of inherited metabolic disorders. He is specialized in disorders of homocysteine transsulfuration pathway, namely in cystathionine beta-synthase deficiency—a disease in which he became interested in 1991-1992 during his fellowship in the laboratory of the late Prof.Jan P.Kraus (University of Colorado School of Medicine in Denver, USA). Professor Kožich has been a tutor of graduate and postgraduate students, he is an author of over 130 publications in international peer reviewed journals, several chapters in books, and of articles and chapters in Czech medical literature. He has been a member of councils of several international learned societies (SSIEM, ERNDIM, and ESHG) and he is a member of the Executive Board of E-HOD since 2013. Prof.Kožich is also interested in newborn screening for inherited metabolic disorders and since 2009 he is a Chairman of the Czech national Coordination Center on Neonatal Screening.

JOÃO VICENTE
LISBON, PORTUGAL

JOÃO B. VICENTE earned his European Label PhD in Biochemistry from NOVA University of Lisbon (Portugal) and Sapienza University of Rome (Italy) in 2007. After a post-doc in Stanford University (USA) and a researcher position at the School of Pharmacy, Lisbon University (Portugal), he started a position as Auxiliary Investigator in the Macromolecular Crystallography Unit at NOVA Instituto de Tecnologia Química e Biológica (Portugal). His research team combines protein biochemistry and biophysics with structural biology to study the molecular mechanisms underlying human hydrogen sulfide (H2S) metabolism. Particularly, his team is interested in understanding how the metabolic pathways leading to the production and breakdown of H2S are regulated by endogenous physiologically relevant modulators. Through a network of collaborators, he aims to position these metabolic pathways and their regulatory mechanisms as key determinants in the context of various human diseases. Moreover, he is committed to the discovery and development of pharmacological and diagnostics tools to improve therapeutics.

SESSION IV
IEM NUTRITIONAL AND PHARMACOLOGICAL TREATMENT

CARY HARDING
WASHINGTON, USA

CARY O. HARDING, MD, FACMG is Professor of Molecular and Medical Genetics and Pediatrics at Oregon Health & Science University (OHSU). He received his medical degree from the University of Washington followed by pediatric and medical genetics training at the University of Wisconsin-Madison. He is board certified in Clinical Genetics and Biochemical Genetics. Dr. Harding is a founding fellow of the American College of Medical Genetics and Genomics (ACMGG) and a member of the American Society for Cell and Gene Therapy (ASGCT). He is the treasurer of the Society for Inherited Metabolic Disorders (SIMD). He is an attending physician on the OHSU clinical genetics service as well as the metabolic clinic at Doernbecher Children's Hospital. He is the Medical Director of the Biochemical Genetics Lab at OHSU. He is also a clinical consultant to the Northwest Regional Newborn Screening Program and to the State of Idaho Genetics Program. He serves as co-chair of the Scientific Advisory Board for the National PKU Alliance (NPKUA) and as the Project Director for the PHEFREE Rare Disorders Consortium. Dr. Harding's basic and clinical research programs are focused upon the development of novel therapies, including gene and cell therapies, for inborn errors of metabolism.

JÚLIO CÉSAR ROCHA
LISBON, PORTUGAL

JÚLIO CÉSAR ROCHA is a Nutritionist, 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 Assistant Professor at NOVA Medical School teaching in the field of Nutrition and Metabolism. He is also Nutritionist member of the clinical team at the Reference Centre of Inherited Metabolic Diseases at Centro Hospitalar Universitário Lisboa Central and he is a researcher at CINTESIS (Center for Health Technology and Services Research), at the Faculty of Medicine, UP. He is council member of the SSIEM (Society for the Study of Inborn Errors of Metabolism) and Chair of the Dieticians Group of the SSIEM (SSIEM-DG). He is also member of the working group of the European Phenylketonuria Guidelines. He is author of more than 50 international, indexed, scientific publications and he has done more than 90 oral presentations/lectures/conferences in more than 12 different countries.

SESSION VI
THE ACETYL-COA CROSS

HANA PAVLU-PEREIRA
LISBON, PORTUGAL

HANA PAVLU-PEREIRA graduated in Biochemistry at the University of Chemistry and Technology in Prague and started her scientific activity in the field of lysosomal storage disorders at the Center of Inherited Metabolic Diseases at Charles University in Prague, where she focused on studies on molecular mechanism of acid sphingomyelinase deficiency. In collaboration with EMBL in Heidelberg, INSERM in Lyon and Institut für Organische Chemie und Biochemie in Bonn she contributed to the classification of the intermediate A/B form of Niemann-Pick disease. After moving to Portugal, she joined the group of Metabolism & Genetics at the Research Institute for Medicines (iMed. ULisboa) and has been involved in the research of pyruvate dehydrogenase deficiency. In collaboration with ITQB in Oeiras she is currently focused on studying molecular mechanisms underlying pathogenic variants and potential therapeutic effect of small molecular weight compounds, namely arginine and thiamine.

ULRIKE STEUERWALD
HANNOVER , GERMANY

ULRIKE STEUERWALD
MD in 1982 and PhD in 1984, both at the University of Freiburg, Germany.
Trained as pediatrician in Osnabrück (Germany), Göttingen (Germany) and Zürich (Schwitzerland).
Registrar in Pediatric since 1992 with main areas neonatology, intensive care and child neurology.
Since 1993 pediatric consultant in the Faroe Islands, presently holding part-time contracts in Tórshavn (National Hospital and Dept. of Occupational and Public Health), Faroe Islands and at the Screening Laboratory in Hannover, Germany
Tight cooperation with D.A. Weinstein, former head of Glycogen Storage Disease Program at Connecticut Children Medical Center Hartford CT, T.G.J. Derks, University Medical Center Groningen, The Netherlands, and Sarah C. Grünert, Children's Hospital at the University of Freiburg, Germany
Honored as Dr. Philip Lee Glycogen Storage Disease Scholar in 2019

PASCAL LAFORÊT
PARIS, FRANCE

PASCAL LAFORÊT, MD, PhD, is a professor of Neurology at the Versailles-Saint Quentin University, consultant specialized in neuromuscular disorders (myasthenia gravis, muscular dystrophies, and metabolic myopathies) in the Neurology department of Raymond-Poincaré hospital, metabolic and coordinator of North/East/Ile de France neuromuscular center and FHU Phenix dedicated to translational research in neuromuscular disorders. He is affiliated to U1179 INSERM-UVSQ laboratory, dedicated to biotherapies of neuromuscular system diseases. Major focus of his research activities are myopathies (pathophysiology and clinical trials), and he coordinates the French registries for mitochondrial disorders, glycogenosis type III, and Pompe disease. He is a member of the French Myology Society (SFM), French Society of Inherited Metabolic Disorders (SFEIM), and boards of the French Glycogenosis Association (AFG) and Garches Fundation.

SESSION VII
LYSOSOMAL DISORDERS

HANS AERTS
LEIDEN, NETHERLANDS

HANS AERTS is Head of the Department of Medical Biochemistry at the University of Leiden since January 2015. Earlier he chaired the Department of Biochemistry at the Academic Medical Center in Amsterdam (2000-2014). His research focuses on glycosphingolipids in health and disease with special attention for inherited lysosomal storage disorders like Gaucher disease, as well as neurodegeneration and the Metabolic Syndrome. Trained in biochemistry at the University of Amsterdam and the National Institutes of Health in Bethesda, USA, he completed his PhD thesis Biochemical studies on glucocerebrosidase in relation to Gaucher disease with honours in 1988. He was involved in the first application of enzyme replacement therapy and substrate reduction therapy of type 1 Gaucher disease in Europe as well as the discovery of now widely used biomarkers. He has been (co)promotor of 42 completed PhD thesis works and published over 430 peer-reviewed papers (H-index: 93, Google Scholar). Professor Aerts is recipient of various governmental research grants, including the ERC Advanced Research Grant 'Chemical Biology of Sphingolipids and the NWO Building Blocks of Life Grant 'Glucosylceramide'. He is founder and honorary president of the European Working Group on Gaucher Disease (EWGGD), a society comprising all major Gaucher clinical and research centres and national patient societies worldwide. He was awarded the Dutch Annual Prize in Biochemistry (1989), the Alan Gordon Memorial Award (2007), the first Gaucher Lifetime Achievement Award (2012) and Cle du Lysosome Award (2016).

MARIA JOSÉ CASTRO LÓPEZ
SANTIAGO DE COMPOSTELA, SPAIN

MARIA JOSÉ CASTRO LÓPEZ
Academic qualifications:
- Graduated in medicine and surgery. Specialist in Pediatrics. University of Santiago de Compostela.
- Master in Hereditary Metabolic Diseases. University of Santiago de Compostela.
- Doctorate in Medicine: NeoSeq: a rapid genetic diagnosis tool for critically ill newborns with suspected genetic disease. University of Santiago de Compostela.

Professional experience:
- Consultant in Pediatric Inherited Metabolic Diseases since 2015
- Co/subinvestigator in phase I-II clinical trials with gene therapy for congenital metabolic diseases (MPSIIA, MPSIIIB, glycogen storage disease Ib, OTC deficiency)
- Investigadora del Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS)

Teaching experience
- Member of the Chair of Hereditary Metabolic Diseases of the University of Santiago de Compostela

SESSION VIII
THE AMMONIA ROUNDABOUT

JOHANNES HÄBERLE
ZURICH, SWITZERLAND

JOHANNES HÄBERLE is a senior metabolic consultant at the University Children's Hospital in Zurich and a trained pediatrician, neonatologist and intensive care specialist. He is an assistant professor for Pediatrics at the University of Zurich, and Head of the Metabolic Laboratory at the University Children's Hospital. His research group focusses on inherited defects of ureagenesis and on the development of novel treatment options for urea cycle disorders. Dr. Häberle is chairperson of the European working group for Guidelines for the Diagnosis and Treatment of Urea Cycle Disorders, and a member of the Executive and Scientific Boards of the European Registry and Network for Intoxication Type Metabolic Diseases (EIMD). He is chair of the SSIEM Education And Training Advisory Committee (ETAC) and Council member of the Society for the Study of Inborn Errors of Metabolism (SSIEM).

ALVARO HERMIDA
SANTIAGO DE COMPOSTELA, SPAIN

ALVARO HERMIDA was born in Bilbao (Basque Country) and studied Medicine at the University of Santiago de Compostela (USC) and graduated in 1998. He received his Ph.D. in Biochemistry from USC, in 2002 and completed his clinical training in internal medicine in 2007. He is currently an assistant physician in the Unit of Diagnosis and Treatment of Inherited Metabolic Diseases of the University Clinical Hospital of Santiago de Compostela (Spain) and a full profesor of the Department of Medicine since 2013.
With a long-standing research experience, he has been co-investigator in more than 20 international clinical trials related to metabolic disorders and cardiovascular morbidity/mortality (Phase II, III and IV). He is author of many research works reported in high-impact scientific journals in the field of oxidative stress, neurometabolism, inherited metabolic disorders and cardiovascular risk.
He is a member of several national and international working groups for various inborn errors of metabolism and member of the Spanish Association for the Study of Inborn Errors of Metabolism (AECOM) and the Spanish Society of Internal Medicine.

SESSION IX
DOWN TOWN MITOCHONDRIA

RITA FERREIRA
AVEIRO, PORTUGAL

RITA FERREIRA has a degree and a PhD in Biochemistry and since 2009 works as Assistant Professor at the University of Aveiro. Alongside teaching activities, RF has developed scientific work in the field of muscle remodeling in pathophysiological conditions, including the evaluation of the impact of wasting conditions and exercise training on mitochondria plasticity using Omics approaches. Most of RF research has been developed within the scope of research projects, from which resulted more than 190 papers published in international peer-reviewed journals.

MARGARIDA COELHO
PORTO, PORTUGAL

MARGARIDA COELHO Paediatrician in the Reference Center for Inherited Metabolic Disorders of Centro Hospitalar Universitário do Porto
Member of Medical Teaching Department - CHUP - Teaching staff for Master Degree in Medicine (Introduction to Medicine I)
PhD Student in Medical Sciences in ICBAS-UP (Metabolomics and Mitochondrial Disorders)

CHARLOTTE ALSTON
NEWCASTLE, UK

CHARLOTTE L ALSTON graduated in 2003 from the University of Aberdeen with a BSc (hons) degree in Genetics, having undertaken her honours project under the supervision of Professor Duncan Shaw. She commenced her Clinical Scientist training in 2004 within the Medical Genetics Laboratory in Aberdeen which she completed just before joining the NHS Highly Specialised Service for Rare Mitochondrial Disorders in Newcastle Upon Tyne in 2007. She obtained her HCPC State Registration in 2008 and spent the next 7 years working as a Clinical Scientist in Newcastle, investigating the genetic basis of mitochondrial diseases. She was awarded a Personal Fellowship from the National Institute of Health Research (NIHR) to undertake her doctorate in the Wellcome Centre for Mitochondrial Research in Newcastle upon Tyne (2013-2017, including a 6 month period of maternity leave) investigating the genetic basis of a cohort of paediatric mitochondrial disease patients using genomic technologies, for which she was awarded the Faculty Doctoral Thesis Prize. In 2018, she was awarded a NIHR Post-Doctoral Fellowship which she commenced in 2019 upon returning from her second period of maternity leave. She is Chief Investigator on a multi-site research study which applies whole exome sequencing of blood DNA samples from patient-parent trios with the aim of demonstrating that muscle biopsy no longer represents the 'gold standard' test for the majority of paediatric patients with suspected mitochondrial disease. Her study also aims to reclassify variants of uncertain pathological significance (VUS) using multi-omic technologies; where patient biopsies are unavailable, CRISPR/Cas9-mediated knock-in of patient variants will generate surrogate tissues for functional experimentation to validate pathogenicity and facilitate access to genetic counselling and reproductive options for at-risk families.

SESSION I

IEM'S MAPPING

CHAIRPERSONS

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CENTRO HOSPITALAR UNIVERSITÁRIO COIMBRA, COIMBRA, PT

DULCE QUELHAS, MSc
CENTRO HOSPITALAR UNIVERSITÁRIO PORTO, PORTO, PORTUGAL, PT

THE EUROPEN REFERENCE NETWORK FOR RARE INHERITED METABOLIC DISEASES, METABERN

MAURIZIO SCARPA

Maurizio Scarpa, Coordinator MetabERN, Regional Coordinating Center for Rare Diseases, University Hospital, Udine, Italy on behalf of the Members of MetabERN.

Up to now about 60.000 diseases are known to affect human beings, among them about 10% are rare diseases. Rare diseases are defined as those affecting less than 5:10.000 citizens in EU, they are rare singularly rare, but it is calculated that about 8-10% of the world population might be cought by a rare disease, in the pediatric or adulthood. Rare diseases are a challenge not only as a public health burden but also at scientific level, since each disease has a different phenotype (even on the same family) and every patient might be considered as a unicum. Indeed, even siblings might have different phenotypes although sharing the same genotype. Indeed there is no genotype-phenotype correlation. For this reason, In order to unravel this medical and scientific challenge it might be important to study a considerable number of patients, which is impossible in a single center. For this reason, the European Commission, as implementation of the Cross Border Directive 2011/24/EU, has launched the European Reference Networks (ERNs).

ERNs are international communities that bring together specialised healthcare providers across the European Union. Their aim is to provide better care, treatment and research (basic and clinical trials) for patients living with complex or rare diseases that require specialised interventions and a concentration of expertise and resources. MetabERN (www.metab.ern-net.eu) is one of the 24 ERNs awarded in 2017; it focuses on rare hereditary metabolic disorders.

The MetabERN is formed at the moment by 78 Health Care Providers in 23 EU Member States and 55 Patient Organizations, closely connected to basic research: the understanding of biological systems, and especially the molecular mechanisms involved in the pathophysiology of metabolic diseases, is crucial to improve the clinical practice. MetabERN is a perfect example of a community of experts (about 2000 medical professionals and researchers) that leverages on the findings of basic research to develop effective therapeutic and diagnostic applications, thus improving the care of rare metabolic patients. MetabERN is following at the moment over 60.000 metabolic patients. The U-IMD pan-metabolic registry (www.u-imd.org), run by the Heidelberg University, Germany, is a major MetabERN tool for the understanding of natural history, newborn screening, early diagnosis and development of new therapies for IMD.

The delay in the diagnosis of a rare disease (average 7.5 years from the first symptom), the limited availability of treatments for only a few diseases, the inappropriate design of clinical trials, which are unable to generate reliable data, the scarce quality of post-market trials that are not able to evaluate the real efficacy of a drug, and the paucity of reliable biomarkers for the evaluation of drug safety and efficacy independently from the clinical answer are crucial gaps. To fill this gaps MetabERN is combining the efforts and points of view of the main representatives of the entire EU metabolic community, assuring a widescale international representation of all stakeholders.

The major activities of MetabERN expert working groups can be depicted as follows

BROADENING THE CONCEPT OF IMD: NOVEL DISORDERS AND INTERNATIONAL CLASSIFICATION

CARLOS R. FERREIRA, MD,

National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA

Inherited metabolic disorders (IMD) have existed since prehistoric times. As an example, a mummy named Harwa, dating from approximately 1,500 B.C.E., was found to have arthropathy, black articular surfaces, and accumulation of homogentisic acid, consistent with alkaptonuria. Alkaptonuria also became the first condition identified as a metabolic disease, in 1902. This lecture briefly traces the historical development of classification systems for IMD, starting with Garrod's tetrad in 1908, and culminating with the establishment of the International Classification of Inherited Metabolic Disorders (ICIMD), a global effort to unify classification systems of metabolic disease, in 2021.

SESSION II

NEWBORN SCREENING FOR IEM

CHAIRPERSONS

HUGO ROCHA, PhD
INSTITUTO NACIONAL DE SAÚDE DR. RICARDO JORGE, PORTO, PT

ANA GASPAR, MD
CENTRO HOSPITALAR UNIVERSITÁRIO LISBOA NORTE, LISBOA, PT

NEWBORN SCREENING IN EUROPE AND INITIATIVES TO DEVELOP PROGRAMMES IN LOW- AND MIDDLE-INCOME COUNTRIES

S JAMES R BONHAM

President, International Society for Neonatal Screening, United Kingdom

Newborn Screening began in many countries in Europe in the late 1960s or early 1970s with the introduction of screening for phenylketonuria. In the intervening 50 years, additional disorders have been added often beginning with congenital hypothyroidism and growing to include congenital adrenal hyperplasia, cystic fibrosis and sickle cell disease.

A real breakthrough came with the introduction of MSMS early in the late 1990s. This changed the model of 'one disorder – one test' and offered the ability to identify a range of up 50 conditions with a single test. Countries had to begin to choose which they would include.

While most countries broadly accepted the Wilson and Jungner criteria as a basis for determining which conditions to include, the evidence required and the way that this choice is made, varies enormously across Europe.

In 2010, partly in response to the 2009 EU Council recommendations on Rare Diseases, a survey was commissioned to examine the situation at the time. This revealed a variety of practice, not just in the conditions screened but also in the way that screening was undertaken. The resulting report submitted to the EC in 2011 made a number of detailed recommendations which were later summarised in a paper in the Eur J Hum Genet in 2013 (https://www.nature.com/articles/ejhg201390) but few of these have been adopted. A subsequent survey reporting in February 2021 (https://www.mdpi.com/2409-515X/7/1/15/htm) made similar observations about the number of conditions screened (ranging from 2-35) and the variety of practice in place about how screening is delivered.

In this presentation some of the reasons for these differences is explored and how initiatives such as Screen4Rare are working with professional groups including the European Reference Networks and politicians and policy makers to work toward a more equitable approach to screening in Europe.

At the same time that we are exploring screening policy in Europe, the ISNS working with the IFCC have established a Global Task Force to promote the growth of newborn screening in low and middle income countries where this is both appropriate and safe. We will present some of the thinking of this group and the initial results of a worldwide survey designed to support a developing global strategy.

NEW TESTS AND FUTURE DIRECTIONS IN THE NBS FOR IEM

GIANCARLO LAMARCA

Department of Experimental and Clinical Biomedical Sciences, University of Florence.
Newborn Screening, Clinical Chemistry and Pharmacology Laboratory,
Meyer Children's Hospital, Florence, Italy

The spread of national newborn screening (NBS) programmes has provided significant benefits in the diagnosis and early treatment of several rare, heritable conditions, preventing adverse health outcomes for most affected infants. New technological developments have enabled the implementation of testing panel covering over 50 disorders. Consequently, the increment of false positive rate has led to a high number of healthy infants recalled for expensive and often invasive additional testing, opening a debate about the harm-benefit ratio of the expanded newborn screening. The false-positive rate represents a challenge for healthcare providers working in NBS systems. We report on the most commonly used strategies for decreasing the adverse effects due to inconclusive screening results. The focus is on NBS performance improvement through the implementation of analytical methods, the application of new and more informative biomarkers, and by using post-analytical interpretive tools. These strategies, used as part of the NBS process, can to enhance the positive predictive value of the test and reduce the parental anxiety and healthcare costs related to the unnecessary tests and procedures.

TREATMENT FOLLOW-UP OF NEONATAL METABOLIC SCREENED DISORDERS

PAULA GARCIA

member of Portuguese Executive Commission of Newborn Screening National Program, Coimbra, Portugal

Portugal begins neonatal screening more than 40 years ago, initially with Phenylketonuria and Congenital Hypothyroidism and expanded to 24 inherited metabolic diseases since 2004. This national public health program has a wide acceptability and covers almost 100% of total births. Until now, over 4 million newborn's were screened. In 2019, birth incidence of the diseases screened in general was 1:1 123.

Phenylketonuria and medium-chain acyl-CoA dehydrogenase deficiency, by far the metabolic disorders more commons have a birth incidence of 1:10 929 e 1:7 303 respectively. Expanded panel includes amino acido disorders, organic acidurias, urea cycle disorders, beta-oxidation defects and since 2019 cystic fibrosis, too.

Once expanded national newborn screening program has been implemented, five Treatment Reference Centers were designated (two in north and south and one in the center region of Portugal). Positive testes are referred to those centers at a median age of 10 days. Confirmatory biochemical and genetic testes are included.

Epidemiologic and clinical data of the follow-up on diagnosed newborns with IMD are presented.

Newborn Screening National Program: President Dr Fernando de Almeida, Coordinator Prof Doutora Laura Vilarinho. Instituto Nacional de Saúde Pública Dr Ricardo Jorge.

Reference Treatment Center representatives: Esmeralda Martins (CHUP), Esmeralda Rodrigues (CHUSJ), Luísa Diogo (CHUC), Ana Cristina Ferreira (CHLC) and Ana Gaspar (CHLN)

SESSION III

FROM CBL TO HCY

CHAIRPERSONS

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PATRÍCIA JANEIRO, MD
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REMETHYLATION DISORDERS: FROM COBALAMIN ABSORPTION, INTRACELLULAR PATHWAYS TO MTHFR DEFICIENCY

MATTHIAS R. BAUMGARTNER

University Children's Hospital Zurich, Zurich, Switzerland

In humans, the folate and methionine cycles are critical pathways to cell survival. Folate, the major cellular carrier of one-carbon units, is required for thymidylate and purine biosynthesis, building blocks of nucleic acid synthesis. The methionine cycle produces methionine and adenosylmethionine (AdoMet), the former an essential amino acid and the latter necessary for the methylation of DNA, RNA, proteins (including histones) and creation of creatine, neurotransmitters and phosphatidylcholine. In line with these crucial roles, severe deficiency due to inborn errors may present as life-threatening metabolic disease in the first days to weeks of life.

The folate and methionine cycles, together called one-carbon metabolism, are connected by the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR). MTHFR catalyzes the irreversible final reduction of folate-carried single-carbon units, which are then exclusively used as a substrate for methionine synthesis. Therefore, MTHFR not only connects the folate and methionine cycles, but dedicates folate-carried carbons to methionine/AdoMet synthesis.

Vitamin B12 (cobalamin, cbl) is required as coenzyme for only 2 reactions, both of which are essential for life. Adenosylcobalamin is the cofactor for mitochondrial methylmalonyl-CoA mutase (MUT), which converts methylmalonyl-CoA to succinyl-CoA, and methylcobalamin is required by cytosolic methionine synthase (MS) for the remethylation of homocysteine (Hcy) to methionine (Met).

Impaired nutritional supply, all inborn errors of Cbl absorption and of the common part of intracellular Cbl processing (the cblC, cblD-MMA/Hcy, cblF and cblJ defects) result in deficient production of both methyl-Cbl and adenosyl-Cbl. In these combined remethylation disorders, Hcy and MMA are increased and Met may be low. Expression of the cblC protein is at least partly mediated by the global transcriptional co-regulator HCFC1 and the transcriptional activators ZNF143 and THAP11. Dysfunction of HCFC1 (cblX) or ZNF143/THAP11 may also lead to elevated Hcy and/or MMA and severe symptoms which however are different from those in the remethylation disorders.

Isolated failure of remethylation results either from defects in the folate or methionine cycle. In these isolated remethylation disorders, homocysteine is elevated; methionine may be low or normal. Finally, dysfunction of MTHFD1, a trifunctional enzyme in the folate cycle results in reduced MS activity but not consistently in elevation of Hcy.

Acquired and inborn Cbl-related disorders and MTHFR deficiency cause multisystem, often severe disease. Failure to thrive, neurocognitive or psychiatric symptoms, eye disease, bone marrow alterations with megaloblastic anaemia, microangiopathy and thromboembolic events are characteristic. MTHFD1 defeciency may additionally present with severe immune deficiency.

INHERITED DISORDERS OF TRANSSULFURATION: CLINICAL PRESENTATION, DIAGNOSIS AND RECENT ADVANCES IN THERAPY

VIKTOR KOŽICH

Department od Pediatrics and Inherited Metabolic Disorders, Charles University-First Faculty of Medicine and General University Hospital in Prague, Prague, Czech Republic

Metabolism of sulfur amino acids (SAA) provides compounds important for many cellular functions. Inherited disorders in the transmethylation and transsulfuration pathway of SAA, and in catabolism of cysteine and hydrogen sulfide are typically severe multisystemic diseases affecting brain, liver, connective tissue or vasculature. Current treatments of these disorders are based on dietary restriction of SAA, provision of lacking products or scavenging of toxic molecules. Recent studies explored novel options such as chaperone and gene therapy, and enzyme replacement therapy. This review summarizes clinical and biochemical presentation of selected disorders, with a focus on the most common disorder- homocystinuria due to cystathionine beta-synthase (CBS) deficiency.

Classical homocystinuria has a wide clinical spectrum, ranging from neurodevelopmental problems, lens dislocation and marfanoid features in early childhood to adult onset disease with predominantly thromboembolic complications. Recent analysis data in 328 patients with CBS deficiency from the E-HOD registry allowed to classify patients into 4 groups of pyridoxine responsivity based on a standardized pyridoxine loading test or surrogate criteria: non-responders (NR), partial, full and extreme responders (PR, FR and ER, respectively). At diagnosis, all groups showed overlapping concentrations of plasma total homocysteine while pyridoxine responsiveness inversely correlated with plasma/serum methionine concentrations. The FR and ER groups had a later age of onset and diagnosis and a longer diagnostic delay than NR and PR patients. Lens dislocation was common in all groups except ER but the age of dislocation increased with increasing responsiveness. Developmental delay was commonest in the NR group while no ER patient had cognitive impairment. Thromboembolism was the commonest presenting feature in ER patients, whereas it was least likely at presentation in the NR group. This study demonstrated that clinical severity of CBS deficiency depends largely on the degree of pyridoxine responsiveness and that an ameliorated phenotype is present in patients with the ER form of the disease. An ongoing analysis of treatment in 328 patients with CBS deficiency shows a large variability of approaches with a varying degree of adherence to the recent guidelines, different extent of natural protein restriction in NR patients in the participating centers, and a good biochemical control of ER and FR patients treated by only pyridoxine administration.

This work was supported by the grant AZV NV19-01-00307 from the Czech Health Research Council, institutional support was provided by Progres Q26 a RVO VFN64165.

THE REVERSE TRANSSULFURATION PATHWAY AT THE CROSSROADS BETWEEN HOMOCYSTEINE AND HYDROGEN SULFIDE METABOLISM: (DYS) REGULATION AND (PATHO) PHYSIOLOGICAL IMPLICATIONS

JOÃO B. VICENTE

Structural Genomics Group, Macromolecular Crystallography Unit, Instituto de Tecnologia Química e Biológica António Xavier, NOVA University of Lisbon, Oeiras, Portugal

In mammalian physiology, the reverse transsulfuration pathway converts homocysteine into cysteine, thereby contributing to maintain homeostatic homocysteine levels and to supply cysteine for different purposes, e.g. glutathione synthesis. This pathway involves two pyridoxal 5'-phosphate (PLP)-dependent enzymes: cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). Notably, through combinations of alternative substrates, both enzymes are major sources of the signaling molecule hydrogen sulfide (H2S) and of other reactive sulfide species (RSS), known to regulate several physiological processes, including cardiovascular function. The relevance of CBS and CSE for the maintenance of critical metabolites (homocysteine and cysteine) and signaling molecules (H2S and RSS) is matched by a complex repertoire of regulatory mechanisms controlling their expression and function.

Functional impairment of the transsulfuration pathway enzymes results in accumulation of homocysteine and depletion of H2S and RSS, with vast pathophysiological implications. While increased plasma homocysteine levels are known to be at the basis of cardiovascular disease, disturbed H2S levels have been growingly linked to many pathologies, from cardiovascular and neurodegenerative diseases to cancer. Therefore, it remains to be established whether cardiovascular complications arise from homocysteine accumulation, depletion of H2S, or both. Herein, the roles of CBS and CSE in homocysteine and H2S metabolism and their link with cardiovascular disease will be discussed, with emphasis on the lessons from the inborn errors of metabolism related to the reverse transsulfuration pathway enzymes.

SESSION IV

IEM NUTRITIONAL AND PHARMACOLOGICAL TREATMENT

CHAIRPERSONS

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SANDRA MEXIA, MSc
CENTRO HOSPITALAR UNIVERSITÁRIO LISBOA NORTE, PT

CURRENT AND FUTURE APPROACHES IN PKU TREATMENT

CARY O. HARDING, MD

Oregon Health & Science University, Portland, OR, USA

Dietary restriction of phenylalanine (Phe) intake continues to be the mainstay of treatment for individuals with phenylalanine hydroxylase (PAH) deficiency, colloquially known as phenylketonuria (PKU). However, several shortcomings of dietary therapy compel the search for novel treatment approaches. Many adolescents and adults with PAH deficiency struggle to maintain blood Phe concentrations below recommended treatment targets using dietary Phe restriction alone. Chronic elevation of blood and consequently brain Phe frequently lead to neuropsychiatric complications including inattention, memory impairment, mood disorders such as depression or anxiety, and altered executive functioning, all manifestations of the so-called 'brain fog' associated with hyperphenylalaninemia. Long term exposure to extreme Phe concentrations may lead to white matter damage and frank neurologic deficits including hyperreflexia, ataxia, and even seizures. Elevated maternal blood Phe during pregnancy is teratogenic, leading to microcephaly and global developmental disability in most affected infants and congenital heart disease in many, the so-called maternal PKU syndrome.

Several novel therapies are available or are in development for individuals with PAH deficiency. Glycomacropeptide (GMP) is a naturally-occurring protein sourced from whey that lacks Phe and is the primary protein included in novel palatable medical foods designed for use in the PKU diet. Sapropterin dihydrochloride treatment allows increased dietary protein intake due to sapropterin's chaperone activity on the PAH enzyme in individuals with some residual PAH activity. Large neutral amino acid (LNAA) supplementation is used to inhibit Phe uptake across the blood-brain barrier and protect the brain from the effects of high Phe concentrations. Subcutaneous pegvaliase injection, a PEGylated recombinant phenylalanine ammonia lyase, is a recently approved successful novel enzyme substitution therapy for individuals with PAH deficiency over 16 years age; pegvaliase therapy is the first therapy that can both control blood Phe concentrations below treatment targets, or even into the normal range, while allowing consumption of unrestricted intact dietary protein. Finally, liver-directed gene addition therapy, which aims to permanently cure PAH deficiency by restoring expression of PAH protein in liver following infusion of a recombinant adeno-associated virus (AAV) gene therapy vector, has entered clinical trial in adults. The future is bright for the development of novel treatments for PAH deficiency.

MICRONUTRIENTS: ROLE IN IEM TREATMENT

JÚLIO CÉSAR ROCHA

Department of Life Sciences, Faculty of Sciences and Technology (FCT),
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Micronutrients constitute an important determinant for an optimal nutrition, preventing deficiency related states, optimizing growth, and maintenance, or even modulating the risk of disease development. Both lipid-soluble and water-soluble vitamins have important roles in metabolism, which underline the importance of preventing deficient and excessive intakes. Also, minerals are required for both physiologic and biochemical functions, and therefore must be provided in the diet.

Increased intake of a specific micronutrient constitutes an effective treatment in some inborn errors that affect micronutrient metabolism. In other patients with inborn errors of metabolism, affected enzymes may lose its functional properties, needing pharmacological administration of specific vitamins to stimulate its residual activity.

In another perspective, in most patients with inborn errors of metabolism, treatment strategies often require personalized nutritional treatment that restrict protein, fat or carbohydrate food sources. These disruptive nutritional treatments may predispose these patients to an increased risk of imbalances, justifying careful monitoring of micronutrient status. More vulnerable groups (growing children and pregnant women) may need careful attention but the usual approaches to assess micronutrient status have important pros and cons, justifying the adoption of a decision tree that combines dietary, clinical, and biochemical assessments.

SESSION V

GENERAL ORAL COMMUNICATIONS

CHAIRPERSONS

HELENA SANTOS, MD
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ANA CRISTINA FERREIRA, MD
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SESSION VI

THE ACETYL-COA CROSS

CHAIRPERSONS

DOMINGO GONZÁLEZ-LAMUÑO, MD, PhD
HOSPITAL UNIVERSITARIO MARQUÉS DE VALDECILLA-UNIVERSIDAD DE CANTABRIA, SANTANDER, SPAIN

M. TERESA CARDOSO, MD
CENTRO HOSPITALAR UNIVERSITÁRIO SÃO JOÃO, PORTO, PT

PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY IN PORTUGAL: PECULIAR CHARACTERISTICS OF ITS CLINICAL, METABOLIC AND MUTATIONAL LANDSCAPES

HANA PAVLU-PEREIRA

Metabolism & Genetics, Research Institute of Medicines, iMed. Ulisboa; Lisboa, PT

Pyruvate oxidation defects and particularly pyruvate dehydrogenase complex (PDC) deficiency represent a significant subgroup among mitochondrial diseases. By catalyzing the irreversible decarboxylation of pyruvate into acetyl-CoA, the pyruvate dehydrogenase complex occupies a prominent position in controlling the energetic metabolism. An alteration in any of the genes encoding its several subunits can affect the highly organized assembly of large multidomain proteins and disturb the sophisticated functional mechanisms. The resulting metabolic disorder affects mainly the tissues with a high demand for ATP. The broad phenotypic spectrum of PDC deficiency, often constrains the achievement of a rapid diagnosis. The detailed knowledge, combining molecular, biochemical and clinical aspects, is fundamental for a correct stratification, for an establishment of the treatment strategy and for a possible design of new therapeutic avenues.

Over the last years we focused on a comprehensive study of PDC and PDC deficiency at diverse levels. We described the clinical, biochemical and genotypic findings from thirteen Portuguese PDC deficient patients, we submitted the clinically relevant variants to detailed structural and functional analyses, and we evaluated the ability of arginine and/or thiamine to restore PDC function in patient-derived cell lines.

This presentation is dedicated to the peculiar characteristics of PDC deficiency in Portugal. The mutational spectrum revealed ten different mutations in the following genes coding for the respective subunits: PDHA1 (PDC-E1α), PDHX (PDC-E3BP), and DLD (PDC-E3). Although the PDHA1 mutations were predominant, there was a striking evidence of a high incidence of E3BP deficiency. All patients from our cohort had the clinical onset between the neonatal period and childhood, manifested different degrees of neurological involvement and, interestingly, most of them reached adulthood. The functional and structural characterization of the mutant protein variants allowed to obtain a molecular insight on the severity of the clinical phenotype and to evaluate the effect of arginine supplementation on recombinant PDC-E1 variants. Extended by a study assessing the impact of arginine and/or thiamine treatment in patient-derived cell lines, our results indicated that the rescue efficacy of these stabilizing molecules is clearly determined by the mutation per se. The data obtained at all levels of this study confirmed our previous observations about the efficacy of arginine treatment in improving the clinical phenotype of the patient carrying the p.R253G mutation, unique to the Portuguese population.

KETOTIC GLYCOGEN STORAGE DISEASES: CURRENT KNOWLEDGE, MANAGEMENT AND MONITORING

ULRIKE STEUERWALD

Nat. Hospital Tórshavn (Faroe Islands)/Screening Laboratory Hannover, Hannover, Germany

In glycogen storage diseases (GSDs), buildup or breakdown of glycogen is impaired. Ketotic GSDs comprise those liver GSDs where gluconeogenesis is intact, i.e. type 0, III, VI and IX. In ketotic GSDs, protein – mostly from muscle – will be transformed into glucose, when blood sugar falls low. As glucose-6-phosphatase and glucose-6-phosphatase transporter are intact in ketotic GSDs (in contrast to GSD I), glucose can be released into circulation by liver cells, thus normalizing blood sugar levels. However, concurrently fatty acids taken up by the liver are converted to ketone bodies, which can serve as alternative energy source for the brain. Typical clinical presentation of ketotic GSDs consist in a combination of symptoms stemming from over-storage of glycogen in liver cells (however, this does not happen in type 0) and from hypoglycemia leading to secondary protein deficiency, ketosis and non-alcoholic fatty liver disease. In GSD type IIIa, debilitating myopathy affecting skeletal and heart muscle often develops during adulthood if condition is not treated optimally.

Treatment consists of a diet with frequent small meals, restriction of carbohydrates (especially of simple sugars) but enriched with protein to replete amino acids used for gluconeogenesis. Fasting should be avoided. To prolong intervals between meals and to stabilize blood sugar slowly released carbohydrates e.g. uncooked cornstarch are added.

Monitoring of blood glucose and blood ketones should be performed regularly at home; continuous glucose monitoring facilitates optimization of metabolic control. Comprehensive laboratory controls are recommended (whole blood count, liver function, protein and pre-albumin, kidney function, bicarbonate and cholesterol/triglyceride, vitamins and minerals). Regular liver ultrasound and/or liver computed tomography (CT) scan or magnetic resonance imaging (MRI), echocardiogram, and dual-energy X-ray absorptiometry (DEXA) scan are needed to screen for complications.

With optimal treatment prognosis is very good for people with GSD type IIIa and outstanding for the other ketotic GSD types.

HOW TO FIND YOUR WAY THROUGH METABOLIC DISORDER IN PATIENTS WITH RHABDOMYOLYSIS.

PASCAL LAFORÊT

Raymond-Poincaré Hospital, APHP, Garches, France

Rhabdomyolysis attacks are one of the most common inaugural symptom and complications of several metabolic myopathies, especially glycogenosis and lipid metabolism disorders. Some mitochondrial disorders may also present with rhabdomyolysis episodes, in association with lactic acidosis. Inherited metabolic disorders should be systematically detected in patients with recurrent rhabdomyolysis attacks triggered by exercise, fever or fasting.

The diagnosis of metabolic disorders has been greatly improved in recent years since implementation of NGS and large gene panels, but the initial diagnostic tools remain biochemical analysis. Acylcarnitine profile allow to detect easily fatty acid oxidation disorders, and assessment of lactate production during a forearm exercise test is a major clue to diagnosis of McArdle disease (glycogenosis type V) and rarer disorders of glycolysis.

We will propose a diagnostic strategy to detect metabolic myopathies revealed by rhabdomyolysis episodes, and we will discuss the main differential diagnosis. However, many cases of rhabdomyolysis remain unsolved despite thorough biochemical and molecular investigations, probably due to still unknown genetic causes or individual susceptibility to exercise-induced rhabdomyolysis during sport activities.

SESSION VII

LYSOSOMAL DISORDERS

CHAIRPERSONS

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CENTRO HOSPITALAR UNIVERSITÁRIO SÃO JOÃO, PORTO, PT

PAULO GASPAR, PhD
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LIPID BIOMARKERS ASSISTING DIAGNOSIS AND MONITORING OF LYSOSOMAL DISORDERS

JOHANNES M, F.G. AERTS

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Relatively common among the inherited lysosomal storage disorders (LSDs) are diseases in which specific glycosphingolipids or other specific lipids accumulate. These disorders are characterized by the presence of lipid-laden storage cells that may release specific proteins or lipids into the circulation. These biochemical abnormalities in plasma can potentially be exploited as biomarkers that assist the confirmation of a diagnosis and assist the monitoring of disease (and response to therapeutic intervention).

A frontrunner in the discovery of biomarkers has been Gaucher disease (GD), an inherited LSD with characteristic lipid-laden macrophages (Gaucher cells). These storage cells are caused by an deficiency in lysosomal beta-glucosidase activity degrading the simplest glycosphingolipid glucosylceramide, as the result of mutations in the GBA gene. For GD meanwhile various therapies have been developed such as ERT (enzyme replacement therapy based on chronic administration of the deficient GCase enzyme) and SRT (substrate reduction therapy based on oral administration of an inhibitor of the synthesis of the accumulating lipid). Other therapy avenues for GD are intensely investigated such as chaperone and gene therapies. In this lecture the discovery of Gaucher-cell derived plasma protein biomarkers will be first discussed and their application in diagnosis and disease monitoring. Next attention will be focused to lipid GD biomarkers, like the massively elevated glucosylsphingosine in plasma and tissues of GD patients, a finding again exploited for diagnosis and disease monitoring. Glucosylsphingosine is actively generated from accumulating primary storage lipid glucosylceramide by deacylation. The same phenomenon occurs also in other LSDs in which sphingolipids accumulate, resulting in massively elevated corresponding deacylated lipids that may serve as biomarkers. The potential toxicity and contribution of deacylated lipids to symptoms in sphingolipid LSDs will be discussed as well as some very recently discovered additional glycolipids in GD. To conclude the present availability of protein and lipid biomarkers for other LSDs than GD will be reviewed.

GENE THERAPY FOR LYSOSOMAL STORAGE DISORDERS: ADVANCES, CHALLENGES AND PERSPECTIVES.

MARIA JOSÉ CASTRO

Reference Center for Inherited Metabolic Disorders of Hospital Clínico Universitario de Santiago de Compostela. ES

The need for long-lasting and transformative therapies for lysosomal storage disorders (LSD) cannot be understated. At the present time, many LSD lack an specific treatment and in other cases current therapies aren't targeting important organs such as the central nervous system. Moreover, the advent of new-born screening procedures may prove a major step forward in early identification and treatment of individuals with LSD. Based on these premises, over the last years novel experimental therapies for LSD have been investigated. Genome therapy holds the promise of a potentially curative treatment for patients with LSD.

Despite recent progress in the field, several considerations should be taken into account. This presentation focuses on the scientific evidence demonstrating that gene therapy provides a promising therapeutic option for LSD and discuss the clinical limitations that remain to be overcome. Recent advances in the field will be highlighted and advantages/disadvantages of gene therapies with a focus on lentiviral and adeno-associated viral vectors will be discussed. Special attention will be paid to gene therapy targeting the central nervous system, potential toxicities and inmunogenicity.

SESSION VIII

THE AMMONIA ROUNDABOUT

CHAIRPERSONS

MARGARIDA SILVA, PhD
FACULDADE DE FARMÁCIA UNIVERSIDADE DE LISBOA, LISBOA, PT

TERESA ALMEIDA CAMPOS, MD
CENTRO HOSPITALAR UNIVERSITÁRIO SÃO JOÃO, PORTO, PT

NEW INSIGHTS INTO THE PATHOPHYSIOLOGY AND MOLECULAR BASIS OF UREA CYCLE DISORDERS

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Urea cycle disorders (UCD) are defects of ammonia detoxification and of arginine synthesis. Patients may be affected by acute development of hyperammonemic encephalopathy, which is associated with high morbidity and mortality despite several recent treatment advances. There are however patients with more subtle onset of disease and neurological complications despite absence of hyperammonemia, and those with a predominantly hepatic phenotype and acute liver dysfunction.

This lecture will start with an examplary review our current understanding of the pathophysiology in patients with argininosuccinic aciduria (ASA). Focus will here be on the high rate of neurological disease in ASA patients even with good metabolic control and on the additional challenges of this condition including the disturbance in nitric oxide homeostasis. We will also review the situation of UCD patients with a predominantly hepatic phenotype, in whom ammonia obviously exerts some direct liver toxicity, resulting in acute liver dysfunction, of which the exact mechanism is not yet understood. We will further explore the role of urea cycle protein abundance in liver cells to understand the challenges of novel therapies for defects of ureagenesis including different gene therapy approaches. Finally, we will discuss some recent insights into genetic alterations affecting regulatory regions of urea cycle genes.

From the aforementioned various aspects, it should become clear that UCDs are not simply conditions with an increased risk for hyperammonemia but have additional pathophysiological characteristics of which some are specific for certain conditions. Some of these specificities can be explained based on the molecular basis but others remain currently not well understood. Since there is often a direct link to therapeutic aspects, further research aiming at a continuously improved understanding of pathophysiology and molecular basis of urea cycle disorders remains our challenge.

POTENTIAL ROLE OF GENE THERAPY FOR OTC DEFICIENCY

ALVARO HERMIDA

Unit of Diagnosis and Treatment of Inherited Metabolic Diseases of the University Clinical Hospital of Santiago de Compostela, ES

The current standard of care for OTC deficiency is to limit dietary protein intake and supplement the diet with a high-energy source. If serum ammonia is not stabilized by dietary restriction alone, ammonia scavengers that promote an alternative pathway of nitrogen removal can be administered. Ammonia scavengers cannot, however, completely prevent individuals from having hyperammonemic crises. Orthotopic liver transplantation can correct OTC deficiency; however, this is limited by donor availability and is associated with significant risk of morbidity and mortality. Thus, there remains a significant unmet medical need for a treatment that allows for sustained ammonia management and prevention of hyperammonemic crises associated with OTC deficiency.

Although gene therapy has suffered clinical setbacks, new vector developments and results from clinical trials have sparked enthusiasm for the development of gene therapy in UCD, particularly OTC deficiency. Multiple vector systems have been investigated, but adeno-associated viral (AAV) vectors have shown impressive safety and long-term efficacy in a number of diseases (AAV8 serotype in particular demonstrates high liver tropism and can achieve efficient liver gene transfer following IV infusion).

Major challenges to translate gene therapy in OTC deficiency into the clinic are; 1) to allocate optimal / effective dose; 2) the prevalence of pre-existing neutralizing antibodies or; 3) the loss of non-integrating vector genomes as a consequence of liver growth.

Currently, no gene transfer product has been approved for the treatment of OTC deficiency. Increasing OTC activity and promoting the removal of ammonia through the urea cycle should allow patients with OTC deficiency to avoid hyperammonemic crises in the context of a well-controlled diet, while reducing or stopping ammonia scavenger therapy. Furthermore, depending on the level of OTC expression and activity achieved, patients may be able to loosen their dietary restrictions, which should greatly improve their quality of life.

SESSION IX

DOWN TOWN MITOCHONDRIA

CHAIRPERSONS

ESMERALDA MARTINS, MD, PhD
CENTRO HOSPITALAR UNIVERSITÁRIO DO PORTO, PORTO, PT

CÉLIA NOGUEIRA, PhD
INSTITUTO NACIONAL DE SAÚDE DOUTOR RICARDO JORGE, PORTO, PT

MITOCHONDRIA PLASTICITY: ROLE IN MUSCLE PATHOLOGY

RITA FERREIRA

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Mitochondrial dysfunction is a hallmark of striated muscle wasting conditions, such as cachexia, and hence a candidate target for intervention. Decreased ability for ATP synthesis, impaired mitochondrial biogenesis, increased oxidative stress, impairment of protein quality control systems, increased susceptibility to mitophagy and to apoptosis were all shown to mediate contractile dysfunction in pre-clinical models of wasting conditions. Moreover, the lower oxidative phosphorylation activity was associated to the accumulation of oxidatively modified mitochondrial proteins and to the decrease of phosphatidic acid, phosphatidylglycerol and cardiolipin content, compensated by increased phosphatidylcholine levels. Therapeutic strategies such as exercise training seem to counteract muscle mass loss by improving, at least in part, mitochondrial functionality, which seems to involve the upregulation of PGC-1alpha. The understanding of the molecular changes that occur in the wasted muscle following exercise and how mitochondrial homeostasis is impacted is essential for the exploration of potential targets for interventions.

RENAL INVOLVEMENTE IN MITOCHONDRIAL DISORDERS

MARGARIDA COELHO

Reference Center for Inherited Metabolic Disorders of Centro Hospitalar Universitário do Porto

In the era of genomics, the number of genes linked to mitochondrial disease has been quickly growing, producing massive knowledge on mitochondrial biochemistry and pathophysiology.

Renal impairment has high prevalence (often underdiagnosed) within mitochondrial disorders being responsible for significant comorbidity. It can be an isolated and/or presenting feature but more commonly it is part of a multiorgan presentation. Genotype–phenotype correlations are often difficult. Awareness between physicians is of major importance considering that clinical suspicion may not be obvious regarding the heterogenicity in presentation and biochemical features of mitochondrial dysfunction.

Kidney disease may have secondary impact in growth and development; hence its early detection becomes of greatest importance. Either in childhood or adulthood it has significant impact on health, quality of life and overall outcome.

Kidney disease in mitochondrial disorders arises in various forms and degrees: either glomerular or tubular: proximal tubulopathy, proximal tubular acidosis, Bartter-like tubulopathy, hypermagnesuria, proteinuria, nephrotic syndrome, tubulointerstitial nephritis, myoglobinuria or renal failure. Of special note, CoQ10 biosynthesis defects can be treated with high-dose CoQ10 supplements. Glomerular and tubular dysfunction are often associated.

From our cohort we hypothesize that a low FENa, the inability to acidify and/or concentrate urine and phosphaturia appear to be early indicators of tubular dysfunction.

Interestingly, other diseases with secondary mitochondrial dysfunction will frequently show the same biochemical findings, often overlooked on the follow-up of these diseases.

THE DIAGNOSIS OF MITOCHONDRIAL DISEASE USING MULTI-OMIC METHODOLOGIES

CHARLOTTE ALSTON

HCPC Registered Clinical Scientist in Molecular Genetics at the Newcastle upon Tyne Hospitals NHS Foundation Trust and NIHR Post-Doctoral Research Fellow at the Wellcome Centre for Mitochondrial Research, Newcastle University, UK

Mitochondrial diseases are clinically and genetically heterogeneous metabolic conditions that occur as a consequence of mitochondrial dysfunction. They can manifest at any age and affect any organ or tissue, in either an isolated or syndromic manner. Although production of ATP through oxidative phosphorylation is their most recognised role, they are involved in numerous cellular pathways and approximately 10% of all human genes (>1100) are predicted to encode proteins with a mitochondrial function. Defects in over 300 mitochondrial genes have already been demonstrated to cause human disease, and this list continues to grow. Some genetic defects involve the mitochondrial genome (mtDNA), but the vast majority occur within the nuclear genome. There remains no cure for mitochondrial diseases, but a genetic diagnosis facilitates access to genetic counselling and reproductive options for at-risk families. Historically, functional data from patient muscle biopsy was crucial for selecting the most appropriate candidate genes for sequencing analysis but this approach has been mostly superseded by genomic technologies using a minimally-invasive blood sample. Even after the application of an unbiased approach to genomic sequencing, there are some patients whose genetic diagnosis remains elusive. For these patients, additional functional investigations are required. In our experience, the application of supplementary 'omics' technologies, including proteomics, metabolomics and – in particular - transcriptomics, can greatly improve diagnostic strategies. For these cases, access to patient biopsy remains crucial for establishing their diagnosis; a skin biopsy has proven sufficient to establish a diagnosis for many patients although for others, muscle remains necessary. The application of multi-omic technologies has been fundamental for assigning function to orphan genes, characterising novel disease genes and for validating pathogenicity of variants of uncertain pathological significance. The analysis of clinically relevant tissues from affected individuals remains crucial for understanding the molecular mechanisms underlying mitochondrial pathology.

ORAL COMMUNICATIONS

OC 01

WHEN THE NEWBORN SCREENING PREVENTS KETOACIDOSIS

Joana Tenente[1], Marisa Rodrigues[2], Ana Marcão[4], Teresa Campos[3], Célia Nogueira[4], Elisa Leão Teles[3], Laura Vilarinho[4], Esmeralda Rodrigues[3]

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Beta-ketothiolase (T2) deficiency is a defect in mitochondrial acetoacetyl-CoA thiolase activity that affects both isoleucine catabolism and ketolysis and can be classified as an organic aciduria or a ketolysis defect. This deficiency results in accumulation of isoleucine catabolic intermediates, presenting with a typical acilcarnitines and urinary organic acid profiles. Some acids are unstable and difficult to detect therefore this disease can be easily undetected in newborn screening test (NBS).

Case report: a male late preterm born at 36 weeks of gestational age for maternal preeclampsia. He was born by c-section, with Apgar score 8/9 and birth weight 3000g. By day 3 he started phototherapy, without any other neonatal complications and was latter discharged. The NBS was performed twice and presented with 3-hydroxybutyrylcarnitine (C4OH), 3-hydroxyisovalerylcarnitine (C5OH) and tiglylcarnitine (C5:1), suggesting a beta-ketothiolase deficiency, with low carnitine levels. Urinary organic acid analysis showed 2-methyl-3-hydroxybutyrate, tiglylglycine and 2-methylacetoacetate. The genetic study found a compound heterozygous mutation: c.4550G>C (p.Gly152Ala)/ c.814C>T (p.Gln272*) in the ACAT1 gene that confirmed the diagnosis.

At 2 months, he was admitted in the hospital for fever. The clinical examination was normal. The laboratory exams showed an increase in protein c-reactive with a normal glucose level, starting ceftriaxone and IV fluids with glucose. On the 3rd day he presented with diarrhea. He completed 5 days of ceftriaxone and was discharged. No occurrence of hypoglycemia, elevation of ketone bodies or metabolic acidosis. He is now 6 months with normal development.

Patients characteristically present with ketoacidotic crises under ketogenic stresses. In most cases the first ketoacidotic crises happens between 6 months and 3 years of age. Neurological manifestations are frequent and can occur without metabolic decompensations. Ketoacidotic crises must be supplemented with glucose. In this case, the previous diagnosis abled to prevent a metabolic decompensation.

Ketolysis defect are rare and only 240 patients have been diagnosed with beta-ketothiolase deficiency. Almost 90% had at least one metabolic decompensation, 22% has some degree of neurological or cognitive impairment. This is the first known Portuguese case and also the first one diagnosed by the NBS. Our aim to aware about this rare condition, focusing the importance of the early diagnosis.

OC 02

TRIFUNCTIONAL PROTEIN DEFICIENCY – CASE REPORT OF NEONATAL ONSET

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INTRODUCTION - Trifunctional protein deficiency (TFPD) is a rare hereditary metabolic disorder (HMD) of mitochondrial beta-oxidation of fatty acids. According to the onset of clinical manifestations, three main phenotypes were described, the neonatal, the infantile and the late adolescent. The neonatal type is less frequent. It is usually a severe and lethal condition.

CLINICAL CASE - A preterm newborn (NB) with 26 weeks of gestational age, born from emergent cesarean section, due to maternal preeclampsia. First child of young, healthy and not related parents. The birth weight was extremely low (690 g). The APGAR score was 5/7/9, needing endotracheal intubation and admission in Neonatal Intensive Care Unit (NICU) in mechanical ventilation (MV). During the first 2 weeks of life, the NB maintained MV and metabolic acidosis along with multiple complications, mostly associated with prematurity. At that time, the result of national newborn screening program was compatible with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) or TFPD, and the NB was transferred to the NICU of our Reference Center, for a multisystemic evaluation, and clinical management. The identification of a homozygotic mutation p.His379GInfs*76 (c.1137delT) in gene HADHB allowed the diagnosis of TFPD. From the multisystemic evaluation performed, two main target organs were identified, heart and lung. The nutritional requirements were reached through personalized parenteral and enteral nutrition, to assure high glucose levels, fat restriction, especially long chain fatty acid, with preferentially use of medium chain fatty acid, and adequate apport of essential amino acids, vitamins and minerals. Although that, there was a progressive deterioration of body functions. The NB continued in MV, and the cardiac function worsens with the development of dilated cardiomyopathy and cardiac failure, deceasing at the 85th day of life from heart failure.

CONCLUSIONS - Few descriptions of neonatal onset of TFPD were presented in the literature. The differentiation between the consequences of prematurity and those caused by the HMD, is a unique challenge but essential to an adequate clinical attitude. The authors highlight the difficulties in managing parenteral/enteral nutritional plans in extreme prematurity, facing metabolic disease. They emphasize the importance of being conscious of the appropriate moment for palliative support, made by the multidisciplinary team, always involving the family.

OC 03

CARNITINE UPTAKE DEFICIENCY IN ASYMPTOMATIC PATIENTS – THE IMPORTANCE OF 5' UNTRANSLATED REGION (UTR) OF SLC22A5

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INTRODUCTION: Carnitine uptake deficiency (CUD) is caused by a defect in the active cellular uptake of carnitine by Na+ dependent organic cation transporter novel 2 (OCTN2). It can cause severe cardiomyopathy and hypoketotic hypoglycaemic crises in children, as well as sudden death in children and adults in periods of high metabolic demands. Newborn screening detects most of these clinical variants, but in addition has identified maternal CUD in asymptomatic women. Pathogenic variants were described in 5' untranslated region (UTR) of SLC22A5 in individuals with possible primary carnitine deficiency in whom only one mutant allele had been found, helping to explain the genetics behind many asymptomatic patients.

We present three asymptomatic patients found by familial screening and their genetic characterization.

CASE REPORTS:

PATIENT 1: Healthy 37 years old woman, with low C0 levels found after a false positive result of her first daughter's newborn screening. No symptoms related to CUD were found after extensive questioning. SLC22A5 gene was sequenced and only one pathogenic variant (c.357C>G p.(F119L)) was found. Sequencing 5'UTR showed c.-149G>A variant confirming CUD genetically. She was stated on levocarnitine supplementation with amelioration of C0 levels.

PATIENT 2: Healthy 44 years old woman, Syrian refugee, screened after CUD diagnosis in one of her sons (SLC22A5 homozygous - c.1354G>A (p.E452K)). She has had 6 children, 3 were dead (one of heart disease). No symptoms were described during any of the gestations or during Ramadan fasting. SLC22A5 gene was sequenced and only one pathogenic variant (c.1354G>A (p.E452K)) was found. Sequencing 5'UTR showed c.-149G>A variant confirming CUD. She was started on levocarnitine supplementation, but did not comply with medication or diet counselling. Lost to follow up after leaving the country.

PATIENT 3: Healthy 15 years old boy, son of case 2 woman, screened after CUD diagnosis in his younger brother. No symptoms described even during Ramadan fasting. He was found to be a SLC22A5 compound heterozygous as his mother - c.1354G>A (p.E452K)/c.-149G>A. He was started on levocarnitine supplementation, but did not comply with medication or diet counselling. Lost to follow up after leaving the country.

DISCUSSION: Sequencing 5' UTR of SLC22A5 in individuals with possible primary carnitine deficiency should always be considered in asymptomatic patients in whom non or only one mutant allele is found.

OC 04

A CASE SERIES OF PAEDIATRIC CLASSICAL HOMOCYSTINURIA – NUTRITIONAL STATUS, DIETARY INTAKE AND METABOLIC PROFILE

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INTRODUCTION: Classical homocystinuria (HCU) is a rare inherited disease in the methionine (Met) catabolism pathway, due to deficiency of cystathionine beta-synthase (CBS) enzyme, resulting in abnormal accumulation of homocysteine (Hcy) and Met, along with a lack of cysteine (Cys). A low-Met diet is crucial to achieve good metabolic profile, providing sufficient amounts of energy, total protein and all the main nutrients and micronutrients for optimal growth and a health nutritional status.

METHODS: In this case series we examine the nutritional status (length/ height, weight, body mass index [BMI], macro and micronutrients intake, biochemical analysis) and metabolic profile (Hcys, Met and Cys concentrations) of a cohort of paediatric patients with classical homocystinuria, followed up at the Centro de Referência de Doenças Hereditárias do Metabolismo of Centro Hospitalar Universitário Lisboa Norte (Portugal).

RESULTS: A four patients' cohort, one of which female, which age ranges from seven months to 17 years (mean=9.21y, SD=6.77y) and diagnosis between one month and 13 years. None of them were born to consanguineous parents and two were siblings. All the patients were under treatment with a low Met diet, supplemented with betaine and pyridoxine. The z-score for length/height, weight and BMI ranges from -0.415 to 0.831, -0.774 to 0.669 and -1.231 to 0.422, respectively. All the patients met their energy, protein and folate requirements; except for essential fatty acids. Cys supplementation was according to recommendations in half of the patients. It was observed, in the older patient, that Met requirements were below recommendations, which is related to a more restricted diet. All the patients presented adequate metabolic profile, with serum Hcy <100 µM, ranging from 8.70 to 55.60 µM (mean= 33.8; SD=21.9) and serum Met <1000 µM, ranging from 36.70 to 552.80 µM (mean= 296.30; SD=255.68).

Consistently with what is suggested in literature, we found a strong, negative correlation between the age at Cys supplementation start and z-score for length/height, nonetheless without significance level, that is strongly influenced by the limited sample size (rspearman=-0.80, n=4, p=0.200).

CONCLUSION: In order to explore this possible relation, a longer study period is necessary for evaluation of dietary intake, Cys supplementation, nutritional status and patient´s metabolic profile.

OC 05

NEW PREDICTIVES FOR LOW VITAMIN B12 IN NEWBORNS

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BACKGROUND: Early diagnosis and intervention of vitamin B12 deficiency (B12D) in exclusively breastfed infants of mothers on a vegetarian/vegan diet with lack of appropriate supplementation, is crucial in preventing possible irreversible neurologic disorders, megaloblastic anemia and failure to thrive. We aim to assess the usefulness of newborn screening program (NBS) to detect presymptomatic B12D related to acquired conditions.

METHODS: Retrospective study of 10 patients' files from 2017 to 2021, with suspected cobalamin deficiency detected by NBS, due to B12 mother's dietary deficiency. Authors described demographic, clinical, and biochemical data and the results after supplementation.

RESULTS: Ten cases (5 males), exclusively breastfed, age range 1-2 months, asymptomatic in the first consultation, were referred to Metabolic Diseases Unit due to abnormalities in NBS biomarkers: abnormal levels of propionylcarnitine and methylmalonic acid. They were followed by second tier measurements of vitamin B12 and homocysteine. They reported a low serum B12 level (<50-202pg/mL, R.V.:195-770), elevated homocysteine (54-163.4umol/L, R.V.:<7, age matched controls), but normal mean corpuscular volumes (88.89-103.7fL, R.V.: 96-108). All mothers (3 from India, 2 from Brazil, 1 from Angola, 4 from Portugal) had a vegetarian, vegan diet or impaired B12 absorption during pregnancy with a low or borderline B12 level (<100-267pg/mL) and high levels of homocysteine (13.4-50.3umol/L). Therapy with intramuscular B12 in infants led to rapid normalization of the metabolic abnormalities with normal psychomotor development. Mothers were also supplemented with oral vitamin B12.

CONCLUSION: The possibility of acquired B12D should be ruled out before proceeding in the differential diagnosis for cobalamin metabolism deficits, methylmalonic acidemia and homocystinuria. The study shows that vitamin B12D can be more severe in breastfed infants than in their mothers. Vitamin B12 levels are not systematically tested during pregnancy, at least in our country, and the described cases showed that it is imperative to evaluate and correct vitamin B12 levels along the pregnancy. Vitamin B12 supplementation is a low risk and a low-cost treatment, bringing potentially large benefit to the infant. Therefore, detecting the infants with B12D is of utmost importance. In our cases, the early detection through NBS allowed to prevent hematological abnormalities and irreversible neurological disorders.

OC 06

TARGETED RNA-BASED THERAPIES FOR MUCOPOLYSACCHARIDOSIS

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Over the last years, most of our work has been focused on the development of alternative, RNA-based therapies for a number of Lysosomal Storage Disorders (LSD), being Mucopolysaccharidosis (MPS) one of the most relevant.

Currently, there are two major research lines being pursued: the first relies on the design of mutation-specific approaches to correct abnormal splicing processes in LSD-related genes whenever they underlie pathology and the second depends upon selective downregulation of one gene involved in the very early stages of the glycosaminoglycans' (GAG) biosynthethic cascade to promote substrate reduction in MPS diseases. There are substantial differences between these two approaches, but they also face common challenges.

Two major possible drug types, depending on the genotype that underlies pathology, are being used: U1snRNA and siRNAs. U1snRNAs are specifically designed to overcome particular splicing mutations. These RNA drugs are, therefore, mutation-specific and constitute patient-tailored approaches. We have already demonstrated in fibroblasts that a modified U1snRNA vector (comprising exon 1 to exon 3) designed to improve the definition of exon 2 5' SDS of the HGSNAT can restore the splicing defect caused by the mutation c.234+1G>A, that leads to MPSIIIC disease (Matos et al., 2014). Currently, our goal is to evaluate in vivo the therapeutic potential of that modified U1 snRNA by testing it in mice expressing the human splicing defect. A preliminary assay was performed and showed promising results.

The second group of RNA drugs, siRNAs, holds a different potential. By acting over the GAGs' biosynthethic cascade, siRNAs will promote an overall decrease of the accumulating substrate. So far, we have already tested this approach in MPSIII patients' fibroblasts and the overall results are quite promising. We observed a high inhibition of the XYLT1 (a gene that encodes an enzyme involved in an early stage of the HS biosynthetic cascade) mRNAs (around 80%) and a decrease in GAGs storage (only assessed for types C and D until now). Currently, we are evaluating the effect of that decrease on the overall GAGs storage 7 days post-transfection, also with promising results.

Here we present an overview on our results with both approaches on MPS diseases.

OC 07

FUNCTIONAL CHARACTERIZATION OF FIVE NOVEL MUTATIONS FOUND IN PATIENTS WITH SUSPICION OF FABRY DISEASE

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Fabry Disease (FD) is a rare, progressive and multisystem disorder, caused by pathogenic mutations in GLA gene. GLA encodes the α-galactosidase A, whose function is to promote the degradation of glycosphingolipids, mainly globotriaosylceramide (Gb3), inside the lysosomes. The α-Gal A deficiency or absence leads to FD. Since GLA was cloned, more than thousand mutations have been reported, and when an undescribed GLA variant is detected in a patient with symptoms common to other diseases, it is difficult to confirm that the symptoms are caused by the mutation, especially in women. In these cases, a system based on damage investigation, as an in vitro functional characterization, is needed. Here, we found five novel mutations in patients with suspicion of FD.

METHODS: HeLa cells were used to transfect the full-length cDNA sequence encoding human α-Gal A (NM_000169) and some α-Gal A mutants. The transfection was confirmed by real-time PCR (mRNA expression) and α-Gal A enzymatic activity by using the artificial substrate 4-MU-α-D-galactopyranoside.

RESULTS: The results showed a twelvefold increase in WT -Gal A mRNA expression and at least tenfold in the mutants when compared to the endogenous expression of GLA in the HeLa cells. The measurement of the α-Gal A enzyme activity showed that the mRNA overexpression of α-Gal A WT promoted a tenfold increase in the endogenous GLA levels when compared to mock transfected HeLa cells. No difference in the activity of the mutants c.59_72dup, p.I133N and p.K168Q was observed when compared with HeLa endogenous activity. The residual activity of these constructs was 0.8% in c.59_72dup and zero in p.I133N and p.K168Q. The results showed that despite the increased mRNA expression, no residual activity of these mutants was observed. The mutant -Gal A containing the p.K140T presented 22% of residual activity when compared to WT, and the p.P323T -Gal A presented 66.7% of activity when compared to WT. Our results confirm the pathogenicity of three mutations studied: p.K140T had only 22% activity and could lead to a late-onset phenotype, and p.P323T had more than 65% residual activity, therefore not causing FD.

OC 08

NIEMANN PICK TYPE C – 26 YEARS OF DIAGNOSIS IN A PORTUGUESE REFERENCE CENTER

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INTRODUCTION: Niemann Pick type C (NPC, MIM #257220) is a rare lysosomal storage disorder with an estimated incidence of 2.2/100 000 live births in the Portuguese population. NPC is characterized by systemic and neurologic deterioration, largely age-dependent, resulting in a shortened lifespan. It is caused by the accumulation of free cholesterol and other complex lipid compounds in lysosomes due to biallelic pathogenic mutations in NPC1 (95%) and NPC2 (5%) genes.

METHODS: Clinical, biochemical and molecular data from NPC patients diagnosed in our Reference Center over the last 26 years were collected and analyzed.

RESULTS: Since 1984, 19 NPC patients were diagnosed in 15 different families with the median age at diagnosis of 10.7 years. Regarding clinical onset and first symptoms: 8 patients (42%) had a juvenile neurological presentation with learning difficulties; 5 (26%) a late infantile neurological onset with developmental delay; 3 (16%) an early infantile neurological onset with hepatosplenomegaly and developmental delay; 2 (11%) had adolescent/adult neurological onset, one with cognitive decline, and another with schizophrenia; and one (5%) with neonatal systemic failure.

Out of the 19 patients, 16 where diagnosed through filipin test, from these 13 had a classic and 3 a variant pattern. One patient was diagnosed through plasma biomarker Lyso-509 quantification and in 2 other patients diagnosis was established by NGS gene panel. Patients genotype revealed 16 different mutations in NPC1 gene: 14 patients were compound heterozygous, and 5 homozygous. Currently, 6 patients are under therapy with Miglustat.

DISCUSSION AND CONCLUSION: Most patients were diagnosed in childhood. The lower prevalence of adult-onset cases may reflect a selection bias since the majority of referrals were from a pediatric hospital. Nevertheless, adults presenting with psychiatric symptoms are probably underdiagnosed.

Diagnosis was established through filipin staining, until the emergence of plasma biomarkers, like Lyso-509. The biomarker should be required as early as possible in patients presenting with NPC signs and symptoms. However, biochemical biomarkers do not deliver a definite diagnosis, so molecular analysis should always be performed.

Raising awareness for this condition is important since it warrants targeted testing for its diagnosis. An early diagnosis is crucial for allowing adequate management and genetic counselling of patients and families.

OC 09

LONG-TERM, SUSTAINED EFFICACY AND SAFETY RESULTS FROM A PHASE 1/2 CLINICAL TRIAL OF AN AAV8-MEDIATED LIVER-DIRECTED GENE THERAPY IN ADULTS WITH GLYCOGEN STORAGE DISEASE TYPE IA

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Glycogen storage disease type Ia (GSDIa) results from a deficiency of glucose 6-phosphatase (G6Pase) which is essential for glycogenolysis and gluconeogenesis. DTX401 is an adeno-associated virus serotype 8 (AAV8) vector expressing the human G6Pase gene (G6PC).

This global, open-label, phase 1/2, dose escalation gene therapy trial (NCT03517085) is evaluating the safety and efficacy of a single DTX401 intravenous infusion in adults with GSDIa.

Three patients in Cohort 1 received DTX401 2.0 x 10^12 gene copies (GC)/kg, and three each in Cohorts 2, 3, and 4 received DTX401 6.0 x 10^12 GC/kg. Cohort 4 was recently enrolled and includes a prophylactic steroid regimen to prevent transaminase elevation related to the immune response to DTX401.

In the nine patients enrolled in Cohorts 1 through 3, mean (SD) total daily cornstarch intake reduction from baseline to Week 52 was 65.5% (22.3) and from baseline to last visit (range: 60 weeks to 131 weeks) was 78.8% (20.1), both p<0.0001. In Cohort 3, where continuous glucose monitoring was implemented, an average 61% cornstarch intake reduction from baseline to Weeks 49 to 52 was associated with a mean 8.4% increase in percentage of time spent in euglycemia (blood glucose 60 mg/dL to 120 mg/dL). Cohort 1 patients reached ≥10 hours in fasting time to hypogcemia in the second year. In most patients, body weight decreased and improvements in everyday life activities were recorded. DTX401 had a consistent safety profile across all patients treated in all cohorts. No infusion related or treatment related serious adverse events (SAEs) were reported. All unrelated SAEs were classified as serious due to hospitalizations, and all resolved.

In conclusion, DTX401 showed a positive and sustained long-term efficacy and safety profile in all treated patients and in favor of the 6.0 x 10^12 GC/kg dose (dose by ddPCR 1.0 x 10^13 GC/kg) as the optimal biological dose for the pivotal phase 3 trial expected to start in the second half of 2021.

OC 10

AAV8 GENE THERAPY AS A POTENTIAL TREATMENT IN ADULTS WITH LATE-ONSET ORNITHINE TRANSCARBAMYLASE (OTC) DEFICIENCY: UPDATED RESULTS FROM A PHASE 1/2 CLINICAL TRIAL

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INTRODUCTION: OTC deficiency is an X-linked urea cycle disorder resulting in episodic hyperammonemia that can cause cumulative neurocognitive damage and even death. DTX301, an AAV8 vector containing the OTC transgene, is currently under investigation for the treatment of OTC deficiency.

METHODS: CAPtivate (NCT02991144) is an ongoing global, multicenter, open-label phase 1/2 dose-escalation trial evaluating safety and preliminary efficacy of DTX301 in adults with late-onset OTC deficiency. The primary endpoint is incidence of adverse events (AEs). Patients received a single IV infusion of DTX301. Cohort 1 received 3.4x10^12 Genome Copies (GC)/kg; Cohort 2, 1.0x10^13 GC/kg; Cohort 3, 1.7x10^13 GC/kg. Data cutoff was 23 December 2020. Complete responders discontinued all ammonia-scavenging drugs and protein-restricted diet. Responders had ≥50% reduction in baseline disease management.

RESULTS: DTX301 dosing of 3 patients in each of cohorts 1, 2, and 3 is complete. No treatment-related serious AEs or dose-limiting toxicities were reported; all AEs were mild or moderate (grade 1, 2) during the study. Seven patients experienced treatment-emergent AEs (TEAEs) that were considered related to study drug. Five patients experienced asymptomatic ALT increases consistent with those seen in other AAV gene transfer clinical trials. ALT increases were managed and resolved with a protocol-specified tapering regimen of oral corticosteroids administered in outpatient setting. Other TEAEs considered related to study drug were photophobia, headache, hypertension, vector-induced hepatitis, and hypophosphatemia.

Overall, 6 of 9 patients responded to DTX301: 3 patients were complete responders, and 3 patients were responders. All 9 treated patients maintained or improved ammonia control. Cohort 1 had one complete responder. Cohort 2 had 1 complete responder and 1 responder. Cohort 3 had 1 complete responder and 2 responders. The longest-treated responders from cohorts 1 and 2 are showing a durable response at 2.5 to 3 years after treatment and remain clinically and metabolically stable with good ammonia control.

CONCLUSIONS: Data from CAPtivate indicate that DTX301 has an acceptable safety profile and may be a potential new therapy with long-term therapeutic benefit for patients with OTC deficiency. Follow up of all patients is ongoing and enrollment in cohort 4 (1.7 x 10^13 GC/kg with prophylactic oral steroid taper) is complete.

OC 11

GENOTYPIC AND PHENOTYPIC FEATURES OF THE MOTHERS OF CHILDREN WITH MTDNA-ASSOCIATED LEIGH SYNDROME

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INTRODUCTION: Leigh syndrome (LS) is a genetic neurometabolic disorder characterized by central nervous system degeneration. Some patients have missense pathogenic variants in mtDNA inherited from the matrilineal lineage. Disease´s expressivity is dependent on the heteroplasmy level at a given tissue. In most cases, healthy mothers of an affected descendent have a lower heteroplasmy level, remaining asymptomatic. Occasionally, they have a substantial proportion of abnormal mtDNA, developing severe symptoms in adulthood. Given this variable expressivity, we reviewed the genotypic and phenotypic features of the mothers of a cohort of patients with mtDNA-associated LS (MALS) and their matrilineal lineage. With this revision, we aim to understand the necessity of a structured follow-up for these women and extended family members.

METHODS: Clinical revision of four families with children with MALS syndrome followed at the Pediatric Metabolic and Medical Genetics Unit, at Centro Hospitalar Universitário do Porto.

RESULTS: All mothers were asymptomatic at the time of their children´s diagnosis. Family I index-case presented the m.3946G>A variant, not detected in the asymptomatic mother´s peripheral blood. Family II index-case presented the m.8993T>G variant, with a heteroplasmy level of 75% in the mother. This woman started complains of fatigue at the age of 40 years, and two of her siblings present with retinitis pigmentosa, features that might be associated with the familial variant. Family III index-case presented m.8993T>G variant, with a heteroplasmy level of 56% in the asymptomatic mother. Family IV index-case presented m.10191T>C variant, not detected in the mother´s peripheral blood. Nevertheless, this woman has headaches and abnormal subcortical white-matter T2 MRI signal. Ongoing studies are trying to understand whether these features are associated with the familial variant.

CONCLUSION: Even if the mothers of children with MALS are asymptomatic at the age of diagnosis, they might develop symptoms later. Family III had second-degree family members by the matrilineal lineage with symptoms compatible with the familial mtDNA variant, stressing the importance of genetic counseling for the extended family of index cases with MALS. Follow-up guidelines should be established for the family members at risk and genetic counseling should be offered to all family members in the matrilineal lineage of an index case.

OC 12

NEW CAUSES OF PERSISTENT OR RECURRENT 3-METHYLGLUTACONIC ACIDURIA – EXPANDING THE DIFFERENTIAL DIAGNOSIS OF SECONDARY 3-MGA-URIA

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3-methylgutaconic aciduria (3-MGA-uria) can be a major finding when persistent, recurrent, isolated or significantly elevated. Significant secondary 3-MGA-uria is a biochemical finding in heterogenous metabolic disorders, largely mitochondrial-related. Here we report four unrelated new causes of significant 3-MGA-uria:

I: 4-year-old female with two cardiorespiratory arrests after viral illness, asymptomatic in between. During acute events she had major excretion of 3-MGA (154 µmol/mmol creatinine) and mild excretion of 3-hydroyisovaleric acid and 3-hydroxymethylglutaric acid (3-HMG). Final diagnosis is ISD11 mitochondrial iron-sulfer cluster deficiency (LYRM4) - published.

II: 5-year-old female with two episodes of ataxia and prostration with consistent isolated excretion of 3-MGA (14,2 µmol/mmol creatinine). She has thiamine-responsive basal ganglia disease (SLC19A3).
III: 5-month-old male with two cardiorespiratory arrests after severe dehydration or viral illness followed by microcephaly and failure to thrive without cardiomyopathy. Both episodes had 3-MGA (35,2 µmol/mmol creatinine) and 3-HMG and moderate excretion of 4-hydroxyphenyllactate (both some days after crises). Diagnosis is COX15 deficiency.

IV: 12-month-old male with abducens nerve palsy, significant bilateral peritrigonal white matter hypersignal and persistent excretion of 3-MGA (28-38,3 µmol/mmol creatinine), 3-HMG and tiglylglycine. He is thriving with normal development. Extensive genetic studies failed to provide a diagnosis. These cases widen the range of possible underlying causes of consistent 3-MGA-uria corroborating mitochondrial dysfunction as a common denominator. Some patients with consistent urinary 3-MGA-uria may remain unexplained despite all efforts as with the last case. However, persistent, isolated or recurrent 3-MGA-uria than can only be present in life-threatening crisis should elicit a wide investigation and follow-up.

SELECT POSTERS

PO 01

CENTRAL RETINAL ARTERY OCCLUSION IN A FEMALE WITH FABRY DISEASE

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INTRODUCTION: Fabry disease (FD) is an X-linked inherited disorder of glycosphingolipid metabolism due to deficient lysosomal α-galactosidase A activity, leading to progressive accumulation of globotriaosylceramide (GB3) in a variety of cell types. Heterozygous females may have an unpredictable phenotype, ranging from mild to severe cardio-cerebro-renal disease. We present a clinical case of an uncommon ophthalmological presentation in a young adult female with FD.

DESCRIPTION: Female patient diagnosed with FD at 21 years of age by family screening of p.S276N mutation in GLA gene, with reduced alpha-galactosidase A activity in leucocytes of 2.4 nmol/h/mg protein (reference range (RR) 36 – 80) and increased plasma lyso-Gb3 of 20 nmol/L (RR <1.3). At diagnosis, the only reported symptoms were mild acroparesthesia and fatigue. During organ involvement assessments, she presented with sudden right monocular visual loss. Ophthalmological examination and transcranial ultrasonography were compatible with central retinal artery occlusion (CRAO), confirmed by angiography. Further neuroimaging studies showed cerebral microangiopathy. Common cardiovascular risk factors (including drugs), autoimmune and thrombophilic disorders were excluded. Despite a normal echocardiogram, magnetic resonance T1 mapping suggested myocardial glycosphingolipid deposition. There was no evidence of renal or gastrointestinal involvement. This patient fulfilled clinical criteria to start treatment for FD, and due to an amenable mutation, she was proposed to initiate migalastat, in addition to antiplatelet aggregation.

DISCUSSION: As in other hereditary metabolic diseases, the eye is a susceptible organ for progressive deposition of glycosphingolipids in Fabry's disease. Most consistent ocular manifestations include cornea verticillata, lens opacities and retinal or conjunctival vascular abnormalities. Ophthalmic emergencies with sudden vision loss, such as anterior ischemic optic neuropathy, ophthalmic artery occlusion, central retinal artery or vein occlusion, are rare but previously reported in small series, more commonly in patients under 30 years of age.

COMMENTS: Central retinal artery occlusion is a rare manifestation of FD. This case reports a heterozygous female with a classic phenotype of FD, who may benefit from early treatment, according to current European guidelines and recommendations.

PO 02

MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY: MITOCHONDRIA CHARACTERIZATION IN PATIENTS-DERIVED FIBROBLASTS

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Multiple acyl-CoA dehydrogenase deficiency (MADD) (OMIM #231680), an inborn error of metabolism, is an autosomal recessively inherited disorder of fatty acid, amino acid and choline metabolism. MADD results from genetic mutations in ETFA, ETFB or ETFDH genes, that lead to defects on electron transfer flavoprotein (ETF), and ETF:ubiquinone oxidoreductase (ETF:QO) proteins. Mutations on their genes will cause distressed mitochondria β-oxidation and impaired energy production. Based on phenotype patients can be divided in severe MADD forms, that result in neonatal death, and mild MADD forms, that present a heterogeneous clinical phenotype with a large spectrum of symptoms and age of onset. Although a relation between genotype/phenotype is partially established for MADD, the pathophysiological mechanisms underlying clinical phenotype development in mild cases, namely at the mitochondrial level, remains unknown.

Aiming to establish a correlation between a disease mutation and mitochondria dysfunction, we have performed a compressive characterization of mitochondria morphology and bioenergetics. Using mild-MADD patient-derived fibroblasts we evaluate mitochondria healthiness by: i) immunofluorescence to characterize mitochondria morphology and mitochondria membrane potential; ii) luminescence assays to assess ATP content and cellular ROS production; iii) measuring cellular respiratory rates; and iv) semi-quantification of electron transport chain (ETC) complexes using antibody detection. Preliminary data showed that patient-derived cells present alter mitochondria morphology and diminished mitochondria membrane potential. We also observed that energy production is impaired in MADD cells, since these cells presented lower ATP content and higher ROS production.

Overall, our data indicate that in MADD-derived fibroblasts mitochondria is compromised, supporting our hypothesis that ETF or ETF:QO missense mutations lead to cellular and mitochondrial impairment.

ACKNOWLEDGEMENTS: This research was supported by the Fundação para a Ciência e Tecnologia (FCT/MCTES, Portugal) through grant PTDC/BIA-BQM/29963/2017 (to B.J.H. and F.S.C. (Research Contract)), SFRH/BD/147202/2019 (to J.V.R.) and center grant UIDP/04046/2020 (to BioISI) and UIDB/04046/2020 (to F.S.C. through BioISI).

PO 03

CLINICAL SPECTRUM OF PEARSON SYNDROME: A CASE SERIES

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BG: Pearson syndrome (PS) is a rare, multi-systemic disorder, caused by large-scale deletions or duplications of mitochondrial DNA. PS manifests typically in infancy with bone marrow failure, sideroblastic anemia and variable exocrine pancreatic insufficiency. Failure to thrive, lactic acidosis or tubulopathy are common additional findings. The mortality rate is significant in early childhood – those who survive may evolve into the Kearns-Sayre Syndrome spectrum. With this case series we aim to illustrate the phenotypic spectrum of PS.

PATIENTS 1&2: Male, monochorionic diamniotic twins presented with severe fetal anemia and intrauterine growth restriction. Born at 31 weeks, they evolved with severe pancytopenia, lactic acidosis, tubulopathy, hyperammonemia and left ventricular hypertrophy with lethal outcome – at 27 and 33 days of age – despite transfusional support, bicarbonate, carnitine and filgrastim. A large deletion in leukocyte mtDNA confirmed PS.

PATIENT 3: Male, 11 months old, presented with failure to thrive, recurrent infections, pancytopenia with macrocytosis and persistent hyperlactacidemia. Bone marrow examination showed vacuolized hematopoietic precursors and ringed sideroblasts. Further metabolic study revealed mitochondrial dysfunction and transient low fecal elastase. A large deletion in leukocyte mtDNA confirmed PS. He required multiple admissions for severe infection and metabolic decompensation. At 3 years old he has normal psychomotor development and requires nutritional supplementation and sporadic transfusional support.

PATIENT 4: Female, 15 months old, presented with failure to thrive, feeding difficulties and fatigue. Pancytopenia with severe macrocytic anemia, hyperlactacidemia and persistent low fecal elastase were present in subsequent evaluations. Bone marrow examination revealed vacuolized precursors and a large deletion of mtDNA in leucocytes confirmed PS. At 20 months of age, she has normal growth and psychomotor development and requires regular transfusional support, pancreatic enzyme and nutritional supplementation.

COMMENTS: This case series illustrates the clinical variability of PS. Aplastic anemia and hyperlactacidemia were present in all cases and thus should raise suspicion for PS, especially if exocrine pancreatic or renal tubular dysfunction are present. Follow-up of patients will be needed to evaluate a possible progression into the Kearns-Sayre spectrum or improvement of hematologic manifestations.

PO 04

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

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Lysosomal storage disorders (LSD) are a group of rare diseases caused mutations in genes that encode lysosomal enzymes, membrane proteins or transporters. This leads to an accumulation of undegraded substrates, which ultimately causes a broad range of highly debilitating clinical symptoms affecting multiple organs/systems, including the central nervous system. Yet, most therapies for LSD are limited to treating non-neurological signs. Thus, there is an urgent need for the development of new ones that can tackle the neuronal pathogenesis. Fortunately, the portfolio of innovative therapeutic approaches under development has been growing tremendously and the need for proper models grows alongside. To address this concern, we propose the development and characterization of innovative patient-derived cell models for early onset neurodegenerative LSD using dental pulp stem cells (DPSC) from deciduous "baby" teeth. DPSCs hold potential to give rise to a variety of cells including functionally active neurons. Nevertheless, to the best of our knowledge, this sort of technology hasn't yet been applied to samples obtained from LSD patients. This will be a total innovation in the field and we believe it holds potential to set a new trend for investigating LSD as it relies on a non-invasive, cost effective approach that can be set as a routine in any lab with standard cell culture conditions. Here we present an update on this project, summarizing its rationale and current results, while giving an overview of the whole protocol and discussing its potential applications. Briefly, over the last months, we have successfully implemented the protocol for the establishment of control DPSC cultures in our lab and are currently working on the differentiation protocol, which will allow the formation of mixed neuronal and glial cultures. We are also actively working with patients' associations and a team of expert pediatricians from the major reference centers for treatment of LSD to identify potential volunteers for baby teeth collection, having already approached several families, who are now actively involved in the project and willing to send us deciduous baby teeth, as soon as they fall.

ACKNOWLEDGMENTS: This work is partially supported by the Portuguese Society for Metabolic Disorders (SPDM - Bolsa SPDM de apoio à investigação Dr. Aguinaldo Cabral 2018; 2019DGH1629/SPDM20181&D) and Sanfilippo Children's Foundation (2019DGH1656/SCF20191&D).

PO 05

PLAIN LANGUAGE, STORYTELLING AND RARE DISEASES: THE 2020'S TOOTH FAIRY EXAMPLE

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Even though they only started a year and a half ago, it is already clear that the 2020s are set to be a disruptive decade. The ripple effects from COVID-19 are likely to be felt for many years, and the social and economic upheaval it triggered are here to last. But this is also a unique time.

Science is now on the front lines, and scientific jargon is all over the media. People started chatting about RNA, vaccines, spike proteins, mutant variants, you name it. At the same time, many scientists realized there is an actual social obligation to help the public navigate through COVID-19 as well informed as possible.

So, times may be confusing, but the grounds are set for a golden decade in Science Communication. And what was already a growing field of knowledge will certainly boom over the next years.

It surely is easy to think this trend will not apply to our field of interest, as these rare diseases do not affect hundreds of thousands of people or humanity. So, ultimately, we may reassure ourselves thinking no one really cares, and skip the hurdles of trying to talk about them to others but our peers. But that would be wrong.

While it may not be that obvious, rare diseases are a major public health issue. They are also the most impressive way of showing how genetics work and how vital and finely regulated is every single metabolic pathway. So, we should be talking about them to the public. And, from our own experience, we know the public wants to hear us. We just have to know how to communicate.

Here, we will use the practical example of the 2020s Tooth Fairy Project, to show you how two of the major Science Communication weapons - storytelling and plain language – may help you deliver your message to the general public. More than that, they may help you get the public's engagement.

By navigating through a simple text, originally written as a call for volunteers, we will give you a hint on what makes good science communication. Ultimately, we want to show that it is actually possible to get people's attention on rare diseases, even when they seem so intrinsically complex that even their names are impossible to pronounce.

ACKNOWLEDGMENTS : "The 2020s Tooth Fairy" project is partially supported by the Portuguese Society for Metabolic Disorders (SPDM - Bolsa SPDM de apoio à investigação Dr. Aguinaldo Cabral 2018; 2019DGH1629/SPDM2018I&D) and Sanfilippo Children's Foundation (2019DGH1656/SCF2019I&D).

PO 06

GLUTAMATE DEHYDROGENASE-DEPENDENT ROLE IS CRUCIAL TO UNVEIL MECHANISMS OF HYPERAMMONEMIA AND ENERGY METABOLISM IN BRAIN DURING LIVER DYSFUNCTION

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Hyperammonemia (HA) may have devastating consequences on neurodegeneration and neurodevelopment.

Glutamate dehydrogenase (GDH) constitutes a major hub of regulation affecting mitochondrial metabolism and the flux of metabolites through the urea cycle (UC). This work aims to investigate metabolome-driven changes of GDH function involved in the pathogenesis of HA, affecting directly or indirectly brain function.

Numerous drug-associated disturbances in the UC may impair ammonia disposal through mechanisms not fully elucidated. In this context, different approaches were used for further evaluation of drug-induced alterations on metabolome, enzyme activity, and acetylated protein modifications. Aminoacids and NAD+ levels were determined in the liver of Wistar rats subjected to Valproate exposure, a paradigm drug that crosses the blood-brain barrier potentially causing HA and hepatic encephalopathy. Considering the reversibility of the GDH-mediated reaction, the activity was studied in both directions, assessed through a spectrophotometric assay. The major mitochondrial acyl-CoA ester of valproic acid was synthesized and used in vitro. Kinetic studies show that valproyl-CoA induced a decrease of GDH activity not only in the reductive amination of ketoglutarate but also in the oxidative deamination of glutamate. The observation of a higher inhibitory effect on the reductive amination direction may provide valuable information to understand the mechanisms underlying valproate-associated HA and the precise tuning of GDH function.

The in-depth knowledge of GDH metabolic links is crucial in brain biology and neuropharmacotoxicology.

This work provides new insights into the pathogenesis of HA or respective drug-associated modulation and will contribute to develop further strategies to overcome, avoid and/or treat Hyperammonemia.

ACKNOWLEDGEMENTS: Financial support was provided by Fundação para a Ciência e a Tecnologia (FCT, Portugal), through Pest UIDB/04138/2020.

PO 07

DISEASE-CAUSING MEDIUM-CHAIN ACYL-COA DEHYDROGENASE VARIANTS: IMPACT ON FAD BINDING

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Medium-chain acyl-CoA dehydrogenase (MCAD) is a homotetrameric flavoenzyme involved in the dehydrogenation of medium-chain acyl-CoAs. MCAD deficiency (MCADD) is an autosomal recessive disorder caused by mutations in the MCAD encoding gene (ACADM). It is considered a loss-of-function conformational disorder as most of the ACADM gene missense mutations lead to variants with a high degree of misfolding. To be fully functional, upon mitochondrial import, MCAD must incorporate one FAD+ molecule per MCAD subunit which contains three structural domains: N- and C-terminal α-domains (αND and αCD) and an intermediary β-sheet domain (βD). Amino acid substitutions in MCAD variants likely impact FAD+ incorporation and consequently protein's function and stability.

Herein we investigated FAD+ incorporation for ten MCAD variants and its impact on enzyme's activity and stability. The wild-type (WT) and variant proteins were expressed in E. coli with a 6xHis tag. After protein purification, FAD+ content and apparent FAD+ binding affinity were determined. Limited proteolysis by trypsin and thermostability were also evaluated (absence/presence of FAD+). Data were further correlated with the protein´s structure and catalytic activity.

Most variants assumed a tetrameric structure, except p.Y372N, which was recovered as dimers. Two variants had lower levels of tetramers due to high levels of dimers (p.Y48C) and high molecular mass forms (p.D143V). Incorporation of FAD+ allowed classification of the variants in four levels (WT as 100%): ≈75% (p. R55G, p.G224R and p.K304E); ≈50% (p.Y48C, p.Y133C, pV264I, and p.G377V); ≈25% (p.A140T) and 0% (p.D143V and p.Y372N). Variants without FAD+ incorporation were highly prone to proteolysis indicating a more flexible conformation. However, with FAD+ proteins assumed more rigid conformations. Concerning thermal stability, a response to FAD+ as for the WT was only observed for five variants. Comparing the variants' catalytic activity with WT, four levels were observed, similarly as for FAD+ incorporation.

The results for each variant are strongly linked to their tertiary/quaternary structural position and proximity to the catalytic pocket. The most unstable variants belong to βD and αCD, both essential for the catalytic pocket formation. This also demonstrates the importance of each domain in the tetramer assembly and how the different steps of this process are linked to the FAD+ incorporation.

Funding: FCT (PTDC/BIA-BQM/29570/2017)

PO 08

CARBOHYDRATE METABOLISM DIAGNOSIS DYSREGULATION DUE TO A GALACTOSEMIA AND TYPE 1 DIABETES: A CASE REPORT.

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INTRODUCTION: Galactosemia is a rare inborn error of carbohydrate metabolism that require a galactose restricted diet. Despite the life-threatening nutritional treatment, long term complications still occur.

CLINICAL CASE: We report a patient with galactosemia and type 1 diabetes. The patient was referred to our reference center for poor weight gain, hypotonia, developmental delay, autism spectrum disorder and proteinuria. In the first appointment with 8 months age, he was still being breastfed and already started food diversification, the weight was 6.025 Kg (p<3) – z-score: -3.053; C: 63.7 cm (p<3) – z-score: -2.821; BMI: 14.9 (p3) – z-score: – 1.906. He started caloric supplementation with carbohydrates and lipids.

He was referred to cardiology, ophthalmology and nephrology consultation. At 18M he was diagnosed with galactosemia confirmed by genetic study and started a galactose restricted diet, supplemented with calcium, vit D, carbohydrates and lipids. He maintained a dietary pattern with pasty consistency, because of the difficulty in introducing solid foods, despite being followed in the speech therapy.

In 2016, at 2 years old, vitiligo was diagnosed. In 2018, at 4 years, he presented a global developmental delay, speech impairment, and autism spectrum disorder with eating difficulties (great sensibility for textures and temperature). Besides the dietary patterns it was noted a recovery in growth with weight: 17 Kg (p41); H: 103.5 cm (p31); BMI: 15.9 (p58).

Throughout follow up it was achieved an excellent metabolic control (Galactose 1P below 7uM/L). In 2020, at 6 years, he was taken to emergency because of polydipsia, pollakiuria and weight loss, with high blood glucose (>500 mg/dl) and ketonemia, being diagnosed with type 1 diabetes.

Currently, with 7 years old, he is eutrophic (w: 21.7 (p31); H: 116.5 cm (p14); BMI: 16 (p61)), well adapted to a galactose-free diet, low in fast-absorbing carbohydrates, although feeding difficulties are still present, the glycemic profile, not yet stable, is trending to lower values.

CONCLUSION: Given this patient's complex dual diagnoses, we proceeded with nutritional treatment trying to respect the nutritional approach for each of the diseases. This case supports the multidisciplinary team for an effective treatment.

PO 09

RETROSPECTIVE REVIEW OF MITOCHONDRIAL GENOME ANALYSIS IN OVER 6600 PATIENTS USING CLINICAL GRADE MTDNA SEQUENCING

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INTRODUCTION: Mitochondrial disease is caused by variants in both the nuclear and mitochondrial genome (mtDNA), but few next-generation sequencing (NGS) panels include mtDNA analysis. Further, reliable detection of heteroplasmy level is necessary for accurate diagnosis. High quality mtDNA analysis alongside panel-based diagnostics has the potential to increase the diagnostic yield and provide a more precise molecular diagnosis in patients with complex presentations. We describe the analytic and clinical validation of a NGS assay which simultaneously analyzes nuclear genes and mtDNA. We evaluated the assay's diagnostic yield in a cohort of >6000 patients.

METHODS: Multiple wet lab protocols and variant calling software were evaluated for low level heteroplasmy detection of single nucleotide variants (SNVs) and insertion/deletions (INDELs). To account for sequence homology of nuclear mitochondrial DNA (NUMT) segments, a sample from cells without mitochondria was used to distinguish mitochondrial from nuclear signal. A mitochondrial quality score that controls for possible low level heteroplasmy (<10%) false positives arising from NUMT segments was developed. Coverage and breakpoint-based analyses were used to detect single large and multiple low level heteroplasmy deletions.

RESULTS: The mean read depth was 18,224x and 100% of base pairs were covered >1000x. Sensitivity to detect SNVs and INDELs was 100% for >10% heteroplasmy level. For SNVs, the sensitivity was 93.3% for 5-10% and 88.9% for <5% heteroplasmy levels. The sensitivity to detect simulated 500bp - 5kb deletions at >10% heteroplasmy level was 99.7%. A total of 188,330 variants were identified in 6,684 patients having undergone mtDNA analysis with this assay. In 76 cases, a likely pathogenic or pathogenic variant was detected. The most common pathogenic variants were m.3243A>G (underlying maternally inherited diabetes, hearing loss and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS; 26 patients) and m.1555A>G (15 patients; aminoglycoside induced hearing loss). The inclusion of mtDNA analysis improved the diagnostic yield, depending on the panel and clinical indication, including the Comprehensive Metabolism panel (4%).

CONCLUSIONS: We demonstrate a comprehensive validation and successful inclusion of mtDNA analysis into a diagnostic NGS pipeline. The addition of mtDNA analysis can increase the diagnostic yield for patients undergoing NGS panel-based testing.

PO 10

LESSENING THE AGGREGATION BEHAVIOUR OF THE P.G46S VARIANT FORM OF HUMAN PHENYLALANINE HYDROXYLASE ASSOCIATED TO THE CLASSIC FORM OF PHENYLKETONURIA

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In human phenylalanine hydroxylase (hPAH) the substitution of a Glycine by a Serine in residue 46 (p.G46S) of the regulatory N-terminal domain leads to a variant protein with a high tendency to in vitro aggregation. This variant has been associated with the most severe phenotype of phenylketonuria (PKU) and is considered an excellent model to the study of the N-terminal PAH instability and aggregation process. The understanding of the underlying mechanism of the hPAH p.G46S self-association and formation of non-amyloid fibrils is fundamental for the development of molecules enhancers of protein stability with a potential to be used in PKU treatment.

In this work, the aggregation process of full-length (FL) and truncated forms, corresponding to the N-terminal regulatory domain (N1-118), of the wild-type (WT) and p.G46S hPAH were studied in the absence and presence of small compounds previously described as hPAH stabilizers (Lopes R et al, Biomolecules 2021, 11:462). The hPAH forms were produced in E. coli in fusion with the maltose binding protein (MBP) to allow further purification by affinity chromatography (amylose). After isolation of the corresponding oligomeric forms (by SEC) protein aggregation was studied by real-time turbidimetry, upon cleavage of the MBP tag, in the presence and absence of studied molecules. In addition, HEK293T cells, transfected with the G46S cDNA, were incubated with compounds showing inhibition of in vitro aggregation and enzymatic and immunocytochemistry assays were performed to follow in cellulo aggregation and enzyme function. Western blot analysis was used to determine protein content.

According the obtained data, molecules inhibiting/delaying p.G46S aggregation in vitro and in cellulo were found. Distinct responses were obtained when studying the FL and N1-118 forms indicating that these molecules bind to different regions of the protein. In preliminary assays, using HEK293T cells expressing thep.G46S, the presence of two of those molecules, induced an enzymatic activity improvement without influencing the protein cellular content, suggesting that enzyme activity is being enhanced by rescuing of protein folding.

In conclusion, among the studied molecules promising compounds were identified that can be used as scaffolds for further structure refinement.

PO 11

PHENOTYPIC VARIABILITY OF MITOCHONDRIAL DISEASE CAUSED BY NUCLEAR MUTATIONS IN COMPLEX I

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Mitochondrial complex I (CI; NADH–ubiquinone oxidoreductase) is the largest complex of the mitochondrial respiratory chain. Therefore, it deficiency is the most frequently observed disorder of the oxidative phosphorylation system (OXPHOS). The complex I is composed of at least 45 subunits of which seven are encoded by the mitochondrial DNA (mtDNA) and the remainder by the nuclear DNA (nDNA). Nuclear-encoded subunits are termed NADH dehydrogenase ubiquinone (NDU), followed by a description of function/location (FS-iron-sulfur protein region, FV-flavoprotein region, FA-subcomplex α, FB-subcomplex β, FC-undefined subcomplex). In addition to these structural subunits, several additional proteins are required for the assembly of the complex I.

Mutations in the nuclear encoded structural or assembly genes of complex I are associated with a wide range of clinical presentations, ranging from lethal neonatal disease to adult-onset neurodegenerative disorders. Phenotypes includes neurological disorders such as Leigh syndrome, cardiomyopathy, liver failure or myopathy. In most centers, complex I deficiency is diagnosed by spectrophotometric assay in biopsied tissue, usually skeletal muscle. However, this assay is only a measure of redox activity within the peripheral arm of the complex. Therefore, mutations of membrane arm subunits, which affect proton pumping rather than electron transfer, may theoretically result in apparently 'normal' enzyme activity.

The high number of genes involved and the phenotypic variability presented make molecular diagnosis and the establishment of a genotype-phenotype correlation a challenge. The next generation sequencing has improved this diagnostic by sequencing all these genes at the same time and in an economical way. Here, we describe seven clinical cases where NGS allowed the identification of mutations in the structural (NDUFV1, NDUFV2, NDUFS1) or assembly (ACAD9) genes of complex I. We can observe that even patients with mutations in the same gene can have highly variable phenotypes and age of onset.

For patients and their families, it is important to obtain a prognosis based on molecular diagnosis. A prognosis includes not only average life expectancy but also predicts the occurrence of symptoms that can severely affect quality of life. It is also indispensable for establishing clinical trials.

PO 12

HYPERTRIGLYCERIDEMIA CAUSED BY HOMOZYGOUS DELETION OF EXON 4 OF THE GPHIBP1 GENE

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Familial chylomicronaemia syndrome is caused by mutations in genes involved in the lipolytic cascade causing an abnormal persistence of chylomicrons (CM) during fasting. The resulting hypertriglyceridemia may lead to recurrent acute pancreatitis, eruptive xanthomata, lipemia retinalis, and hepatosplenomegaly. Mutations in the lipoprotein lipase (LPL) gene account for more than 80% of the cases of FCS reported in literature; other causes include the deficit of APOA5, GPIHBP1, APOC2 and LMF1.

A three-month-old girl, second child of Pakistani consanguineous parents, with irrelevant personal and familial history, presented to the emergency department with a 1-week history of isolated episodes of vomiting. Clinically she had mild hepatosplenomegaly, no abdominal tenderness and no xanthomata. The initial blood draw had a milky appearance, with high levels of total cholesterol - TC (669 mg/dL), low-density lipoprotein cholesterol – LDL (181 mg/dL), and triglycerides – TG (6744 mg/dL); and low high-density lipoprotein cholesterol - HDL (6 mg/dL). The levels of apolipoprotein A1, apolipoprotein B, amylase and lipase were in the reference range. Ophthalmology evaluation was normal and abdominal ultrasound described liver, spleen and kidney in their normal superior size for age. Breastfeeding was replaced by a low-fat formula with subsequent decrease in 48 hours of TC (245 mg/dL) and TG (692 mg/dL) and normalization of HDL and LDL cholesterol. Complementary feeding was started at 6 months of age, with restriction of dietary fat of the total energy intake. We perform the genetic test by target sequencing where the genes associated with FCS were included. We found a homozygous deletion of exon 4 of the GPHIBP1 gene, under confirmation by long PCR. No point pathogenic variants or small deletions were found in these genes.

Presently, at 12 months of age, she has adequate growth and psychomotor development, with normal plasmatic levels of CT, LDL and HDL and TG sustainably under 2000 mg/dl, without hepatosplenomegaly or pancreatitis.

We believe exon homozygous 4 deletion in GPIHBP1 is the probable genetic cause in this case, as previously reported in a consanguineous Pakistani family by Berge et al, 2014.

PO 13

ON THE TRACK OF MITOCHONDRIAL AMINOACYL-TRNA SYNTHETASE-RELATED NEUROLOGICAL DISEASES - EXPRESSION AND PURIFICATION OF EARS2 PROTEIN

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Mitochondrial diseases (MD) are associated with defects that affect mitochondrial protein synthesis genes, mitochondrial structural proteins, or proteins crucial for energy production [1]. Due to the ubiquitous nature of mitochondria, the pathophysiology of these diseases can be complex, and just about any system or organ is vulnerable.

In recent years the interest around mitochondrial aminoacyl tRNA synthetases (mt-aaRS), a family of enzymes that ensure proper translation by adding the appropriate amino acid into the correct tRNA molecule, is emerging due to the increasing number of MD patients with mutations on these enzymes, including in Portugal [2]. So far, more than 150 mutations have been reported to affect the constitutive functional domains of mt-aaRS leading to diseases with pleiotropic effects and remarkable tissue specificity, affecting primarily the central nervous system [3].

Although the knowledge around these disorders has been increasing, there are still open questions including the fact that the characterization of human mt-aaRS at the protein structural, conformational, and functional levels is scarce. To address these features will fill a gap in the field and will help to elucidate the molecular mechanism of disease behind mt-aaRS.

Here we present our recent work on glutamyl-tRNA synthetase (EARS2). We have used protein biochemical methodologies to make for the first time, to our knowledge, structural characterization of human EARS2 protein.

We have implemented and optimized the recombinant protein expression of human EARS2-wild-type in E. coli. And were able to purify the recombinant human protein at a yield of ~ 0.5 mg per g cell pellet. Further, we used different biophysical methodologies to characterize protein secondary and tertiary structure and to measure EARS2 stability. Preliminary data indicate that the purified protein presented a typical α/β fold with an apparent thermal melting temperature of ~68°C.

Our results bring a new perspective to the field and open new avenues to study mitochondrial diseases associate with mt-aaRS defects through structural characterization of disease variants.

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Financial support by FCT/MCTES PT: PTDC/BIA-BQM/29963/2017 to B.J.H. and center grant UIDP/04046/2020 to BioISI

PO 14

α-IDURONIDASE AS A SURROGATE MARKER OF HOSPITALIZATIONS IN HEART FAILURE

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INTRODUCTION: Heart failure (HF) is a multifactorial syndrome with continuously increasing prevalence and huge mortality rates and hospitalizations seem to contribute largely to this. Specific protein biomarkers have been associated with HF and some authors advocate the use of a "multi-omics" approach pursuing a comprehensive profile of the omics, including genomics and proteomics.

OBJECTIVE: We intent to evaluate the proteomic and genomic profile on HF outcomes.

Material and Methods: Prospective cohort study of patients followed up in outpatient HF appointment during one year. Lysosomal enzymes activity (LEA) evaluated at dried blood sample (DBS) were the independent variables and the mortality, emergency department readmission and re-hospitalization rates were the outcomes. Numeric variables were compared using T-test or Mann-Whitney as appropriate and the categorical using the Chi-square. The impact of the independent variables on the outcomes was studied by logistic regression and survival curves were obtained by Kaplan-Meier and cox-regression. Statistical significance was considered for $p<0.05$.

RESULTS: We followed up 50 patients with a median age of 76 years and female predominance (64%). Ischemic heart disease (14%), dilated cardiomyopathy (14%), systemic hypertension (12%) and valve disease (10%) were the main HF etiologies. Most of them had preserved left ventricle systolic function (42%). The most common comorbidities were hypertension (78%) and hyperlipidaemia (68%). The majority (n=32, 64%) had unspecific inflammatory patterns on serum protein electrophoresis, as well as abnormal LEA in DBS (n=31, 62%), although no significant correlation was found between these alterations. Additionally, we found that patients who had higher rates of re-hospitalization also had higher levels of α-iduronidase (IDUA) (15.5 vs. 4.2pmol/h/punction, p=0.041). None had mutations on IDUA gene.

DISCUSSION AND CONCLUSION: Glycans have been studied as biomarkers for HF and were shown to be associated with left ventricle hypertrophy, while high mannose N-glycans are associated with high activity of IDUA. In this cohort, with pronounced inflammatory burden, the higher the IDUA, the higher the hospitalization rate. Hence, we hypothesise that instead of using ancillary mass spectrometry to analyse glycans, we may use IDUA evaluation in DBS as a surrogate marker of HF severity and prognosis.

PO 15

LYSOSOMAL BETA-GLUCOSIDASE CORRELATES WITH HEART FAILURE MORTALITY

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INTRODUCTION: Gaucher disease is the most prevalent lysosomal disease and it has been described a cardiac involvement. In experimental studies, beta-glucosidase deficiency was correlated with accumulation of glucosphingolipids in myocardiocytes and further development of heart failure (HF). In humans, HF is a multifactorial syndrome with continuously increasing prevalence and huge mortality rates despite therapeutic innovations. Specific protein biomarkers have been associated with HF and some lysosomal enzymes/substrates may play a role.

OBJECTIVE: We intent to evaluate the proteomic and genomic profile on HF outcomes.

MATERIAL AND METHODS: Prospective cohort study of patients followed up in outpatient HF appointment during one year. Lysosomal enzymes activity (LEA) evaluated at dried blood sample (DBS) were the independent variables and the mortality, emergency department readmission and re-hospitalization rates were the outcomes. Numeric variables were compared using T-test or Mann-Whitney as appropriate and the categorical using the 2. The impact of the independent variables on the outcomes was studied by logistic regression and survival curves were obtained by Kaplan-Meier and cox-regression. Statistical significance was considered for $p<0.05$.

RESULTS: We followed up 50 patients with a median age of 76 years and female predominance (64%). Ischemic heart disease (14%), dilated cardiomyopathy (14%), systemic hypertension (12%) and valve disease (10%) were the main HF etiologies. Most of them had preserved left ventricle systolic function (42%). The most common comorbidities were hypertension (78%) and hyperlipidaemia (68%). The majority (n=32, 64%) had unspecific inflammatory patterns on serum protein electrophoresis, as well as abnormal LEA in DBS (n=31, 62%), although no significant correlation was found between these alterations. We found that beta-glucosidase was significantly lower among patients who died (3.04 vs. 3.98pmol/h/punction, p=0.026) and Cox regression showed a trend to a protective effect (OR = 0.39, 95% IC 0.051-2.936). Discussion and conclusion: Beta-glucosidase is deficient in Gaucher disease and it has been associated with HF. Nonetheless, this enzyme may play a role in HF pathophysiology even in the absence of Gaucher disease, as a trend towards mortality rate reduction was apparent with the enzyme activity increase. A larger cohort will be needed to confirm this trend, but these are promising findings.

PO 16

STRUCTURAL IMPACT OF SUCCINYLATION ON ELECTRON TRANSFER FLAVOPROTEIN FUNCTION

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Non-enzymatic post-translational acylations have been emerging as key players in mitochondrial metabolism. And, it is known that the frequency of these non-enzymatic PTMs correlate with the cellular accumulation of intermediate metabolites such as succinyl-CoA and glutaryl-CoA, a condition commonly associated to pathophysiological states [1]. Despite of the current available knowledge on acylations, the structural and functional consequences of these types of modifications on mitochondrial proteins remain to be elucidated.

Here we report our studies on the effect of succinylation on electron transfer flavoprotein (ETF), using a multidisciplinary approach combining in vitro and in silico methods. ETF is a key mitochondrial protein that participates in fatty acid, amino acid and choline metabolic pathways. Mutations on ETF coding genes cause multiple acyl-CoA dehydrogenase deficiency (MADD, OMIM #231680), an inherited metabolic disorder [2].

We have generated in vitro a model of succinylated ETF, and we showed that the modification abolishes the enzymatic activity of the protein. Further, succinylation altered the typical thermal denaturation profile of ETF, increasing protein stability at the level of secondary structure, without causing major structural changes on the native structure. Tryptic digestion of ETF allowed the identification by LC-MS/MS of around 8 lysine residues succinylated, and among them were Lys208 and Lys283 which are in the region surrounding the flavin binding pocket. Carrying out a mutagenesis analysis on these two lysine residues, we confirmed that succinylation in these sites led to functional impairment of ETF, suggesting that the introduction of a negative charge in this region alters the flavin cofactor's properties. Resorting to in silico studies, we identify the major structural impacts of succinylation on ETF, and showed that when the protein is succinylated, its interaction with MCAD (biological partner) samples different conformational regions compared to the wild-type simulations, which indicates that the modification impacts ETF interactions.

Overall, our results display a molecular rationale for the effects of succinylation on protein function, and corroborate previous reports on the importance of non-enzymatic PTMs as fine regulators of mitochondrial energy metabolism.

1. Trub AG, Hirschey MD. Sci. 2018 May;43(5):369-379.

2. Henriques BJ, et al. Gene. 2021 Apr 15;776:145407.

PO 17

ADAR-RELATED NEUROLOGICAL DISEASE - A MULTIDISCIPLINARY APPROACH

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INTRODUCTION: ADAR-related pathologies encompasses a group of rare inflammatory diseases with a type I interferon signature. The described phenotypes include classic Aicardi-Goutières syndrome (AGS), spastic dystonic syndrome, bilateral striatal necrosis and non-syndromic spastic tetraparesis. Both autosomal dominant and recessive inheritances have been reported. We described the case of autosomal dominant bilateral striatal necrosis diagnosed in a boy with severe psychomotor development regression.

CASE PRESENTATION: We present a 2-year-old boy, the second son of healthy, non-consanguineous parents. Irrelevant family history and uneventful pregnancy and delivery. Psychomotor development and growth were normal until 18 months of age, when he presented with great prostration and developmental regression with loss of ambulation, in the context of multiple minor infections. He evolved with macrocephaly, axial hypotonia followed by peripheral lower limb hypertonia, pyramidal signs and spastic tetraparesis. At 20 months of age, CE-MRI revealed discrete bilateral signal changes in the basal ganglia and myelination delay. Genetic testing with arrayCGH and trio-exome sequencing were negative and extensive metabolic evaluation was normal. CE-MRI reevaluation at 26 months demonstrated a slight putamen volume reduction and increased levels of neopterin in liquor suggested an inflammatory condition. Whole exome sequencing (WES) reanalysis for AGS and Alexander diseases was performed showing the presence of a de novo heterozygous pathogenic variant in ADAR gene: c.3019G>A, p.(Gly1007Arg).

CONCLUSIONS: This variant was previously described in the literature in about 12 patients associated with a broad phenotypic spectrum, ranging from a more severe classical AGS syndrome to non-syndromic tetraparesis. Our case, corresponds to a bilateral striatal necrosis. The dominant negative effect of this variant and its lack of association with dominant inheritance in OMIM database, prevented its report in the first exome analysis. Fortunetly, WES reanalysis based on a multidisciplinary approach and high clinical suspicion were crucial to reach the diagnosis. Since then, benefits and challenges of potential therapies have been the main focus in this setting.

PO 18

PHYSICAL MEDICINE AND REHABILITATION CONTRIBUTE ON MPS CHILDREN'S CARE

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Mucopolysaccharidosis are a group of rare disorders of lysosomal accumulation of undegraded glucosaminoglycans. Independently of the disease's rate of progression, it leads to cummulative disability, namely to accumulation of mucopolysaccharides in the connective tissue which contribute to decreased motor abilities. The degree and type of motor impairment is specific to the child's diagnosed disease.

Thus, the integration of Physical Medicine and Rehabilitation (PM&R), as an obligatory baseline approach, has to be a reality to these patients. Presently, amongst the several treatment guidelines to the various types of MPS, only some of them refer to PM&R. In this presentation, the author focuses on the role of PM&R on early detection of musculoskeletal and neurological problems and highlights the need of a planned timeframe for patient PM&R observation, as well as of a planned timeframe for imaging/ electrophysiology studies, in order to detect the presence of early signs of peripheral nerve entrapment and myelopathy. The last part of the talk will focus on the PT and OT interventions and environmental adaptations to increase the patient's participation in society.

Finally, a snapshot of today's functionality of the MPS patients on outpatient clinic at Hospital Dona Estefânia (Lisbon's pediatric Hospital), will be presented, as well as of the Portuguese healthcare network for disabled children.

PO 19

MTO1 GENE MUTATIONS: INTRAFAMILIAL PHENOTYPIC VARIABILITY

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Mithocondrial diseases are a group of rare multi-systemic disorders that may result from pathogenic mutations of the mitochondrial or nuclear genome. Since the mitochondria is present in virtually all cells, any organ can be affected. Mutations in the MTO1 gene that encodes the mitochondrial tRNA translation optimization 1 protein are associated with a mitochondrial disease, the MTO1 deficiency, first described in 2012, essentially characterized by lactic acidosis, global developmental delay and hypertrophic cardiomyopathy. Here we describe two siblings with a 13 year age gap and mitochondrial cytopathy with distinct outcomes. The index case, a female born in 1989, presented hypotonia and lactic acidosis since birth. She developed a severe myoclonic encephalopathy, alterations of movement and psychomotor and growth delay. Respiratory chain enzyme complex measurement in muscle revealed a partial deficiency of complex III and IV. Throughout the years she had multiple decompensations with severe acidemia and died at age of 16, due to a respiratory infection. Cardiac evaluation was always normal. The younger sibling, a male, had a less severe clinical presentation. He was born premature at 32 weeks of gestational age and presented hypotonia and lactic acidosis. Metabolic study performed at 8 days of life revealed elevated plasma alanine, hiperlactacidemy and a lactate to pyruvate elevated ratio. At 8 months of age a partial deficiency of complex IV was reported. He had a mild persistent hiperlactacidemy, psychomotor development delay and generalized hypotonia. At 19 months of age a dilated cardiomyopathy was detected, he started diuretic therapy at age 6 and remained asymptomatic until now. Between 4 and 6 years of age he had simple febrile seizures. At 11 years old, after a surgical correction of an acute appendicitis and peritonitis, he had status epilepticus, and started anti-epileptic drugs. In 2017, at 15 years of age, a genetic study (Next Generation Sequencing panel) confirmed the mitochondrial disease with identification of two new MTO1 likely pathogenic variants [c.413delT (p.M138fs) / c.1450C>T (p.R484W)] not described to that date. Currently, he is clinically stable maintaining a multidisciplinary follow up. The same genotype was confirmed in his sister's stored DNA. The aim of this clinical case is to emphasize mithocondrial diseases phenotypic heterogeneity, even in the same family, and the significance of the new genetic diagnostic techniques.

PO 20

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

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INTRODUCTION: Glycogen Storage Disease type IX (GSD IX), known since 1966, with a prevalence of 1:100.000, accounts for 25% of all GSD. It is caused by phosphorylase kinase (PhK) deficiency, the activator of glycogen phosphorylase, impairing glycogen hydrolysis into glucose. The PhK molecule is composed of four subunits encoded by different genes, including PHKA2. This is associated to GSD IXa (MIM #306000), the most frequent (75% of GSD IX cases) and the only X-linked form. GSD IX has a great clinical variability, and the liver is often an affected organ.

CASE REPORTS: A three-year-old previously healthy boy was referred with suspected symptomatic morning hypoglycaemia episodes, in the previous 9 months. Symptoms subsided after eating. An overnight 12h fasting investigation, including glycemia, aminotransferases, insulin and cortisol, had been inconclusive. Similar symptoms were noticed in his ten-month-old brother. Physical examination, aminotransferases and lipid profile were normal in both. Home monitoring capillary blood glucose and beta-hydroxybutirate evidenced ketotic hypoglycaemia in both: glycemia 1,6 and 1,9 mg/dl; ketonemia 2,6 and 1,7 mmol/L, respectively in the eldest and the youngest. The hemizygous variant, c.721A>G (p.Ile241Val), in PHKA2 gene was identified in the eldest.

A two-year-old boy presented at 12 months with persistent asymptomatic elevated aminotransferases (AST 208; ALT 141 UI/L). Abdominal distention, visible collateral circulation and hepatomegaly ensued. Glycemia was normal. Dyslipidaemia (total cholesterol 230; LDLc 193; HDLc-16; triglyceride 272 mg/dl) and abnormal urine organic acid profile, including ketones were found. Abdominal ultrasound showed hyperreflective and enlarged liver. His dizygotic twin brother presented a similar clinical picture. The hemizygous variant c.1210C>T (p.Gln404*) in PHKA2 gene was detected in both. Home monitoring disclosed occasional subnormal glycaemia (≥2,8 mmol/l) and elevated ketones (≤0,7 mmol/l). Fasting avoidance, adequate protein intake and nocturnal maltodextrin supplement were implemented in all, with regression of clinical and laboratorial abnormalities. Family genetic studies are in progress.

CONCLUSION: GSDIXa is a condition with great variability both on clinical presentation and severity. It should be considered in all cases of ketotic hypoglycaemia and/or hypertransaminasemia with dyslipidaemia, particularly in male patients. Dietetic measures prevent disease complications.

PO 21

METABOLIC CONTROL OF PATIENTS WITH PHENYLKETONURIA DURING THE COVID-19 PANDEMIC

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INTRODUCTION: The COVID-19 pandemic caused changes in the population's lifestyle, particularly in terms of food consumption. Moreover, although face-to-face consultations continued to occur, there was an increase in the use of teleconsultations. These changes may have influenced the metabolic control of patients with phenylketonuria.

OBJECTIVE: To evaluate the effect of the COVID-19 pandemic on the metabolic control of patients with phenylketonuria.

METHODS: 53 children and 83 adults followed at the Reference Centre for Inherited Metabolic Diseases of the Centro Hospitalar e Universitário do Porto were included. Phenylalanine levels were measured from fasting dried blood spots taken by patients/caregivers and analysed using a tandem mass spectrometry. All blood spots collected between 1 January 2019 and 29 February 2020 (n=2853) were considered to be in the period before the pandemic; and blood spots collected between 1 March 2020 and 30 April 2021 (n=2721) in the period during the pandemic. For each patient, in each of the periods, the median of their phenylalanine levels was estimated. The difference between the phenylalanine levels before and during the pandemic was evaluated using the t-student test for paired samples.

RESULTS: The children had a mean age of 10.1 years (min 2 months; max 17 years), being 47.2% male. On the other hand, the mean age of adults was 28.1 years (min 18 years; max 59 years), with 45.8% male. There were no significant differences in the number of blood spots collections taken before and during the pandemic either in children (before: 25.8±24.0 vs. during: 25.6±19.7; p=0.928) or in adults (before: 17.6±16.1 vs. during: 15.4±15.5; p=0.138). During the pandemic, a statistically significant decrease in phenylalanine levels was observed in children (-0.31±0.99 mg/dL, p=0.031; before: 5.64±2.11 mg/dL vs. during: 5.33±1.99 mg/dL) as well as in adults (-0.54±2.14 mg/dL, p=0.023; before: 9.75±4.54 mg/dL vs. during: 9.21±4.57 mg/dL). This decrease seems to have been more pronounced in patients with classic phenylketonuria (children: -0.86±2.73 mg/dL; adults: -1.24±2.25 mg/dL).

CONCLUSION: During the COVID-19 pandemic, the patients with phenylketonuria maintained the number of blood spots collections, and even improved their metabolic control. These results show that the follow-up of patients with phenylketonuria in our Centre was adequate despite all the limitations implied by the COVID-19 pandemic.

PO 22

PEX10 - PEROXISOME BIOGENESIS DISORDERS: EXPANDING PHENOTYPE

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BACKGROUND: Peroxisome biogenesis disorders in the Zellweger spectrum are a heterogeneous group caused by mutations in PEX genes, which can manifest a complex spectrum of clinical phenotypes, ranging from patients presenting in the neonatal period who die within the first year of life to patients presenting in adulthood with minor symptoms. Due to these facts, disease diagnosis and medical management are challenging. We aim to describe atypical clinic and genetic features of a seven-year-old boy, with peroxisome biogenesis disorder related to PEX10 mutation.

CASE REPORT: Second child of Brazilian non-consanguineous couple, unremarkable family history and uneventful gestation, was affected by progressive cerebellar ataxia since he was 5 years old and learning difficulties. Neurological examination showed mild mental retardation, gait instability with frequent falls, intention tremor and hypertonic lower limbs, without other pyramidal signs. Ophthalmologic evaluation showed horizontal nystagmus. Serial cerebral MRI exhibited progressive cerebellar atrophy. Extensive laboratory evaluation revealed elevations of C26:0 and C26:1, pristanic, phytanic and pipecolic acids. Due to that, the sequencing of the PEX 10 gene was requested, which revealed compound heterozygous mutations: (NM_153818.2) exon 1 c.2T>C (p.M1?) and exon 4 c.815_816delAC (p.H272fs), consistent with a peroxisome biogenesis disorder in the Zellweger spectrum, and both parents were confirmed to be heterozygous carriers. After the diagnosis he started dietary restriction of phytanic acid and supplements of the fat-soluble vitamins, ADEK, which have been reported at some extent to be effective. Currently, he is able to walk independently with some difficulty, he shows mild dysartria, intension tremor and dysmetria. He also has hepatomegaly de novo and mildly elevated transaminases, without cholestasis.

DISCUSSION: Our case suggests that peroxisome biogenesis disorders should be considered in the differential diagnosis of autosomal recessive progressive ataxia with early onset. The current guidelines are meant to provide a starting point for the management of these complex conditions. The early diagnosis should prompt evaluation of appropriate treatments, such as bile acid supplementation and dietary restriction of phytanic acid.

PO 23

THREE CASES, THREE PHENOTYPES OF ASSOCIATED TO MMADHC GENE

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BACKGROUND: Intracellular metabolism of cobalamin is a multiple step pathway leading to the synthesis of its active forms adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl), cofactors respectively for mithocondrial methylmalony-CoA mutase and cytoplasmatic methionine synthase. Deficiency in AdoCbl leads to accumulation of methylmalonic acid in body fluids while deficiency in MeCbl results in elevated levels in homocysteine.

The MMADHC gene encodes a protein involved in one of the steps of intracellular cobalamin metabolism. Depending on the nature and location of mutations within the protein, 1 of 3 biochemical phenotypes can occur: 1- Isolated Methylmalonic aciduria (MMA), 2- Isolated Homocystinuria (HC), and 3- Combined Methylmalonic Aciduria and Homocystinuria (MMA/HC).

Here we report 3 clinical cases referred to our clinic, representing the 3 distinct phenotypes associated to MMADHC gene.

CASE REPORT: CASE 1 (CBID-MMA): 5-year-old girl presenting with feeding difficulties, failure to thrive, developmental delay and an episode of severe metabolic acidosis with MMA. Genetic investigation revealed a region of loss of heterozigosity 2q22.1q31.1 which included MMADHC gene and simultaneously a c.228dup (p.Asn77Glufs*5) variant in homozygosity in MMADHC gene.

CASE 2 (CBID-HC): 16-month-old boy presenting with microcephaly, eating difficulties, hypotonia, severe developmental delay and failure to thrive. Metabolic investigation revealed HU and genetic studies detected the homozygous pathogenic variant c.746A>G (p.Tyr249Cys) in MMADHC gene.

CASE 3 (CBID-MMA/HC): 20-year-old girl presenting at the age of 4 years with developmental delay, frequent vomiting, axial hypotonia, absent language and unsteady gait, whose metabolic investigation revealed MMA and HC. Genetic studies revealed a homozygous c.748C>T mutation in MMADHC gene.

COMMENTS: The presented cases reflect a genotype-phenotype correlation, which means the location of pathogenic variants within MMADHC correlates with the type of enzyme deficiency. cbID-MMA typically results from pathogenic nonsense and frameshift variants found in exons 3 and 4, which encode the region of the protein necessary for AdoCbl synthesis. cbID-HC usually results from pathogenic missense variants found in exons 6 and 8, which encode the region of the protein necessary for MeCbl synthesis. cbID-MMA/HC typically results from pathogenic variants which occur in exon 5, exon 8 and intron 7, encoding the middle of the protein.

PO 24

REYE-LIKE CLINICAL PRESENTATION IN AN INFANT – A CHALLENGING DIAGNOSIS

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Reye syndrome is a rare acute metabolic rapidly progressive encephalopathy that affects children and teenagers. A "Reye-like" picture due to different etiologies such as inborn errors of metabolism can make the final diagnosis a challenge.

10-year-old boy, with no prior relevant family and medical background, with normal neonatal screening. With 11 months he was admitted in the emergency department of a local hospital with fever and vomiting.

He was, initially, discharged with the diagnosis of acute gastroenteritis. After being asymptomatic for 3 days he was re-admitted for several times for recurring symptoms. At day 8 of disease he was admitted with significant clinical deterioration and septic shock suspicion, being transferred to the PICU of central hospital.

It was registered severe metabolic acidosis without hyperlactacidemia, hypoglycemia with moderate ketone bodies, hyperammonemia, abnormal liver tests and no other analytical findings. At physical examination non-reactive midline pupils, global hypotonia and enlarged liver were described. The initial screening at admission showed elevation in 2 and 3-hydroxyglutaric acid levels in urine sample. It was not evaluated acycarnitine profile due to prior plasma transfusion. It was started high doses of intravenous glucose, and afterwards a hypoproteic, hypolipidic diet, supplemented with carbohydrates and fasting eviction.

Meanwhile, molecular and enzymatic studies for glutaric aciduria type 1, molecular study for glutaric aciduria type 2, muscle biopsy and study of the mitochondrial respiratory chain complexes were all normal. The nutritional plan was adapted, increasing the protein intake. During the period of 10 years follow-up, the boy had a positive evolution, without significant hypoglycemia episodes and improvement of all metabolic profiles, presenting however attention deficit/ hyperactivity disorder. He is now 10 years old and attending normal school. Recently, it was performed a clinical exome study with the identification of a pathogenic variant correlated with 3-hydroxy-3-methylglutaryl-CoA synthase deficiency.

Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency gives rise to failure of ketogenesis in liver, during illness or fasting. It is an extremely rare entity being this case the first one reported in Portugal. The importance of an early diagnosis relies in the eviction of severe hypoglycemic crisis which can lead to cumulative brain damage or even to death.

PO 25

TANGO2-RELATED METABOLIC ENCEPHALOPATHY AND ARRHYTHMIAS, A DIAGNOSIS TO KEEP IN MIND

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INTRODUCTION: TANGO2, is often encompassed in 22q11.2 deletion syndrome, and is also associated with the autosomal recessive entity MIM #616878: metabolic encephalomyopathic crises, recurrent, with rhabdomyolysis, cardiac arrhythmias, and neurodegeneration (MECRCN).

MECRCN is an important metabolic condition to consider, since encephalomyopathic crisis have several known triggers, namely infection, prolonged fasting and dehydration, and may cause regression and irreversible sequelae. When onset is not acute, it may present with developmental delays hard to distinguish from non-metabolic causes. CK is classically only increased during decompensation, and crises may be life-threatening due to torsade-de-pointe arrhythmia and secondary kidney failure.

CLINICAL CASE: A 12yo boy referred to Genetics consultation for moderate intellectual disability, tetrapyramidal syndrome, orobuccal apraxia, and paroxystic metabolic crisis associated with fever. Parents were healthy and non-consanguineous. He had a post-crisis regression after a frenulectomy with general anesthesia at 10 mo. He had no diagnosis-specific dysmorphic features and normal anthropometry. Previous investigations included extensive biochemical and metabolic workup, CGH-array, several single-gene molecular studies and MRI with spectroscopy. Serum pyruvate was increased during crisis, with maximum reported value 174 µmol/L at 3yo.

NGS-based study of a multigene panel for intellectual disability identified an intragenic deletion-c.(56+1_57-1)_(c.*1_?)del – and a pathogenic splice-site variant – c.605+1G>A in compound heterozygosity in TANGO2, thus establishing the diagnosis of MECRCN. Management was adjusted and measures to avoid triggers were initiated. Subsequent Cardiology evaluation was normal. Genetic counselling was provided for several relatives. The younger brother underwent molecular testing and was unaffected.

DISCUSSION: MECRCN should be suspected in children with trigger-induced crises, particularly when parents are consanguineous. It is also a diagnosis to keep in mind in unusually severe 22q11.2 deletion syndrome. Despite its progressive course, prognosis can be improved with crisis-avoidance and kidney-protecting attitudes. Parents and other family members benefit from condition-specific genetic counselling in order to consider reproductive options. Pre-symptomatic testing may be life-saving for at-risk children.

PO 26

AUTISM SPECTRUM DISORDER AS INITIAL PRESENTATION OF INBORN ERROS OF METABOLISM

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INTRODUCTION: Neurodevelopmental and psychiatric manifestations can be seen as initial presentation in almost all Inborn Errors of Metabolism (IEM) that affect the central nervous system. In some cases, psychiatric symptoms are compatible with well-defined disorders, such as autism spectrum disorder (ASD). ASD is a well-known form of presentation of some IEM but only a small percentage of cases with ASD will have an IEM. The authors intend to review the cases of IEM that initially presented as ASD in our center, but had a final diagnosis of IEM.

METHODS: Consultation of the patient database of the Pediatric IEM outpatient clinic and clinical record review of patients referred to the outpatient clinic with the diagnosis of essential/nonsyndromic ASD.

RESULTS: The data of 8 male patients were analyzed, with a current median age of 14.1 years. None of them had a family history of consanguinity or IEM disease. ASD diagnosis was established at a median age of 5 years. Most patients were referred from the Child and Adolescent Psychiatry (37,5%) and Pediatric Neurology outpatient clinic (37,5%), with a median age of referral of 5 years. Half of the patients did not show any other clinical manifestation. Only one patient had dystonia, extrapyramidal signs and a history of neurological regression. One patient was referred due to an elevated ammonia value. During follow-up, 3 patients (37,5%) were diagnosed with epilepsy and another patient later developed dystonia, extrapyramidal signs and ataxia. The subsequent investigation revealed an IEM disease in all patients at a median age of diagnosis of 6 years and 9 months. Those who did not show any other comorbidity or physical alterations were later diagnosed with creatine transporter deficiency (3 patients) and mitochondrial cytopathy- complex I deficiency (1 patient). All other patients were diagnosed with either Niemann Pick Disease type C (3 patients) or RFT 1 congenital disorder of glycosylation (1 patient).

CONCLUSION: Routine testing for IEM is not indicated in all patients as the percentage of metabolic disorders in children diagnosed with ASD is less than 5%. However, as suggested by our review, a careful history and physical examination and a close follow-up looking for comorbid clinical findings, such as seizures, developmental regression or neurological examination findings, may guide the investigation of a metabolic disorder.

PO 27

RECURRENT RHABDOMYOLYSIS – WHAT LIES BENEATH

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INTRODUCTION – Exercise intolerance and leg pain are common complaints in adolescents but, if associated with rhabdomyolysis, critical care may be needed. In recurrent episodes, a metabolic myopathy must be considered.

CASE REPORT – A 13-year old girl was hospitalized with intense myalgia after physical activity. Serum creatine kinase (CK) was 39.765 UI/L, myoglobinuria was detected and acute rhabdomyolysis was diagnosed. Personal history highlighted two previous hospitalizations due to dark urine, interpreted as hematuria, associated with urinary tract infection and mononucleosis, respectively. In both episodes, the adolescent recalls muscle pain that was not appreciated, so CK was not assessed. Further investigation revealed normal CK between crisis, acylcarnitine elevation and multi-gene panel for rhabdomyolysis identified two mutations in heterozygosity: p.Ser113Leu and p.Arg296Gln, establishing the diagnosis of Carnitine Palmitoyl Transferase II (CPTII) deficiency.

DISCUSSION – Myoglobinuria associated with mild rhabdomyolysis may be unnoticed or mistaken for hematuria, leading to delayed diagnosis. Investigation of recurrent rhabdomyolysis should include causes of metabolic myopathy. CPTII deficiency is the most common inherited disorder of the long-chain fatty acid oxidation. Although the pattern of inheritance is known to be autosomal recessive, 30-40% of patients are compound heterozygotes, carrying the most common mutation (p.Ser113Leu) in combination with a rare second pathogenic variant.

CONCLUSION – Recurrent rhabdomyolysis and myoglobinuria, if left undiagnosed, may lead to progressive muscle weakness. Although rare, metabolic myopathies should be considered earlier in the investigation. Genetic testing is now regarded as the gold standard in the diagnosis of CPTII deficiency.

PO 28

MUCOPOLYSACCHARIDOSIS TYPE I - THE IMPORTANCE OF EARLY DIAGNOSIS AND TREATMENT. CASE REPORT

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INTRODUCTION: Mucopolysaccharidosis type I (MPS I) is an autosomal recessive disorder caused by alpha-L-iduronidase deficiency, characterized by glycosaminoglycans (dermatan and heparan sulphate) accumulation in tissues and multisystemic damage. It is a life-threatening condition with severe disease burden and premature death. Haematopoietic stem cell transplantation (HSCT) is effective in preventing disease progression, namely the neurologic involvement and improving quality of life.

CASE REPORT: A 15 months old girl was referred because of motor delay (unable to sit without support at nine months). Family history was irrelevant. The gestation and labour were normal. At birth, umbilical, bilateral inguinal and linea alba hernias were noticed. Since her first year of life, she had recurrent otitis media, rhinitis and wheezing. Physical examination showed coarse facial features, dolichocephaly, mild thoracolumbar kyphosis, hepatomegaly and umbilical hernia.

MPS was suspected and corroborated by elevation of urinary glycosaminoglycans (GAG). Alfa-L-iduronidase enzymatic activity was low, suggesting MPS I. The diagnosis was confirmed by the finding of p.W420X (c. 1205 G>A) and p.P533R (c. 1598 C>G) mutations, respectively in exons 9 and 11 of IDUAgene.

She started enzyme replacement therapy (ERT) at 29 months old and was submitted to HSCT at 33 months old. Full-donor chimerism was achieved at 4 months and ERT stopped at 7 months after HSCT. Graft-vs-host disease and other HSCT complications were resolved by current protocols.

Currently, at 5-years-old, urinary GAG excretion is normal, (with abnormal electrophoretic profile) and leukocyte alfa-L-iduronidase activity is low normal. She is clinically stable, with softening of the coarse features. Neurodevelopment, normal at 26 months, is moderately delayed.

CONCLUSIONS: MPS should be suspected in a child with hernias, recurrent ear, nose and throat Infections infections and/or coarse facial features. Early diagnosis allows prompt ERT implementation and timely manner HSCT, which are available/effective for some MPS subtypes, namely MPS I. Therefore, neonatal screening for MPS I is now a matter of debate. Opportune diagnosis is of utmost importance for adequate family counselling.

PO 29

TANGO2-RELATED METABOLIC ENCEPHALOPATHY AND ARRHYTHMIAS SYNDROME WITH BASAL GANGLIA INVOLVEMENT AND A NOVEL MUTATION

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BACKGROUND: TANGO2-related metabolic encephalopathy and arrhythmias is a rare metabolic autosomal recessive disease caused by mutations in TANGO2 gene, which plays a dual role in endoplasmic reticulum-Golgi apparatus interaction and at the mitochondria. Patients usually present with acute metabolic crises (hypoglycaemia, mild hyperammonaemia, rhabdomyolysis, arrhythmias) and neurodegeneration.

CASE REPORT: A 19-year-old female patient, of Syrian origin, presented with a history of developmental delay since she was 18 months, followed by recurrent encephalopathic episodes of variable duration with hypoglycaemia since she was 10 years of age. One episode was accompanied by cardiac failure, leading to ICU admission. There was parental consanguinity and a family history of early deaths by undetermined cause (brother and cousins). Neurological evaluation showed global developmental and speech delay (able to comprehend simple orders), complex ophthalmoparesis, spastic tetraparesis with bilateral Babinski sign, generalized dystonia (trunk and limbs), generalized muscular atrophy, pes cavus and scoliosis. These findings suggested progressive and cumulative neurological impairment, as encephalopathic episodes recurred. Brain CT showed bilateral and symmetric basal ganglia and cerebral peduncle hypodense lesions, and cerebral, cerebellar and midbrain atrophy. NGS gene panel for metabolic diseases identified a novel, likely pathogenic, homozygous variant in the TANGO2 gene, p.Leu197Phe (c.589C>T). Both parents were heterozygous for this variant. Nutritional intervention was started, leading to improved alertness and better interaction with her family, and also prevented further metabolic crises.

DISCUSSION: We describe a novel mutation in the TANGO2 gene, leading to a typical clinical presentation of acute recurrent encephalopathy, developmental delay and neurodegeneration. However, basal ganglia lesions are not yet described in this disease , further expanding the radiological phenotype. Early diagnosis is important, since nutritional intervention, trigger avoidance and early treatment of metabolic crises are essential to improve quality of life and to minimize progression and neurological impairment.

PO 30

MOGS - CONGENITAL DISORDER OF GLYCOSYLATION TYPE IIB: A NEW CASE WITH EARLY FATAL OUTCOME

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BACKGROUND: MOGS-CDG is a congenital disorder of N-linked glycosylation that results from mannosyl-oligosaccharide glucosidase (MOGS) deficiency. MOGS is expressed in the endoplasmic reticulum and plays a critical role in processing N-glycans by removing the distal α-1,2-linked glucose from the Glc3Man9GlcNAc2 oligosaccharide precursor. Recessive mutations in MOGS gene are responsible for multisystemic manifestations with prominent central nervous system (CNS) involvement, microcephaly, typical dysmorphisms, immunological dysfunction, respiratory difficulty, and other abnormalities (cardiac, gastrointestinal, ophthalmological, endocrine). Only 11 cases were reported so far with poor prognosis (most with life span of less than a year).

CASE REPORT: We report a neonate female with a polymalformative syndrome. Second child of non-consanguineous healthy parents with no relevant familial medical history. Prenatal findings included fetal growth restriction, retrognathia, short femur, non specific changes in fetal MRI and normal array. The patient was born at 39 weeks with respiratory distress that required mechanical ventilation and was transferred to the neonatal intensive care unit. At our observation she showed generalized hypotonia, feeding problems, microcephaly, dysmorphisms (low hairline, low set ears, short palpebral fissures, corneal opacity, deviated nasal septum, bulbous nasal tip, cleft palate, retrognathia, clenched hands with overlapping fingers, metatarsus adductus and anterior anus). MRI showed extensive CNS malformations and EEG abnormal activity. Echocardiogram revealed an interventricular communication and abdominal imaging identified hepatomegaly. Absence of peristalsis since birth led to abdominal distension and a laparotomy was performed with a poor outcome. She died at 40 days old.

Exome sequencing identified two variants in MOGS gene, a truncating likely pathogenic variant c.882del (p.(Glu295Asnfs*10)) and a missense variant of uncertain significance c.2225A>G (p.(Asn742Ser)). Parental segregation confirmed that the variants were in trans and with reverse phenotype provided a diagnosis for this patient and family.

COMMENTS: CDG should be included in the differential diagnosis of any multisystemic congenital abnormalities.

Our patient's phenotype showed many overlapping features with the previous reported MOGS-CDG cases.

Only exome sequencing provided an explanation for the clinical findings and allowed genetic counselling to the family.

PO 31

CLINICAL MIMICS: A STUDY OF THREE PATIENTS PREVIOUSLY DIAGNOSED WITH MITOCHONDRIAL DISEASE

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INTRODUCTION: Primary mitochondrial disorders (PMD) are a complex group of diseases with challenging diagnosis arising from its nonspecific presentation and absence of reliable mitochondrial biomarkers. Mitochondrial Disease Criteria (MDC) using clinical, metabolic, imaging, and histopathologic features and biochemical investigations of skeletal muscle has been used for the diagnosis of PMD in infants and children prior to genetic testing or when mutation analysis is inconclusive. Nonetheless, it should be noted that many genetic disorders might present in a similar way, mimicking PMD. Aim: We present a cohort of three cases previously diagnosed as "probable PMD" according to consensus MDC, in which alternative primary diagnoses have recently been identified.

CASE REPORTS:

P1: An infant male presented with developmental delay, pyramidal and extrapyramidal signs, severe gastroesophageal reflux, failure to thrive, increased serum lactate, pyruvate and alanine, and decreased activity of pyruvate dehydrogenase complex. **P2:** A two-year-old female came to attention for developmental delay, microcephaly, strabismus, failure to thrive, recurrent prostration episodes and chronic vomiting, with elevated lactate and lactate/pyruvate ratio; MRI showed a cerebellar atrophy; respiratory chain studies in muscle biopsy revealed complex V and II+III deficiency. **P3:** A female with a 22q11.2 microdeletion presented with a severe rhabdomyolysis episode at age three. In recent years, she developed episodic weakness, epilepsy and hypothyroidism; muscle biopsy identified a complex II and IV deficiency.

Recently, genomic evaluation revealed a non-mitochondrial diagnosis for each case: P1, Allan-Herndon-Dudley syndrome (SLC16A2, MIM#300523); P2, mental retardation and microcephaly with pontine and cerebellar hypoplasia (CASK, MIM#300749); P3, metabolic encephalomyopathic crises, recurrent, with rhabdomyolysis, cardiac arrhythmias, and neurodegeneration (TANGO2, MIM#616878).

CONCLUSION: Recent advances in molecular diagnosis such as chromosomal microarray and next generation sequencing have shown that some patients with clinical and biochemical features suggestive of PMD may have other genetic disorder. When a PMD is suspected, genomic testing should be considered as first or second line test in the diagnostic workflow. Furthermore, the recognition of mitochondrial mimics and establishment of an accurate diagnosis is important for patient management and genetic counselling.

PO 32

"IDIOPATHIC" COPPER DEFICIENCY MYELOPATHY AND LEUKODYSTROPHY: SHOULD GENETIC CAUSES BE CONSIDERED?

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BACKGROUND: Copper is a component of metalloenzymes that have a critical role in the structure and function of the nervous system and bone marrow. Copper deficiency has hematologic (anemia and neutropenia) and neurologic manifestations (the most common are myelopathy and peripheral neuropathy). It can be a result of genetic (Menkes syndrome) or acquired (previous gastric surgery, celiac disease, inflammatory bowel disease and excessive zinc ingestion).

CASE REPORT: A 51-year-old female patient presented with a history of progressive gait impairment and developmental delay since nine years-old. At the age of 18, she developed microcytic anemia, without any identifiable cause despite a broad etiological study. At the age of 30, she presented with a marked worsening of gait and developed bladder dysfunction, which led to multiple admissions in neurology wards.

Neurological evaluation showed impulsive and inappropriate speech, cognitive impairment, bilateral optic atrophy, spastic paraparesis, diminished vibratory sense in the lower limbs and generalized hyperreflexia.

She had no relevant familial history. Extended blood tests were normal, including autoimmunity, serologies, folic acid, vitamin B12 and zinc. However, serum ceruloplasmin and copper levels were markedly reduced to 0.09g/L and 0.65mg/L, respectively. Cerebrospinal fluid analysis was normal. Brain MRI showed confluent T2 hyperintense lesions in the periventricular white matter, predominantly frontoparietal, suggestive of leukodystrophy. Nerve conduction studies showed sensorimotor mixed axonal and demyelinating polyneuropathy. Extended genetic testing excluded, among other diseases, Menkes disease, aceruloplasminemia, the most frequent hereditary spastic paraplegia and X-linked adrenoleukodystrophy.

DISCUSSION: We report a patient with progressive pediatric-onset myelopathy and leukodystrophy associated with copper deficiency, suggesting a genetic etiology not yet identified despite extensive testing. About 20% of copper deficiency myelopathy are idiopathic despite extensive study, suggesting that possible genetic causes of copper deficiency should be further studied.

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