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SOCIEDADE PORTUGUESA
DE DOENÇAS METABÓLICAS

16th INTERNATIONAL SYMPOSIUM OF THE PORTUGUESE SOCIETY FOR METABOLIC DISORDERS

New Horizons in Inborn Errors of Metabolism

11th . 12th . 13th November 2020

VIRTUAL SYMPOSIUM

WELCOME ADDRESS

Dear Colleagues,

Dear Colleagues,
I have a great pleasure to welcome you all to the **16th International Symposium of the Portuguese Society for Metabolic Disorders (SPDM)**, this year in a virtual format.

Starting a new decade and under the motto "New Horizons in Inborn Errors of Metabolism", we aim to bring you the most recent scientific advances and to inspire new approaches to Inherited Metabolic Disorders, with the participation of world experts. The programme includes topics that are transversal to clinicians, scientists and dietitians thereby creating opportunities to build bridges. We would like to encourage you to participate in the sessions by posing questions to the speakers in the live chat.

The first day will be dedicated to selected oral communications and selected e-posters communications. E-posters can be viewed in the specific section anytime during the symposium. As a novelty, delegates will be allowed to evaluate these works. Grants will be attributed to the highest classified works in each category. Thank you very much for joining us "digitally".

Daniel Costa Gomes
2020 Symposium Chairperson

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VIRTUAL SYMPOSIUM

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Past SPDM GA - President

SPEAKERS BIO-NOTES

Session IV

CROSSTALK AMONGST INTRACELLULAR ORGANELLES

RONALD J. A. WANDERS
Amsterdam, NL

RONALD JA WANDERS holds a Professorship in Clinical Enzymology of Metabolic Diseases at the University of Amsterdam and was Head of the Laboratory Genetic Metabolic Diseases, Academic Medical Center, UMC Amsterdam from 01.01.2003 until his formal retirement on 01.02.2018. He studied Chemistry with Biochemistry as subspecialization, did his PhD at the E.C.Slater Institute for Biochemical Research, Science Faculty, University of Amsterdam under supervision of Prof. Dr. JM Tager, did postdoctoral work at the same Institute and started to work as Postdoc in 1983 in Laborotory Genetic Metabolic Diseases where he has been > 35 students did their PhD under his supervision. He has received numerous awards, gave >250 lectures worldwide and has been Member of a large number of national and international scientific Review Boards and patient organisations. After his formal retirement he continues to work in the Laboratory Genetic Metabllic Diseases as Senior Scientist on a project basis.

UTE SPIEKERKOETTER
Freiburg, DE

UTE SPIEKERKOETTER, chair of Pediatrics and Medical Director of the Department of Pediatrics and Adolescent Medicine, University Children's Hospital Freiburg, Germany
Medical Schools in Aachen, Goettingen and Muenster, Germany, from 1986 to 1993.
Specialisation in Pediatrics at University Children's Hospital in Essen, Germany and University Children's Hospital in Duesseldorf, Germany, from 1994 to 2001.

FRÉDÉRIC M. VAZ
Amsterdam, NL

FRÉDÉRIC VAZ, he got his master in chemistry at the University of Utrecht in 1997, specializing in biochemistry. In the Laboratory Genetic Metabolic Diseases in Amsterdam, headed by Professor Wanders, he performed his Ph.D. project and wrote his thesis on carnitine biosynthesis. As a postdoc, research on carnitine biosynthesis continued but the new subject of Barth syndrome got him interested in lipids, especially cardiolipin. During this period he was trained as a clinical biochemist specializing in inborn error of metabolism.
He now is head of the metabolite section of the Laboratory Genetic Metabolic Diseases in Amsterdam, responsible for diagnostics of inborn errors of metabolism as well as the head of the Core facility metabolomics of the AMC. His main goals are the continuing development of advanced lipidomics/metabolomics platforms - including the required bioinformatics- and their application for both research and diagnostic purposes.
Amsterdam UMC, location Academic Medical Center, Department of Clinical Chemistry, Laboratory Genetic Metabolic Disease, Amsterdam, The Netherlands.

Session IV

CROSSTALK AMONGST INTRACELLULAR ORGANELLES

JEAN-MARIE SAUDUBRAY
Paris, FR

JEAN-MARIE SAUDUBRAY, MD, born in 1937, trained in the Hopitaux de Paris (AP-HP). He was promoted Professor and Chairman of Paediatrics at the Université Paris Descartes in 1975 and spent in 1980 a sabbatical year at University California san Diego in the metabolic diseases screening laboratory of Southern California (chairman Prof Nyhan).

He set up an international metabolic reference centre in Necker Enfants Malades hospital Paris and trained in his ward many metabolic physicians who are now head of metabolic department in their respective institutions. He has designed many metabolic training courses in France, Europe, North Africa, middle East, Pacific Asia, North and South America.

He has published about 440 peer-review papers, 200 invited chapters on IEM and described several new entities. He is editor of the reference textbook "Inborn metabolic diseases diagnosis and treatment" (6th edition 2016) and wrote the chapter on the clinical approach and algorithms for IEM published in the Metabolic and Molecular Bases of Inherited Disease (Scriver). He gave about 400 plenary international conferences among them many keynote lectures and the Komrower lecture at 3 SSIEM symposium. He is honorary lifetime member of the SSIEM and member of the Scriver family visiting professorship in genetic medicine. He is currently the executive editor of the North American Metabolic Academy training programme and member of the scientific committee of the Recordati rare disease foundation. He is appointed as senior consultant advisor of the adult neurometabolic Unit, Pitié-Salpêtrière Hospital, University Pierre and Marie Curie, Paris France.

Session V

NEW INSIGHTS IN CARBOHYDRATE DISORDERS

RENÉ SANTER
Hamburg, DE

RENÉ SANTER, he is a full professor of Pediatric Metabolic Medicine at the Dept of Pediatrics, Univ Medical Center Eppendorf in Hamburg, Germany, an SSIEM / UEMS -accredited training center for Pediatric Metabolic Medicine. He received his medical education at LMU University Medical School in Munich, Germany, got his training in Pediatrics in Kiel, Germany, and was a fellow in Pediatric Gastroenterology at the Children's Hospital of the State University of New York (SUNY) at Buffalo, NY, USA before he specialized in Pediatric Metabolic Medicine. His main interests in clinic and research are inborn errors of metabolism, particularly those of carbohydrate metabolism. He has a specific interest in congenital disorders of cellular glucose transport and for several such defects the molecular bases have been elucidated in his lab. Further topics of interest are mitochondrial disorders, urea cycle disorders, and liver transplantation in metabolic diseases. As a director of the Northern German Screening laboratory, he also has an interest in the scientific evaluation of such programmes. Prof Santer is the President of the German Society for the Study of Inborn Errors of Metabolism (APS) and thus involved in training programmes of young clinicians and clinical scientists.

ESTELA RUBIO-GONZALBO
Maastricht, NL

ESTELA RUBIO GOZALBO is a pediatrician metabolic diseases and researcher, Head of the metabolic diseases department and full professor of inborn errors of metabolism with focus on galactosemia at the Maastricht University Medical Center in The Netherlands. After a happy childhood in Spain she moved to the United States where she did part of her secondary school and moved back to Spain. Shortly thereafter she moved to The Netherlands where she completed her MD degree at the University of Amsterdam and her Pediatric residency at the Emma's Children Hospital in Amsterdam. She moved to the Nijmegen University to obtain a degree in metabolic diseases (fellowship metabolic diseases) and performed her PhD on mitochondrial disorders. Since 2000 she works at the Maastricht University Medical Center as a staff member of the Pediatric Department and Laboratory Genetic Metabolic Diseases combining clinical care with research (patient-related and basic research). She leads a multidisciplinary, successful Galactosemia research line with many national and international collaborations. She is the founder and chair of the international Network for Galactosemia (GalNet). Prof. Rubio Gozalbo has been the recipient of many national and international grants and has published >100 peer reviewed papers and several book chapters. She is proud to be an Honorary Member of the Dutch Galactosemia Society (patient organization).

Session VI

NEW TREATMENT PERSPECTIVES

ANIA MUNTAU
Hamburg, DE

ANIA MUNTAU, she is Professor of Molecular Pediatrics at the Faculty of Medicine of Munich University, Munich, Germany since 2006 and she was appointed Chair of Pediatrics and Director of the Children's Hospital at University Medical Center Hamburg-Eppendorf, Hamburg, Germany, in 2014.
She was awarded her MD degree from the Ludwig-Maximilians-University (LMU), Munich, Germany in 1992, where she also received her postgraduate training in Pediatrics until 1997 and became board-certified in 2000. From 1997-2004, she did her subspecialty training in Biochemical Genetics and Molecular Biology. In 1999, she started to work as at the Dr. von Hauner Children's Hospital in Munich, Germany, where she headed the Departments of Molecular Pediatrics and Inborn Errors of Metabolism from 2004 to 2014.
Prof. Muntau's studies first focused on the identification of genes causing severe inborn errors of metabolism and on the understanding of the function of related proteins. Her research group currently works on the elucidation of the molecular basis of different genetic protein misfolding diseases and on the development of therapeutic strategies for patients suffering from these severely disabling diseases.
In 2015, she was elected into the German National Academy of Science (Leopoldina).
Prof. Muntau has (co-)authored almost 100 articles in peer-reviewed journals and contributed to 15 books or book chapters.

LAKSHMINARAYAN RANGANATH
Liverpool, UK

LAKSHMINARAYAN RANGANATH is a consultant at the Royal Liverpool University Hospital in the UK. Professor L. Ranganath established an NHS Highly Specialised Services funded National Alkaptonuria Centre (NAC), employing off-label use of nitisinone. Professor L. Ranganath is the inaugural Director of the NAC. Patients in the NAC are able to access nitisinone free of charge and access a multidisciplinary team of experts. Professor L. Ranganath has carried out a national survey that identified 81 UK, 450 European and 1000 patients worldwide. He has pioneered an assessment of Alkaptonuria patients. Professor L. Ranganath is also co-ordinating DevelopAKUre, a European Union-funded international research programme, which involves 3 studies in Alkaptonuria. This will bring advances to all patients with Alkaptonuria worldwide.

Session VI

NEW TREATMENT PERSPECTIVES

ANS VAN DER PLOEG
Rotterdam, NL

ANS T. VAN DER PLOEG MD, PhD is Chair of the Center for Lysosomal and Metabolic Diseases at the Erasmus MC University, Rotterdam, and chair of department for metabolic diseases of Leiden University Medical Center, the Netherlands. The center for lysosomal and metabolic diseases is a joined initiative of the departments of Pediatrics, (Child)Neurology, Internal Medicine, Clinical Genetics and Hospital Pharmacy. It aims to improve treatment, care and diagnosis of children and adults, to stimulate translational research, to provide education and to disseminate information. The center has is a designated rare disease expert center for Pompe disease, Lysosomal storage disorders, hypophosphatasia, urea cycle defects and organic acidurias and Porphyria. It serves as the national reference center for treatment with enzyme replacement therapy of patients with MPS I, MPS II, MPS VI, NCL and Pompe disease. The research performed by the center has an important focus on lysosomal storage disorders and in particular Pompe disease, and includes clinical research (long term effects of ERT, dosing, immunomodulation, patient reported outcome) as well as development of innovative therapies (Gene, RNA based and cell based therapies) and implementation of next generation (enzyme) therapies. The center collaborates since 2002 with the International Pompe Association on the IPA survey. The Center is governor of the international Pompe mutation database www.pompecenter.nl. Van der Ploeg is vice-coordinator of the European Pompe Consortium (EPOC), metabERN and coordinator of the LSD subnetwork of metabERN, and member of EuroNMD (European reference network for Neuromuscular Disorders). She is member of several scientific advisory boards, member of the council of the Society of the Study Group of Inborn Errors of Metabolism (SSIEM). She was Chair and organizer of the SSIEM symposium named “building bridges” held in the Doelen in Rotterdam in 2019, which was attended by more than 3000 participants from 86 countries. The symposium focused in particular on innovative therapies such as gene therapy, regenerative medicine, RNA based therapies and potential benefits from other fields.

Van der Ploeg received her MD “with honors” in 1985 at the Erasmus University. From 1985 till 1989 she worked at the Department of Cell Biology and Clinical Genetics on the feasibility of enzyme replacement therapy in cellular models for Pompe disease. Since then she has been involved in the multiple steps leading to the development of enzyme replacement therapy: Cloning of the gene, biotechnological production of recombinant human alpha-glucosidase (the enzyme deficient in Pompe disease) in milk of transgenic mice and rabbits, and in CHO cells; development of a knock-out model for Pompe disease; feasibility studies in mice and finally the first clinical trial in infants; and later the international multicenter placebo controlled trial that showed effects of therapy in adults. The current work of the center on implementation and development of new therapies builds on this experience. Van der Ploeg received several awards for her work such as “The Research award for young investigators” from Erasmus University Rotterdam, the “Pharming therapy award”, and “het kroontje” from the Prinses Beatrix Spierfonds. She has published over 200 publications in peer reviewed international journals and books.

Session VII

EXPANDING THE CONCEPT OF NEUROMETABOLIC DISORDERS

ANGELES GARCÍA-CAZORLA
Barcelona, ES

ANGELES GARCÍA-CAZORLA is a Paediatric Neurologist at the Sant Joan de Déu Barcelona Children's Hospital, in Barcelona. She is an expert in rare neurometabolic and neurogenetic disorders.

Dr Cazorla obtained her degree in Medicine from the University of Barcelona. She then completed her clinical and scientific training in Inborn Errors of Metabolism and Neurometabolic Disorders at the Hospital Necker, in Paris, and at the University of Columbia in New York (post-doctoral fellowship). She has been an Associate Professor at the University of Barcelona since 2012.

She is currently the coordinator of the Neurometabolic Disorders Unit and the Lead Researcher at the Neurology Laboratory (Synaptic Metabolism Lab). She is a member of the CIBERER Network, a member of Scientific Committee of the Recordati Rare Diseases Foundation, and the coordinator of the subgroup of neurotransmitter related disorders at the MetabERN. Her research interests include neurotransmission mechanisms and neuronal communication in inborn metabolic diseases as well as the development of new therapies for these rare diseases.

BARBARA PLECKO
Graz, AT

BARBARA PLECKO, MD, has studied medicine at the University of Graz, where she also achieved her medical license as a paediatrician in 1995, and trained as a fellow in child neurology and inborn errors of metabolism. From 1999 to 2001 she has been a research fellow at the metabolic unit of the Univ. Childrens Hospital in Vienna (Sylvia Stockler) and received her habilitation in 2003. In 2007 she worked as an associate professor at the neurometabolic clinic at the Childrens and Womens Hospital of the University of British Columbia. From 2011 to 2017 she has been chair of child neurology at the Univ. Childrens Hospital in Zurich. Since 2017 she has returned to Austria and is head of the Division of General Pediatrics including the services of child neurology and metabolic disorders at the Department of Pediatrics and Adolescent Medicine of the Medical University of Graz. Fields of Clinical Interest and Research: Infantile epileptic encephalopathies and neurometabolic disorders. Memberships and Chairs: Chair of the Scientific and Research Comittee of the EPNS (European Society for Pediatric Neurology).Co-Editor of Neuropediatrics.

Session VII

EXPANDING THE CONCEPT OF NEUROMETABOLIC DISORDERS

RÚBEN RAMOS
New York, USA

RÚBEN RAMOS, he received his MSc degree in Pharmaceutical Sciences from the Faculdade de Farmácia da Universidade de Lisboa, Portugal, in 2008. In the same year he started working as a senior technician at the Research Institute for Medicines and Pharmaceutical Sciences (iMed.ULisboa), in the Metabolism and Genetics group. There he received training in the field of rare metabolic diseases, being especially involved in the diagnosis and follow-up of patients with inborn errors of metabolism disorders. In 2009 he started a second MSc degree, in Clinical Chemistry, in the Faculdade de Farmácia da Universidade de Lisboa. He graduated in 2013, under the supervision of Prof. Isabel Tavares de Almeida. His work, in collaboration with Prof. Cornelis Jakobs and Dr. Mirjam Wamelink from the Free University of Amsterdam, the Netherlands, allowed the characterization of a new metabolic disorder in the pentose phosphate pathway. In 2014 Rúben started his PhD studies in the department of Genetics at the University Medical Center Utrecht, under the supervision of Prof. Nanda Verhoeven-Duif and Dr. Judith Jans. His studies focused on understanding the intracellular metabolic consequences of vitamin B6 deficiency and on improving the diagnostic tools and treatment options of patients with vitamin B6 deficiency. Additionally, his work allowed the characterization of a new B6-responsive inborn error of metabolism: GOT2 deficiency. Rúben currently works as a senior research assistant in the department of Cell Metabolism at Memorial Sloan Kettering Cancer Center in New York, United States of America. His current field of research focuses on understanding the impact of the human intestinal microbiome and its metabolome on treatment-related complication and mortality of cancer patients that underwent allogenic hematopoietic stem cell transplantation (allo-HCT).

VANESSA MORAIS
Lisboa, PT

VANESSA A. MORAIS, PhD, is head of Mitochondrial Biology and Neurodegeneration laboratory at iMM | Instituto de Medicina Molecular, and is Associate Professor at the Faculty of Medicine University of Lisbon.

Session VIII

EXPANDING TREATMENT TARGETS – WHAT'S FOR THE FUTURE OF GENETIC THERAPY?

NICOLINA CRISTINA SORRENTINO
Naples, IT

NICOLINA CRISTINA SORRENTINO is faculty researcher and head of translational Unit at TIGEM "Telethon Institute of Genetics and Medicine" of Naples.
She received the bachelor degree cum laude in Biological Sciences at "Federico II" University of Naples. In 2008, she started her PhD research at the Telethon Institute of Genetics and Medicine of Naples in the laboratory of Prof. Andrea Ballabio. She worked on a research project based on "Developing a Systemic AAV-Medicated Gene Therapy Approach to Cross the Blood-Brain Barrier and Treat a CNS Pathology in Mucopolysaccharidosis Type IIIA". This approach has been published in 2013 (Sorrentino NC et al. EMBO Mol Med. 2013) and is also under a patent (WO 2012085622 A1). In 2012 she received the Ph.D. degree in in Life and Bio-molecular Sciences from Open University of Oxford. In 2013, she joined the research group of Dr. Alessandro Fraldi at Tigem to start a postdoctoral position. During this period, she was responsible of a project in collaboration with" Shire Pharmaceutics" based on the Intrathecal AAV-mediated Gene Therapy approaches to treat the SNC pathology in large and small animal models of lysosomal storage disorders (Sorrentino NC et al. Molecular Therapy 2016; Sorrentino NC et al. Mol Ther Methods Clin Dev. 2019). In 2018, she started the new position as Head of translational unit and faculty researcher at Telethon Institute of Genetics and Medicine. The main aims of her research are the study and development of new gene and drug therapy approaches for the treatment of somatic and in particular CNS pathology in lysosomal storage disorders

JEAN-CHARLES DEYBACH
Paris, FR

JEAN-CHARLES DEYBACH, MD, PhD, (born 1947 07 31) was for 25 years Professor of Biochemistry and Molecular Biology at the Faculty of Medicine, University Denis Diderot Paris 7. From 2000 to 2016 he was the head of the Department of Biochemistry and Molecular Genetics at Hospital Louis Mourier – Assistance Publique Hôpitaux de Paris (APHP), Director of the French Reference Center for Porphyrias (CRMR Porphyrias). He launched in 2001 the European Porphyria Initative (EPI) to built a dedicated porphyria network in Europe. Funded as a pilot project by EU Commission he became from 2007until 2013 the main coordinator of the European Reference Porphyria Network (EPNET) which links 36 European countries. From 2014 to 2018 as the interim President of EPNET he helped to set up a new independent EPNET association which was officially launched in Oct 2018. As member of a research unit INSERM U 1149 located in Faculty of Medicine University D. Diderot Paris 7, he has more than 35 years experience on research in heme biosynthesis and related disorders and mainly contributed to the recognition of enzymatic, genetic defects and pathophysiology in human porphyrias (up to 240 peer reviewed publications). As a MD and with his team in Louis Mourier Hospital he had in charge the management of the porphyria patients from France through the unique Reference Center for Porphyrias (CRMR Porphyrias). Since September 2016 he is Professor emeritus at the University Denis Diderot Paris 7 and as the head of the Biochemistry Department at Louis Mourier Hospital. However he still act as a part time medical consultant at the French Porphyria Center, as a member of the research unit Inserm U1149 and is still active at the European level as Honorary President of the European Porphyria Network association (EPNET).

Session VIII

EXPANDING TREATMENT TARGETS – WHAT'S FOR THE FUTURE OF GENETIC THERAPY?

SHAMIMA RAHMAN
Liverpool, UK

SHAMIMA RAHMAN is Professor of Paediatric Metabolic Medicine at the UCL Great Ormond Street Institute of Child Health (ICH), and honorary consultant at Great Ormond Street Hospital for Children (GOSH), London, UK. She trained in Medicine at Oxford University, and in Paediatric Metabolic Medicine at GOSH. Professor Rahman established the Mitochondrial Research Group at ICH in 2000, with a mission to improve the diagnosis and outcomes for children affected by mitochondrial and other rare metabolic diseases. Her group focusses on discovering mitochondrial disease genes, developing novel computational diagnostic strategies, and investigating therapeutic approaches where there are currently no disease-modifying treatments.
Professor Rahman is an Editor of the Journal of Inherited Metabolic Disease and a Senior Editor of the Annals of Human Genetics. She has a passion for education and until 2019 was Senior Adviser to the Society for the Study of Inborn Errors of Metabolism's Education and Training Advisory Committee. Professor Rahman sits on the Scientific Advisory Board of the French Muscular Dystrophy Association (AFM-Telethon), the Australian Mitochondrial Disease Foundation's Clinical and Scientific Review Panel, and the Medical Advisory Boards of the Lily Foundation for Mitochondrial Disease and the Freya Foundation for Pyruvate Dehydrogenase Deficiency, and acts as a special adviser to the UK Human Fertilisation and Embryology Authority.

FRANCISCA COUTINHO
Porto, PT

MARIA FRANCISCA COUTINHO, she is a researcher at the National Institute of Health Doutor Ricardo Jorge in Portugal. Ever since she finished her degree in Biology, in 2007, her scientific activity has been focused in the field of Health Sciences, particularly in the Human Genetics area.
Maria Francisca has been working in Lysosomal Storage Disorders (LSDs), one of the major groups of genetic disorders, affecting both children and adults. Over these years she has actively contributed to the development of new methods for diagnosis/characterization of LSD patients and to the study of the pathophysiological mechanisms of these diseases. Currently, and taking advantage on the practical skills and theoretical background that those previous works have given her, she is working on a fully molecular approach to promote substrate reduction in a subgroup of LSDs, the Mucopolysaccharidoses, as potential therapeutic approach for these disorders, where no solution exists for the neurological phenotype.

Session IX

AGEING IN INHERITED METABOLIC DISORDERS

FANNY MOCHEL
Paris, FR

FANNY MOCHEL is an associate professor of genetics at Sorbonne University. She received her MD in Genetics in 2005 at the University Paris Descartes, her PhD in Neuroscience in 2010 at Sorbonne University and is board certified in inborn errors of metabolism. Dr Mochel leads the French reference center on Neurometabolic diseases in adults and runs a Neurometabolic research group at the Brain and Spine Institute (ICM) of La Pitié-Salpêtrière University Hospital in Paris. She is chair of the Adult Metabolic Group of the Society for the Study of Inborn Errors of Metabolism (SSIEM), council member of the SSIEM, and co-chair of the French society for inborn of errors of metabolism in adults. Her research is focused on the characterization and treatment of brain energy deficiencies in neurometabolic and neurodegenerative diseases. Her major areas of expertise are the identification of neurometabolic biomarkers in vitro(metabolomics) and in vivo (metabolic imaging) as well as therapeutic approaches targeting the Krebs cycle.

LEONOR GUEDES
Lisboa, PT

LEONOR CORREIA GUEDES obtained her degree in Medicine at the Medical School of the University of Lisbon and made her Residency in Neurology at the Neurology Department of the Hospital de Santa Maria. Performed an internship in genetics at the Department of Clinical Genetics, Erasmus Medical center, University of Rotterdam, and a Master Degree in neuroscience and a PhD in Neurology at the University of Lisbon on the subjects of Motor Fluctuations and Genetics of Parkinson's disease, respectively. Prof. Dr. Leonor Correia Guedes Is the founder of the Neurogenetics in Movement Disorders outpatient clinic at her hospital and a collaborator of the Reference Center of Adult Metabolic Disorders of the Hospital de Santa Maria.

PATRÍCIO AGUIAR
Lisboa, PT

PATRÍCIO AGUIAR graduated at Lisbon University Medical School in 2006 and completed his trainee in Internal Medicine, in 2017, at Centro Hospitalar e Universitário Lisboa Norte (Lisbon, Portugal). He completed his PhD at Lisbon University Medical School, in 2018, in the field of inborn errors of metabolism (biomarkers of Anderson-Fabry disease) in partnership with the University College of London. He performs the evaluation and follow up of patients with inherited metabolic disorders, mainly lysosomal storage disorders. He is also member of the core team of one of the national reference center in inherited metabolic disorders (Centro Hospitalar e Universitário Lisboa Norte), as well as member of the board of the rare diseases study group of the Portuguese Society of Internal Medicine. His main research areas are Lysosomal Storage Disorders and Autonomic Nervous System Diseases, with several ongoing research projects on Anderson-Fabry disease (AFD) biomarkers, autonomic manifestations of AFD, immunogenicity against recombinant proteins used for enzyme replacement therapy, factors affecting quality of life in patients with type 1 Gaucher disease and neuroimaging prodromic signs of Parkinsonism in Gaucher disease. In 2014, he was awarded the prize of the Federation for the Development of Internal Medicine in Europe for research in the field of rare disorders. He is invited lecturer of Lisbon University Medical school and has published 28 articles in national and international peer-reviewed journals and presented more than 100 communications in national and international conferences and meetings.

PROGRAM

SCIENTIFIC PROGRAMME

WEDNESDAY, NOVEMBER 11TH

14:50 - 15:00	**SYMPOSIUM PREOPENING**
15:00 – 16:30	**SESSION I - SELECTED FREE COMMUNICATIONS** *Chairpersons: Ana M. Gaspar, Lisboa, PT / Hugo Rocha, Porto, PT*
16:30 – 17:30	**SESSION II – SELECTED E-POSTERS COMMUNICATIONS** *Chairpersons: Teresa Campos, Porto, PT / Margarida Silva, Lisboa, PT*
17:30 – 19:00	**SESSION III - SELECTED FREE COMMUNICATIONS** *Chairpersons: Paula Garcia, Coimbra, PT / Anabela Bandeira, Porto, PT*

THURSDAY, NOVEMBER 12TH

8:15 – 8:30	**SYMPOSIUM OPENING - WELCOME ADDRESS ON BEHALF OF THE SPDM** *Daniel C. Gomes – Symposium Chairperson*
	SESSION IV – CROSSTALK AMONGST INTRACELLULAR ORGANELLES *Chairpersons: Isabel Tavares de Almeida, Lisboa, PT / Hugo Rocha, Porto, PT*
8:30 – 8:50	Fatty acid metabolism: intracellular interactions *Ronald J. A. Wanders | Amsterdam, NL*
8:55 – 9:15	New perspectives in fatty acid disorders *Ute Spiekerkoetter | Freiburg, DE*
9:20 – 9:40	Disorders of complex lipid metabolism *Frédéric M. Vaz | Amsterdam, NL*
9:45 – 10:05	A clinical approach to complex lipid disorders *Jean-Marie Saudubray | Paris, FR*
10:10 – 10:20	Session IV discussion
10:20 – 10:50	**Networking break**
	SESSION V – NEW INSIGHTS IN CARBOHYDRATE DISORDERS *Chairpersons: Elisa Leão Teles, Porto, PT / Patrícia Janeiro, Lisboa, PT*
10:50 – 11:10	Glycogen storage disorders revisited *René Santer | Hamburg, DE*
11:15 – 11:35	Update in disorders of galactose metabolism *Estela Rubio-Gozalbo | Maastricht, NL*
11:40 – 11:50	Session V discussion
11:50 – 12:50	**Networking break**
	SESSION VI – NEW TREATMENT PERSPECTIVES *Chairpersons: Domingo González-Lamuño, Santander, ES / Ana Paula Leandro, Lisboa , PT*
12:50 – 13:10	Enzyme substitution therapy: Pegvaliase for the treatment of PKU *Ania Muntau | Hamburg, DE*
13:15 – 13:35	Expanding the use of substrate reduction therapy: nitisinone for the treatment of alkaptonuria *Lakshminarayan Ranganath | Liverpool, UK*
13:40 – 14:00	Future directions in the treatment of lysosomal disorders *Ans van der Ploeg | Rotterdam, NL*
14:05 – 14:15	Session VI discussion

FRIDAY, NOVEMBER 13TH

	SESSION VII – EXPANDING THE CONCEPT OF NEUROMETABOLIC DISORDERS *Chairpersons: Sofia Duarte, Lisboa, PT / João Durães, Coimbra, PT*
08:30 – 08:50	Cellular neurometabolism: a tentative to connect cell biology and metabolism in neurology *Angeles García-Cazorla | Barcelona, ES*
08:55 – 09:15	Neonatal Seizures: Are we there yet? *Barbara Plecko | Graz, AT*
09:20 – 09:35	GOT2 deficiency: a novel malate-aspartate shuttle disease that leads to early-infantile encephalopathy (invited short communication) *Rúben Ramos | New York, USA*
09:35 – 09:50	Mitochondrial bioenergetics in neurodegeneration (invited short communication) *Vanessa Morais | Lisboa, PT*
09:50 – 10:00	Session VII discussion
10:00 – 10:30	**Networking break**
	SESSION VIII – EXPANDING TREATMENT TARGETS – WHAT'S FOR THE FUTURE OF GENETIC THERAPY? *Chairpersons: Dulce Quelhas, Porto, PT / Isabel Rivera, Lisbon, PT*
10:30 – 10:50	Recent advances in gene transfer therapy to the CNS in lysosomal storage diseases *Nicolina Cristina Sorrentino | Naples, IT*
10:55 – 11:15	Emerging therapies in the hereditary porphyrias *Jean-Charles Deybach | Paris, FR*
11:20 – 11:40	Emerging therapies for mitochondrial disease *Shamima Rahman | London, UK*
11:45 – 12:00	Genetic substrate reduction therapy for mucopolysaccharidosis; Toward a siRNA-containing nanoparticle targeted to brain cells (SPDM Aguinaldo Cabral Grant) *Francisca Coutinho | Porto, PT*
12:00 – 12:10	Session VIII discussion
12:10 – 13:10	**Networking break**
	SESSION IX - AGEING IN INHERITED METABOLIC DISORDERS *Chairpersons: Anabela Oliveira, Lisboa, PT / Álvaro Hermida, Santiago de Compostela, ES*
13:10 – 13:30	Age related metabolic changes in Inherited Metabolic Disorders (TBC) *Fanny Mochel | Paris, FR*
13:35 – 13:55	Inborn metabolism defects in common neurodegenerative disorders *Leonor Guedes | Lisboa, PT*
14:00 – 14:20	Ethical issues in the treatment of elderly patients *Patrício Aguiar | Lisboa, PT*
14:25 – 14:35	Session IX discussion
14:35 – 15:00	Awards / Closing Remarks *M. Teresa Cardoso – Past SPDM President*
	End of the Symposium

11TH NOVEMBER 2020

Session I – 15h00 – 16h30

SELECTED FREE COMMUNICATIONS

OC 01	Structural insights into the allosteric substrate activation of the human enzyme phenylalanine hydroxylase. *J.B. Vicente, Lisboa, PT*
OC 02	Synaptic mitochondria: In search of its functional fingerprint. *A. Faria-Pereira, Lisboa, PT*
OC 03	Mucolipidosis type II/III: rare diseases that should be addressed in different clinical settings. *P. Pinto, Lisboa, PT*
OC 04	Clinical, biochemical, histological, imaging and molecular features of a cohort of 37 patients with mitochondrial disorders in a neuro-metabolic tertiary center. *S. Jorge, Lisboa. PT*
OC 05	Prevalence and incidence of metabolic syndrome in Portuguese patients with phenylketonuria: the 10 year-longitudinal TNSPKU study. *J. C. Rocha, Porto, PT*
OC 06	X-Linked Combined Methylmalonic Aciduria and Hyperhomocysteinemia - A Case Report. S. *Ferreira, Coimbra, PT*

Session II – 16h30 – 17h30

SELECTED E-POSTERS COMMUNICATIONS

POC 01	Broad clinical spectrum in a family with glucose transporter type 1 deficiency. J. *Martins, Porto, PT*
POC 02	Chorea as a neurological manifestation of mitochondrial cytopathy caused by TSFM gene mutation. *J. Lourenço Rosa, Lisboa, PT*
POC 03	Two siblings with NEMMLAS, a Neurodevelopmental Disorder, Mitochondrial, With Abnormal Movements and Lactic Acidosis, With or Without Seizures. *A.L. Rodrigues, Lisboa, PT*
POC 04	Long term follow up in patients with Hyperphenylalaninemia and mild Phenylketonuria – nutritional status and cognitive outcomes. *A. S. Freitas, Porto, PT*
POC 05	ImmunoCDGQ: Immunology and CDG Questionnaire for Patients and Caregivers. *R. Francisco, Lisboa, PT*
POC 06	Glycomacropeptide in Patients with Phenylketonuria: A Long-Term Nutritional Landscape. *M. J. Pena, Porto, PT*
POC 07	Multiple acyl-CoA dehydrogenase deficiency (MADD) and its mimicries – a case series. I. *Ayres Preira, Porto, PT*
POC 08	Old and new biomarkers in LSD. *F. Laranjeira, Porto, PT*

Session III – 17h30 – 19h00

SELECTED FREE COMMUNICATIONS

OC 07	Staled lysosomes prime cell proliferation. *C. Ramos, Lisboa, PT*
OC 08	Inhibition of the mitochondrial complex I by a Parkinson's Disease-inducing neurotoxin leads to a NPC1 phenotype in neuronal cells. *E. Rodrigues, Lisboa, PT*
OC 09	Clinical profile of organic acidurias in children from a single center: an 18-year retrospective study. *P. Pinto, Lisboa, PT*
OC 10	Does the protein intake in PKU patients adhere to the European PKU Guidelines? *M. Gomes, Porto, PT*
OC 11	Effect of small compounds on the activity and stability of recombinant human phenylalanine hydroxylase: in vitro and in cellulo studies. *R. Lopes, Lisboa, PT*
OC 12	Biochemical and molecular characterization of carriers of pathogenic variants in the GLA gene, the cause of Fabry disease. *F. Larajeira, Porto, PT*
OC 13	Treatment of Fabry disease with migalastat: experience in clinical practice. *F. Gonçalves, Lisboa, PT*

SESSION I

SELECTED FREE COMMUNICATIONS

CHAIRPERSONS

ANA M. GASPAR, MD

Reference Center of Metabolic Diseases, C.Hosp.Univ. Lisboa Norte – HstaM, Lisboa, PT

HUGO ROCHA, PhD

Instituto Nacional de Saúde Dr. Ricardo Jorge, INSA, Porto, PT

STRUCTURAL INSIGHTS INTO THE ALLOSTERIC SUBSTRATE ACTIVATION OF THE HUMAN ENZYME PHENYLALANINE HYDROXYLASE

OC-01

J.B. Vicente[1], C.S. Tomé[1,2], R.R. Lopes[2], PMF Sousa[5], M.P, Amaro[4], H.D.T. Mertens[3], P. Leandro[2]

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Plasma homeostatic L-phenylalanine (L-Phe) levels are maintained via the human enzyme phenylalanine hydroxylase (hPAH). L-Phe is an essential precursor for neurotransmitters biosynthesis, although elevated L-Phe levels become neurotoxic.

Phenylketonuria (PKU), the most common hereditary disorder of the amino acid metabolism, is caused by dysfunctional hPAH, resulting from mutations in the PAH gene, which can affect protein folding, catalysis or regulation [1]. Knowledge on the molecular mechanisms behind PKU is essential to develop therapeutic options, mostly restricted to an L-Phe-free diet.

hPAH assembles as a homotetramer. Each subunit comprises an N-terminal regulatory domain, a central catalytic domain and a C-terminal oligomerization domain. The complex regulatory mechanisms modulating hPAH function involve transition between oligomeric states, conformational changes, substrate activation and cofactor inhibition. A combination of biochemical and biophysical methods were herein employed to investigate the L-Phe allosteric regulation of hPAH. We report the first structural model of the full-length wild-type activated hPAH determined by small-angle X-ray scattering [2]. Regulatory domains adopt an auto-inhibitory conformation in the basal state, which block the entrance to the catalytic pocket. L-Phe binding promotes displacement and dimerization of the regulatory domains, allowing substrates to access the active site. The activated protein becomes more compact and more resistant to thermal denaturation and proteolytic cleavage.

References

1 PAHvdb: http://www.biopku.org/home/pah.asp.

2 Tomé CS, Lopes RR, Sousa PMF, Amaro MP, Leandro J, Mertens HDT, Leandro P, Vicente JB (2019) Structure of full-length human phenylalanine hydroxylase by small angle X-ray scattering reveals substrate-induced conformational stability, Sci Rep 9(1):13615.

SYNAPTIC MITOCHONDRIA: IN SEARCH OF ITS FUNCTIONAL FINGERPRINT

OC-02

Andreia Faria-Pereira[1], Marco Spinazzi[2], Katleen Craessaerts[2], Jeffrey Savas[3], Bart De Strooper[2], Vanessa A. Morais[1,2]

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The physiological relevance of mitochondria has been increasingly appreciated in the last years, as mutations in mitochondrial proteins have been linked to a wide range of pathologies. Mitochondria are dynamic organelles commonly known as the "cell powerhouse", as they are responsible for the majority of ATP needed for cells.

Neurons are high energy-demanding where around 80% of those energy requirements are provided by mitochondria.

Additionally, neuronal activity is highly dependent on a balanced mitochondrial homeostasis. This correlation is supported by the fact that patients with mitochondrial disorders present neurological impairments, underlined by either neurotransmitter production/release defects. Hence, mitochondrial activity has a crucial role in neuronal survival and function.

Neurons are morphologically polarized cells and mitochondria have been observed in all their sub-compartments. Additionally, these compartment-specific mitochondria appear to have different morphological features, raising the question of whether these are functionally similar or actually have specialized functions. Therefore, we aim at understanding if synaptic mitochondria are devoted to different functions than other neuronal mitochondria that do not reside at the synapse.

To address this question we assessed the proteomic profile of synaptic mitochondria compared to non-synaptic mitochondria. For this, we performed quantitative Mass Spectrometry of isolated mouse brain mitochondria (synaptic and non-synaptic), followed by unbiased bioinformatics analysis. From the 3120 identified proteins, we were able to pinpoint 211 proteins differentially expressed between both mitochondrial pools, clearly indicating that both mitochondrial pools (synaptic and non-synaptic) present significant proteomic differences. Additionally, when performing the bioinformatics analysis, we were able to identify several proteins of different metabolic pathways to be differentially expressed in each mitochondrial pool. These findings suggest that distinct functions and metabolic processes may be at play in these two different mitochondrial pools.

Additionally, through enzymatic activity assays we observe that some respiratory chain complexes present different catalytic rates, suggesting that synaptic mitochondria present a different bioenergetics profile than non-synaptic mitochondria. Currently, we are assessing how the modulation of this functional differences impact in neuronal activity.

MUCOLIPIDOSIS TYPE II/III: RARE DISEASES THAT SHOULD BE ADDRESSED IN DIFFERENT CLINICAL SETTINGS

OC-03

Patrícia Lipari Pinto[1], Ariadna Borràs[2], Noelia Rivera[2], Leticia Pías[2], Daniel Natera[2], Judith Armstrong[3], Ana Fernández- Marmiesse[4], Carmen Fons[2], Mercè Pineda[2], Laura Gort[5], Maria Josep Coll[5], Andrés Nascimento Osorio[2], Àngels García-Cazorla[2], Maria del Mar O'Callaghan[2]

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BACKGROUND: Mucolipidosis (ML) II and III are rare lysosomal diseases caused by mutations in GNPTAB/GNPTG genes, which encode the α/β-precursor and Ɣ-subunit of N-acetylglucosamine (GlcNAc)-1-phosphotransferase. The deficiency of this enzyme results in missorting of lysosomal enzymes and an accumulation of non-degradable macromolecules in lysosomes, impairing cellular function. Clinical manifestations of ML II and III have not yet been fully elucidated. They are multisystemic and varied among affected individuals.

OBJECTIVE: Characterize clinical manifestations of children with ML II and III followed at a referral center for neurometabolic diseases, in Barcelona, between 2000-2019, to better recognize and anticipate diagnosis.

METHODS: Six patients with ML and clinical data were analyzed.

RESULTS: 6 patients (ML II=2, ML III α/β=2, ML III Ɣ=2) were recruited from 5 unrelated families. Predominant clinical presentations were skeletal symptoms. In neither case were ML considered the first diagnostic hypothesis. All showed high levels of lysosomal acid hydrolase enzymes in plasma and/or reduced in fibroblasts. ML II/III were confirmed by identification of pathogenic variants in GNPTAB/GNPTG. ML II patients showed the most severe clinical phenotype and the diagnosis were sooner, with initial symptoms in early childhood: low weight at birth, joint restriction in all limbs, scoliosis, coarse facial features, gingival hypertrophy and moderate developmental delay (DD). Both with cardiologic manifestations, only 1 with hypoacusis, craniosynostosis and hepatosplenomegaly, the same who had a fatal outcome at 4, due to cardiorespiratory failure. In ML III α/β patients, first symptoms were at 2-3 years-old with progressive stiffness, pain in multiple joints and coarse facial features. Other skeletal alterations were progressive kyphoscoliosis and pectus carinatum and both were submitted to adenoidectomy. Only 1 showed mild DD. ML III Ɣ patients were sisters from consanguineous parents with subtle coarse facial features, gross motor DD detected at 2 and 4 years-old and developed non-painful joint stiffness in multiple joints. They underwent surgery of bilateral carpal tunnel syndrome.

CONCLUSION: ML should be considered in cases of joint stiffness. The differential diagnosis of ML II or III is based on age of onset, clinical findings and degree of severity. The early diagnosis especially in ML II is an opportunity to bone marrow transplant, a promising therapy.

CLINICAL, BIOCHEMICAL, HISTOLOGICAL, IMAGING AND MOLECULAR FEATURES OF A COHORT OF 37 PATIENTS WITH MITOCHONDRIAL DISORDERS IN A NEURO-METABOLIC TERTIARY CENTER

OC-04

S Jorge[1,2]; T Painho[1]; Rita Lopes Silva[1]; A I Dias[1]; A Pereira[1]; A Moreira[1]; E Calado[1]; D Antunes[5]; M Amorim[5]; A Cordeiro[3]; P Kjöllerström[4]; R Maia[4]; C Conceição[6]; S Sequeira[7]; S Duarte[1,7]; J P Vieira[1]; S Jacinto[1,7]; A C Ferreira[7]

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BACKGROUND: Mitochondrial disorders are multisystemic diseases characterized by a wide spectrum of clinical manifestations and complex molecular etiology. Contributing to its persistence as underdiagnosed disorders is the failure to recognise children with "nonclassic" phenotypes. Biochemical tests, muscle histology and respiratory chain enzymatic activity, although frequently performed, can be inconclusive and often misleading. The "gold standard" for a definitive diagnosis is genetic studies, nowadays more accessible with NGS gene panels.

METHODS: We performed a retrospective study of clinical, biochemical, histological, imaging and molecular features of a cohort of patients with mitochondrial disorders followed for the last 20 years at our neuro-metabolic centre.

RESULTS: Thirty-seven patients from 33 unrelated families, with a median age of 10-years (min 18mo – max 22y) were included. A definitive diagnosis of a mitochondrial disorder was established in 28 patients either by genetic confirmation or according to consensus Mitochondrial Disease Criteria (MDC), and a probable diagnosis in 9 by the same criteria. Most patients presented in the first 2 years of life (n=24) with neurologic manifestations: encephalopathy (56%), muscular symptoms (10%) and neonatal seizures (5%). Extra-neurological manifestations at presentation were cardiac AV block, deafness and haematological (1 patient each). Classical clinical presentations were Leigh syndrome (n=4), Kearns-Sayre syndrome (n=3) and Pearson syndrome (n=1). Cerebral MRI findings were diverse but Leigh syndrome and leukoencephalopathy were among the most frequent. Twenty-three patients (62%) had a muscle biopsy, with 82% showing abnormalities either in histological or/and RC enzyme activity. Molecular diagnosis was achieved in 22 patients, involving nuclear genes in 14 (64%): PDHc (n=3), LYRM7 (n=3) and MFN2 (n=2), and in POLG, TMEM70, SERAC1, BCS1L, COQ8A and RMND1 (one patient each). In the mtDNA, m.8993 T>C and m.8993T>G (n=4) and large-scale deletions (n=2) were the most frequent findings.

CONCLUSIONS: This cohort, although small, reflects mitochondrial disorders clinical heterogeneity, different inheritage patterns. Advances in molecular testing have improved diagnostic yield, although establishing a definitive genetic diagnosis remains challenging. We stress that clinical awareness is the first crucial element of a multistep approach leading to a definitive diagnosis.

PREVALENCE AND INCIDENCE OF METABOLIC SYNDROME IN PORTUGUESE PATIENTS WITH PHENYLKETONURIA: THE 10 YEAR-LONGITUDINAL TNSPKU STUDY

OC-05

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BACKGROUND: There is recent discussion about whether the special phenylalanine restricted diet, the PKU disease itself or both, may increase the risk of comorbidities in adult patients. The 10 year-longitudinal study TNSPKU aimed to investigate the prevalence and incidence of metabolic syndrome (MetSyn) in a Portuguese patient's cohort.

METHODS: 61 PKU patients (18.6 ± 6.6 y; 54% females) who have completed at least an annual nutritional status evaluation (ANSE), every 2 years (biennium), from 2009 to 2018, were studied. Data was collected for anthropometry, body composition, blood pressure and biochemistry. MetSyn was classified according to International Diabetes Federation criteria defined by the combination of central obesity plus any two of the following: raised triglycerides (TG), reduced high density lipoprotein cholesterol (HDL-c), raised blood pressure (BP) or raised fasting plasma glucose (GLU). Additionally, we also calculated the homeostasis model assessment of insulin resistance (HOMA-IR) for ANSE's performed since 2013.

RESULTS: MetSyn prevalence (%) evolution was the following: 4.9 (2009-2010), 6.6 (2011-2012), 4.9 (2013-2014), 3.3 (2015-2016) and 14.5 (2017-2018). Comparing the 1st and the last biennium we found 36.1% vs. 58.1% patients with increased waist circumference, 8.2% vs. 12.9% patients with increased TG and 29.5% vs. 37.1% patients with decreased HDL-c. No patients revealed increased GLU during the study period. From the 1st to the last biennium, 7 patients showed criteria for MetSyn (11.5%). Out of 11 patients who became overweight/obese from the 1st to the last biennium, 63.6% showed increase of waist circumference, 18.2% increased TG and 27.3% decreased HDL-c. In the last three biennium's, HOMA-IR was significantly higher in overweight patients vs. normal weight patients: 2.35 vs. 1.66, p=0.012; 2.50 vs. 1.74, p=0.001; 2.24 vs. 1.49, p=0.001; respectively.

DISCUSSION: The 10y incidence of MetSyn in PKU patients older than 10y was 11.5%. The most important components of MetSyn were the increased waist circumference and dyslipidaemic markers (TG or HDL-c). Although the increased fasting glucose component was not identified, the increased HOMA-IR in overweight patients underlines the importance of the long-term follow-up of this population.

X-LINKED COMBINED METHYLMALONIC ACIDURIA AND HYPERHOMOCYSTEINEMIA - A CASE REPORT

OC-06

Sara Ferreira[1], Fátima Martins[1], Ângela Almeida[2], Elisa Cardoso[2], Altina Lopes[3], Célia Nogueira[3], Laura Vilarinho[3], Luísa Diogo[1]

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Cobalamin-X disorder (cblX) - MIM #309541 - is an ultrarare X-linked recessive inborn error of intracellular cobalamin (cbl) metabolism caused by mutations in HCFC1 (Host Cell Factor C1) gene. This gene encodes a chromatin-associated transcriptional regulator that affects the expression of several genes, including MMACHC, associated to the more frequent cblC disorder.

CASE REPORT: This 9-month-old boy is the first child of non-consanguineous parents, with irrelevant family history. Pregnancy was uneventful, except for maternal treated hyperthyroidism. Delivery and extrauterine life adaptation were normal. Weight, length and head circumference at birth were on the 50th, 15-50th and 50th centiles, respectively. Expanded neonatal screening was negative.

He presented with global hypotonia, gastroesophageal reflux and failure to thrive from the first months of life. At 6 months, weight and length ≤ 3rd centile, moderate global developmental delay, predominantly motor, axial hypotonia and limb hypertonia were recorded. Investigation showed increased plasma total homocysteine (101 mol/L; r.v.<14) and urinary methylmalonic acid 300 mol/mmol creat; r.v.<6,2), with normal plasma methionine (10 mol/L; r.v.9-41) and vitamin B12 levels. Under hydroxocobalamin and betaine moderate clinical improvement and normalization of homocysteine and methylmalonic acid levels were observed. Currently, weight and length are on the 3rd and head circumference is on the 50-85th centiles. A new genetic variant, p.Pro1078Pro (c.3234G>A), interfering with the splicing, in hemizygosity in the HCFC1 gene, suggestive of cblX disorder was identified by NGS.

COMMENTS: CblC disorder (MIM #277400) the most frequent inborn error of intracellular cbl metabolism, can be detected by newborn screening.

The gene HCFC1, formerly associated to X-linked intellectual disability, was connected to cblX deficiency in 2013. HCFC1 mutations have been found in more 14 patients, who presented in the first months of life, similarly to cblC, but with milder metabolic and more severe neurologic abnormalities.

To our knowledge, this is the first case of cblX disorder identified in Portugal. Evolution is promising, although cbl metabolism abnormalities are probably not the only consequences of the HCFC1 mutation.

SESSION II

SELECTED E-POSTERS COMMUNICATIONS

CHAIRPERSONS

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BROAD CLINICAL SPECTRUM IN A FAMILY WITH GLUCOSE TRANSPORTER TYPE 1 DEFICIENCY

POC-01

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Glucose transporter type 1 (GLUT1) deficiency syndrome leads to a decreased glucose transport to the brain. It is caused by mutations in the SLC2A1 gene and, although most cases are sporadic, families with autosomal dominant mutations have been described. Early diagnosis is essential since it is potentially treatable with ketogenic diet.

We present a family with GLUT1 deficiency syndrome, and its phenotypical particularities.

An 9-year-old girl, with normal motor and cognitive development, developed episodes of sudden involuntary choreo-dystonic movements at the age of 2. These occurred daily, lasting 30, and triggers were not identified. Brain MRI was normal. CSF-to-blood glucose ratio was reduced (0.45). SCL2A1 gene sequencing disclosed a missense mutation (c.971C>T). Ketogenic diet was tried in multiple occasions, but her mother refused this treatment.

Her twin sister (dizygotic, bicorionic), with normal motor and cognitive development, developed absence seizures at the age of 7. CSF-to-blood glucose ratio was reduced (0.46), and SCL2A1 gene sequencing disclosed the same mutation. Epilepsy is currently controlled with antiepileptic drugs and mild learning disability is evident.

Their mother had had learning difficulties, and at the age of 18, developed paroxysmal episodes of segmental dystonia, triggered by continuous exercise. Extensive diagnostic workup was negative, and therapeutic drug trials with antiepileptic drugs and levodopa were unsuccessful. At 48 years of age, the SCL2A1 gene sequencing confirmed the same mutation.

This family highlights the variability of clinical phenotypes associated to GLUT1 deficiency syndrome, even within the same family. It should be considered in the presence of paroxysmal movement symptoms and/or epileptic seizures, even in the absence of the typical triggers. Also, it raises the question wether asymptomatic siblings should be tested.

CHOREA AS A NEUROLOGICAL MANIFESTATION OF MITOCHONDRIAL CYTOPATHY CAUSED BY TSFM GENE MUTATIO

POC-02

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INTRODUCTION: TSFM gene related encephalopathy is part of Leigh syndrome and is characterized by mitochondrial energy deficiency. Clinical phenotype is variable and with multisystem involvement. Main clinical features comprise early encephalopathy and cardiomyopathy. Neurological manifestations are variable and usually include hypotonia, ataxia, dystonia, myopathy, peripheral neuropathy, optic nerve atrophy and neurosensory hearing loss.

CLINICAL CASE: We present two siblings of non-consanguineous healthy parents, a girl and a boy of 8 and 4 years old, respectively. Both gestations had no complications and they had a caesarean (due to placenta praevia) and an eutocic delivery, respectively. Psychomotor development was uneventful during the first year, after which impairment in speech and walk slowly started to be noticeable in both children. Additionally, both developed the same neurological features: dysarthria, gait imbalance with broad base, appendicular hypotonia, brisk tendon reflexes and choreatic movements of all limbs. Brain MRI showed symmetric T2 hyperintensities in globus pallidus, midbrain and substantia nigra in the girl, whereas no abnormality was observed in the boy. Further diagnostic work-up revealed hypertrophic cardiomyopathy and redox potential abnormalities in both children. After mitochondrial and nuclear disease panel analysis with next-generation sequencing technology, the same two variants of TSFM gene in compound heterozygosity were identified in both patients – p.Cys64Tyr (c.191G>A) and p.Cys261Ser (c.782G>C).

CONCLUSIONS: Twelve cases of TSFM mutations have been described in the literature, with child-onset chorea being found only in one of them. Next generating sequencing has revolutionized the diagnosis of mitochondrial diseases in recent years, enabling a definitive diagnosis in a much faster and less invasive way.

TWO SIBLINGS WITH NEMMLAS, A NEURODEVELOPMENTAL DISORDER, MITOCHONDRIAL, WITH ABNORMAL MOVEMENTS AND LACTIC ACIDOSIS, WITH OR WITHOUT SEIZURES

POC-03

AL Rodrigues[1,2]; J Coelho[1]; J Alves[3]; P Janeiro[2]; A Gaspar[2]; T Moreno[1]

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BACKGROUND: WARS2 nuclear gene encodes a tryptophanyl tRNA synthetase, which is involved in mitochondrial protein synthesis. Mutations in WARS2, first described in 2017, are rare and have been associated with a spectrum of clinical presentations, including neurodevelopmental disorder with abnormal movements, lactic acidosis with or without seizures - NEMMLAS (OMIM #617710). Here we report two siblings with mitochondrial disease due to compound heterozygous mutations in WARS2 gene.

CASE REPORT: A ten-month old boy was admitted at Pediatrics Department due to febrile seizures during a viral infection. Since 9 months old was noted a global neurodevelopment delay, hypotonia and action and resting tremor. Curently, at 13 months, he is not able to transfer objects from one hand to another, roll or sit, and has poor vocalizations. EEG showed theta-delta rhythms indicating diffuse brain dysfunction with no paroxistic activity. Cerebral MRI was normal. Lactate was increased.

A patients brother died at 10 years old due to respiratory failure after a respiratory infection. He had global development delay, microcephaly, hypotonia and myoclonic epilepsy. Clinical manifestations progressed throughout childhood to complete aphasia, visual impairment, and spastic tetraparesis. Lactate was increased. Cerebral MRI showed ventricular enlargement presumably due to white matter loss, cerebral and cerebellar atrophy. EEG revealed diffuse changes in electrogenesis and multifocal paroxysmal elements. Muscle biopsy and respiratory chain enzyme activities in muscle were normal. Frequent mutations responsible for MELAS, MERFF and NARS2 were not found.

Whole exome sequencing (WES) on genomic DNA samples of both subjects and their parents was performed identifying two compound heterozygous variants c.37T>C p.(Trp13Arg) e c.568C>T p.(Gln190*) in the WARS2 gene as potential disease causing variants in both siblings.

CONCLUSION: We emphasize the importance of WES to ensure early diagnosis for rare pediatric disorders. An extensive investigation, more invasive and slow was necessary for the older brother that included muscle biopsy and mitochondrial enzyme assay, that were useless for the definitive diagnosis. The WES allowed an early diagnosis in the case index, however no specific treatment is currently available. The younger patient is being trialed on idebenona e ubidecarenona. Longitudinal monitoring will help establish the natural history of this condition.

LONG TERM FOLLOW UP IN PATIENTS WITH HYPERPHENYLALANINEMIA AND MILD PHENYLKETONURIA – NUTRITIONAL STATUS AND COGNITIVE OUTCOMES

POC-04

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BACKGROUND: The primary goals of Phenylketonuria (PKU) treatment are to allow normal neurocognitive development and growth by decreasing blood [Phe]. Patients with hyperphenylalaninemia and mild PKU may not achieve optimal nutritional status and cognitive outcomes. Our aim was to evaluate nutritional status, cognitive outcomes and metabolic control in early treated patients with hyperphenylalaninemia or mild phenylketonuria and to compare these outcomes with classical PKU patients.

METHODS: A retrospective study was developed in 63 early treated patients divided into two groups: 29 patients with hyperphenylalaninemia and mild PKU (Mild Group) and 34 classical PKU patients (Classical Group) under follow-up. Age, gender, blood [Phe] at newborn screening, metabolic control, genotype, treatment strategy, nutritional status data, cognitive outcomes, sociodemographic and psychosocial characterization were collected.

RESULTS: Metabolic control was significantly better in Mild Group, with lower median blood [Phe] in Mild Group vs. Classical Group (3,75 [3,02-4,75] vs. 8,00 [6,20-10,80] mg/dL; p<0,001). A higher Phe intake was also detected in Mild Group (4586 [3654-5375] vs. 1153 [777-1528] mg/day; p<0,001) and lower protein equivalent from protein substitutes intake (0,00 [0,00-0,00] vs. 0,85 [0,73-0,98] g/kg/day; p<0,001). Adult height (m) was significantly higher in Mild Group (1,69 ± 0,09 vs. 1,62 ± 0,09; p=0,032) but this difference did not remain when analysed by gender. In Mild Group significantly lower levels of uric acid (4,68 ± 0,18 vs. 4,92 ± 0,13 mg/dL; p<0,001), prealbumin (250,00 [242,30-274,00] vs. 291,00 [278,00-320,00] mg/dL; p<0,001) and folic acid (7,97 ± 0,75 vs. 13,50 ± 0,95ng/mL; p<0,001), and significantly higher levels of urea (25,94 ± 0,91 vs. 21,69 ± 0,81 mg/dL; p=0,001), calcium (2,42 ± 0,08 vs. 2,38 ± 0,06 mmol/L; p=0,042) and phosphorus (1,21 [1,05-1,37] vs. 1,12 [0,94-1,15] mmol/L; p=0,010) were found. No significant differences related to the mean IQ, dropout from school/college or need for additional psychological monitoring for an anxiety/disorder or depression were found.

DISCUSSION: Anthropometry, body composition, biochemical markers and IQ data showed that some patients from Mild Group were outside the target range for the analysed variables. Long term follow-up is needed irrespective of disease severity.

KEYWORDS: Phenylketonuria; Nutritional status; Cognitive outcomes.

IMMUNOCDGQ: IMMUNOLOGY AND CDG QUESTIONNAIRE FOR PATIENTS AND CAREGIVERS

POC-05

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INTRODUCTION: Congenital Disorders of Glycosylation (CDG) are a heterogenous family of rare, metabolic diseases with over 130 types.23 CDG types with immunological involvement have been described.Although glycans play diverse immunological roles, the nature and prevalence of immune-related signs and symptoms remain largely undetermined [1,2,3,4].

OBJECTIVES: To address and fill this knowledge gap, a multi-disciplinary team developed an online questionnaire directed at CDG patients and caregivers: the Immunology and CDG Questionnaire (ImmunoCDGQ).

METHODS: The medical and scientific content was based on a literature revision [3] conjugated with the advice from CDG clinical experts.5 CDG families reviewed language and adequacy.A pilot study with 9 participants was carried out to fine-tune the tool.Finally, ImmunoCDGQ was translated in 6 languages.Recruitment campaigns based on email and social media were developed.

RESULTS: 210 participants completed the ImmunoCDGQ. Reported CDG patients were from 31 countries and covered 36 CDG types.PMM2-CDG was the most represented CDG type (122/210).162 reported at least one immune-related manifestation.122 participants described relevant issues associated with infections, while 79 identified allergies. Contrastingly,only a minority of respondents reported confirmed autoimmune diseases(9/210).Regarding vaccination,the great majority of patients were vaccinated(172/210),however a subset of CDG patients(19/210) exhibited an altered response to vaccination.

CONCLUSION: This innovative,multidisciplinary and patient-centred approach allowed the systematic collection of clinical data on a poorly studied topic in a rare population.Opening avenues for new research projects and improved clinical management of immunological problems in CDG.

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GLYCOMACROPEPTIDE IN PATIENTS WITH PHENYLKETONURIA: A LONG-TERM NUTRITIONAL LANDSCAPE

POC-06

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BACKGROUND: In phenylketonuria (PKU), casein glycomacropeptide (CGMP) is used as an alternative to the traditional phenylalanine (Phe)-free L-amino acid mixtures (L-AA). To ensure suitability for PKU, CGMP is modified and supplemented with rate-limiting amino acids (CGMP-AA) but studies focusing on the long-term nutritional status of CGMP-AA are lacking.

METHODS: This retrospective study evaluated the long-term impact of CGMP-AA in 18 patients aged 10-42 years [11 females; hyperphenylalaninaemia (HPA)=3, mild=4 and classical PKU=11]; it evaluated metabolic control, anthropometry, body composition and biochemical parameters.

RESULTS: Overall, biochemical biomarkers remained unchanged. When compared to baseline, median blood Phe [8.6 (5.1-12.3) mg/dL vs 8.7 (5.0-14.1) mg/dL; p=0.006] and Tyr [49.3 (38.9-61.3) µmol/L vs 56.5 (42.3-75.3) µmol/L; p=0.004] increased. Protein equivalent from protein substitute (PS) was reduced with CGMP-AA [0.84 (0.69-1.09) g/kg/day vs 0.74 (0.64-0.94) g/kg/day; p=0.039]. Tyrosine (Tyr) intake from PS was significantly decreased [4.76 (3.75-6.68) g/day vs 3.85 (3.29-4.89) g/day; p=0.018] whilst leucine intake increased [5.76 (4.46-7.39) g/day vs 8.26 (6.85-8.91) g/day; p=0.004] on CGMP-AA.

CONCLUSIONS: This study shows that CGMP-AA introduction in PKU patients did not significantly alter nutritional status. The slight blood Phe control deterioration, even in the presence of increased blood Tyr concentrations, warrants further studies to understand the metabolic impact of using CGMP-AA in patients with PKU.

KEYWORDS: casein glycomacropeptide; amino acids; nutritional status; phenylketonuria; phenylalanine; tyrosine

MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY (MADD) AND ITS MIMICRIES – A CASE SERIES.

POC-07

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MADD results from genetic defects in Electron transfer flavoprotein (ETF) and ETF-coenzyme ubiquinone-oxireductase (EFT-QO), compromising electron transfer from FAD-dependent dehydrogenases to respiratory chain disrupting fatty-acid, aminoacid and choline metabolism. The acylcarnitine profile usually reveals variable elevation of multiple C4-18 compounds.

Comprehensive analyses of the six cases referred from newborn screening with the suspicion of MADD based on acylcarnitine elevation of multiple C4-18 compounds.

At evaluation, patients 1-3 were asymptomatic; patient 4 had been admitted in a neonatal unit since 48h of life with seizures and hypoglycaemia with hyperlactacidaemia and CK elevation; patient 5 had poor weight gain; patient 6 had been discharged home after admission at birth with transient tachypnea of the newborn associated with anaemia, and revealed hypotonia, poor weight gain, dysmorphic facial features and severe iron deficiency anaemia (min Hb 6,8g/dL). All patients started riboflavin.

Genetic investigation confirmed MADD in cases 1,2,4 and 5, with mutations found in the ETFDH gene (ETF-QO); in two cases an intronic variant, and in one case a new variant (p.V324M ;c.970G>A - probably pathogenic) was found in heterozygosity along with a known mutation. At follow-up none has cardiac, renal or ophthalmologic involvement, two have hypotonia and one has autism spectrum disorder.

For patients 3 and 6 no mutations were found in ETFA/B and ETFDH genes. Because of the possible biochemical overlap of MADD and riboflavin transport defects and the progressive neurologic dysfunction noted in case 6 - horizontal nystagmus, neurosensorial deafness, poor eye contact, generalized hypotonia, apneas and progressive axonal motor neuropathy - investigation was extended to riboflavin transporter (RFVT) deficiency; a mutation in SCL52A2 gene (RFVT2) was found in homozygosity allowing the diagnosis of Brown-Vialetto-van Laer Syndrome. For patient 3, RFVT mutations were also negative and at this point he is 2 years-old, has normal neurodevelopment with no organ specific dysfunction and awaits exclusion of other riboflavin metabolism defects.

By impairing β-oxidation of fatty-acids, riboflavin transport/metabolism defects may share a similar blood acylcarnitine profile with MADD, making diagnosis difficult. These should be kept in mind whenever the initial suspicion of MADD is not confirmed, with an active search always sought, while maintaining riboflavin.

OLD AND NEW BIOMARKERS IN LSD

POC-08

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INTRODUCTION Lysosomal storage disorders (LDS) is a group of inborn errors of metabolism characterized by lysosomal disfunction, caused either by enzyme or carrier protein defects.

Most LSD are caused by single enzyme deficiency, like Fabry disease (FD) and Gaucher disease (GD).

Regarding carrying defects, the most prevalent is type C Niemann-Pick disease (NPC). The diagnosis of this disorder always placed difficulties due to the lack of specific testing and the demand for specific biomarkers has been pursued from longtime ago.

With the arise of therapies for LDS the quest for biomarkers for the various disorders became more relevant in order to evaluate therapeutic efficacy.

Following scientific and technical evolution our Centre implemented the study of the most recently described biomarkers for several LSD - GD, FD and NPC.

MATERIALS AND METHODS Measurement of plasma concentrations of several metabolites was performed by liquid chromatography and tandem mass spectrometry (LC-MS/MS) both in healthy controls and patients affected by several LSD, at diagnosis and during therapy follow-up: glucosylsphingosine (Lyso-Gb1) in GD patients; globotriaosylsphingosine (Lyso-Gb3) in FD patients; lyso-sphingomyelin-509 (Lyso-SM-509) and its carboxilated analogue lyso-sphingomyelin (Lyso-SM) in NPC patients.

Comparison with biomarkers previously used for these disorders was also performed to evaluate the benefits.

RESULTS Clear distinction was observed between controls and patients at diagnosis. In GD and FD patients under therapy it was observed convergence to the normal values towards therapy's follow-up.

Combined use of Lyso-SM and Lyso-SM-509 also allowed for the distinction between NPC patients from those affected by types A or B Niemann-Pick diseases (acid sphingomyelinase deficiency).

CONCLUSION Lyso-GB1 and Lyso-GB3 biomarkers are reliable both for diagnosis and therapy follow-up monitoring in GD and FD patients, respectively.

Lyso-SM and Lyso-SM-509 improve the diagnosis of NPC as they are more specific than filipin staining test. Regarding therapy monitoring, however, no conclusions can be made even because clinical efficacy is not clear for the one currently used for this disease.

SESSION III

SELECTED FREE COMMUNICATIONS

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STALED LYSOSOMES PRIME CELL PROLIFERATION

OC-07

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Lysosomes play a critical role in autophagy, and their dysfunction is associated with Parkinson's disease (PD).

Heterozygous mutations in the GBA1 gene, which encodes for the lysosomal enzyme b-glucocerebrosidase (Gcase), are a risk factor for PD.

To investigate the effect of Gcase loss-of-function on cellular metabolism, we exposed cultured cells to CBE, which is a specific inhibitor of Gcase. Transcriptomic analysis of human primary fibroblasts treated with CBE revealed an up-regulation of genes involved in cell division. Consistent with this result, CBE increased the proportion of cells in S phase and the total number of cells in the cultures.

We further observed that CBE stimulated the proliferation of neural stem cells isolated from the subventricular zone of new born mouse brains. Neural stem cells were cultured as neurospheres for 7 days with or without 5µM CBE. Compared to mock-treated cultures, the cultures treated with CBE showed a higher proportion of cells in S-phase, and an increase in the total number of cells. An increase in the diameter of neurospheres was also observed.

Taken together our results suggest that inhibition of b-glucocerebrosidase activity leads to increased cell proliferation. These observations raise the possibility that loss-of-function mutations in the GBA1 gene may interfere with the cell-cycle control of neural stem cells in the human brain.

INHIBITION OF THE MITOCHONDRIAL COMPLEX I BY A PARKINSON'S DISEASE-INDUCING NEUROTOXIN LEADS TO A NPC1 PHENOTYPE IN NEURONAL CELLS

OC-08

Elsa Rodrigues[1]; Inês Caria[1]; Maria João Nunes[1]; Andreia Neves Carvalho[1]; Margarida Castro-Caldas[1,2]; Maria João Gama[1]; Jorge Ruas[1,3]

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Cholesterol has a key role in neuronal function and alterations in brain cholesterol homeostasis correlate with neurodegeneration. While disruptions in cholesterol homeostasis have been clearly associated with neurodegenerative disorders such as Alzheimer's disease, the role of cholesterol in Parkinson's disease (PD) remains controversial. To address this question, we characterize changes in cholesterol intracellular localization and levels using N2a mouse neuroblastoma cells treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxic metabolite, 1-methyl-4-phenylpyridinium (MPP+). Filipin III staining showed a increase in lysosomal accumulation of free cholesterol 16 hours after treatment with 1mM MPP+. Moreover, quantification of cholesterol levels revealed a significant increase in intracellular cholesterol. The increase in total cholesterol levels, occurred concomitantly with a significant decrease in the mRNA levels of NPC1 and lipase A (LIPA), and to a significant decreased in the mRNA levels and transcriptional activity of sterol regulatory element-binding protein (SREBP) 1 and 2 proteins, which are the main regulators of fatty acids and cholesterol synthesis. In parallel, there is a down-regulation in the mRNA levels of SREBP-target genes, such as fatty acid synthase and hydroxymethylglutaryl-CoA reductase. To further investigate this issue, we proceeded to characterized changes in cholesterol homeostasis in the brain of MPTP-treated mice (40mg/mL, i.p). Interestingly, we observed a significant decrease in NPC1 and LIPA mRNA levels 6 hours after MPTP administration, in both the midbrain and striatum. Thus, our results show that the inhibition of mitochondrial complex I leads to lysosomal accumulation and reduced NPC1 expression, further supporting previous reports that suggest PD as lysosomal storage disorder.

CLINICAL PROFILE OF ORGANIC ACIDURIAS IN CHILDREN FROM A SINGLE CENTER: AN 18-YEAR RETROSPECTIVE STUDY

OC-09

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BACKGROUND: Organic acidurias (OAs) are an important group of rare inherited metabolic disorders, with a broad clinical spectrum, caused by defects in intermediary metabolic pathways, namely, those of amino acids.

OBJECTIVE: Report and integrate clinical, radiological and genetic data of children with OAs.

METHODS: Retrospective study by clinical chart review of patients with OA followed at a reference center for metabolic diseases between 2002-2020.

RESULTS: 39 patients were reviewed, 14 with glutaric aciduria type 1 (GA1), 8 with methylmalonic aciduria (MMA), 5 with propionic aciduria (PA), 4 with 3-methylcrotonyl coenzyme A carboxylase deficiency (3MCCD), 4 with 3-hydroxy-3-methylglutaric aciduria (3HMGD), 3 with isovaleric aciduria (IVA) and 1 with multiple carboxylase deficiency (MCD).

The mean age at clinical presentation was 5 months. Consanguinity was present in 7.7% (3/39) of the cases. Death was reported in two cases (1-PA, 1-MMA). Neonatal tailored clinical signs were observed in 18% and recurrent crisis in 23.1% of the survivors. Neurodevelopmental issues (25.6%) and metabolic crisis (43.6%) were common presenting features. The majority of IVA (66%), all patients with 3MCCD and MCD presented a normal neurological examination which was observed only in 12.5% of MMA cases. Concerning the whole group of patients, 67% (26/39) were diagnosed by newborn screening (NBS). All 3HMGD patients were asymptomatic except 1 (1/4) who was not diagnosed by NBS and showed several decompensations and severe intellectual disability. All GA1 patients, who were not diagnosed by NBS (5/14) presented neurological abnormalities (NA), but only 55% (5/9) of those diagnosed by NBS (9/14) presented NA. All the PA patients showed NA whether they were diagnosed or not by NBS.

Imaging was available for 21/39 patients including 13/14 GA1 (1 with normal exam), 1/4 3MCCD without abnormalities, and 1/3 IVA, 4/8 MMA (1 with normal exam) and 2/5 PA (1 with normal exam). The diagnosis was confirmed by mutation analysis in 26/39 patients.

CONCLUSION: PA had the most severe neurological prognosis and medical care anticipation through NBS was not enough to prevent neurologic comprise, which is in agreement with previous data of the literature. GA1 was the most frequent OAs and GA1 as well as 3HMGD patients with earlier medical intervention due to NBS diagnosis had a better neurological outcome.

DOES THE PROTEIN INTAKE IN PKU PATIENTS ADHERE TO THE EUROPEAN PKU GUIDELINES?

OC-10

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BACKGROUND: With PKU patients the correct protein intake is critical for nutritional management. We evaluated protein intake in PKU, one-year post publication of the European PKU Guidelines 2017 (EPG).

METHODS: In 2018, a sample of 99 early treated PKU patients (46% females; 24 HPA, 48 mild PKU and 27 classical PKU), aged 19.3±8.2 y, who had an annual nutritional status evaluation (ANSE) was studied. The majority of patients were on exclusive dietary treatment (83%), while 17% of patients were on diet with BH4 therapy. Data on anthropometry and nutritional intake [total protein (TP, g/kg), natural protein (NP, g/kg), protein equivalent from PS (PE, g/kg)] was collected. TP adequacy (TPA) was calculated as a % of WHO (2007) safe levels of protein intake. Results were compared with recommendation #30 of the EPG (WHO+20% to compensate digestibility losses from L-aminoacids and +20% to improve blood phenylalanine metabolic control effect).

RESULTS: Median NP intakes were 0.69 g/kg [0.43-1.4]; PE from PS was 0.7 g/kg [0g/kg-0.91g/kg]. Median [P25-P75] % NP of TP intake was 53% [31%-100%]. Median TPA [P25-P75] was 171% [146%-203%], with 79% [51%-165%] for NP and 84% [0%-109%] for PE from PS. Only 6 patients had a TPA <100%: 1 child, 1 adolescent and 4 adults. The majority of patients (N=77; 78%) had a TPA above 140% (43 paediatrics and 34 adults). A TPA of 100-120% was seen in n=5 (1 child and 4 adults), while in n=11 patients, it was 120-140% (4 children and 7 adults). TPA was not significantly different with BH4 therapy compared with diet treatment only: 182% [155%-206%] vs. 168% [144%-202%]; p=0.55, respectively.

DISCUSSION: The majority of PKU patients (78%) were above of EPG recommendation for TP intake on our audit. Some patients (n=22) showed TP intake above the recommendations, were 7 of 22 patients were children. Especially in children and adolescents, our results underline that adequate protein should be prescribed and intake monitored in order to optimize outcome.

EFFECT OF SMALL COMPOUNDS ON THE ACTIVITY AND STABILITY OF RECOMBINANT HUMAN PHENYLALANINE HYDROXYLASE: IN VITRO AND IN CELLULO STUDIES

OC-11

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Phenylketonuria (PKU) is an autosomal recessive inborn error of metabolism caused by a deficient activity of human phenylalanine hydroxylase (hPAH) a nonheme iron homotetrameric enzyme that hydroxylates phenylalanine (L-Phe) into tyrosine (L-Tyr) in the presence of O2 and tetrahydrobiopterin (BH4). PKU is considered a conformational disease with loss of function, as the majority of hPAH variants present with misfolding that decrease protein stability. Recent alternative approaches with therapeutic potential for PKU aim at correcting the hPAH misfolding, and in this respect, pharmacological chaperones are focus of increasing interest.

In order to modulate hPAH stability and/or activity, 12 small molecules were designed based on the properties and structural characteristics of the catalytic hPAH ligands (L-Phe, BH4 and Fe). From this in-house library, four molecules were considered the most promising candidates based on their effect over the activity, thermostability and conformation of the recombinant hPAH. They presented a slight inhibitory effect (17 to 40% enzyme activity decrease) but allowed further activation by L-Phe (1.3 to 1.7-fold). Two of the selected molecules stabilized the hPAH regulatory domain (Tm1: +8.3 and +4.0 ºC). These molecules were further studied on a cellular context for their effect on hPAH activity and stability. The hPAH was transiently expressed in HEK293 cells (as they do not constitutively express hPAH) for 24 hours in the absence or presence of 50 µM of either compound. Cells were harvested and steady-state PAH activity and protein amount (western blotting) were measured. Pulse-chase assays were also performed to follow enzyme activity and protein levels at different time points after protein synthesis blockage with puromycin. An in vitro/in cellulo correlation was found for two of the four tested compounds as an increase in hPAH intracellular levels were observed indicating an effect as a hPAH stabilizer thus preventing hPAH intracellular proteolysis. These molecules are hit compounds for further structural refinement aiming the development of new therapeutic approaches to PKU treatment.

Work funded by FCT: project PTDC/MED-QUI/29712/2017

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF CARRIERS OF PATHOGENIC VARIANTS IN THE GLA GENE, THE CAUSE OF FABRY DISEASE

OC-12

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INTRODUCTION Fabry disease (FD) is an X-linked progressive inborn error of metabolism originated by mutations in the alpha-galactosidase A gene (GLA) causing a deficiency of the lysosomal acid hydrolase α-galactosidase (α-Gal) activity. In consequence, the breakdown of glycolipids occurs, mainly globotriaosylceramide (Gb3) and its deacylated form, globotriaosylsphingosine (lyso-Gb3), among other sphingolipids. The lysosomal substrate accumulation is the onset of this lysosomal storage disorder that can lead to life-threatening complications.
Male patients usually present very low α-Gal activity and marked substrate accumulation, and at least some of the clinical hallmarks. In females, however, there is a wide range of phenotypes – from completely asymptomatic to severe "men-like" – leading to great controversy regarding clinical diagnosis and therapeutic decisions. Non-random X chromosome inactivation pattern is the most common hypothesis to explain such variety of phenotypes. Nevertheless some authors state that most heterozygous carriers end up presenting clinical signs, only with some years delay when compared to males.

PATIENTS AND METHODS Data concerning leukocytes and plasma α-Gal activity together with plasma and urine substrate accumulation for 140 males and 209 females were obtained. Correlation among biochemical parameters and with the molecular defect was evaluated.

RESULTS The most prevalent genetic variant is c.337T>C p.(F113L) found in 50% of males and females.
Males usually present a correlation of reduced α-Gal activity with increased substrate accumulation. In this group it was possible to identify some genetic variant-specific patterns. One interesting finding is that for the c.827G>A p.(S276N) genetic variant the plasma α-Gal activity is perfectly normal in plasma although the very low activity in leukocytes.
Heterozygous females show less pronounced effect on substrate accumulation and enzyme deficiency. The variability of patterns within carriers of the same genetic variant is also noticed.

CONCLUSIONS Some genetic variants whose pathogenicity is somehow questioned are indeed associated with milder biochemical phenotypes.
Plasma α-Gal activity is not recommended as only diagnosis testing due to the possibility of false negatives.
Heterogeneity among heterozygous females should be further evaluated with relation to X chromosome inactivation profile.

SESSION IV

CROSSTALK AMONGST INTRACELLULAR ORGANELLES

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FATTY ACID METABOLISM: INTRACELLULAR INTERACTIONS

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Fatty acids serve a variety of different functions in human metabolism. First, FAs are an important source of energy both under fed as well as fasted conditions with FAs being the main oxidizable substrate for the heart under all conditions. Secondly, FAs are important building building blocks for a variety of different lipid species including triglycerides, phospholipids, sphingolipids, cholesterol esters, cardiolipin, among others. Furthermore, FAs are important signaling molecules for instance by acting as anchoring units turning certain proteins from soluble to membrane-bound proteins. FAs are derived from dietary sources but except from linoleic acid and linolenic acid, these FAs can also be synthesized endogenously from acetyl-CoA units via the FAS-complex. Subsequent steps of elongation and desaturation will then allow the formation of a wide variety of acyl-CoA species which can subsequently be incorporated into multiple lipid species. Acyl-CoA esters are also obligatory for the formation of long-chain alcohols required for the synthesis of ether-linked phospholipids by means of the two acyl-CoA reductases FAR1 and FAR2. All these lipids also undergo breakdown in the lysosome or otherwise as part of their life cycle and by doing so FAs are released and will fill up the intracellular fatty acid pool to start a new cycle of acyl-CoA ester formation or-if deemed necessary- these acyl-CoAs undergo breakdown via beta-oxidation either in the mitochondrion or in the peroxisome. Proper fatty acid homeostasis requires the concerted action between different subcellular compartments each equipped with different enzyme systems and transporters and multiple genetic deficiencies affecting FA homeostasis have been identified through the years.This includes the mitochondrial fatty acid oxidation deficiencies in which different acyl-CoAs and acylcarnitines accumulate with all its detrimental consequences. Our own recent work in cardiomyocytes to be presented at the meeting shows that the toxicity is caused foremost by the accumulating acylcarnitines. Furthermore, detailed studies in a patient with elevated very-long-chain-fatty acids led to the discovery of a new player involved in the intracellular metabolism of FAs (see Ferdinandusse et al.(2017) J.Med.Genet.54,330-338).The protein involved is a peroxisomal membrane-bound protein exposed to the cytosol which interacts with the ER-bound protein VAPB and by doing so, it brings the two organelles i.e. the peroxisome and endoplasmic reticulum close together so that lipids can move from one to the other organelle. The ACBD5-VAPB-complex is just one of a growing group of so-called tethering proteins which serve to bring organelles together for the greater benefit of metabolite exchange and specific interaction between organelles rather than through unspecific metabolite transfer through the cytosol.

NEW PERSPECTIVES IN FATTY ACID OXIDATION DISORDERS

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Fatty acid oxidation disorders are a heterogeneous group of diseases presenting with different phenotypes also in case of the same underlying enzyme defect and genotype. In addition, many asymptomatic individuals with confirmed disease have been identified through newborn and family screening. They require an early risk assessment of the expected phenotype and an individualized treatment starting from the time of diagnosis.

Current therapeutic interventions are still mainly based on dietary modifications. Experimental dietary therapies including odd-chain medium fatty acids and ketone bodies have been recently studied in clinical trials in order to prove their efficacies. New studies on ketone body treatment in very long-chain acyl-CoA dehydrogenase deficiency prove its effectiveness as well as respective studies in multiple-acyl-CoA dehydrogenase deficiency and ketone synthesis defects. The effects of odd-chain medium chain fatty acids have been studied in patients in comparison to even-chain medium chain fatty acids and it remains challenging to identify clear clinically meaningful differences.

Overall, the main treatment goal is still to improve energy expenditure and to avoid catabolic stress. Since fat restriction generally results in higher carbohydrate loads and often weight gain in patients with LC-FAODs, increasing the protein intake is a new dietary approach which maintains metabolic control and helps to preserve lean body mass according to first data.

In conclusion, individualized therapy concepts need to be brought on its way. In order to realize these, an international interdisciplinary fatty acid oxidation guideline group has been formed in 2019. The aims are defining the current varying standards on treatment, diagnostic procedures and monitoring for the group of FAO disorder patients in different countries.

Guidelines are overdue, despite the fact that evidence for different treatment measures is still scarce. With a personalized treatment approach, consensus may be easier and does take individual compensatory mechanisms and individual environmental conditions modulating disease into account.

AN OVERVIEW OF COMPLEX LIPID SYNTHESIS AND RELATED DISORDERS

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Fatty acids serve a variety of different functions in human metabolism. First, FAs are an important source of energy both The number of inborn errors of metabolism caused by defects in complex lipid metabolism is expanding rapidly. This relatively uncharted part of metabolism is increasingly explored and this has been greatly stimulated by the identification of new groups of patients by next generation sequencing which are characterized by combination with lipidomics translational research efforts. Our aim is provide a general overview of the main lipid synthesis pathways and relate this to the clinical symptomatology of the disorders while also presenting examples of how a lipidomics approach can contribute to biomarker discovery and to the understanding of pathology for these type of disorders.

A CLINICAL APPROACH TO COMPLEX LIPID SYNTHESIS DISORDERS

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Complex molecules disorders have been for a long time restricted to lysosome storage disorders linked to catabolic defects of Sphingolipids (SPL), Oligosaccharides and Glycoproteins. From another hand, defects of long, very long and ultra long chain fatty acid (VL/ULCFA) synthesis and remodeling and their incorporation into and release from phospholipids (PL), and SPL were almost ignored. Peroxysome including plasmalogens defects were considered as an independent group of diseases despite they share many pathways and clinical findings with complex lipid synthesis and remodeling disorders. Within the last 10 years over one hundred diseases related to inherited defects of PL and SPL synthesis and remodeling were reported. They also included several new non mitochondrial FA metabolism disorders like elongases defects and the already well known peroxysome defects.

While the associated clinical phenotype is currently difficult to outline, with only a few patients identified, it appears that all organs and systems may be affected. The main clinical presentations that can be isolated or diversely combined are :

1) Diseases affecting the central and peripheral nervous system. Complex lipid synthesis disorders produce prominent motor manifestations due to upper and/or lower motoneuron degeneration. Motor signs are often complex, associated with other neurological and extraneurological signs. Four neurological phenotypes, spastic paraplegia, neurodegeneration with brain iron accumulation, peripheral neuropathies,and movement disorders deserve special attention.

2) Muscular/ cardiac presentations presenting as congenital muscular dystrophy, or Barth and Sengers syndromes

3) Skin symptoms mostly represented by syndromic (neurocutaneous syndrome like Sjogren Larsson , ELOV 1,4, Refsum disease) and non syndromic autosomal recessive congenital ichthyosis (ARCI)

4) Retinal dystrophies with syndromic and non syndromic retinitis pigmentosa, Leber congenital amaurosis, cone rod dystrophy, Stargardt disease.

5) Congenital bone dysplasias are seen in defective synthesis of Plasmalogen like Chondrodysplasia punctata types 1-3, Cholesterol like in Smith Lemli Opitz syndrome, Phosphatidyl inositides like Opsysmodysplasia and Phosphatidyl serine like in Lenz Majewski hyperostotic dwarfism syndrome.

6) Liver presentations characterized mainly by transient neonatal cholestatic jaundice and non alcoholic liver steatosis with hypertriglyceridemia are seen in neutral lipid defects like in the Chanarin Dorfmann syndrome .

(7) Other presentations like Renal ,immune or intestinal signs may also be seen

Lipidomics and molecular functional studies could help to elucidate the mechanism(s) of dominant versus recessive inheritance observed for the same gene in a growing number of these disorders

SESSION V

NEW INSIGHTS IN CARBOHYDRATE DISORDERS

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UPDATE IN DISORDERS OF GALACTOSE METABOLISM TYPE 1 AND TYPE 2.

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Type 1 galactosemia, GALT deficiency:

The current cornerstone of treatment, diet, has proven effective to treat the neonatal manifestations but not in preventing burdensome complications. Most affected patients develop brain impairments (85.0%), primary ovarian insufficiency (79.7%) and a diminished bone mineral density (26.5%) according to data from 509 patients included in the GalNet registry. Newborn screening, age at onset of dietary treatment, strictness of the galactose-restricted diet, p.Gln188Arg mutation and GALT enzyme activity influence the clinical picture. Detection by newborn screening and commencement of diet in the first week of life are associated with a more favorable outcome. A homozygous p.Gln188Arg mutation, GALT enzyme activity of ≤ 1% and strict galactose restriction are associated with a less favorable outcome.

The pathophysiology is complex and not yet fully elucidated. Gal-1-P is necessary but not sufficient for expression of the human phenotype. Other currently described contributing factors include galactose metabolites accumulation, UDP-hexoses alterations and subsequent impaired glycosylation, ER stress with subsequent UPR induction and signalling pathway alterations, and oxidative stress. Structural and functional studies show that some GALT disease-causing mutations give rise to misfolded variants with a higher propensity for aggregation and altered stability.

Several animal models have been developed that (partly) mimic the phenotype that allow us to advance our comprehension of the complex playing field of the hereditary metabolism of galactose. Different expression of the Leloir pathway components and alternative galactose disposal routes in the different tissues, combined with specific tissue demands, epigenetic and environmental factors, urge to revisit our understanding.

Recently several animal models have been developed that (partly) mimic the phenotype that allow us to advance our comprehension of the complex playing field of the hereditary metabolism of galactose. New therapeutic approaches are currently being evaluated which can be divided in 2 groups: i) treatments to restore the enzyme GALT activity and ii) treatments focused on reduction of accumulated metabolites or downstream phenomena. The group targeting the GALT enzyme deficiency includes human GALT messenger RNA and GALT modulators. The group aimed at influencing the metabolic reactions triggered by this deficiency consists of GALK and AR inhibitors, and ER stress reducers. For these strategies to be optimal, the window of opportunity for treatment needs to be well-characterized, e.g. neonatal and early infancy, or through adolescence or lifelong treatment.

Type 2 galactosemia, GALK deficiency:

Beyond cataract, the phenotypic spectrum s questionable. Data from the GalNet registry in 33 patients show that neonatal illness is repeatedly reported, with elevated transaminases being the most frequently observed abnormality. Diet treatment is effective and NBS has a beneficial effect. Neurological complications have been reported in patients. Yet, they are rare and it is unclear whether they are related to the GALK deficiency. Other information on follow-up is so limited among the patients that no conclusions can be drawn. Many patients with this entity are not regularly seen in follow-up.

SESSION VI

NEW TREATMENT PERSPECTIVES

CHAIRPERSONS

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ENZYME SUBSTITUTION THERAPY: PEGVALIASE FOR THE TREATMENT OF PKU

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Treatment for life is recommended for patients suffering from phenylketonuria (PKU) arising from phenylalanine hydroxylase deficiency. However, the dietary treatment is burdensome and it is associated with significant psychosocial consequences. Many adolescent and adult PKU patients show long-term metabolic derangement despite existing management. A study among in-clinic adult patients with PKU actively managed with diet in the US showed that 72% of patients have blood phenylalanine concentrations above 600 µmol/l, while 18% of patients even display blood phenylalanine concentrations above 1.200 µmol/l. These high phenylalanine levels may lead to neurocognitive and neuropsychiatric symptoms such as lack of comprehension, trouble of focusing, inability to complete tasks, sleeping problems, depression, and anxiety.

This demonstrates that alternative treatments for PKU patients are urgently needed. In 2019, Pegvaliase, the first enzyme substitution therapy for patients suffering from phenylalanine hydroxylase deficiency, was approved for the treatment of patients 16 years or older with unsatisfactory metabolic control. Compared to the current dietary treatment this therapy is invasive and requires a lot of efforts from both parents and specialized PKU treatment teams. Here we show how to set up the clinic for the introduction of pegvaliase treatment, how to evaluate patients eligible for treatment, and how to prepare the patient for this new therapeutic approach.

Since October 2019 21 patients were treated with pegvaliase. Several case studies will demonstrate that pegvaliase treatment needs an individualized approach for every single patient. We will discuss different courses to efficacy and the sometimes challenging safety profile of this new drug including injection site reactions, arthralgia, and acute systemic hypersensitivity reaction that need particular attention from treating physicians.

EXPANDING THE USE OF SUBSTRATE REDUCTION THERAPY: NITISINONE FOR THE TREATMENT OF ALKAPTONURIA

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Alkaptonuria (AKU) is an autosomal recessive disorder characterised by a lack of homogentisate 1,2 dioxygenase enzyme leading to accumulation of homogentisic acid (HGA). Deposition of HGA as melanin-like pigment in connective tissues of the body results in the multisystem damage in AKU. The clinical features of AKU include spondyloarhtropathy, aortic & mitral valve valve disease, osteopenia, fractures, ruptures of tendons/muscle/ligaments, hearing impairment, and lithiasis (renal, prostate, salivary, gall bladder). The diagnosis is suspected by external ochronosis in eyes and ears, early arthritis and dark urine, and confirmed by measurement of urine homogentisic acid. Clinical assessment involved multiple modalities to track the multisystem involvement in this condition with a variable phenotype. Generating a composite score namely alkaptonuria severity score index is useful for prognostic and monitoring purposes. Nitisinone inhibits p-hydrozyphenylpyruvate dioxygenase and prevents the formation of HGA from tyrosine. However, all patients receiving nitisinone therefore exhibit increased circulating and tissue tyrosine levels. Tyrosinaemia can cause visual impairment due to build-up in the cornea. Patients receive regular slit-lamp examination to ensure safe usage. In September 2020, European Medicines Agency authorised the use of nitisinone in adult AKU, following the successful report of the phase III randomised clinical trial of nitisinone in AKU, called Suitability of nitisinone in alkaptonuria 2 (SONIA 2).

SESSION VII

EXPANDING TREATMENT TARGETS – WHAT'S FOR THE FUTURE OF GENETIC THERAPY?

CHAIRPERSONS

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RECENT ADVANCES IN GENE TRANSFER THERAPY TO THE CNS IN LYSOSOMAL STORAGE DISEASES

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Lysosomal Storage Disorders (LSD) are a group of inherited metabolic diseases caused by the deficiency of hydrolytic enzymes involved in the degradation of macromolecules including sphingolipids, glycosaminoglycans, glycoproteins and glycogen that accumulates into the lysosomes. The storage of undegraded materials triggers a cascade of secondary events in different tissues and organs causing a complex phenotype of these disorders characterized by the variable association of systemic manifestations. The metabolic defect can primarily affect the Central Nervous System (CNS) leading to the neuronal death, microglial and astroglial activation, inflammatory processes, and axonal degeneration. The treatment of neurodegeneration represents the main goal of therapeutic approaches development for the LSDs. Currently, there is no effective therapy for the treatment of CNS pathology and existing clinical approved protocols try to alleviate the somatic symptoms rather than treat the causes of the disease. The most available therapeutic strategies are based on the delivery of functional lysosomal enzymes by using the enzyme replacement therapy (ERT) or Adeno Associated Viral (AAV) vectors mediated gene therapy protocols. These treatments rely on the mechanism of cross-correction that allows the spreading of functional enzyme into affected cells by mannose-6-phosphate receptor mediated endocytosis. However, due the limitations of ERT in terms of half-life of the human enzyme, treatment costs and inability to cross the blood brain barrier (BBB), the AAV-gene therapy approach potentially represents the more achievable strategy for the treatment of neuropathology in LSD. The AAVs efficiently infect dividing and non-dividing cell types, persist as episomes, and guarantee a long-term efficacy. During the last decade, we and other researchers developed new AAV gene therapy protocols by the designing more efficient and safe AAV administration routes, selecting specific AAV capsids with high CNS tropism and engineering the lysosomal enzymes in order to enhance their activity. All these strategies allowed to bypass the BBB obstacle, minimize the immune response, improve the CNS targeting and biodistribution and rescue the CNS storage pathology in neuropathic LSDs such as Mucopolysaccharidoses. A special mention has to be done to the important results obtained by the intrathecal AAV9-mediated gene therapy that at moment represents one of most powerful gene therapy approach for different neurodegenerative LSDs. In conclusion, thanks to these exciting and recent advances, AAV-gene therapy protocols achieved both successful preclinical and clinical trials results overcoming previous limitations and moving forward in several directions in the LSDs treatment.

EMERGING THERAPIES IN THE HEREDITARY PORPHYRIAS

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Porphyrias are rare inherited disorders caused by specific enzyme dysfunctions in the haem synthesis pathway, which result in abnormal accumulation of specific pathway intermediates. Porphyrins are photoreactive and cause photocutaneous lesions on sunlight-exposed areas in most of the porphyrias, whereas accumulation of the early porphyrin precursors d-aminolevulinic acid (ALA) and porphobilinogen (PBG) is related to acute neurovisceral attacks in acute hepatic porphyrias (AHPs). The various symptoms of the different porphyrias depend on the chemical characteristics of the pathway intermediates that accumulate. Their variable clinical expression depends on the severity of the underlying enzymatic abnormality and the activity of the first and rate-limiting ALA synthase enzymes of the pathway, through induced expression of the housekeeping *ALAS1* gene in the liver and tissue specific induced expression of the *ALAS2* gene in the bone marrow.

Activation of AHPs results from induction of hepatic *ALAS1* by exogenous or endogenous factors in the presence of inherited deficiency of 1 of 4 downstream enzymes in the pathway. This results in the accumulation of porphyrin precursors, ALA, PBG and porphyrins, leading to neurological symptoms. A therapeutic interference RNA (givosiran, Alnylam Pharmaceuticals) that targets hepatic *ALAS1* mRNA production is currently undergoing clinical trials phase 3 in AHPs and just recently approved for clinical use (Givlaari®) by the EMA and FDA. Meanwhile, trials aimed at rescuing the metabolic blockage by gene or mRNA therapies are ongoing for acute intermittent porphyria (AIP) the most prevalent AHP.

Porphyrins accumulate and may cause typical cutaneous photosensitivity lesions in porphyria cutanea tarda (PCT), the most common and non acute porphyria. PCT is readily treated with repeated phlebotomies or low-dose hydroxychloroquine, which are equally effective. Nowadays hepatitis C is a major susceptibility factor in many patients with PCT, and the possibility that DAAs can be effective as initial treatment for both conditions is under investigation.

In erythropoietic porphyrias resulting from dysfunctions in other pathway enzymes, com- pensatory induction of erythroid *ALAS2* or gain- of-function *ALAS2* mutations, lead to porphyrin accumulation and variable types of cutaneous photosensitivity depending on the site of the enzymatic defect.

Protection from sunlight exposure is the cornerstone of management for these two conditions. Increased skin melanin production after treatment with afamelanotide has increased significant light tolerance and quality of life in EPP patient and a treatment approved by EMA (Scenesse®, Clinuvel Pharmaceuticals). However additional novel therapies based on the etiologies and pathogenesis of these conditions, ie. protoporphyrin hyperproduction and accumulation by either *ALAS2* induction or FECH mutations, are under development, such as the antisense oligonucleotide (AON) strategy to increase expression of the hypomorphic FECH allele (IVS3-48 T>C, found in the great majority of patients with EPP), which is now in preclinical development. Alternatively, inhibitors of erythroid *ALAS2* and suppression of erythroid haem synthesis are additional innovative approaches with potential for treating all erythropoietic porphyrias.

EMERGING THERAPIES FOR MITOCHONDRIAL DISEASE

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Primary mitochondrial diseases are devastating genetic disorders affecting the structure and/or function of the mitochondria, dynamic organelles that act as cellular power stations and signalling hubs. At present, curative therapies are lacking for the vast majority of mitochondrial diseases, with the possible exception of some disorders of the biosynthesis of coenzyme Q_{10}, an essential mitochondrial electron carrier and antioxidant. A plethora of emerging therapies is at a preclinical or early clinical stage of development. These therapies can be divided broadly into pharmacological and genetic approaches. Pharmacological therapies can be subdivided further into disease-specific and disease-agnostic approaches. An example of a disease-specific therapy is nucleoside supplementation for thymidine kinase 2 (TK2) deficiency; disease-agnostic approaches target reactive oxygen species, mitochondrial membrane lipids, mitochondrial biogenesis and mitophagy. Genetic approaches can also be subdivided, into those that target the mitochondrial DNA and those aiming to cure nuclear gene defects. The former include methods to selectively destroy mutant mitochondrial DNA, e.g. using zinc finger nucleases or mitochondrial TALENs, or by allotopic expression of mitochondrially encoded proteins. AAV and/or lentiviral mediated gene therapy has now been reported in mouse models of a handful of mitochondrial disorders caused by nuclear gene defects. The recent failure of a Phase 3 trial of elamipretide, a Szeto-Schiller tetrapeptide that aims to stabilize cardiolipin, highlights the extreme challenges in demonstrating efficacy of any of the above emerging therapies in a field blighted by heterogeneity and complexity at every level (clinical, biochemical and genetic).

GENETIC SUBSTRATE REDUCTION THERAPY FOR MUCOPOLYSACCHARIDOSES TYPE III: TOWARD A SIRNA-CONTAINING NANOPARTICLE TARGETED TO BRAIN CELLS

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ABSTRACT: The classical therapeutic approach for LSDs, enzyme replacement therapy, would hardly rise as a potentially successful tool to reduce the disease burden in MPS III patients, as it is long known to have no impact on neuropathology. A tempting alternative, however, would be to block substrate accumulation upstream, by decreasing its synthesis. That concept is known as substrate reduction therapy (SRT).

Having this in mind, we designed an RNA-based strategy based upon the selective downregulation of one gene involved in the very early stages of the glycosaminoglycans' (GAG) biosynthethic cascade. Our goal is to promote an effective reduction of the accumulating substrate, ultimately decreasing or delaying MPS' symptoms. As tools to achieve substrate reduction, we are evaluating a specific type of antisense oligonucleotides, able to trigger a naturally-occurring post-transcriptional gene silencing process called RNA interference: the small interfering RNAs (siRNAs). So far, the obtained results are quite promising with marked decreases of the target mRNA levels in fibroblast cell lines for all the different MPS III disease subtypes. Initial studies addressing the overall storage of sulphated GAGs used either the routine alcian blue or a modified, more sensitive 1,9-dimethylmethylene blue assay at different time points. Nevertheless, the low confluency levels required for siRNA transfection did not allow detection of GAGs excreted to the culture media. Similar problems have been noted by other authors, including over- and under-estimation of sulphated GAGs. This is particularly relevant in small samples, like the ones we have been using. In fact, even the direct assessment of the intralysosomal suphated GAGs on those samples, while more reliable, does show some limitations. That is why we are currently implementing a novel, more sensitive method for GAG detection by liquid chromatography and quantification with electrospray ionization–tandem mass spectrometry (Saville et al., 2018). Thus, additional data on the effect of the designed siRNAs on substrate accumulation will be collected over the next months and other methods will be used to further address this issue.

Here we present an overview on the current results of this project, while discussing its' next steps, namely the development and evaluation of vectors for in vivo delivery. Our goal is to develop targeted stable nucleic acid lipid particles (t-SNALPs) coupled with different ligands, which promote cell uptake of the 'anti-GAG' siRNAs in a variety of cells, including neurons.

ACKNOWLEDGMENTS: This work was partially supported by the Portuguese Society for Metabolic Disorders (Sociedade Portuguesa de Doenças Metabólicas, SPDM - Bolsa SPDM de apoio à investigação Dr. Aguinaldo Cabral 2018).

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SESSION VIII

EXPANDING THE CONCEPT OF NEUROMETABOLIC DISORDERS

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CELLULAR NEUROMETABOLISM: A TENTATIVE TO CONNECT CELL BIOLOGY AND METABOLISM IN NEUROLOGY

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Inborn errors of metabolism (IEMs) are particularly prevalent as diseases of the nervous system. This is especially true now that a broader, more inclusive definition of IEM is accepted. Moreover, the whole group of neurometabolic disorders, many of them lacking biomarkers, is expected to experience substantial growth in the near future as a result of advanced genetic diagnostic techniques. In fact, we could easily find major neurological symptoms in around 80% of the whole group of IEMs. Because this implies an important challenge for both neurologists and metabolic physicians, it is necessary to redefine our approach to these diseases.

The "Cellular Neurometabolism" aims to integrate biochemistry and cell biology based on the peculiarities of the nervous system, which is highly compartmentalized. In fact, different cell compartments (neuronal soma, axons, synaptic terminal, glia-connections…) behave as unique microenvironments with specific metabolic properties.

During the talk, a tentative approximation to this integrative overview will be developed. For this purpose we will consider how the main categories of IEM, according to the recently published simplified classification, have an impact in the different cell compartments of the nervous system. This means to explore how disorders of simple, complex molecules and energy defects behave in the brain from a cellular and biochemical perspective. Conversely, we will assess the metabolic behavior of major cellular processes such as trafficking and signaling and their related neurological symptoms and diseases.

Although this is a complex and incomplete tentative, it could be a starting point to train the clinical thinking for a multiple level approach that includes metabolism and cell biology. Increasing knowledge about the pathophysiology of disorders as well as big data application will probably help to better define this and similar approaches in the future.

NEONATAL SEIZURES - ARE WE THERE YET ?

BARBARA PLECKO

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Recent years have brought major progress in the field of epileptic encephalopathies that are caused by inborn errors of metabolism (IEM). Though rare, their early recognition is crucial to enable specific treat- ment and avoid irreversible brain damage. Hypoglycemia and electrolyte imbalance including hypomag- nesemia must be ruled out before screening for IEM. Diagnostic work-up for IEM is based on age of on- set, seizure semiology, EEG characteristics, concomitant clinical or radiologic findings and the identifica- tion of specific biomarkers in body fluids. The largest group of IEM manifesting with neonatal seizures is that of cofactor deficiencies, including vitamin B6 dependent epilepsies, dihydrofolate reductase and mo- lybdenum cofactor deficiency, that are all amenable to specific treatment.

Every newborn with myoclonic seizures that are resistant to common anticonvulsants should be investi- gated for vitamin B6 dependent epilepsies and receive a standardised cofactor trial with pyridoxine 30 mg/kg/d in two single dosages, if ineffective followed by add-on of folinic acid 3-5 mg/kg/d and a switch to PLP, 30-60mg/kg/d. Vitamine B6 dependent epilepsies can occur due to different IEM, that can be sepa- rated by distinct biomarkers (Table 1). These include Antiquitin deficiency, Pyridox(am)ine 5'-phosphate oxidase deficiency, severe forms of congenital hypophosphatasia and Pyridoxal 5'-phosphate binding protein (PLPBP-formerly PROSC) deficiency. In Antiquitin deficiency add-on treatment with a lysine re- stricted diet and arginine supplementation might improve cognitive outcome. Recent research has unrav- eled a considerable pyridoxine response in patients with mutations in the potassium channel KCNQ2. As PLP is a cofactor of the glycine cleavage system, elevated glycine may be present in plasma and CSF of patients with B6 dependent epilepsies and mislead to the diagnosis of non-ketotic hyperglycinemia (NKH). Megaloblastic anemia or pancytopenia along neonatal seizures and rapidly progressing micro- cephaly should alert clinicians to think about folate cycle defects, such as dihydrofolate reductase defi- ciency. Patients with isolated sulfite oxidase or molybdenum cofactor deficiency present with poor feeding before the onset of bilateral tonic-clonic seizures. MRI shows brain edema, followed by multicystic grey and white matter degeneration. Supplementation of precursor Z is available for molybdenum cofactor deficiency type A but needs early recognition to benefit the patient.

Some rare organoacidurias, mitochondriopathies and peroxisomal disorders may also present with neo- natal seizures with or without dysmorphic signs but lack specific treatment options.

Biomarkers for neonatal seizures due to IEM table 1

Disorder	Plasma	CSF	Urin	others
Antiquitin deficiency	Pipecolic acid		AASA	
PNPO deficiency	Pyridoxamine		Vanillactate	
Cong. hypophosphatasia	Alkaline phosphatase			
PLPBP deficiency	-	-	-	No biomarker
NKH	aminoacids	aminoacids		Glycine CSF/plasma ratio
MOCOD and ISOD			sulfocysteine	
Zellweger Syndrome	VLCFA			
Mitochondriopathies	lactate	lactate		
Dihydrofolate reductase def.		Methyltetrahydrofolate		megalobl. ery.
Organoacidurias (eg D2OHGA)			Organic acids	

GOT2 DEFICIENCY: A NOVEL MALATE-ASPARTATE SHUTTLE DISEASE THAT LEADS TO EARLY-INFANTILE ENCEPHALOPATHY

RÚBEN RAMOS

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Early-infantile encephalopathies with epilepsy are devastating conditions that demand accurate diagnosis to guide proper management. Whole exome sequencing in four children from three independent families with intellectual disability and epilepsy revealed loss-of-function mutations in *GOT2*. *GOT2* encodes the mitochondrial glutamate oxaloacetate transaminase (GOT2), a pyridoxal 5'-phosphate-dependent enzyme. GOT2 plays an essential role in the malate aspartate shuttle (MAS) by catalyzing the reversible interconversion of oxaloacetate and glutamate into aspartate and α-ketoglutarate. The MAS provides a mechanism for net transfer of NADH reducing equivalents across the inner mitochondrial membrane, regulating glycolysis and lactate metabolism. Biochemically, all GOT2-deficient patients presented with hyperlactatemia and hyperammonemia, while the most severely affected patient presented also low plasma serine and hypercitrullinemia. We characterized the functional consequences of the observed mutations by measuring the enzyme activity and by cell and animal models. In vitro analysis of GOT2 activity in the patients' fibroblasts revealed strong decreases in the enzyme activity. We performed stable isotope analysis in the patients' cells and found an impaired serine de *novo* biosynthesis. To further investigate the causal relationship between GOT2 deficiency and serine production, we generated GOT2-knockout HEK293 cells and confirmed a strong impairment in serine de novo biosynthesis. Hypothesizing that the defect in producing serine was secondary to increased cytosolic NADH/NAD+ ratio, we tested the effect of glycerol and pyruvate on serine biosynthesis and found that pyruvate restores serine production in the *GOT2*-knockout cells. Additionally, knockdown of *got2a* in zebrafish resulted in a brain developmental defect associated with seizure-like electroencephalography spikes, which could be rescued by supplementing pyridoxine and serine to the fish water.

Although future research on this MAS defect is needed to fully characterize the phenotypic and genotypic spectrum of the disease, to identify biomarkers and evaluate therapeutic interventions, our findings strongly suggest that serine, vitamin B6 and pyruvate supplementation may be important therapeutic options in correcting the clinical and biochemical abnormalities observed in GOT2 deficiency. Nowadays, the two most severely affected patients are under serine and vitamin B6 treatment, showing a complete control of their seizures.

MITOCHONDRIAL BIOENERGETICS IN NEURODEGENERATION (INVITED SHORT COMMUNICATION)

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Mitochondria are organelles known as the "powerhouses" of the cells, as they are responsible for producing 90% of the energy necessary to maintain and support cell survival. When mitochondria fail to produce enough energy to sustain cell life, degeneration of the cells, the tissue and ultimately the organ occurs. Mitochondrial dysfunction can be caused by mitochondrial or nuclear DNA mutations or can be acquired due to metabolic, aging or environmental stress. Diseases associated with mitochondrial dysfunction are very common, including aging-associated diseases and metabolic disorders. Despite significant advances in recognizing, diagnosing and treating patients with mitochondrial diseases, effective treatments that are targeted to the specific deficit in a patient are still lacking.

To address the unmet diagnostic and therapeutic needs of patients affected by mitochondrial disorders we will apply a precision medicine approach that integrates the development of novel tools for non-invasive testing that will define the genetic and bioenergetics profile of mitochondria present in afflicted individuals. For this, we have attained biological samples (blood and skin fibroblasts) from patients afflicted with an apparent metabolic disorder phenotype. To determine the mitochondrial genome, we have extracted mtDNA from these biological samples and performed whole genome sequencing (WGS). We used the Applied Biosystems™ Precision ID mtDNA Whole Genome Panel in conjunction with the Ion Torrent™ Ion S5™. Performing bioinformatics analysis, we are able to determine mutation rate and heteroplasmy levels. To determine the mitochondrial bioenergetics profile, we are assessing oxygen consumption rates and determining mitochondrial morphology. These assays enable the determination of the bioenergetics profile of each biological sample through comparison to age-match healthy controls.

Thus, defining the bioenergetics profile of each individual, compiled with their genetic and clinical profile will allow appropriate treatment and prognosis for these individuals.

SESSION IX

AGEING IN INHERITED METABOLIC DISORDERS

CHAIRPERSONS

ANABELA OLIVEIRA, MD
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AGE RELATED METABOLIC CHANGES IN INHERITED METABOLIC DISORDERS

FANNY MOCHEL

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An increasing number of adult-onset inherited metabolic diseases (IMD) have now been recognized, as new metabolomics and molecular diagnostic techniques have become available. I will discuss possible mechanisms underlying phenotypic variability in adult versus children with IMD. Specifically, phenotypic severity and age of onset is expected to be modulated by differences in residual protein activity possibly driven by various genetic factors. Phenotypic variability may also occur in the context of similar protein expression, which suggests the intervention of environmental, ontogenic and ageing factors.

Likewise, environmental factors play an important role in the precipitation of IMD at any age, but triggers of IMD mainly seen in adults encompass prolonged fasting, hypercatabolic status, certain diets or drugs and pregnancies. The long-term accumulation of toxic metabolites can also be at stake in some typical adult-onset IMD. Ontogenic factors may underlie important phenotypic variability in IMD as metabolic networks and inter-organ interactions evolve with age. Ageing itself may influence the expression of IMD due to additional cellular modifications. This is especially true for the maintenance of energy homeostasis in the nervous system that is challenged with cellular ageing, as well as the accumulation mitochondrial DNA mutations accumulate with age, increasing somatic mutations, defective autophagy and increased risk for protein aggregation. In the era of expanded newborn screening, understanding mechanisms underlying pediatric versus adult phenotypic differences is even more critical. Metabolomics and lipidomics stand out among omics as the study of the end products of cellular processes, therefore are more likely to be representative of clinical phenotypes than genetic variants or changes in gene expression.

INBORN METABOLISM DEFECTS IN COMMON NEURODEGENERATIVE DISORDERS

LEONOR CORREIA GUEDES

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The cause of common neurodegenerative diseases, as Parkinson's disease, are still largely unknown, but as the genetic basis of rare subgroups is uncovered, also related metabolic defects are associated with these largely late adult onset neurodegenerative disorders. Having Parkinson's disease as a paradigm, various now related metabolic changes in neurodegenerative disorders will be presented, as well as new developments in therapeutic strategies having the correction of the metabolic defect as target.

Additionally, for the discussion on possible common pathways, clinical cases will be presented for illustrating the phenotypical overlap and difficult differential diagnosis between more common neurodegenerative diseases and rare metabolic disorders.

ETHICAL ISSUES IN THE TREATMENT OF ELDERLY PATIENTS

PATRÍCIO AGUIAR

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The improvement in the health care, the approval of new treatments and the recognition of late-onset / attenuated phenotypes led to an increased survival in patients with inherited metabolic disorders (IMD). This fact generated new challenges in the follow-up of adult patients with IMD, including treatment related ethical issues.

The presence of cognitive impairment in several IMD imposes ethical issues related go treatment initiation and discontinuation, because most of the available treatments do not cross the blood-brain barrier and the patients may experience improvement or stabilization of somatic manifestation, with concurrent progression in cognitive disabilities. Mucopolysaccharidosis affecting the central nervous system are examples of these types of disorders and few expert consensus statements have been published about this ethical issue.

In disorders not affecting the cognitive function other treatment related ethical issues have emerged, because the patients present a near normal life expectancy and most of the available treatments have not been tested in this age group and in patients with late-onset phenotypes. Moreover, the presence of irreversible organ damage or other conditions clearly reducing life expectancy may affect the treatment efficacy. Therefore, several guidelines have been published about treatment initiation and termination criteria.

Concluding, the development and approval of new treatments is warranted in IMD, but ethical issues about treatment of an ageing population with irreversible damage should be systematically addressed.

SELECTED POSTERS

SELECTED POSTERS | AMINO ACID DISORDERS

EXPRESSION, PURIFICATION AND FUNCTIONAL CHARACTERIZATION OF THE HUMAN TYROSINE AND TRYPTOPHAN HYDROXYLASES

PO-35

R. Sousa[1], D. Nunes[1], R. Padanha[2], R. Russo[2], P. M. P. Góis[2], P. Leandro[1], C. S. Tomé[1]

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The aromatic amino acid hydroxylases (AAAHs) phenylalanine hydroxylase (PAH), tryptophan hydroxylase (TPH) and tyrosine hydroxylase (TH) catalyze the initial steps in the biosynthesis of catecholamine and serotonin neurotransmitters. These homotetrameric enzymes share an identical structure, and use non-heme iron, the cofactor (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4), and O2 to hydroxylate their respective amino acid substrate. Mutations in the genes that codify these enzymes can affect their folding, catalysis and/or regulation, leading to physiological changes with severe clinical outcomes such as neurological and motor disabilities. These Inborn Errors of Metabolism (IEM) are rare and albeit the clinical advances accomplished in the last decades, pharmacological approaches to the treatment of hPAH, hTH and hTPH are still very limited or even unavailable.

The use of small molecules that stabilize (pharmacological chaperones) or activate (activity chaperones) the dysfunctional enzymes have emerged as a therapeutic approach for IEMs. Our team has designed a compound library towards the human AAAHs in order to identify hit compounds for the development of new treatments for AAAHs deficiency. This library has been tested and validated as modulators of hPAH. Herein we present the production, purification and functional characterization of the isoform 1 of recombinant human TH (hTH1) and isoform 2 of TPH (hTPH2). The cDNAs were cloned into the pHTP1 cloning vector, comprising a His6 and MBP tags at the protein N-terminus. The effect of culture media, induction time and temperature were investigated in order to optimize the heterologous expression of hTH1 and hTPH2 in E. coli BL21(DE3) cells. Affinity chromatography based on the His6 or MBP tags and size exclusion chromatography were optimized for protein purification. Enzymatic activities and kinetic parameters were determined through fluorimetric detection of the reaction products L-Dopa (hTH1) and 5-hydroxytryptophan (hTPH2). Preliminary assays to study the ability of our compound library to modulate the enzymes' stability and activity, as well as their specificity towards hPAH, hTH1 and hTPH2, are ongoing.

This work was supported by Fundação para a Ciência e Tecnologia (FCT), project PTDC/MED-QUI/29712/2017.

SELECTED POSTERS | AMINO ACID DISORDERS

MODIFICATIONS OF LYSINE RESIDUES OF HUMAN PHENYLALANINE HYDROXYLASE: EFFECT ON PROTEIN FUNCTION AND STABILITY

PO-39

A Dias[1]; R. R. Lopes[1]; P. Leandro[1]; L. Gonçalves[2]; M. L. Corvo[2]

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Phenylketonuria (PKU) is the most prevalent genetic disorder of the amino acid metabolism, and is due to mutations affecting the PAH gene which encodes for the liver human phenylalanine hydroxylase (hPAH). This protein is responsible for the conversion of phenylalanine (L-Phe) into tyrosine (L-Tyr). Presently, more efficient therapies are still lacking, being dietetic restriction and cofactor supplementation the main available approaches to treat PKU patients. Human PAH is a homotetramer with each subunit (50 kDa) presenting: an N-terminal regulatory domain, a catalytic domain and a C-terminal oligomerization domain responsible for dimerization and subsequent tetramerization. An enzyme reposition therapy (ERT) involving hPAH administration is challenging mainly due to the protein instability (high sensitivity to environment conditions), high molecular mass (200 kDa) and necessary maintenance of the biologic tetrameric state. An attractive approach to overcome these constraints involves the formulation of the protein as well design drug delivery system, namely in enzymosomes. This approach has already been developed for other enzymes as for example for superoxide dismutase (SOD) where it has been proved to efficiently maintain the enzymatic activity and structural stability while inducing a lower immune response (Corvo M. L. et al, Pharm Res (2015)32:91-102).

In this work, we performed optimization of the assay conditions for hPAH modification for further covalent attachment of the enzyme to liposomes (enzymosome). To this end, recombinant hPAH was produced and purified using a previously optimized prokaryotic expression system. The homotetrameric species, isolated by size exclusion chromatography, were modified using different ratios of hPAH:modifier agent (1:1; 4:1 and 8:1). The sucessful modified hPAH was isolated and tested for enzyme activity, L-Phe pre-activation, thermostability and quantification. By comparing the obtained data with those found for the non-modified hPAH, we observed no significant changes in the enzymatic activity and a slight increase in the melting temperature (Tm) of the modified hPAH. These data indicate that the modified protein preserves the catalytic function and stability. It is now possible to continue our studies and covalently attach the modified hPAH to liposome preparations.

This work was supported by Fundação para a Ciência e Tecnologia (FCT), project PTDC/MED-QUI/29712/2017.

SELECTED POSTERS | AMINO ACID DISORDERS

MODULATION OF THE IN VITRO AGGREGATION OF THE FULL-LENGTH AND A TRUNCATED FORM OF P.G46S HUMAN PHENYLALANINE HYDROXYLASE BY SMALL MOLECULES

PO-41

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Phenylketonuria (PKU; OMIM #261600) is an autosomal recessive disorder, caused mostly by missense mutation in the PAH gene, which encodes phenylalanine hydroxylase (hPAH). The majority of these mutations result in misfolded hPAH variants which, in the cellular context, will form soluble aggregates targeted for degradation, leading to a loss-of-function phenotype. Among these variants, the p.G46S is considered as an excellent model to the in vitro study of the hPAH aggregation process, as it presents a high tendency to self-associate and form non-amyloid fibrils, when overexpressed in E.Coli, and to be rapidly degraded in eukaryotic systems. The presence of the maltose binding protein (MBP) N-terminally to p.G46S stabilizes it and soluble metastable tetramers are obtained. Upon MBP tag cleavage with FXa protease the hPAH p.G46S aggregates in a process efficiently followed in vitro.

Our aim was to identify, among an in-house compound library, molecules able to modulate this behavior in vitro, and to characterize the underlying mechanism by studying the full length (FL) and a truncated form of p.G46S representing the unstable N-terminal regulatory domain (N1-118) harboring the allosteric site. For this, the p.G46S forms were expressed in a prokaryotic system in fusion with the MBP tag and purified (affinity and size exclusion chromatography). The rate of self-association of the isolated FL tetramers and N1-118 dimers were studied by real-time turbidimetry, upon cleavage of the MBP tag, in the presence and absence of studied molecules. The effect of these compounds on the FXa activity was firstly screened in order to rule out an inhibitory effect that could influence the cleavage rate.

From the tested molecules at least 2 delayed the p.G46S-FL aggregation, 3 inhibited and 3 had no influence on the aggregation behavior. Interestingly, when studying the effect on the p.G46S-N1-118, all combinations were obtained (FL aggregation/N1-118 aggregation inhibition; FL aggregation inhibition/N1-118 aggregation; etc). These data suggest that molecules might be binding to the regulatory domain in different locations (allosteric site or in another site). Identification of a binding site for molecules stabilizing the hPAH protein at a site different from the catalytic center or allosteric site (regulatory domain) could lead to a new class of molecules designed to act as hPAH stabilizers (pharmacological chaperones).

Work supported by FCT, project PTDC/MED-QUI/29712/2017

SELECTED POSTERS | AMINO ACID DISORDERS

MAPLE SYRUP URINE DISEASE (MSUD): THE INTERPLAY OF PLASMA BIOMARKERS

PO-72

João Caio[1], Cristina Florindo[1], Patricia Janeiro[2], Rita Jotta[2], Ana Gaspar[2] and Isabel Tavares de Almeida[1]

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MSUD is a metabolic disease caused by a deficiency in the branched-chain α-keto acid dehydrogenase complex, leading to the accumulation of branched-chain keto acids (BCKAs) and their corresponding branched-chain amino acids (BCAA) in tissues and bloodstream. The associated neurotoxic mechanisms have been attributed to the accumulation of leucine (Leu) and of keto-isocaproic (KIC). Leu and other large neutral amino acids share the same transporter through the blood brain barrier (BBB), having Leu a much higher affinity. Imbalance transport of LNAT1 substrates across the BBB alters protein turnover and monoamine neurotransmitter metabolism. Moreover, chronic elevation of LEU / KIC disturb cerebral transamination fluxes thus depleting the brain of glutamate/glutamine and may induce mitochondrial dysfunction through the inhibition of α-ketoglutarate and pyruvate dehydrogenase complexes, with repercussion on the Krebs cycle flux as well as on the oxidative homeostasis.

To provide new insights in the metabolic interplay of BCAAs and respective BCKAs with other metabolic players for long-term disease process in MSUD, we evaluated the correlation between BCCAs/BCKAs and between those and other non-essential amino acids (Ala, Gln) and neurotransmitter substrates (Tyr). For this purpose 11 MSUD patients were included in this study and the selected metabolites were evaluated in the plasma samples collected for treatment monitoring. BCKAs were analysed as quinoxalinol-TMS derivatives by GC-MS and amino acids were evaluated by GC-FID after extraction / derivatization using EZ:faast amino acid analysis kit or as OPA-derivatives by HPLC.

The results showed: strong reciprocal relationship (SRR) between Leu and KIC (Spearman correlation coefficient (SCC) =-0.9419; $p<0.0001$). We observed that even when Leu is within the normal range, in MSUD, KIC (25 - 42 μM) is above the control value (N.D. or vestigial).The pairs Val/KMV and Ile/KIV do not show a significant correlation, probably because the diet is Val and Ile supplemented, but the effect of high levels of KMV and KIV is not well known; KIC and Leu present a SRR with Ala (SCC= -0.8901; $p<0.001$ and SCC= -0.8241; $p<0.001$) and Gln (SCC= -0.7586; $0.001<p<0.005$ and SCC= -0.7186; $0.001< p<0.005$); SRR between KIC or Leu with Tyr has less significance (SCC=-0.541758; $p<0.01$).

These observations point to the potential relevance of the metabolites relationship for amino acids levels adjustments and indices of metabolic response.

SELECTED POSTERS | CARBOHYDRATE DISORDERS

GALACTOSE INTRACELLULAR METABOLITES: ITS ROLE FOR GALACTOSEMIA DIETETIC TREATMENT MANAGEMENT

PO-69

C Florindo[1], R Jotta[2], P Janeiro[2], A Gomes[1], J Caio[1], A Gaspar[2], I Rivera[1], IT Almeida[1]

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Galactosemias are inborn errors of galactose (Gal) metabolism caused by a deficiency in one of the enzymes that participate in the catabolic pathway of Gal: galactokinase (GALK), galactose-1-phosphate uridyltransferase (GALT) and UDP-galactose 4-epimerase (GALE). The blockage of the metabolic pathway leads to the accumulation of Gal and its derivatives: galactose-1-phosphate (Gal-1-P) and galactitol (Gti). Currently, the only treatment is restriction of galactose. Classic galactosemia (CG) is caused by GALT deficiency and presents long-term complications even on a galactose restricted diet. This is probably due to the interplay of accumulated Gal-1-P with other metabolic pathways. GALK deficiency (GALK-D) has a milder phenotype with the major clinical outcome being the formation of cataracts due to galactitol accumulation.

To provide a deeper understanding of the biomarkers that may reflect a dietary compliance in a long-term disease process, as well as a marker for differential biochemical diagnosis, CG (n=9) and GALK-D (n=2) patients on galactose-restricted diet, were regularly monitored for: red blood cells (RBC) Gal-1-P and Gti, plasma Gal and urinary Gti and Gal. Gal and Gti were quantified by stable isotope-dilution GC/MS-SIM and GAL-1-P was assessed by a standard spectrophotometric enzymatic assay.

Our results showed that:

(1) for CG and GALK-D groups, on a galactose-restricted diet, the urinary Gti levels, as well as the RBC Gti content, were not significantly different between both groups: urinary Gti for GALK-D and CG patients was, respectively 196.0 mmol/mol Crn (mean, n=3 range 164.5-212.8) and 185.4 mmol/mol Crn (mean, n=9; range 117.9- 333.6); age-paired healthy controls 9.8 (mean, n=12; range 2.1 - 31.0). RBC Gti mean content for GALK-D and CG patients was 6.5±0.1 (n=4) and 4.9±1.7 µmol/L (n=19), respectively; age-paired normal controls 1.1±1.0 µmol/L (mean, n=15); (2) for CG patients, RBC Gal-1P and Gti, although without significant correlation, Gli corroborate the role of Gal-1-P in diet monitoring;

(3) urinary Gti was greatly increased in GALK-D patients (>2400 µmol/mmol Crn), concomitantly with normal RBC Gal-1-P levels and high Gal, in a lactose containing diet.

Our results clearly point to the usefulness of urinary Gti as a predict marker of GALK-D. Urinary and RBC Gti, in GALK-D, reflect the dietary compliance. In CG, it may have a role in diet management since it responds earlier than Gal-1-P to a diet imbalance.

SELECTED POSTERS | CLINICAL STUDIES

BIOPSYCHOSOCIAL PROFILE OF 149 PKU PATIENTS FROM NEWBORN SCREENING FOLLOWED–UP AT CGM/CHUP THROUGHOUT LIFE

PO-19

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INTRODUCTION: Studies on patients with phenylketonuria (PKU) who started diet after newborn screening showed that the global intellectual impairment may be prevented by the initiation of a diet strictly phenylalanine (Phe)-reduced and supplemented with Phe-free amino acid mixture to maintain low Phe levels during childhood. However, a slightly decreased intellectual quotient (IQ) and specific cognitive impairments have been reported in early treated patients. Over the years, we tried to investigate these evidences also observed in the regular follow up of our patients. The aim was to understand risk factors and redefine the blood Phe values that may be considered as good dietary control to achieve the best global development of our patients.

METHODS: We studied 149 PKU patients (71 females and 78 males) aged 1 to 38 years early diagnosed at the neonatal screening. The neonatal screening blood Phe concentrations were considered as independent variable: according to PKU classification, we have three groups - hyperphenylalanimemia (screening values from 2,5 mg/dL to 5,9 mg/dL), moderate PKU (screening values from 6,0 mg/dL to 20 mg/dL) and classical PKU (screening values > 20 mg/dL).We considered Patient's outcome was evaluated according to the global quotient development (QD)/ intelligence quotient (IQ) value on the Griffiths Mental Development Scales, Wechsler Intelligence Scale for Children (WISC-III) and Wechsler Adult Intelligence Scale (WAIS-III), the quality of dietetic control (QDC), as well as educational level, school curriculum, and comorbidities. Differences between groups were analysed.

RESULTS: We observed significant differences between the PKU groups according PKU classification on DQ/IQ global values and QDC after the age of 6, with classical PKU patients having the lower mean DQ/IQ global values, the worse dietary compliance, the bigger percentage of individuals with adapted curriculum or special education and more severe comorbidities. Patients with hyperphenylalaninemia were the only ones showing a good QDC in all groups of age, with annual medians of Phe < 6 mg/dL. Patients born after 1992 have a better metabolic control with consequential better performance on the neurocognitive evaluations. Conclusion: These results point to the need for a regular psychological follow-up in the mild and classical forms of the disease with an intervention in different contexts to optimize a good metabolic control throughout life.

SELECTED POSTERS | CLINICAL STUDIES

ARTIFICIAL INTELLIGENCE (AI) IN RARE DISEASES: IS THE FUTURE BRIGHTER?

PO-44

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INTRODUCTION: The amount of data collected and managed in (bio)medicine is increasing. Thus, there is a need to efficiently collect, analyze and characterize all this information. Artificial intelligence (AI) holds a great promise in this area and is being successfully applied to basic research, diagnosis, drug discovery and clinical trial development. Rare diseases (RDs) which are severely underrepresented in basic and clinical research can particularly benefit from AI technologies. The ability of AI technologies to integrate and analyze data from different sources can increase diagnosis, the knowledge of disease pathophysiology and boost therapy development in RDs.

OBJECTIVES: This review aims to collect and summarize the AI approaches already being applied to RDs, with a special focus on Congenital Disorders of Glycosylation (CDG), a particularly orphan RD.

METHODS: A double and triple combination of specific keywords related to RDs, CDG, AI and Medicine/Pharmaceutics was used to search the Medline database, using PubMed, through its API interface Entrez Programming Utilities. To use API we wrote a Python programming language script using libraries from the biopython project. A total of 615 articles were found and after selection, 72 were included in this review.

RESULTS: We have found 44 AI and machine-learning methods used in RDs for: 1) mutation detection, prediction and classification; 2) genotype-phenotype integration; 3) medical decision support systems (DSS); 4) biochemical fingerprinting; 5) disease classification and characterization and 6) Therapeutic approaches (drug repositioning, clinical trials, patient registries, etc.). For CDG, AI has been used for: 1) disease mechanisms elucidation; 2) diagnosis, classification and characterization and 3) therapy discovery.

CONCLUSIONS: RDs face multiple challenges but the ability of AI technologies to integrate knowledge from several (bio) medical areas will ultimately boost therapeutic approaches in RDs.

SELECTED POSTERS | DIETETICS AND NUTRITION

SPECIAL LOW PROTEIN FOODS AVAILABLE IN PORTUGAL: WHAT WE KNOW?

PO-01

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BACKGROUND Inherited metabolic disease (IMD) are characterized by genetic defects that affects protein synthesis. These genetic defects leads to changes in nutrients metabolism leading to intoxication disorders. IMD are divided into groups accordingly to affected metabolism. Aminoacidopathies affects amino acid (AA) and protein metabolism, where essential AA are neither degraded or produced endogenously leading to the accumulation of some substances and deficit in others.

The management of these disorders is based on nutritional care, i.e. through a long-term natural protein restriction. Sothese patients require a special low protein (SLP) food products, designed to provide enough macro and micronutrients to ensure metabolic control and normal growth.

The study aim was to compare the differences of macronutrients (energy, proteins, fat, saturated fatty acids, carbohydrates, sugars and sodium) between SLP and regular food products.

METHODS In April 2019 data on the nutritional composition of SLP food products available in Portugal were collected from the information provided by the brands. The information led to the creation of an SPSS® database. The nutritional composition of regular food products was obtained from the Portuguese Food Composition Table, except some products whose information was collected from their labels. The products were divided into subgroups. Nutritional composition is expressed in weight per 100g or 100ml of the product.

RESULTS The fat content in SLP breads is higher than in regular ones (4.74g and 2.77g/100g, respectively, p=0.040). Low protein milk replacers have more fat (p=0.043) and saturated fatty acids (p=0.02) (2.68g and 1.53g/100ml, respectively) when compared with plant-based beverages (1.4 and 0.38g/100ml, respectively, p=0.02). SLP breakfast cereals have a higher content in carbohydrates (CHO) (92.23g to 77.27g/100g, p=0.034) and sugars(35.6g vs. 19.38g/100g, p=0.05) than regular ones. Meat substitutes show a significant difference in CHO (p=0.046) and sugars (p=0.043). Deserts have a significant difference in CHO (p=0.021) and sugars (p=0.02) content. Most SLP food products identified a limited number of mineral contents and didn't declare vitamin content.

DISCUSSION In SLP food products there is a need for a more detailed and complete label. Although these products have a low protein content the other macronutrients may be higher than in the regular food products which may prompt changes in nutritional.

SELECTED POSTERS | DIETETICS AND NUTRITION

MICRONUTRIENTS INTAKE FROM PROTEIN SUBSTITUTES IN PATIENTS WITH PHENYLKETONURIA: EFSA RECOMMENDATIONS ADEQUACY ASSESSMENT

PO-20

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BACKGROUND: The severe dietary restrictions used in PKU treatment, may lead to micronutrient imbalance. Optimal mineral and vitamin supplementation is not well defined in PKU. Considering that most protein substitutes (PS) are a well established source of micronutrients, we aimed to analyse the micronutrient adequacy % (MA) from PS according to EFSA recommendations.

METHODS: A sample of 64 early treated PKU patients, taking prescribed PS, who completed the annual nutritional status evaluation (ANSE) in 2018 was studied. They were aged 20.6±9.1y (52% females; 1 HPA, 38 mild PKU, 25 classical PKU). 51 patients were on a low phenylalanine diet treatment only whilst 13 were on BH4 and diet treatment. Patient's weight (kg), PS intake (g of protein equivalent/day) and micronutrient intake from PS was collected and compared with EFSA micronutrient recommendations. Deficient or excessive MA was considered when intakes were < 90% or > 110%. Micronutrient intake was not calculated from natural protein sources.

RESULTS: Mean weight of patients was 54.8±19.4 kg and median intake of protein equivalent from PS was 0.86 g/kg/day (45.3±18.2 g/day). More than 50% of patients exceeded the MA for Ca (n=42), Fe (n=55), Zn (n=48), P (n=37), I (n=42), choline (n=38), vitamin A (n=37), B1 (n=54), B2 (n=35) and B7 (n=39). At least 50% of patients had a deficient MA for Cu (n=52), Se (n=41), K (n=64), Mg (n=36), F (n=64), Mn (n=54), Mo (n=43), vitamin D (n=42), E (n=37), K (n=32), C (n=32) and B12 (n=41).

DISCUSSION: Since PS is prescribed according to protein needs, age, weight, PS composition and disease severity it will alter protein equivalent intake and compromise MA. More robust data on full dietary/nutrient intake in PKU is needed in order to better interpret micronutrient status in PKU.

SELECTED POSTERS | DIETETICS AND NUTRITION

CHARACTERIZATION OF FOOD PATTERNS IN PATIENTS WITH PHENYLKETONURIA

PO-21

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BACKGROUND: The dietary PKU treatment imposes a special food pattern. We aimed to analyse the food pattern in PKU patients: type/daily distribution of protein substitute (PS) used and natural protein (NP) sources.

METHODS: A sample of 99 early treated PKU patients (46% females; 24 HPA, 48 mild PKU and 27 classical PKU), aged 19.3±8.2 y, with an annual nutritional status evaluation in 2018 was studied. In the sample, 82 patients were on diet treatment and 17 on diet+BH4 therapy. Intakes of total protein (TP, g/kg), NP (g/kg) and protein equivalent from PS (PE, g/kg) were collected. Sources of NP, type of PS and its administration form/meal distribution were recorded from patient's dietary files.

RESULTS: Most patients were taking PS (n=64); 53 (83%) exclusively from Phe-free L-amino acid supplements (L-AA) and 10 (16%) from glycomacropeptide ± L-AA. PS powdered was used by 48% of the patients; liquid PS by 8% and 42% were on powdered and liquid PS. PS was taken with breakfast, afternoon snack and bedtime supper (97%, 84% and 78% of the patients, respectively). Most patients (N=57) took the PS in at least 3 doses. NP (g/kg) contributed a median of 34% [25%-51%] whilst PS contributed 66% [49%-75%] of TP intake. Potato, fruit, rice and vegetables were consumed by >91% of the 99 patients (99%, 98%, 95% and 90% respectively). Milk/yogurt was consumed by 70%, while pulses (30%), cheese/ham (33%) and meat/fish/egg (44%) were the least ingested food groups, these patients had a median NP (g/kg) of 1.4; 1.4; 1.5, respectively. High biological protein containing foods were prescribed in > 58% of the HPA patients, and in BH4 treated patients it was > 47%.

DISCUSSION: Most patients were taking PS, mainly in liquid form and divided at least in 3 meals. BH4 patients showed higher intakes of high biological protein, although not as liberal as in HPA patients. In PKU, further research is needed to clarify the best food protein sources and its impact on nutritional status.

SELECTED POSTERS | DIETETICS AND NUTRITION

THE CHALLENGES OF INTERPRETING MICRONUTRIENTS ADEQUACY IN PHENYLKETONURIA USING DIFFERENT NUTRITIONAL RECOMMENDATIONS

PO-22

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BACKGROUND: In PKU recent United States and European guidelines follow distinct recommendations, Food and Nutrition Board (FNB) and European Food Safety Authority (EFSA) respectively. We report in PKU patients, the micronutrient adequacy (MA) from protein substitutes (PS) using FNB or EFSA recommendations.

METHODS: A sample of 64 early treated PKU patients aged 20.6±9.1 y (52% females; 1 HPA, 38 mild PKU and 25 classical PKU) was studied. All patients were prescribed a PS and completed the annual nutritional status evaluation (ANSE) in 2018. 51 patients were on a low phenylalanine diet treatment only whilst 13 were on BH4 and diet treatment. Patient weight (kg), protein equivalent (g/kg/day) and micronutrient intake from PS were collected. MA was considered deficient or excessive when micronutrient intakes were < 90% or >110% of the FNB or EFSA recommendations, respectively.

RESULTS: Mean weight of patients was 54.8±19.4 kg and median protein equivalent intake from PS was 0.86g/kg/day (45.3±18.2 g/day). For Cu, Se, Mo, Vit. D and Vit. B12, the mean/median % of MA was systematically high when using FNB and low according to EFSA, respectively: Cu (145;65), Se (112;77), Mo (122;71), Vit. D (115;77) and Vit. B12 (153;77). There were inconsistent results for the other micronutrients: P (108;123), Mg (96;80), choline (99;119), Vit. K (63;93), Vit. C (114;90), Vit. B5 (116;107), Vit. B6 (143;103) and Vit. B9 (83;106).

DISCUSSION: A different MA from PS was observed in patients with PKU according to if FNB or EFSA recommendations were used. FNB generally recommended lower intake of micronutrients, resulting in increased MA for Cu, Se, Mo, Vit. D and Vit. B12. Our results suggest that consensus about nutritional requirements should be within the European PKU guidelines, preventing misleading interpretation of MA.

SELECTED POSTERS | DIETETICS AND NUTRITION

CHARACTERIZATION OF THE ADULT MSUD POPULATION FOLLOWED AT CENTRO HOSPITALAR UNIVERSITÁRIO LISBOA NORTE - HOSPITAL DE SANTA MARIA

PO-23

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Maple Syrup Urine Disease (MSUD) is a hereditary disorder of the branched-chain amino acid (AA) metabolism resulting from a deficit in the branched-chain alpha-keto acid dehydrogenase complex, responsible for the metabolism of leucine, isoleucine and valine. Early diagnosis is essential to prevent neurological damage. The treatment consists of diet with natural protein restriction, supplemented with branched-chain-free amino acid mixture, in order to satisfy protein, mineral and vitamin needs. Some patients are also responsive to the enzyme co-factor thiamine.

At our center, 6 adult patients with MSUD are currently under follow-up, 1 male and 5 females. The average ages are 29.3 ± 8.2 years. They have an average weight of 58kg ± 10.2kg. There average Body Mass Index (BMI) is 23kg / m2 ± 3.6kg / m2. The mean last LEU serum level was 5.6mg/dl (426.1µm/L) ± 1.8mg / dl (136.9 µm/L). Of all the patients, only one female with thiamine-responsive MSUD is on an unrestricted diet, with no need for supplementation with AA mixture. The remainder have an average of 51.4g, ± 8.3g of synthetic protein intake, which is equivalent to 0.8g/kg ± 0.2g of synthetic protein.

CONCLUSION: Our MSUD population has a normal BMI. According to the latest guidelines, LEU measurements in the last appointment are above the recommended (300 µm/L). Patient adherence to diet throughout life is fundamental to prevent MSUD decompensations, therefore adult patients need to be encouraged to maintain protein restriction even though they were stable during adolescence.

SELECTED POSTERS | DIETETICS AND NUTRITION

SERUM VITAMIN D LEVELS IN PATIENTS WITH PHENYLKETONURIA

PO-24

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BACKGROUND: Data on vitamin D (VitD) status in PKU population is scarce. This study aimed to evaluate VitD status in PKU patients compared with age-matched non PKU controls, using serum 25(OH)D levels. We also looked at the influence of the anthropometry, body composition and season variation on VitD levels in both groups.

METHODS: This is a Portuguese retrospective cross-sectional study, including 80 early treated patients (14.0 ± 6.5 years; 45.0% females) and age-matched 75 controls (16.5 ± 8 years; 60.0% females). They performed a nutritional status evaluation during 2010 or 2011, collecting data on anthropometry, body composition and serum 25(OH)D. Participants were classified with VitD adequacy (VitDa) when serum 25(OH)D levels were ≥ 20 ng/mL (50.0 nmol/L) according to the Institute of Medicine (IOM). When serum 25(OH)D was < 20 ng/mL they were considered with VitD inadequacy (VitDi). Additionally, in PKU patients, data on VitD intake [from amino acid mixtures (AAM) (n=80) and multivitamin/mineral supplements (MVS) (n=25)] was also collected.

RESULTS: We found higher mean serum 25(OH)D (ng/mL) and lower VitDi prevalence in patients compared with controls (25.67 ± 8.74 vs 17.89 ± 7.30, p<0.001 and 27.5% vs 65.3%, p<0.001, respectively). There were no differences in serum 25(OH) levels between normal weight and overweight/obese patients (26.39 ± 9.53 vs. 24.25 ± 6.89; p=0.304). In controls, a similar trend was found (17.34 ± 6.95 vs. 19.51 ± 8.25; p= 0.265). Also, body fat % was not different between participants with VitDa or VitDi (patients: 23.55 ± 9.89 vs. 19.86 ± 9.59, p=0.137; controls: 23.62 ± 12.10 vs. 22.80 ± 8.30, p=0.761). Our results showed a trend for a higher serum [25(OH)D] in controls between August and November. In contrast, a seasonal variation of serum 25(OH)D levels was not observed in PKU patients. There was a positive association between the total VitD intake from AAM and MVS, and the serum [25(OH)D] in patients with PKU (r=0.249, p=0.026).

DISCUSSION: Our results showed that VitD status in PKU patients is better than in controls. Weight, body fat and season did not influence serum [25(OH)D] of PKU patients. Nutritional monitoring is essential in PKU, and the use of AAM and MVS plays an important role in micronutrient adequacy.

SELECTED POSTERS | DIETETICS AND NUTRITION

PREVALENCE AND INCIDENCE OF OVERWEIGHT AND OBESITY IN PATIENTS WITH PHENYLKETONURIA: THE 10 YEAR-LONGITUDINAL TNSPKU STUDY

PO-26

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BACKGROUND: In phenylketonuria (PKU), there is limited data on overweight (OVW) and obesity (OB) trends. We aimed to describe the prevalence and incidence of OVW+OB of PKU patients of a Portuguese reference centre.

METHODS: 94 PKU patients, aged 14.0±7.8 y (48.9% females; 18 HPA, 43 mild PKU, 28 classical PKU, 5 late treated) have completed at least an annual nutritional status evaluation (ANSE), every 2 years (biennium), from 2009 to 2018. Nutritional intake, metabolic control and anthropometry data was collected. Out of the 94 patients, 62 were exclusively diet treated and 32 started BH4 treatment in 2015 (19 remained on BH4 until 2018).

RESULTS: In the 1st vs. last biennium mean dietary intakes were: natural protein (g/kg) 0.62 ± 0.51 vs. 0.87 ± 0.64; protein equivalent (g/kg) 1.08 ± 0.40 vs. 0.56 ± 0.43; total protein (g/kg) 1.70 ± 0.63 vs. 1.42 ± 0.41; and phenylalanine (Phe; mg/day) 800.7 ± 486.2 vs. 2434.6 ± 1778.1, respectively. Annual median blood [Phe] ranged between 5.0 [3.7-8.3] mg/dl in 2009 and 7.0 [4.8-9.9] mg/dl in 2018. OVW+OB prevalence along each biennium was: 24, 30, 32, 32 and 33%. In adults (>18y in 2009) OVW+OB prevalence showed a trend of: 33, 29, 38, 38, 38%. In each biennium, OVW+OB prevalence (%) seemed to be systematically higher in females vs. males: 28 vs. 21; 37 vs. 23; 35 vs. 29; 37 vs. 27; 37 vs. 29. Comparing the 1st and the last ANSE from each patient we found that 12% of normal weight patients developed OVW or OB. Out of 19 patients under BH4 therapy, OVW+OB prevalence in 2015-2016 and 2017-2018 was: 26 vs. 21%. Only 2/19 (11%) became OVW from the 1st to the last ANSE.

DISCUSSION: A trend for an increased prevalence of OVW+OB throughout the 10y study was found, with higher expression in adults and females. The 10y incidence of OVW+OB was 12%. Although the incidence was not higher in BH4 treated patients, studies with a longer drug exposure are warranted.

SELECTED POSTERS | DIETETICS AND NUTRITION

KETOGENIC DIET AND DRUG-RESISTANT EPILEPSY IN ADULTS: THE EXPERIENCE OF A TERTIARY-CARE CENTRE IN PORTUGAL

PO-49

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BACKGROUND: Drug-resistant epilepsy represents about 25% of all epileptic patients. Surgery may be considered, but other non-pharmacological treatments are possible, such as ketogenic diet. This is mainly used in paediatric patients, but is becoming an alternative in adults. Further experience and data on its effectiveness and safety is needed.

OBJECTIVE: To describe the experience of a tertiary-care epilepsy centre in Portugal in the management of adult patients with drug-resistant epilepsy on ketogenic diet.

METHODS: We performed a descriptive study and included 4 adult patients with drug-resistant epilepsy and frequent seizures. These patients were refused for surgery and a ketogenic diet was initiated.

RESULTS: Patient 1: 27-year-old female with bilateral parieto-occipital focal cortical dysplasia (FCD) and daily seizures (2-3/day) despite a polydrug regimen with zonisamide (ZNS), phenobarbital, rufinamide (RUF), levetiracetam (LVT), extended-release carbamazepine and clobazam (CLB). Vagus nerve stimulation (VNS) had no benefit. On ketogenic diet, there was > 50% reduction in seizure frequency. She showed an early and significant clinical and electrographic benefit and maintained the diet for 3 years. She is now on an adapted low-carb, low-calorie diet, maintaining good seizure control (max. 5 days without seizures). Patient 2: 25-year-old female with a left parieto-occipital FCD and daily seizures (3-4/day) despite treatment with ZNS, oxcarbazepine and LVT. Refused for surgery after invasive study, she is on a ketogenic diet for 1 year, with > 50% reduction in seizure frequency. However, parents are considering to stop the diet given an effort/benefit evaluation. Patient 3: 32-year-old male with hypoxic-ischemic ulegyria and daily seizures (2-3/day) despite treatment with LVT, lamotrigine (LTG), eslicarbazepine and clonazepam. He showed no significant clinical benefit with the ketogenic diet, probably due to poor compliance. Patient 4: 22-year-old male with extensive bilateral polymicrogyria and daily seizures despite treatment with RUF, LVT, valproate, LTG and CLB, and also VNS for 5 years. He started ketogenic diet a month ago and will be revaluated next week. In all patients the diet was well tolerated.

DISCUSSION: Ketogenic diet appears to be an effective and well tolerated non-pharmacological approach in adults with drug-resistant epilepsy, even without a metabolic aetiology. Adapted diets in adults may help improve compliance.

SELECTED POSTERS | DIETETICS AND NUTRITION

PSYCHOSOCIAL FACTORS AND METABOLIC CONTROL IN PKU PATIENTS FOLLOWED-UP AT CHUP BEFORE AND AFTER FULL SUBSIDY OF DIETARY PRODUCTS USED IN THE NUTRITIONAL TREATMENT

PO-52

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BACKGROUND: A natural protein and phenylalanine (Phe)-restricted diet, supplemented with protein substitutes and special low protein foods (SLPF) remains the mainstay treatment of phenylketonuria (PKU). High Phe levels are associated with neurotoxic effects that may interfere with neurocognitive development and socio-affective outcomes. To facilitate diet compliance, SLPF were fully reimbursed in Portugal since June 2005. Our aim was to compare PKU patients before and after the full reimbursement of SLPF taking into account their metabolic control, cognitive outcomes and psychosocial aspects.

METHODS: A retrospective observational study was conducted on 52 PKU patients considering two age groups: Group A - <12 years and group B - ≥12 years at 01/01/2006, in two periods (01/01/1997-31/12/2005 before reimbursement and 01/01/2006-31/12/2014 after reimbursement). The inclusion criteria were: PKU patients early diagnosed at the neonatal screening, followed-up at Centro Hospitalar Universitario do Porto (CHUP), with blood Phe levels at screening ≥6 mg/dL, following a restricted Phe diet supplemented with SLPF. Gender, age, PKU classification, metabolic control, global quotient development (QD)/intelligence quotient (IQ), school curriculum and comorbidities were evaluated.

RESULTS: We observed significant differences between the two age groups, with the group A (<12 years) having a better metabolic control than the group B in both periods of time (p<0,005). Considering the differences in metabolic control in both periods of time, we observed a significan t decreased in average annual medians of Phe between the two periods (before/after reimbursement) in the group B (≥12 years) (p<0,005). We also found significant improvement in IQ between the two periods in group A (p<0,005), despite no significant differences were observed on IQ values between the two groups. Comorbidities were more frequent in the group B (≥12 years) (A:20% vs B:54%). A normal school curriculum was more frequently observed in both groups (A:73% and B:65%).

DISCUSSION: Full reimbursement of SLPF improved significantly the dietary compliance resulting on a better metabolic control and allowing a better neurocognitive and socioaffective development. Studies taking into account others variables that may interfere with these outcomes should be done.

SELECTED POSTERS | DISORDERS OF FATTY ACID OXIDATION AND KETONE BODY METABOLISM

PROTEIN CONFORMATIONAL ANALYSIS – A KEY TOOL TO UNDERSTAND MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY (MADD)

PO-11

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Multiple-CoA dehydrogenase deficiency (MADD) is an inborn disorder of fatty acid and amino acid metabolism caused by mutations in the genes encoding for human electron transfer flavoprotein (ETF) and its partner electron transfer flavoprotein:ubiquinone oxidoreductase (ETF:QO). Albeit a rare disease, extensive newborn screening programs contributed to a wider coverage of MADD genotypes. However, the impact of non-lethal mutations on ETF:QO function remains scarcely understood from a structural perspective.

Here we revisit a riboflavin responsive-MADD (RR-MADD) clinically relevant variant, the ETF:QO-p. Pro456Leu, in order to clarify how the mutation affects enzyme structure and folding. Taking advantage of the higher expression levels of the bacterial Rhodobacter sphaeroides (Rs) ETF:QO in E. coli, and its 67 % sequence identity with the mature human ETF:QO protein, we performed a detailed in vitro investigation on Rs ETF:QO-p.Pro389Leu variant (homolog of the human RR-MADD variant) as a proxy for the effects on the human protein.

The in vitro biochemical and biophysical investigations showed that, while the mutation does not significantly affect the protein α/β fold, it introduces tertiary structure and flavin cofactor plasticity. Moreover, this variant presents a higher rate of FAD release under thermal stress as in fever episodes and increased flavin thermolability. Therefore, although this clinical mutation occurs in the ubiquinone domain, its effect likely propagates to the nearby FAD binding domain, probably influencing electron transfer and redox potentials.

Overall, our results provide a molecular rational for the decreased enzyme activity observed in patients and suggest that compromised FAD interactions in ETF:QO might account for the known riboflavin responsiveness of this variant.

SELECTED POSTERS | DISORDERS OF FATTY ACID OXIDATION AND KETONE BODY METABOLISM

NEPHROTIC SYNDROME IN AN ADOLESCENT WITH MULTIPLE ACYL-COA DEHYDROGENASE DEFICIENCY - A COINCIDENTAL FINDING OR A LATTER COMPLICATION?

PO-33

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INTRODUCTION: Multiple acyl-CoA dehydrogenase deficiency (MADD) is a rare inborn disorder of electron transport flavoprotein metabolism, due to ETFA, ETFB or ETFDH gene mutations, with impairment of fatty acid oxidation and the catabolism of a few amino acids. Little renal impact has been described in these patients, except for congenital renal cyst dysplasia in severe forms.

CASE DESCRIPTION: We present an 18YO boy diagnosed with MADD at the age of 4YO, in the sequence of global developmental delay detected on the 2nd semester of life, leukodystrophy and intermittent vomiting. Diagnosis, based on metabolite and enzymatic analysis, was confirmed by the detection of a c.1601C>T mutation in the ETFDH gene in homozygosity. He was treated with a low-fat diet with moderately restricted protein, riboflavin and levocarnitine. Evolution was marked by several episodes of metabolic decompensation (vomiting, prostration with or without hypoglycemia and myalgia) and moderate intellectual deficit, with stabilization of brain MRI lesions. Growth was normal. At 17 YO, he presented with anorexia and progressive asthenia of 3-month length which caused significant weight loss (21% in 1 year) and inability to walk. Later on, edema emerged. Investigation revealed nephrotic proteinuria (88mg/m2/h; protein/creat=5,6mg/mg), hypoalbuminemia and hypercholesterolemia. Glomerular filtration rate (GFR), C3, C4, cardiac and renal ultrasound were normal. No evidence of metabolic crisis, rhabdomyolysis or metabolic acidosis was found. Kidney biopsy revealed focal and segmental mesangial proliferative glomerulonephritis, with a predominance of C3 deposits. After 4 weeks on prednisolone 60mg id and coenzyme Q10 100mg id, appetite improved and he could walk again. Along with proteinuria improvement, prednisolone was gradually reduced. Enalapril was introduced at 10 weeks, reaching non-nephrotic proteinuria (protein/creat=0,9mg/mg). Currently, on prednisolone 20mg on alternate days, enalapril 10mg/day, he presents non-significative proteinuria and maintains normal GFR.

DISCUSSION: To our knowledge, this is the first MADD patient with nephrotic syndrome. Although the association could be coincidental, there are reports of MADD due to ETFDH gene mutations with secondary CoQ10 deficiency. Interestingly, nephrotic syndrome is one of the clinical presentations of CoQ10 synthesis deficiency. Further studies are needed to clarify whether the nephrotic syndrome is a latter complication of MADD.

SELECTED POSTERS | DISORDERS OF FATTY ACID OXIDATION AND KETONE BODY METABOLISM

INSIGHTS INTO THE STRUCTURE OF VARIOUS MEDIUM-CHAIN ACYL-COA DEHYDROGENASE VARIANTS

PO-34

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Medium-chain acyl-CoA dehydrogenase deficiency (MCADD), the most common inborn error of mitochondrial fatty acid β-oxidation (mFAO), results from an impaired activity of medium-chain acyl-CoA dehydrogenase (MCAD). This homotetramer flavoprotein, involved in the first step of mFAO, catalyse the dehydrogenation of medium-chain acyl-CoAs (C6-C12). Each human MCAD (hMCAD) monomer (43.6 kDa) is arranged in three structural domains: two alpha-helical domains in both N- and C-terminus and an intermediary beta-sheet domain. Many of the identified hMCAD amino acid substitutions are associated with protein misfolding and a loss-of-function phenotype affecting MCADD patients during metabolic stress episodes. As MCADD is considered a conformational disorder without an available pharmacological treatment, identification of small molecules as stabilizers of the hMCAD structure will have the potential to rescue protein function, representing an attractive therapeutic strategy to decrease the morbidity and mortality associated with MCADD. To identify this class of compounds a deep understanding of the impact of the identified amino acid substitutions on hMCAD structure and response to its cofactor (FAD+) is necessary.

With this aim, various hMCAD variants were recombinantly produced and structurally studied. After protein purification, the oligomeric profile, proteolysis susceptibility, thermostability, FAD content and apparent FAD+ binding affinity were determined. Interestingly from the studied variants, the p.Y372N was recovered mostly as dimers (67%) while the p.Y48C also presented a high content of dimers (21%). Two variants were unable to respond to FAD+ (p.D143V and p.Y372N). Resistance of hMCAD variants to protease degradation was not significantly affected, except for the p.D143V and p.Y372N suggesting a destabilized tertiary structure. FAD+ induced a more rigid conformation (except for the mutants unable to incorporate FAD+). The p.Y48C and p.Y372N also demonstrated a lower thermal stability.

Among the studied hMCAD variants the p.Y48C, p.D143V and p.Y372N are the variants presenting higher structural changes. Taken together our data indicate that different structural events are leading to hMCAD impaired activity and that probably not all the studied variants present a lower stability. For these variants other mechanisms must be investigated.

Work supported by FCT, project PTDC/BIA-BQM/29570/2017

SELECTED POSTERS | DISORDERS OF FATTY ACID OXIDATION AND KETONE BODY METABOLISM

FUNCTIONAL EVALUATION OF VARIOUS HUMAN MEDIUM CHAIN ACYL-COA DEHYDROGENASE (HMCAD) VARIANTS USING THE NATURAL ELECTRON ACCEPTOR: INSIGHTS INTO THE INTERACTION WITH THE ELECTRON TRANSFERRING PROTEIN

PO- 40

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Medium-chain acyl-CoA dehydrogenase (MCAD) belongs to the acyl-CoA dehydrogenase family involved in the mitochondrial fatty ?-oxidation pathway. Human MCAD (hMCAD) is a flavoenzyme responsible for the dehydrogenation of medium-chain acyl-CoA (chain length C6-C12) and contributes to the production of energy by feeding the respiratory chain. This communication involves hMCAD interaction with the matrix soluble electron transferring protein (ETF), a flavoprotein which in turn interacts with ETF-QO, a mitochondrial internal membrane enzyme that finally transfers the electrons to ubiquinone (respiratory chain). MCAD deficiency (MCADD) is an autosomal recessive hereditary disease caused by mutations in the ACADM gene. About 90% of MCADD patients present the p.K304E mutation (mature protein numbering). In the remaining MCADD patients rare mutations have been identified but their characterization is still missing. Treatment of MCADD relies on a dietetic approach to avoid hypoglycaemia, which can lead to coma and death.

Due to the identified high number of missense mutations resulting in misfolded hMCAD variants, MCADD is considered a loss-of-function conformational disorder, being a candidate for the treatment with small molecules able to stabilize conformationally unstable variants.

In this work we aimed to clarify the molecular mechanism of ten hMCAD variants by studying their interaction with ETF. For this, recombinant hMCAD variants and the heterodimeric ETF were produced in a heterologous expression system and further purified. The hMCAD variants were characterized by enzyme activity and kinetic parameters (Km, Vmax and Kcat) determination using an UV-spectrophotometric assay, the octanoyl-CoA as the substrate and the artificial redox pair (PMS/DCPIP). The T1/2 values were also determined as the thermal enzyme inactivation profiles strongly correlates with protein conformational changes. When compared to the wild-type form, a reduced specific enzymatic activity was observed for all hMCAD variants with the p.Y372N and p.G377V presenting no activity. The hMCAD variants presented lower Vmax but different effects on the catalytic efficiency due to different affinities for the substrate (lower or higher). The majority of the studied variants also presented lower T1/2. Enzyme activity and pull-down assays in the presence of ETF are ongoing in order to study hMCAD/ETF interactions.

Work supported by FCT, project PTDC/BIA-BQM/29570/2017

SELECTED POSTERS | DISORDERS OF FATTY ACID OXIDATION AND KETONE BODY METABOLISM

LONG CHAINS MITOCHONDRIAL FATTY ACID OXIDATION DISORDERS (MFAOD) – ONE PATHWAY, DIFFERENT STORIES

PO-65

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Long chains MFAOD have a wide spectrum of clinical presentations, ranging from severe neonatal hypoglycaemia with lactic acidosis, cardiomyopathy and hepatopathy, to milder forms presenting at later ages as chronic weakness, recurrent rhabdomyolysis or cardiomyopathy.

Retrospective analyses of the Long-chain β-Oxidation-Defects and Carnitine Cycle Defects evaluated in a National Reference Center for Inborn Errors of Metabolism.

Thirteen patients were included, four Carnitine palmitoyltransferase II deficiency (CPTII-D), one Carnitine aylcarnitine translocase deficiency (CACT-D), four Very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD-D), and four Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD-D). CACT-D presented at 48h of life with severe hypoglycaemia and hyperammonaemia dying within 5 days. Of the CPTII-D, one had severe neonatal onset with hiperammonaemic coma and progressive hypertrophic cardiomyopathy and myopathy, dying at 4 years-of-age, two presented in the first 3-years-of-age as intermediate forms, and one at adolescence as recurrent rhabdomyolysis. All VLCAD-D were diagnosed pre-symptomatically on neonatal screening. Two LCHAD-D patients were detected on neonatal screening, one with rhabdomyolysis and hepatitis at evaluation; one patient presented in the 2nd year-of-life with heart failure and other at 4-years-of-age with recurrent rhabdomyolysis.

At follow-up, none of the surviving CPTII-D (1, 13 and 16 years-old) or VLCAD-D (10, 2 years and 7 months-old; one lost to follow-up) has cardiac, neurologic or ophthalmological involvement; three patients have mild developmental disorders. Of the LCHAD-D (6,10-16 years-old), 75% developed cardiomyopathy with ventricular dysfunction and sensitive axonal polyneuropathy (10-16 years-old) and all retinopathy; 50% have development disorders and 50% psychiatric comorbidities; obesity is present in 75%. The majority of patient had multiple metabolic crises with rhabdomyolysis.

The authors highlight the variable presentation and natural history of Long chains MAFOD influenced by residual enzyme activity, age of presentation and exposure to stress. CACT-D and neonatal forms of CPTII-D are particularly severe with a high mortality risk. LCHAD-D is associated with significant long-term morbidity, with cardiac, neurologic and ophthalmological dysfunction. VLCAD-D and later forms of CPTII-D frequently have a milder phenotype with recurrent rhabdomyolysis being the main concern.

SELECTED POSTERS | DISORDERS OF PURINES, PYRIMIDINES, NUCLEIC ACIDS AND PORPHYRIAS

PORPHYRIA: THE NEGLECTED DIAGNOSIS

PO-15

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The porphyrias are a group of eight rare mostly inherited metabolic disorders resulting from a variable of eight catalytic defect enzymes involved in the heme biosynthesis pathway, depending on the specific enzyme involved. Porphyrias are classified into two major categories: 1) the acute or inducible porphyrias and 2) the chronic cutaneous porphyrias. Acute attacks of porphyria, namely acute intermittent porphyria (AIP), porphyria variegate (VP), coproporphyria hereditary (HCP), and porphyria due to severe deficiency of delta-aminolevulinic acid (ALA) dehydratase (ALADP), although they are multisystem diseases with a variety of clinical features or symptoms, there are no pathognomonic signs, but these acute porphyrias have in common the abdominal pain.

Many different environmental factors or pathological conditions often play a key role in triggering the clinical exacerbation (acute porphyric attack) of these diseases. Prognosis is good if the condition is recognized early and treated aggressively, otherwise could be fatal.

The patterns of porphyrin accumulation in erythrocytes and plasma and excretion of the heme precursors in urine and feces allow for the detection and differentiation of the porphyrias. In order to obtain an accurate diagnosis of acute porphyria, the knowledge and the use of appropriate diagnostic tools are mandatory. Random urinary porphobilinogen (PBG- Hoesch test) is the most important rapid and easy test for diagnosis an acute porphyria. Then, in the setting of a positive urinary PBG screening test, the diagnosis of an acute porphyria is confirmed by measuring quantitative ALA, and also screening the porphyrins profile from the same urine sample that was used for the initial rapid screening test. High-performance liquid chromatography (HPLC) is the standard sensitive method for differentiating the types of porphyria.

In Portugal, there is little or none knowledge about porphyria prevalence in our population. We intend with this study, first alert for the necessity of thinking porphyria in presence of an unexplained severe abdominal pain, then, screening for the acute attacks of porhyria, providing some recommendations for the diagnostic steps of an acute porphyria and finally, the more we know about these diseases the more we can contribute to the understanding of porphyric neuropathy pathogenesis.

Keywords—Acute porphyria; Abdominal pain; Haem metabolism, Diagnostic tools

SELECTED POSTERS | DISORDERS OF PURINES, PYRIMIDINES, NUCLEIC ACIDS AND PORPHYRIAS

ACUTE INTERMITTENT PORPHYRIA PRESENTING AS SUBARACHNOID HAEMORRHAGE

PO-38

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Acute intermittent porphyria (AIP) (OMIM # 176000), an autosomal dominant disorder, occurs in heterozygotes for an HMBS pathogenic variant that causes reduced activity of the enzyme porphobilinogen deaminase.

It's very low penetrance and nonspecific neurovisceral symptoms can often mislead to wrong diagnosis.

We present the case of a previously healthy 38-year-old female patient who carried a pathogenic variant on HMBS gene. It was identified when she was 10 years old following a family screening study. She had no history of porphyria attacks, wasn't on any regular medications and didn't have any medical follow-up.

After two weeks of a carbohydrate restricted diet to lose weight, she presented to the emergency department with abdominal pain, nausea, vomiting and unusually dark urine. She underwent an abdominal CT which was normal and was discharged with the diagnosis of gastroenteritis. Two days later she was taken to the emergency department with a generalized tonic-clonic seizure. On admission she presented with GCS 12, with neck stiffness, severe transient hypertension and tachycardia. Laboratory findings of euvolemic hyponatremia (129 mEq/L), elevated transaminases (aspartate transaminase of 245 U/L and alanine transaminase of 75 U/L) and rhabdomyolysis (creatine kinase of 15579 IU/L). A Hoesch test was performed in the emergency department, with a positive result. Craniocerebral CT revealed a subarachnoid haemorrhage of the Sylvian fissure. Angiography confirmed the diagnosis of an aneurysm of the left middle cerebral artery trifurcation. She underwent a craniotomy with clipping of the aneurism and was admitted to the intensive care unit for neurological surveillance. She was medicated with a 4-day course of hemin and was hydrated with dextrose in normal saline, with no recurrence of abdominal pain, hypertension and seizures. The chromatographic profile of porphyrins in the urine with HPLC-fluorescence detection demonstrated a uroporphyrin of 861 ug/L (R.V. < 20 ug/L), a coproporphyrin of 1650 ug/L (R.V. < 50 ug/L) and total urine porphyrins of 2750 ug/L (R.V. < 100 ug/L).

This rare presentation demonstrates the importance of the early diagnosis of AIP attacks. In this specific case, we propose that the transient hypertension during the attack may have caused the rupture of the intracranial aneurysm. The previous knowledge of the carrier state for an HMBS pathogenic variant was essential because it leaded to adequate treatment of the AIP attack.

SELECTED POSTERS | DISORDERS OF VITAMINS, COFACTORS AND TRACE ELEMENTS

NEONATAL PRESENTATION OF THIAMINE METABOLISM DYSFUNCTION SYNDROME 2 - A CASE REPORT

PO-58

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INTRODUCTION: Thiamine metabolism dysfunction syndrome-2 (THMD2; MIM#607483), is a rare autosomal recessive disorder caused by mutations in the SLCN19A3 gene. It is characterized by acute episodes of encephalopathy and symmetric lesions in basal ganglia, thalami or periaqueductal grey matter, and less frequently in the cortex, with a dramatic response to biotin and/or thiamine supplementation.

CASE REPORT: We describe a female newborn, the first child of non-consanguineous healthy parents, born after uneventful pregnancy and delivery. Extended newborn screening was normal. Poor feeding and failure to thrive were a complaint since birth. She presented to the hospital with irritability and episodes of lethargy from the age of 25 days. Absence of visual interest, weak cry, and generalized hypotonia with periods of opisthotonos and seizures were noticed. Brain MRI revealed extensive and relatively symmetrical lesions with restricted diffusion, involving all cerebral lobes, with posterior predilection, the splenium of corpus callosum, the lentiform nucleus and internal capsule, with extension to cerebral peduncles and protuberance. MR spectroscopy suggested the presence of a lactate peak. Electroencephalogram disclosed encephalopathic baseline rhythm and multifocal paroxysmal activity. Initial investigation, including amino acids, organic acids and acylcarnitines was inconclusive, except for moderate hyperlactacidemia (3.7 mmol/L; r.v.<2,1). An oral multivitamin cocktail (thiamine, biotin, riboflavin and co-enzyme Q10) was started on day 30 of life. She improved dramatically after 1 to 2 days (consciousness recovered and seizures and feeding difficulties ceased) and was discharged on day five of multivitamin treatment. Genetic analysis revealed a previous reported pathogenic homozygous mutation of SLC19A3 (c.74dupT).

Currently she is three months-old and is on thiamine (30mg/kg/day) and biotin (20mg/day) supplementation. Despite the initial improvement and adequate weight gain, generalized hypertonia, notion of impaired vision and inconstant eye pursuit are present.

CONCLUSION: Although rare, vitamin-responsive disorders like THMD2 should be early addressed and treated, even before diagnostic confirmation, since symptomatic treatment can be achieved and final diagnosis is not delayed. THMD2 should be considered in any infant with acute epileptic encephalopathy.

SELECTED POSTERS | DISORDERS OF VITAMINS, COFACTORS AND TRACE ELEMENTS

HYPOPHOSPHATASIA: FROM THE CLINICAL PRESENTATION TO THE DIAGNOSIS

PO-59

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Hypophosphatasia is a rare inherited disorder characterized by abnormal mineralization of bones and/or teeth, in the presence of reduced activity of serum alkaline phosphatase. The real prevalence of this disease is not known, but the prevalence of the severe form has been estimated in 1/300.000 births in Europe.

The clinical spectrum is very wide and six different clinical forms have been described: i) prenatal benign form; ii) perinatal lethal form; iii) childhood-onset form; iv) adult form; v) odontohypophosphatasia; and vi) pseudohypophosphatasia. Each form has a different clinical presentation, different levels of severity and different prognosis.

The diagnosis is based on laboratory and molecular testing of ALPL gene to detect mutations.

The authors present a case of a 44 years old female with osteoarticular pain, since her childhood, which has been increased along the years; she reported an episode of muscle paralysis at age of 17, multiple bone fractures with minimal trauma (fist, humerus, ribs, metatarsus, coccyx and knee) and documented osteoporosis at the age of 30. She was observed by different medical specialties and had performed an exhaustive investigation, which was not conclusive. The diagnosis of hypophosphatasia was achieved after twenty-eight years of clinical symptoms. At the moment, this patient is under supportive symptomatic treatment, with analgesic and orthopedic management, and in evaluation for enzyme replacement therapy.

The authors expose the case, discuss the differential diagnosis and explain the particularities of hypophosphatasia.

SELECTED POSTERS | DISORDERS OF VITAMINS, COFACTORS AND TRACE ELEMENTS

MENKES DISEASE - DIAGNOSTIC CHALLENGE AND THE NEED FOR EARLY TREATMENT

PO-70

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INTRODUCTION: Menkes disease is a rare, x-linked recessive disease, that affects copper levels in the body, caused by mutations in the ATP7A gene. It is characterized by sparse, kinky hair; failure to thrive and deterioration of the nervous system. Weakened bones (osteoporosis) are common and can result in fractures Supplementation with copper histidine showed improvement in prognosis, particularly if started before the 10th day of life. It is intended to review cases of Menkes disease followed in consultation of Metabolic Diseases.

METHODS: Observational retrospective study of patients with Menkes disease followed in CHUP, between 2002 and 2019. The clinic presentation, biochemical parameters and treatment were analyzed.

RESULTS: 4 cases were diagnosed. In three cases, clinical suspicion arose from fractures in the neonatal period and epilepsy and in a patient for epilepsy at 3 months, associated with dysmorphia and typical hair. All had hypotonia, low weight and psychomotor delay, but only 3 had typical hair. None of them had family history. The diagnosis was established between the 1st and 6th month of life by decreased serum copper and ceruloplasmin and confirmed by molecular study. A mother carries the mutation; in another case the mother refused the genetic study. Two children began treatment with copper histidine, between the 3rd and 5th months of life. They're both alive, with 20 months and three years old. They have severe psychomotor delay. The two untreated cases died at 22 and 23months.

CONCLUSION: The diagnosis of Menkes' disease in the neonatal period is very difficult, making the start of treatment away for the ideal period. The most specific signs of the disease appear later in life, contributing for the delay in diagnosis and this way compromising he prognosis.

SELECTED POSTERS | DISORDERS OF VITAMINS, COFACTORS AND TRACE ELEMENTS

CBLG REMETHYLATION DEFECT – A RARE ETHIOLOGY OF MACROCYTIC ANEMIA

PO-73

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BACKGROUND: Cobalamin G (CblG) defect is a rare inborn error of intracellular cobalamin metabolism caused by deficiency of methionine synthase (MIM 250940), leading to an impairment in homocysteine to methionine remethylation. Patients usually present in infancy with failure to thrive, feeding problems, developmental delay, muscular hypotonia, cognitive impairment and macrocytic anemia with megaloblastic features.

CASE REPORT: We report the case of a 15 month-old-girl, first child of a healthy unrelated couple, with uneventful full-term pregnancy, delivery and neonatal period and exclusively breastfed until 5 months, who presented at 6 months of age with poor weight gain, global developmental delay and hypotonia. Laboratory investigation showed severe arregenerative macrocytic anemia (Hb 5,2 g/dL, MCV 102 fL), moderate neutropenia (610 x10^6) and elevated LDH. Vitamin B12 and folic acid deficiency were excluded. Bone marrow biopsy excluded dyserythropoiesis.

Further investigation showed markedly elevated homocysteine (130 µmol/l), low plasma methionine and normal urinary methylmalonic acid, suggesting a CblE/CblG disorder of intracellular cobalamin metabolism.

Treatment was started with hydroxocobalamin, betaine, folic acid and L-carnitine There was an excellent hematological and biochemical response and significant improvement on neurocognitive development. Genetic testing (Next-Generation Sequencing panel for genes associated vitamin B12 deficiency) revealed two variants in heterozygosity in the MTR gene: a pathogenic frameshift c.2101del variant and an uncertain clinical significance (missense) c.1228G>A variant supporting CblG deficiency. Parental studies are ongoing

COMMENTS: Cobalamin metabolism defects are a very rare cause of megaloblastic anemia and a high level of suspicion is needed, especially after excluding the main causes, as vitamin B12 and folic acid deficiency. In the presented case the classic triad of hyperhomocysteinemia with normal levels of methylmalonic acid and low levels of methionine strongly suggested the diagnosis, leading to prompt treatment.
In previously reported cases early treatment corrects cytological abnormalities, improves biochemical parameters but has limited effect on neurocognitive impairment and ophthalmologic and CNS changes tend to progress despite it. In the presented case a developmental improvement was noted in the beginning, but long term follow-up is required.

SELECTED POSTERS | GLYCOSYLATION DISORDERS/CDG, PROTEIN MODIFICATION DISORDERS

A NOVEL VARIANT OF SSR4 CONGENITAL DISORDER OF N-LINKED GLYCOSYLATION: A CASE REPORT

PO-31

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BACKGROUND: SSR4-CDG is a congenital disorder of N-linked glycosylation (CDG-N-linked) caused by mutations in the gene SSR4 (Xq28) that encodes one of four signal sequence receptor proteins involved in the translocon (TRAP) complex, which aids in the translocation of proteins across the membrane of the endoplasmic reticulum. SSR4 mutation reduces expression of TRAP, induces ER stress and leads to underglycosylation. Only 9 cases were reported so far.

CASE REPORT: We present an 11-year-old male, referred to genetic outpatient clinic for investigation of facial dysmorphism (deep set eyes, large ears, large mouth, with widely spaced teeth), microcephaly, failure to thrive, global developmental delay, intellectual disability, hypotony, strabismus and hyperopia.

Extensive genetic investigation terminated in molecular analysis with exome sequence that revealed a de novo variant in SSR4 gene involving exon 4 in hemizygozity. This mutation was not described in the literature, however consist in the replacement of arginine with a stop-codon, c.268C>T p.(Arg90*), originating a truncate protein. Database analysis suggested the variant is pathogenic and predict a loss of SSR4 function. Another mutation was identified in KCNH2 gene responsible for long QT syndrome (AD), to this point asymptomatic.

Further characterization of SSR4-CDG diagnosis included: normal transaminases, coagulation and endocrinologic tests; thin corpus callosum; normal echocardiogram and prolonged QTc. Investigation of transferrin isoform pattern, usually abnormal in SSR4-CDG, is ongoing.

COMMENTS: Our patient phenotype shows many overlapping features with the previous nine SSR4-CDG cases reported in the literature. However, these clinical features are commonly seen in many CDG subtypes as well other genetic diseases. Only exome sequencing techniques provided an explanation for the clinical findings, improved medical follow-up and treatment of complications, and gave genetic counselling to the family.

KEYWORDS: Congenital disorders of N-linked glycosylation, signal sequence receptor subunit 4, translocon-associated protein, neurological abnormalities.

SELECTED POSTERS | GLYCOSYLATION DISORDERS/CDG, PROTEIN MODIFICATION DISORDERS

CDG DIAGNOSIS: A SIMPLIFIED GUIDE FOR DIFFERENT STAKEHOLDERS

PO-43

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Congenital disorders of glycosylation (CDG) and their diagnosis are continuously updated research topics. CDG diagnosis is most of the times a challenge due to the fact that CDG are a rapidly growing family of genetic diseases of over 130 different types but also because there is huge intra and inter clinical heterogeneity. Moreover, the classical screening test, serum transferrin isoelectrofocusing, has some pitfalls as it cannot be used for every CDG type and can also generate false negatives even for PMM2-CDG, the most frequent N-glycosylation defect.

The objective of this work was to address the challenges of CDG diagnosis by representing an overview of the different approaches that help CDG diagnosis. We performed a literature review and identified the clinical and biochemical hallmarks of these diseases as well as the biochemical and genetic testing available. A list of symptoms on different identified CDG is also provided.

This work resulted in the following practical tools: (1) Clinical manifestations reported in CDG per organ/system; (2) Biochemical and laboratory findings reported in CDG per organ/system; (3) Clinical features suggestive of distinct CDG; and (4) a diagnostic decision tree in face of a patient with a suspicion of CDG.

We hope that this work can act as: (i) a CDG diagnosis simplified guide for healthcare professionals but also for families and researchers; (ii) an awareness and lobbying tool to help in the effectiveness and promptness of CDG diagnosis. (Work published as scientific work: Mol Genet Metab. 2019 Jan; 126(1):1-5. doi: 10.1016/j.ymgme.2018.11.003.)

SELECTED POSTERS | GLYCOSYLATION DISORDERS/CDG, PROTEIN MODIFICATION DISORDERS

SIX MONTH TRIAL OF ORAL D-GALACTOSE IN A PATIENT WITH SLC35A2-CDG

PO-57

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BACKGROUND: Variants in the SLC35A2 gene (MIM#300896), on the X chromosome, codes for the transporter of UDP-galactose to the Golgi apparatus and results in a N-linked congenital disorder of glycosylation (CDG). The phenotype includes developmental delay, early epileptic encephalopathy with hypsarrhythmia, hypotonia, dysmorphic features, skeletal abnormalities and cortical visual impairment.

As some CDGs have been successfully treated with hexose supplementation (e.g., galactose for PGM1-CDG, fucose for SLC35C1-CDG, or mannose for MPI-CDG) it was been considered a possibility in SLC35A2-CDG. The rational is that galactose might be able to increase the cytosolic UDP-galactose concentration and by this way increase galactose transport and use in the Golgi apparatus. We report our experience with a six-month trial of oral D-galactose supplementation in a female patient with SLC35A2-CDG.

CASE REPORT: A 2-year-old female patient with a diagnosis of SLC35A2-CDG, presented at 2 months of age with epileptic encephalopathy with hypsarrhythmia, developmental delay, axial hypotonia, limb hypertonia and slight dysmorphic features. Epilepsy was difficult to control with multiple trials of medication including ACTH, topiramate, vigabatrin and levetiracetam. With the association of ketogenic diet, partial control of seizures was achieved.

Due to promising reports on the use of D-galactose, she started on the dose of 1g/Kg/day, 4qid, with adjustments on her ketogenic diet.

After six months of supplementation, the mother reported slight improvement in attention, social interaction and head control. Seizures control was difficult to compare as there were temporal increase during respiratory infections. The only side effect reported was transient abdominal pain, without diarrhea. As the patient had several controls with abnormal type 2 transferrin isoelectric focusing (TIEF), it was used as a biomarker for therapy efficacy monitoring. In spite of transferrin glycosylation focusing pattern completely normalized after therapy, no improvements on EEG were noted.

COMMENTS: In our case, oral D-galactose was well tolerated, and improvement in attention, social contact and head control was observed, whilst dubious effect on epilepsy. As reported previously, TIEF completely normalized. Combining galactose supplementation and ketogenic diet was a challenging issue. A longer follow-up is needed to ascertain therapeutic efficacy.

SELECTED POSTERS | INBORN ERRORS OF METABOLISM IN ADULTS

ACUTE METABOLIC DECOMPENSATION IN ADULT POPULATION: REVISION OF 10 YEARS

PO-62

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The hereditary disorders of metabolism have a hide spectrum of clinical manifestations. The type intoxications and energy deficiencies may have acute events of metabolic decompensation. These episodes can be severe and could leave permanent sequela or even cause death.

During the last 10 years our center treated several adults with acute metabolic decompensations. Most of these patients had a known metabolic disorder but in some patients the diagnosis was made in the setting of the acute event.

The revision of all these events is a needed reflection, to optimize the care of this patients that face this critical event in their life.

The national reference centers must be responsible for the adequate management of this acute events. They must provide clinical guidelines and clinical guidance to other hospitals that have do deal with these situations.

SELECTED POSTERS | LYSOSOMAL DISORDERS

LONG-TERM EFFICACY AND SAFETY OF VESTRONIDASE ALFA ENZYME REPLACEMENT THERAPY IN SUBJECTS WITH MUCOPOLYSACCHARIDOSIS VII <5 YEARS OLD

PO-02

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Vestronidase alfa (recombinant human beta-glucuronidase) is an enzyme replacement therapy for Mucopolysaccharidosis (MPS) VII, a highly heterogeneous, ultra-rare disease. UX003-CL203 was an open-label trial designed to investigate the safety and efficacy of vestronidase alfa (4mg/kg IV every other week) in subjects with MPS VII <5 years old. Eight subjects (1.6-5 years) enrolled; 7 completed the 48-week Treatment Period and continued up to 2.6 years (44-136 weeks; median 95.1 weeks). Urinary glycosaminoglycan (uGAG) dermatan sulfate decreased significantly ($p<0.0001$) from baseline (BL) by LS mean of 60% at Week 4, with reduction sustained at Week 48 (61%) and throughout the study. All subjects achieved ≥50% reduction in uGAGs. Improvement in height z-scores (mean (SD) z-score change of 0.196 (0.30) from -2.630 (1.17) at BL to -2.045 (0.27) at Week 48) and a positive trend toward increased growth velocity from a mean (SD) z-score of 2.59 (1.49) at BL to 0.39 (2.10) post-BL ($p=0.27$), were observed. BL hepatomegaly resolved in 3/3 subjects assessed by ultrasound and 5/6 subjects assessed by physical examination (PE) by end of study; BL splenomegaly resolved in 1/3 subjects assessed by ultrasound and 2/2 subjects assessed by PE. All subjects experienced at least one treatment-emergent adverse event (TEAE); 5 subjects (62.5%) experienced TEAEs assessed as related to study drug. Three subjects (37.5%) experienced serious TEAEs; most were known complications of MPS VII. Four subjects (50.0%) experienced mild or moderate infusion-associated reactions (IARs). No subjects discontinued due to AEs or IARs. All subjects developed anti-drug antibodies and 3 developed neutralizing antibodies, although no association with uGAG reduction was apparent. Safety with long-term exposure was consistent with known safety profile of vestronidase alfa; no new safety concerns were identified. Long-term vestronidase alfa treatment demonstrated sustained reductions in uGAGs, continued growth, and improved hepatosplenomegaly in children with MPS VII <5 years old.

SELECTED POSTERS | LYSOSOMAL DISORDERS

DIFFERENTIAL DIAGNOSIS IN DIAGNOSTIC CRITERIA BASED DISEASES

PO-16

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Niemann Pick Disease (NPD) type A or neurologic acid sphingomyelinase deficiency is a rare autosomal recessive metabolic disorder characterized by lysosomal lipid storage and results from pathogenic variants in SMPD1 gene. Hemophagocytic lymphohistiocytosis syndrome (HLH) is characterized by a hyperinflammatory state and phagocytosis of blood cells by macrophages. It can be primary, caused by pathogenic variants in HLH-associated genes or secondary, due to an inability of the immune system to adequately restrict stimulatory effects of various triggers, including inherited metabolic diseases. Both disorders can have similar symptoms, so differential diagnosis is essential for therapeutic, prognostic and counselling issues.

CASE REPORT: We report the case of a 7-month-old boy, with uneventful prenatal and neonatal background. He had Influenza A at 3 months of age. No other relevant pathological history. During an acute illness episode, he presented with diagnostic criteria for hemophagocytic lymphohistiocytosis syndrome: fever, hepatosplenomegaly, pancytopenia, hypertriglyceridemia, hyperferritinemia and mild bone marrow hemophagocytosis. Under this context, he was admitted in the Hemato-oncology unit. The fever solved within the first 24 hours of admission and the hematologic parameters quickly improved. However, hepatosplenomegaly with hepatic cytolysis, hypertriglyceridemia and mild thrombocytopenia and anemia persisted. Molecular genetic test for HLH didn't disclose pathogenic variants. Upon follow-up, developmental delay (Global Development index 22) and failure to thrive were noticed. Therefore inborn metabolic disease diagnosis was considered. Bone marrow and liver biopsies were performed at 13 months, suggesting Niemann Pick Disease. The diagnosis was confirmed by low acid sphingomyelinase enzyme activity in leukocytes (0,09 nmol/h/mg protein; RR 0,39 a 1,38), and a new potential pathogenic variant (c.1100G>A/p.G367E), in apparent homozygosity in SMPD1 gene, was found.

COMMENTS: This case illustrates the importance to maintain high suspicion in differential diagnosis especially when the course of disease is atypical. In our case, despite fulfilment of HLH diagnostic criteria during an acute febrile illness, a meticulous search for underlying diseases was performed, leading to NPD type A definitive diagnosis. To our knowledge, there is only one case report in the literature of HLH features associated with NPD type A.

SELECTED POSTERS | LYSOSOMAL DISORDERS

THE 2020S TOOTH FAIRY: FROM LOOSE TOOTH TO NEURONAL CELL CULTURES, A METHOD TO ESTABLISH PATIENT-DERIVED MPS III NEURONAL CELL LINES

PO-18

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Mucopolysaccharidosis type III is a subgroup of rare, neuronopathic LSD, which has been one of our research focuses over the last decade. During these years, we have molecularly characterized the Portuguese MPS III population. More recently, we have also designed RNA-based methods to either correct specific mutations or actively promote substrate reduction by inhibiting its biosynthesis. Whatever the case, the therapeutic potential of the RNA drugs under development has been addressed in patients' fibroblasts with promising results. Nevertheless, those cell lines hardly qualify as the most suitable ones to address the drugs' full potential. Ideally, we should test each drug on its target cells/tissues. Thus, taking into account that our major goal is to achieve brain delivery of therapeutic RNA drugs, we recognize that it would be crucial to test those same molecules not only in patient fibroblasts and model neuronal cell lines, but also in patient neuronal cells. Still, the inability to access live neurons from affected individuals remains a challenge.

Here we present an alternative approach to generate patient-derived MPS III neuronal cell lines through a non-invasive, cost effective approach using dental pulp stem cells (DPSC). The plasticity of DPSCs has been demonstrated by several independent teams, who have shown that they self-maintain through several passages, holding 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. Our goal is to establish one such protocol in house, to generate MPS III neurons from normally shed deciduous "baby" teeth. Once established, those neuronal cell lines will constitute a valuable platform to address further address RNA drugs' efficiency in vitro.

Our current goal is to drive our peers' attention to this project and promote it among the Portuguese medical community on inherited metabolic disorders. In summary, this is a call for collaborators: if you're a clinician following up MPS III children who are currently losing their baby teeth, please consider contacting us.

Acknowledgments:

This work is partially supported by the Portuguese Society for Metabolic Disorders (Bolsa SPDM de apoio à investigação Dr. Aguinaldo Cabral 2018; 2019DGH1629/SPDM2018I&D) and Sanfilippo Children's Foundation (2019DGH1656/SCF2019I&D).

SELECTED POSTERS | LYSOSOMAL DISORDERS

FROM BENCH TO BIOTERIUM AND BACK AGAIN: DEVELOPMENT OF A U1 SNRNA-BASED THERAPEUTIC STRATEGY FOR MUCOPOLYSACCHARIDOSIS IIIC

PO-47

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Splicing is an essential cellular process to generate mature transcripts from pre-mRNA. One of the most important factors for mRNA transcription is the U1snRNA, a spliceosomal component that recognizes 5' splice site (ss) at specific regions in pre-mRNA. Splicing mutations represent one of the most frequent (~20%) genetic defects in Mucopolysaccharidosis IIIC (MPS IIIC), a LSD caused by mutations in the HGSNAT gene, encoding an enzyme involved in heparan sulphate degradation. Exon-skipping has been demonstrated as probably the most frequent aberrant splicing defect, and occurs due to mutations in the 5'ss. Application of modified U1snRNAs to improve recognition of mutated 5'ss represent a potential therapeutic strategy to recover the normal splicing process.

c.234+1G>A is a frequent mutation among patients from the Mediterranean basin (Portugal, Spain, Morocco and Tunisia). It's located in the + 1 position of intron 2 of HGSNAT gene and leads to the skipping of exon 2. We demonstrated in fibroblast cells that a modified U1snRNA vector (comprising exons 1 to 3) designed to improve the definition of exon 2 5'ss of the HGSNAT can restore the splicing defect caused by this mutation (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. We generated full-length (HGSNAT cDNA sequence plus part of introns 1 and 2) splicing competent constructs of wild-type (wt) and c.234+1G>A HGSNAT by cloning the wt or the mutated HGSNAT splicing-competent cassettes into the pcDNA 3.1 backbone. Then, we transfected the constructs into COS-7 cells to check if the splicing pattern was reproduced. We proceeded for in vivo studies using the constructed plasmid vectors to promote transient expression of the human HGSNAT wt or mutant in c57bl/6 mice. We administrated the pHGSNAT or pHGSNAT+1G constructs in mice by hydrodynamic injection (using the tail vein). After 48h animals were sacrificed, the liver was collected and the analysis by molecular biology performed. Unfortunately, no evidence of effective pHGSNAT cDNA gene expression was observed. We will now repeat the protocol of in vivo procedures with some alterations, such as a different mice strain, an optimized injection volume (increasing for 8-9% of the mice body weight instead 7%) and the inclusion of a commercial delivery solution in the protocol to enhance the transfection efficiency.

SELECTED POSTERS | LYSOSOMAL DISORDERS

CYSTINOSIS - A RARE BUT CRUCIAL DIAGNOSIS

PO-53

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Nephropatic cystinosis is a rare and autosomal recessive disease, caused by CTNS gene mutations, with a consequent early lysosomal cystine accumulation in virtually all tissues. The kidney is the first affected organ and infantile nephropathic cystinosis is the most frequent and severe variant.

Seventeen months-old girl with a normal height-weight ratio evolution until 14 months old, when parents noted polydipsia, polyuria and height-weight stagnation. She was admitted on hospital and blood tests showed chronic kidney disease (stage IIIa) with hyponatraemia, hypokalaemia, hypophosphoremia, hypocalcemia hypouricaemia and hyperchloremic metabolic acidemia. Urinary tests showed glycosuria, proteinuria and low sodium and potassium. The diagnostic of Fanconi Syndrome was raised. X-ray of long bones showed changes compatible with rickets. Cardiological evaluation and renal ultrasound were normal. That time, there were no crystals of cystine in the eyes. The hypothesis of diagnosis of infantile nephropatic cystinosis was raised and the determination of intraleukocytic cystine revelead a high value of 0.46γmol/g protein. Genetic study showed the mutation delACG, confirming the disease. Tretament with cysteamine was started (150mg, every 6h) as well as conservative renal therapy, with successive adjustements.

At 21 months, the ophthalmological evaluation showed already cystine crystals in the left eye and therapy with cysteamine eye drops (3.8mg/mL, 1 drop every 6h) was started. She is currently 3 years old, in the 3rd percentile of weight, under the 3rd percentile of height, clinically and analytically stable, without progression of rickets and with asymptomatic cystine crystals only in the ocular epithelium, without other important systemic manifestations.

It is necessary to have a high degree of suspicion for this disease, in order to start targeted therapy early and an adequate multidisciplinary follow-up of these patients. To improve adherence to therapy and quality of life, without disturbing the quality of night sleep, the use of therapy with greater dose comfort is an important factor.

SELECTED POSTERS | MITOCHONDRIAL DISORDERS

THE TOP TEN RESEARCH PRIORITIES FOR RARE MITOCHONDRIAL DISEASES: RESULTS OF A PATIENT/HEALTH PROFESSIONAL PRIORITY SETTING PARTNERSHIP

PO-12

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BACKGROUND: Primary mitochondrial diseases are severe inherited disorders in which the function of the mitochondria is affected by a nuclear or mitochondrial genetic mutation. These disorders currently lack effective disease-modifying therapies and are frequently life-limiting and/or disabling. The Priority Setting Partnerships (PSPs) are an established and collaborative methodology that brings patients, patient organisation representatives and clinicians together to try to establish the most pressing and unanswered research priorities for a specific disease.

AIM: To identify the unanswered questions about rare, primary, mitochondrial diseases from patient, carer and clinical perspectives; and to prioritise those that patients, carers and clinicians agree are the most important questions for research to address.

METHODS: The top ten research priorities were established through a structured process of web-based questionnaires, literature searching and face-to-face workshops. At every stage we actively sought to be inclusive of people with visual impairment and from minority ethnic groups.

RESULTS: An initial web-based questionnaire, asking all patients affected by primary mitochondrial disease, their carers and clinicians to pose their important research questions, yielded 709 questions. These 709 questions (from 50 patients, 47 carers and 50 clinicians) were grouped into themes and filtered to exclude statements that were personal stories rather than answerable questions. Extensive literature searching then determined which of these questions are unanswered based on current evidence, resulting in a list of 42 discrete, answerable questions. All patients, carers and clinicians were invited to prioritise these 42 questions and rank them from 'important' to 'less important' using a web-platform. This identified 24 questions that were taken forward to a face-to-face workshop attended by a diverse range of patients, carers, charity representatives and clinicians, where the relative merits and strengths of each question was debated to create a definitive 'top 10 of unanswered research questions for primary mitochondrial disorders'.

DISCUSSION: The Mitochondrial Disease Priority Setting Partnership hopes that by identifying the priority questions for research, we will ensure that future research is focused on the issues that matter most to people with mitochondrial disease, their carers and relatives and the healthcare professionals who support them.

SELECTED POSTERS | MITOCHONDRIAL DISORDERS

LEIGH SYNDROME AND MITOCHONDRIAL DNA – REINFORCING THIS RELATION IN GENETIC SCREENING

PO-13

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Mitochondrial disorders of energy generation are the most frequent group of inborn errors of metabolism frequently manifesting as Leigh Syndrome. This syndrome is the most common presentation phenotype in early years of life, although may manifest at any age group. Leigh syndrome classically presents predominant neurological symptoms such as neurodevelopment delay, hypotonic tonus, seizures, ophthalmological impairment and progressive apnea crisis. Brain imaging investigation displays symmetrical basal ganglia and brain stem lesions which are associated to this syndrome. The investigation pathway towards diagnosis includes biochemical and molecular analysis to confirm the suspicion. Besides techniques progresses in genetic evaluation, an important major step in performing molecular analysis is the mitochondrial DNA content. In these presentations some variants are strikingly important to be checked. Unlike nuclear DNA the mitochondrial nucleic acid material is composed by a double circular molecule containing 37 genes that have already been detailed. Recent investigations have proven to correlate already described mutations to more than one phenotype. This expanding knowledge of association has enhanced the need to analyze mitochondrial DNA when investigating mitochondrial disorders and if associated to biochemical studies effective results may be given in optimal time. The most frequent associated variant is the m.8993 T>C/G in ATP6 which represents a Neuropathy, Ataxia and Retinitis Pigmentosa (NARP)/ Maternally Inherited Leigh Syndrome (MILS) phenotypes. This mutation may produce in biochemical amino acid analysis hypocitrullinemia, which may contribute to the investigation. The clinical picture presents basically according to mutation load of this variant, expecting MILS phenotype in higher rates and NARP phenotype in lower rates. So far, nearly 30 mutations already described in mitochondrial DNA are associated to Leigh syndrome in isolated manner or associated to other disorder. The mutations most frequently relating Leigh syndrome to another mitochondrial phenotypes are: m.3243 A>G, m. 3697 G>A, m.9176 T>C/G, m.10191T>C, m.13513 G>A. The increase in diagnosed cases genetically underlined by mitochondrial mutations proves the importance of analyzing mtDNA in Leigh syndrome in first molecular approach and proceeding in complexity analysis whenever required in each case.

SELECTED POSTERS | MITOCHONDRIAL DISORDERS

NOVEL NUBPL VARIANTS REVEALED BY WHOLE-EXOME SEQUENCING IN A CHILD WITH ATAXIC GAIT

PO-28

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With the advance of genetic technics, mainly of next generation sequencing, there is an increasing number of patients with a previously not recognized disease or syndrome that is being diagnosed.

We present an 11 years-old boy with non-consanguineous healthy parents and one younger healthy brother. The child was born at term after an uneventful pregnancy with normal birth somatometry. He had an apparently normal psychomotor development and growth, stature and occipitofrontal circumference until 5 months of age, when he started with feeding difficulties (vomiting and food refusal). Global development delay was noticed at 8 months. He started to walk at 3,5 years of age with ataxic gait and had expressive language delay. Psychometric evaluation revealed a low-average score. Brain MRI performed at 4 years of age showed cerebellar atrophy and a cervical syringomyelic cavity at C6-C7 level without mass effect. There was no involvement of internal organs. During development he is showing progressive improvement in his motor skills, with less severe gait ataxia, and dysarthria.

Whole-exome sequencing (WES) was performed after extensive etiologic investigation, revealing compound heterozygous variants, both classified as of uncertain significance, in NUBPL gene, encoding an assembly factor for mitochondrial complex I. One of the variants - c.270_275del - is novel and leads to the deletion of two amino acids (p.(Leu92_Ala93del)); the other variant - c.897G>A, p.(Glu299=) - is not found in any database of human variation and is predicted to affect a donor splice site. Respiratory chain enzyme assay in muscle is underway to investigate complex I deficiency.

Autosomal recessive NUBPL pathogenic variants were first reported as being associated with complex I deficiency in 2010. Since then, 13 patients (10 families) have been described, presenting leukoencephalopathy with ataxia, dystonia, spasticity and dysarthria. Recently, one patient was reported with multisystemic involvement. Most of these patients exhibit a distinct pattern in brain MRI involving the cerebellar cortex, deep white matter and corpus callosum at early stage, progressing to severe cerebellar atrophy and brainstem involvement at a later stage.

This case report demonstrates the relevance of WES in diagnosing patients with rare diseases and brings further insight into the NUBPL genetic and clinical knowledge, by showing a child with a less severe phenotype that is progressively improving.

SELECTED POSTERS | MITOCHONDRIAL DISORDERS

AMINOACYL-TRNA SYNTHETASE DISORDERS: AN EMERGING GROUP OF MITOCHONDRIAL DISEASES

PO-30

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BACKGROUND: Pathologies associated with mitochondrial dysfunctions involve a large number of genetic defects with different inheritance mechanisms, either maternally or mendelian, caused by mutations in mitochondrial and nuclear genome, respectively. The nucleus-encoded mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) are key components of the mitochondrial translation apparatus. Mutations in these enzymes can lead to vastly diverse diseases, occurring at different stages in life, and involving different tissues. The first description of a series of mutations in an mt-aaRSs causing a particular leukodystrophy dates back to 2007. In only 13 years, by Next Generation Sequencing (NGS) technology, mutations in each of the nuclear genes coding for 19 mt-aaRSs have been correlated to human diseases, with pleiotropic clinical manifestations, although predominantly affecting the central nervous system.

PATIENTS AND METHODS: In this study we present 10 patients, mainly children, with leukoencephalopathy, high lactate, feeding difficulties, hypotonia, myopathy, dystonia, development delay, cerebral calcifications, epilepsy, ophthalmoparesis, hyperuricemia, among others.

Pathogenic variants in mt-aaRSs genes (DARS2, EARS2, LARS2, SARS2, VARS2 and WARS2) were identified by NGS, which was performed in a MiSeq Illumina instrument using a custom gene panel with 209 nuclear genes involved in mitochondria metabolism, applying SureSelect QXT target enrichment system from Agilent.

RESULTS: The studied patients harbored 18 pathogenic variants in mt-aaRSs, being 11 previously reported in the literature and seven novel variants probably pathogenic. The identified variants were confirmed by Sanger sequencing in the index cases and in their relatives.

DISCUSSION AND CONCLUSION: In the new era of genomics, reported mutations in these genes are increasing fastly, having this study contributed to expand the mutational landscape of pathogenic variants in mt-aaRSs genes. Defects in mitochondrial translation are associated with combined mitochondrial respiratory chain defects and produce diverse clinical phenotypes from neonatal period to adulthood, with the most severe presenting in infancy as metabolic disorders. However, neurological features tend to manifest later in life. Thus, while treatments for mitochondrial diseases are currently scarce, the molecular characterization will be important for an accurate genetic counseling and a prenatal diagnosis for the affected.

SELECTED POSTERS | MITOCHONDRIAL DISORDERS

SDHA RELATED DISORDER ASSOCIATED WITH CONTINUOUS SPIKE AND WAVES DURING SLOW-WAVE SLEEP SYNDROME WITHOUT NECROTIZING LEUKOENCEPHALOPATHY

PO-45

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INTRODUCTION: Leigh syndrome is a subacute necrotizing leukoencephalopathy with different genetic backgrounds. It is characterized by neurological and/or multiorganic decompensations with acute illness, developemental delay, hypotonia, ataxia, epilepsy and a variety of extraneurological manifestations. It can be mitochondrial or nuclear gene associated, although in the last case genes are usually involved in mitochondrial function and maintenance.

CASE REPORT: A 30 month old girl, daughter of healthy parents, with no known consanguinity, was observed in neuropediatric outpatient department by toewalking. She had a slight developmental delay and inadequate reciprocal interaction. She was started on occupational therapy. At 45months she initiated partial crises of left upper arm during sleep; at 51 months continuous spike wave of sleep (CSWS) pattern was diagnosed, needing prednisone treatment for 9 months. Clinically she had worsening gait disturbance and severe tantrums, with no other manifestations. Complementary exams showed slight blood lactate elevation and traces of glutaric acid in urine, with posterior normalization, and 3Tesla cerebral MRI with spectroscopy was normal. She was observed in genetic outpatient department and an epilepsy genetic panel showed a homozygous c.1786G>C variant, non-described, probably pathogenic, in exon 13 of SDHA gene, usually associated with Leigh syndrome. Both parents were heterozygous for the pathogenic variant. Mitochondrial respiratory chain enzyme assay revealed an isolated 22% partial complex II defect, confirming biochemically the genetic results.
She is now 6 years old, has a moderate developmental delay, a slight ataxia, dysarthria and mild behavioural disturbance. She was started on acetazolamide when CSWS pattern reinitiated (parents refused prednisone) along with valproic acid and clobazam, with normalization of EEG. Until now, she has not had any episode of acute decompensation, but has never faced any significant illness. She has speech and occupational therapy, psychological and special education support and was initiated on methylphenidate with good behavioral response.

DISCUSSION: The patient has genetic and biochemical features of nuclear gene encoded Leigh syndrome, although she does not fullfil the complete clinical or imaging criteria. To our knowledge, this is the first case of SDHA related disorder without leukoencephalopathy with continuous spike and waves during slow-wave sleep syndrome.

SELECTED POSTERS | MITOCHONDRIAL DISORDERS

PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY IN PORTUGAL: CLINICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION, PROSPECT OF GENOTYPE-PHENOTYPE CORRELATION

PO-51

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The pyruvate oxidation route constitutes a fundamental pathway in aerobic energy metabolism, bridging the glycolytic pathway in the cytosol with the tricarboxylic acid cycle and oxidative phosphorylation in the mitochondria. Pyruvate is irreversibly converted to acetyl-CoA by the Pyruvate Dehydrogenase Complex (PDC), consisting of multiple copies of three catalytic and one structural components, respectively: pyruvate dehydrogenase (E1), dihydrolipoyl-lysine-residue acetyltransferase (E2), dihydrolipoyl dehydrogenase (E3) and E3 binding protein (E3BP).

Impaired PDC activity leads to cellular energy deprivation and inadequate removal of pyruvate and lactate results in lactic acidemia. The heterogenous clinical presentation of PDC deficiency ranges from progressive neurological and neuromuscular degradation to chronic neurological dysfunction.

The precise diagnosis, including the identification of the genetic defect, is important and decisive for the appropriate selection of the therapeutic strategy, which may target the metabolic pathway via ketogenic diet, or the dysfunctional complex through its regulatory system (dichloroacetate) or its stimulation by cofactor(s).

Herein we describe and discuss the profiles of 13 PDC deficient patients, combining information on the associated clinical, biochemical, enzymatic and genotypic spectra. The cohort represents all regions of Portugal. The clinical features were evaluated by the physicians following the patients, PDC activity was assayed by a standard method of [1-14C]-pyruvate decarboxylation and sequence analysis was performed by Sanger sequencing. In silico impact of the mutations was studied using the PolyPhen-2 server and SWISS-MODEL.

All patients had the clinical onset between the neonatal period and infancy and manifested different degrees of neurological involvement. All patients displayed elevated levels of plasma lactate and pyruvate and significantly reduced PDC activity. Interestingly, most of the patients reached adulthood. Ten different mutations affecting three genes, PDHA1 (54 %), PDHX (38 %) and DLD (8 %) have been identified. The most striking evidence is a relatively high incidence of E3BP deficiency. According to the data from this subset of patients, the severity of the phenotype roughly resembles the genotype, depending on the mutation type and the affected PDC-subunit.

strategic project UID/DTP/04138/2019, grant SFRH/BD/91729/2012), iNOVA4Health Research Unit (LISBOA-01-0145-FEDER-0

SELECTED POSTERS | MITOCHONDRIAL DISORDERS

COULD ARGININE ASPARTATE BE A PROMISING TREATMENT FOR FUMARIC ACIDURIA?

PO-55

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BACKGROUND: Fumaric aciduria is a rare autosomal recessive metabolic disease, characterized by developmental delay, hypotonia, seizures and dysmorphic facial features. No specific treatments are available and many children don't survive childhood. Smith and Robinson constructed a model of fumarase deficiency showing ATP production improvement by amino acids involved in malate-aspartate shuttle.

CASE REPORT: Authors report a 7-year-old boy's case with fumarase deficiency on arginine aspartate supplementation and low glycose index (GI) specific diet. Symptomatic manifestation started in postnatal period with tachypnoea and asphyxia. By 6 months it was noted global developmental delay, microcephaly, hypotonia, hyperlaxity and distal dystonic postures. Metabolic studies showed persistent elevated urinary fumaric and succinic acids. Molecular FH gene test revealed compound heterozygosity c.431G>A; c.1431_1433dupAAA confirming the diagnosis. Fumarase enzymatic activity showed reduced level (11.7%). At 22 months he was hospitalized with a severe metabolic acidosis and started a low glycaemic index diet supplemented by MCToil, DHA, L-carnitine and arginine aspartate 5-10g/day.
He maintained the treatment plan for three years and but he maintained static developmental delay and dystonia, and metabolic decompensations did not improved.

DISCUSSION: There are few published reports of dietary interventions in FHD. Low protein diet showed no efficacy (Baştuğ et al. 2014; Kimonis et al 2012). High glucose intake caused minimal increase in ATP production but large increase in lactate (Smith and Robinson 2011). According to Ewbank 2011, ketogenic diet is not recommended. Supplementation with aspartate and a low GI diet were suggested by Smith and Robinson (2011) due to a positive effect observed in ATP production in vitro.
Ryder et al (2017) started a high fat low carbohydrate diet on a girl with 9% fumarase activity and a genotype previously linked to severe phenotype. Although, our case did not show any improvement.
Further studies are necessary to evaluate the clinical impact of this therapy.

SELECTED POSTERS | ORGANIC ACIDURIAS

MOLECULAR RATIONALE FOR RIBOFLAVIN TREATMENT IN GLUTARIC ACIDURIA TYPE I PATIENTS

PO-07

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Glutaric Aciduria type I (GA-I) is an autosomal recessive neurometabolic disorder caused by mutations in the GCDH gene. This gene encodes for glutaryl-CoA dehydrogenase (GCDH), a mitochondrial homotetrameric flavoprotein, with a non-covalently bound flavin adenine dinucleotide (FAD) cofactor per subunit, which is involved in lysine, hydroxylysine and tryptophan metabolism [1]. The severity of GA-I is very broad, varying from lethal cases to milder forms of the disease depending on the nature of the mutations [2]. In milder cases disease symptoms can be triggered by stress conditions, like fever or fasting, suggesting an important role for environment and cellular factors in disease onset [3]. Furthermore, it is known for other metabolic disorders associated with defects in flavoproteins that FAD cellular availability can also modulate the folding and activity of disease variants [3]. Also, there are some reports of GA-I patients showing a positive response to riboflavin supplementation [4].

To elucidate on the molecular mechanism behind the therapeutic effects of riboflavin supplementation on GA-I patients, we used as a model the GCDH-p.Val400Met variant, that has been previously described with impaired FAD binding [2]. First, we observed that E. coli recombinant expression of human GCDH-p.Val400Met results only in the production of GCDH apo form, completely depleted of FAD cofactor. Even so we were able to purify GCDH-p.Val400Met apo form, and investigate, employing several biochemical and biophysical methodologies, the effects of adding external flavin on the conformational quality of this variant. We observed an increase in the protein's stability and proteolytic resistance. Furthermore, adding external flavin also rescued the GCDH-p.Val400Met variant enzymatic activity. Our results suggest that some misfolded GCDH disease-variants can be rescued by increasing cellular FAD content through riboflavin supplementation, as observed in other metabolic diseases.

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SELECTED POSTERS | ORGANIC ACIDURIAS

ISOLATED GLYCEROL KINASE DEFICIENCY: A RARE CAUSE OF METABOLIC KETOACIDOSIS

PO-36

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BACKGROUND: Glycerol kinase deficiency (GKD, MIM 307030) is a rare X-linked disorder that may occur as an isolated form caused by a pathogenic variant in GK gene, or as part of Xp21 contiguous gene syndrome involving GK and NR0B1 genes with or without DAX1, IL1RAPL, and DMD genes. Individuals with isolated GKD may be asymptomatic (only detected by pseudo hypertriglyceridemia) or symptomatic with episodes of vomiting, acidosis and lethargy that may progress to coma. Individuals with complex GKD may also have features of congenital adrenal hypoplasia, Duchenne muscular dystrophy, or developmental delay.

CASE REPORT: Our case is a 6 year-old boy, the second child of non-consanguineous parents, followed in metabolic and neurology outpatient clinic for hypertriglyceridemia and borderline psychomotor development. He had no history of adrenal crisis, no dysmorphic features, no muscle symptoms, and normal creatine kinase. At 4 years of age, after a mild infection, he was admitted for vomiting, somnolence and dehydration. Laboratory analysis showed marked metabolic ketoacidosis (pH 7.07, pCO2 18 mmHg, HCO3- 8.9 mmol/L, urinary ketones 80 mg/dL) with normal glycaemia. Intravenous fluids and bicarbonate were initiated and the patient progressively improved. Organic acids analysis in crisis showed elevated 3-hydroxybutyric acid, 2-hydroxybutyric acid, 2-hydroxyisovaleric acid, and high levels of glycerol. Glycerol was confirmed in plasma outside crisis (25.5 µmol/L). GK gene sequencing revealed a previously unreported hemizygous variant in exon 3 (c.213_214del, p.Cys72Ter), classified as likely pathogenic. This nonsense variant results in a premature stop codon at amino acid 72, which originates a truncated protein.

COMMENTS: Our patient presentation suggested the isolated GKD diagnosis, since he had metabolic ketoacidosis episodes, glycerolemia and hypertriglyceridemia, without manifestations of Xp21 contiguous gene deletions, such as dysmorphic features, motor deficiency (DMD gene) or adrenal insufficiency (NR0B1 gene). GK gene sequencing confirmed our clinical hypothesis. In GKD patients, genetic analysis is important to identify the underlying molecular mechanism, which is critical for patient management, prognosis and genetic counseling.

SELECTED POSTERS | ORGANIC ACIDURIAS

COMBINED MALONIC AND METHYLMALONIC ACIDURIA – A CASE OF LATE DIAGNOSIS WITH MILD PHENOTYPE

PO-46

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BACKGROUND: Combined malonic and methylmalonic aciduria (CMAMMA, OMIM # 614265) is a rare recessive disorder, with a calculated incidence of 1:30.000, caused by homozygous or compound heterozygous mutations in the ACSF3 gene which encodes an acyl-CoA synthetase. Phenotype is extremely variable, ranging from asymptomatic patients identified as a result of newborn urine screening program to children with developmental delay and failure to thrive. In those who were diagnosed as adults, symptoms may include neurological and psychiatric features that can mimic Alzheimer's disease.

CASE REPORT: We present a 22-year-old female patient referred from another hospital for investigation of cognitive development delay. She is the first child of gipsy consanguineous parents and has 2 healthy brothers. Her neonatal period was complicated by sepsis. She had regular motor skills development but had mild learning difficulties (couldn't finish her seventh grade) and behavior disturbances. She also had grade 3 obesity, type 2 diabetes and hypertension. A screening for inherited metabolic conditions performed when she was 10 years age revealed a moderate to high excretion of methylmalonic acid (204-835 mmol/mol Cr; R.V. < 14). Plasma homocysteine, vitamin B12 and folic acid were within the respective reference ranges. Taking into account the biochemical pattern a deficiency of the mutase (mut- , with residual activity) or a defect of its cofactor AdoCbl (CblA, CblB or CblD-MMA genes) were evaluated by gene sequencing but no pathogenic variants were found. Finally, a NGS panel identified p.Glu359Lys (c.G1075A) mutation in homozygosity in ACSF3 gene, previously described in CMAMMA patients.

DISCUSSION AND CONCLUSIONS: The biochemical diagnosis of CMAMMA can be easily missed. The excretion of malonic acid (MA) may be normal or just slightly elevated. Furthermore, in the urinary organic acids GC-MS analysis, MA may be overlapped by methylmalonic acid (MMA) due to their similar chromatographic behavior. Indeed, in the analyzed urine samples no MA was detected. The diagnosis of CMAMMA was finally confirmed by NGS which is in agreement with the patient's clinical picture and the scarce cases published. Long-term follow-up is needed to understand the natural history of this disorder.

SELECTED POSTERS | ORGANIC ACIDURIAS

CBLD - MMA – A RARE CASE OF ISOLATED METHYLMALONIC ACIDEMIA

PO-48

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BACKGROUND: Isolated methylmalonic acidemia is caused by complete or partial deficiency of the enzyme methylmalonyl-CoA mutase (mut0 or mut–), a defect in the transport or synthesis of its cofactor, adenosyl-cobalamin (cblA, cblB, or cblD-MMA), or deficiency of the enzyme methylmalonyl-CoA epimerase. The knowledge of the underlying enzymatic defect and genotype (MUT, MMAA, MMAB, MMADHC or MCEE) is of great importance since residual enzyme activity and vitamin-B12 responsiveness influences the clinical course and can be associated with a better long term outcome. The cblD-MMA is among the rarest of the disorders of intracellular cobalamin metabolism, with only seven cases reported so far.

CASE REPORT: We report the case of a 5-year-old girl, 2nd child of consanguineous healthy parents from Bangladesh, born in Portugal after an uneventful gestation, delivery and neonatal period. The metabolic newborn screening was negative. She had poor weight gain, feeding difficulties with protein aversion and moderate motor and language delay. At four years of age she was admitted for vomiting, fever, prostration, ataxia, and dehydration, severe metabolic acidosis (pH 7,101, pCO2 16 mmHg, HCO3- 5,1 mmol/L) with increased anion gap 35,7 mmol/L, hyperlactacidemia and hypermonemia. After correction with intravenous fluids, there was complete clinical and metabolic recovery.

Laboratory investigation in crisis showed massive amounts of urinary methylmalonic acid (3,745 micromol/mmol creatinine), elevated propionylcarnitine (C3), normal plasma total homocysteine and B12 vitamin. Microarray shown a region of loss of heterozigosity 2q22.1q31.1 (141,348,339-170,445,880) which included MMADHC gene. NGS analysis revelead a c.228dup variant in homozygosity in MMADHC gene, confirming the diagnosis of cblD–MMA.

Meanwhile, response to hydroxicobalamin was confirmed by the test according to Fowler, and hydroxicobalamin intramuscular weekly was started with good clinical and metabolic evolution.

COMMENTS: We report a rare case cblD-MMA presenting with an intermediate phenotype with feeding difficulties, failure to thrive, developmental delay and an episode of severe metabolic acidosis. As these children are at risk for a catastrophic decompensation it is of extremely importance to make a correct and timely diagnosis.

This case was not detected in newborn screening probably because it was a milder phenotype and because the C3 cut-off at that time was higher than that currently

SELECTED POSTERS | PEROXISOMAL, STEROL, BILE ACID, LIPID AND LIPOPROTEIN METABOLISM

A CLINICAL CASE OF ZELLWEGER SPECTRUM DISORDER

PO-66

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BACKGROUND: Zellweger spectrum disorder (ZSD) is a group of rare autosomal recessive disorders caused by mutations in more than 12 genes that encode a group of proteins essential for the formation and normal function of the peroxisomes. Mutations in the PEX1 gene are found in nearly 70% of affected individuals. The ZSD phenotypic spectrum is broad, ranging from mild manifestations to the most severe form, characterized by a distinctive facial appearance, visual and hearing impairment, hypotonia, developmental delay, seizures, hepatic dysfunction, renal cysts and bone abnormalities.

CASE REPORT: Here we report a case of a 9-month-old male referred to our clinic by developmental delay, dysmorphic facial features and visual deficit. The proband is the first child of a non-consanguineous couple from Cape Verde, born after 39 weeks gestation by eutocic delivery with adequate somatometry. He had seizures on the first months of life. At clinical observation he had distinctive facies (epicanthus, flattened face, broad nasal bridge, high forehead, low-seat ears), large anterior fontanel with wide sutures, convergent strabismus with nystagmus movements, global hypotonia with lower limb spasticity and hepatosplenomegaly. Diagnostic investigation showed increased transaminases with otherwise normal liver function; normal echocardiogram, renal ultrasound and skeleton X-ray. Ophthalmologic evaluation showed persistent hyperplastic primary vitreous. EEG demonstrated slow anomaly with moderate epileptiform activity over the left posterior temporal region. Brain MRI evidenced bilateral temporal polar sequel injuries of hemorrhagic vascular nature. Metabolic investigation revealed elevated hexacosanoic acid on very long chain fatty acids study and peroxisomal dysfunction profile on urinary organic acids. Next Generation Sequencing (NGS) panel identified 2 pathogenic variants in PEX1 gene, suggesting the diagnosis of Zellweger spectrum disorder. He started on early intervention program. Parents' genetic study is ongoing to confirm the compound heterozygosity.

COMMENTS: We emphasize the importance of NGS sequencing in the diagnosis of rare diseases as well as the importance of a multidisciplinary approach.

SELECTED POSTERS | UREA CYCLE DISORDERS

CITRIN DEFICIENCY: UNCOMMON PRESENTATIONS IN INFANCY

PO-60

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BACKGROUND: Citrin deficiency is an autosomal recessive inborn error of metabolism. Three main phenotypes of have been reported, including neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) during infancy, failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD) in older children, and recurrent hyperammonemia with neuropsychiatric symptoms in citrullinemia type 2 (CTLN2).

CASE REPORT: We present two unrelated patients, both born at term to Portuguese non consanguineous parents, with an appropriate birth weight.

Patient 1 was admitted at 4-month-old due to a prolonged non cholestatic jaundice and failure to thrive. He had mild elevated hepatic enzymes and normal coagulation profile and blood count. Infectious causes and α-1 antitrypsin deficiency were excluded. Plasma amino acids revealed increased citrulline and methionine. Urinary organic acids showed persistent excretion of p-hydroxyphenyllactic and p-hydroxyphenylpyruvic acids. Blood galactose was normal. Jaundice resolved at 5-month-old but failure to thrive maintained, despite increased caloric intake. Due to persistent biochemical results, citrin deficiency was suspected. The molecular analysis showed two new pathogenic variants (c.1056_1060delA and c.1231-1G>A) in SLC25A13 gene.

Patient 2 was referred at 6-month-old after a symptomatic hypoglycemia episode associated with failure to thrive, raised liver enzymes, dyslipidemia and diffuse liver steatosis without hepatomegaly. Ammonia and blood galactose were normal and there was no history of jaundice. Plasma amino acids showed citrulline, methionine and threonine elevation.

The diagnose of citrin deficiency was confirmed by molecular analysis (homozygous for the c.1231-1G>A variant). Dietary intervention in both patients included increased fat and protein intake, restriction of carbohydrates and medium-chain triglyceride supplementation, with growth curves normalization and biochemical improvement. Now at 13 and 2 years old respectively, they remain under dietary treatment and asymptomatic.

DISCUSSION: During the period after NICCD recovery and CTLN2 onset, several clinical manifestations have been reported including fatigue, fatty liver, growth impairment/failure to thrive, hypoglycemia, dyslipidemia and pancreatitis. We present two patients with similar manifestations, but that begun during infancy and with no NICCD history, which contributes to elucidate the clinical spectrum of this disease.

SELECTED POSTERS | OTHER

OSTEOPOROSIS: GENE INTERACTION BETWEEN POLYMORPHISMS IN GENES RELATED TO IRON METABOLISM

PO-14

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Osteoporosis is a common metabolic bone disease characterized by reduced bone mass and increased risk of fragility fractures. The pathogenesis of this disease is complex and influenced by multiple risk factors, where genetic factors play an important role. Osteoporosis and iron metabolism have an important relationship. Iron overload suppresses osteoblast formation and also stimulates osteoclast resorption of bone, suggesting that polymorphisms in genes affecting iron homeostasis can increase the susceptibility for the development of osteoporosis.

In the present study, we aimed to analyse the epistatic relationship between two iron metabolism related genes - haptoglobin (Hp) and HFE – in osteoporosis. We also studied the BMP2 gene. To achieve this, 313 patients with osteoporosis and 450 controls with normal bone mineral density were enrolled. Haptoglobin phenotype was determined by polyacrylamide gel electrophoresis (PAGE) and HFE polymorphisms (H63D and C282Y) were evaluated by PCR-RFLP. BMP2 polymorphism (c.570A>T) was evaluated by PCR-ARMS. All statistical tests were performed with SPSS 24.0 software. Results showed that, no significant differences were found between the two populations (patients vs controls) concerning Hp phenotypes or HFE (H63D and C282Y) genotypes. However, individuals that have co-inherited the Hp 2.2 and the HFE_H63D HH have an increased risk for developing osteoporosis [p=0.049; OR (95% CI) = 2.509 (1.003-6.279)] (adjusted for age and body mass index).

In summary, a significant epistatic interaction was detected between haptoglobin and HFE and osteoporosis, where Hp 2.2 in combination with HFE_H63D HH genotype appears to increase the risk for developing osteoporosis. Since these genes are related to iron metabolism, the results of this study reinforce an important action of this metabolism in the development of osteoporosis.

SELECTED POSTERS | OTHER

METABOLIC MAPPING AS A TARGET FOR OPTIMIZED HEART FAILURE THERAPY

PO-68

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Heart failure's incidence is still increasing worldwide and it's mortality rate is a matter of concern, despite continuing therapeutic innovations. Inflammation is a well-known pathophysiological mediator as is, recently, metabolic modulation. Even though, there's no clearly stablished metabolic or inflammation pattern which accurately predicts heart failure (HF) prognosis. Additionally, in spite of proteomics evolution, classic 2-dimensional electrophoresis (2-DE) continues a recognized method in screening analysis of protein's pattern.

Among a cohort of 250 HF patients followed in our external consultation we used to see (in approximately 28% of them) a seric protein electrophoretic pattern which has been related to worse prognosis. This pattern is characterized by low albumin and transferrin and high orosomucoid and haptoglobin. In face of this we hypothesised that it could be partially explained by some unrecognized lysosomal disorders or other unknown metabolic disease. So, we studied these patients analysing certain enzymatic activities on peripheral blood, in order to roughly ascertain this thesis.

We investigated 50 patients and almost 67% of them had alterations, at least in one of the next key enzymes: alpha-glucosidase, beta-galactosidase, beta-d-quitotriosidase, alfa-iduronidase and beta-glucosidase. Although not fulfilling criteria for major lisozomal diseases as Gaucher, Pompe or Fabry, in most of them, investigation proceeded to genomic DNA analysis.

These patients have clearly a different metabolic pattern when compared to HF patients with normal seric electrophoresis. The problem is that we really don't know it yet, or in which way it interferes in HF's pathophysiology. At the end of this study, we intent to clarify this, and, hopefully, to contribute with another valuable therapeutic target.


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