23rd Annual Meeting 2019

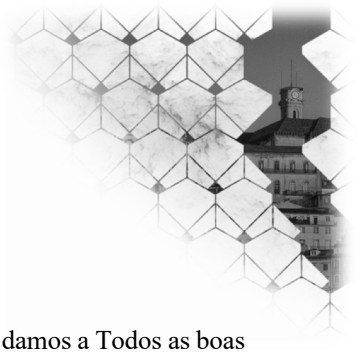
Abstract Book

Sociedade Portuguesa de Genética Humana

SPGH
Sociedade Portuguesa de Genética Humana

14th to 16th November

Fundação Bissaya Barreto
Bencanta – Coimbra



Caros Colegas,

Em nome da Direção da SPGH e da sua Comissão Científica damos a Todos as boas vindas à 23ª Reunião Anual 2019, em Coimbra.

Como já vem sendo habitual procurámos trazer especialistas de várias áreas da Genética Humana de modo a apresentarmos um programa com temas emergentes e com impacto na investigação, no diagnóstico, prognóstico e saúde pública, bem como, na discussão de aspetos éticos associados. Procurámos construir um programa atrativo em que todos possamos aprender e discutir os tópicos mais relevantes da Genética Humana do momento e, dispor de um bom ambiente, para que Todos os que trabalham nesta área queiram voltar à reunião anual, não só para estabelecer novas sinergias, mas também para encontrar velhos amigos e fazer novos.

Uma palavra muito especial à Direção e à Comissão Científica que tiveram a sabedoria de construir um programa que, não só nos permitiu reunir as condições para sermos considerados pela nossa congénere Europeia, a European Society of Human Genetics, como *Official Partners*, como também o de selecionar os trabalhos submetidos mais desafiantes para discussão. O patrocínio científico de diferentes entidades a nível nacional são também a demonstração da difusão da SPGH. A procura do evento anual da Sociedade e o apoio por diversas empresas nacionais e internacionais espelham também essa disseminação e representam um enorme contributo para a qualidade do congresso. Finalmente, o empenho, a dedicação e o rigor da Comissão Organizadora Local foram, sem dúvida, ao longo de meses, um pilar significativo deste evento nacional.

A Todos, um bem-haja por terem vindo ao nosso congresso e à "Coimbra dos Estudantes" e, portanto, como manda a tradição aqui vai o desafio...
E para a SPGH não vai nada, nada, nada?

TUDO!

Isabel Marques Carreira,
Presidente da SPGH

Dear Colleagues,

On behalf of the SPGH Board and Scientific Committee, we welcome you All to the 23rd Annual Meeting 2019, in Coimbra.

As in previous years, the main purpose of our annual meeting is to invite the best speakers from various fields of human genetics, to come and share with us excellent science, in the areas of diagnosis, prognosis, public health as well as to discuss ethical issues. We aimed to put together a challenging and attractive scientific program where we can all learn and discuss the most relevant topics in Human Genetics of today. We also want to create the environment for Everyone, working in this field, to want to return to the annual meeting to establish new synergies, find old friends and make new ones.

A very special word of thanks to the Board and to the Scientific Committee that had the insight to construct a program that allowed us to meet the conditions to be considered *Official Partners* by our European counterpart, the European Society of Human Genetics. The Scientific Committee, also had the ability to select the most challenging abstracts for the presentations and awards. Scientific sponsorship by different entities, demonstrates the diffusion of our Society. Support from various national and international exhibitors also reflect this dissemination and represents a significant contribution to the quality of our meeting. Finally, the commitment, dedication and rigor of the Organizing Committee were undoubtedly, over the last months, a fundamental pillar of this national event.

Thank you All for attending our congress and coming to the city known in Portugal as the “city of the students” because of its University and its academic traditions.

We trust you will enjoy these 3 days!

Isabel Marques Carreira,
President of SPGH

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SCIENTIFIC PROGRAM

PROGRAMA CIENTÍFICO / SCIENTIFIC PROGRAM

Day 1 **Thursday, November 14th**

09h00-09h45 Registration

09h45-11h45 SPGH Club meetings (concurrent)
CLUB 1: Cytogenetic and Molecular Genetics Club
Chairs: Joana Barbosa de Melo and Celeste Bento
CLUB 2: Dysmorphology and Clinical Genetics Club - Genetic syndromes and the mTOR/PIK3CA pathway
Chairs: Jorge Saraiva and Sérgio B. Sousa

11h45-12h45 Workshop: European Reference Networks, what is in there for me?
Chairs: Carla Oliveira and Lina Ramos
11h45-11h55 Brief ERNs introduction
Sérgio B. Sousa (Centro Hospitalar e Universitário de Coimbra-CHUC, Portugal)
11h55-12h10 Selected ERN: EpiCARE
EpiCARE-ERN for rare and complex epilepsies: overall organization and what is in there for the geneticist
Gaetan Lesca (Lyon University Hospital, France, France)
12h10-12h20 Questions and Answers
12h20-12h30 EpiCARE in Portugal
Francisco Sales (Centro Hospitalar e Universitário de Coimbra-CHUC, Portugal)
12h30-12h45 General discussion

12h45-13h15 Lunch box

13h15-13h45 Corporate Symposium - Sophia Genetics: Molecular Diagnosis of Neurometabolic Diseases Using SOPHIA Clinical Exome Solution
Sofia Isabel Gouveia (Hospital Clínico Universidad de Santiago de Compostela, Spain)

14h00-14h15 Opening & Welcome
Isabel Carreira, Eunice Matoso, Rosário Pinto Leite, Conselho Diretivo da FMUC

14h15-14h50 Keynote Lecture 1: "Implementing whole-genome sequencing in routine - consequences to the NHS"
Lucy Raymond (Cambridge Institute for Medical Research, UK)

Chairs: Carla Oliveira

14h50-16h20 Invited Symposium I (ISI): "Therapy for Genetic Diseases"
Chairs: Sérgio B. Sousa and Ana Berta Sousa
14h50-15h20 ISI-1: "From gene discovery to gene therapy in 20 years: the choroideremia case"
Miguel Seabra (Centro de Estudos de Doenças Crónicas - CEDOC, Nova Medical School, Lisbon, Portugal)
15h20-15h50 ISI-2: "From disease mechanism to new treatments in X-linked hypophosphatemia"
Helena Gil Peña (Hospital Universitario Central de Astúrias, Oviedo, Spain)
15h50-16h20 ISI-3: "Targeted therapy in patients with PIK3CA-related overgrowth syndrome"
Guillaume Canaud (Necker Hospital, Paris, France)

16h20-17h00 Coffee-break / Poster viewing and Discussion

17h00-18h00 5 Selected Oral Presentations I (Basic Research)
Chairs: Susana Fernandes and João Gonçalves

18h00 SPGH General Assembly
Chairs: Isabel Carreira, Eunice Matoso, Rosário Pinto Leite

09h00-10h00 5 Selected Oral Presentations II (Clinical Research)
Chairs: Sérgio B. Sousa and Luísa Romão

10h00-11h00 Panel Discussion I (PDI): "SPGH and SPO-Portuguese Society of Oncology: Building bridges"
Chairs: Sofia Maia and Sara Meireles
10h00-10h20 PDI-1: "Somatic testing of *BRCA* genes in tumours: implications for germline testing: The Oncologist perspective"
Gabriela Sousa (Portuguese Institute of Oncology, IPO, Coimbra, Portugal)
10h20-10h40 PDI-2: "Somatic testing of BRCA genes in tumours: implications for germline testing: The Geneticist perspective"
Ana Berta Sousa (Hospital Santa Maria, Lisboa, Portugal)
10h40-11h00 Discussion

11h00-11h30 Coffee-break / Poster viewing and Discussion

11h30-13h00 Invited Symposium II (ISII): "Mosaicism: Old but Hot Topic"
Chairs: Joana Barbosa de Melo and Lina Ramos
11h30-12h00 ISII-1: "Mosaicism in human blastocysts: incidence, prevalence and diagnostic capabilities in PGT-A cycles"
Antonio Capalbo (Igenomix, Rome, Italy)
12h00-12h30 ISII-2: "Somatic Mosaicism in Tumour Genetics"
Stefan Aretz (University of Bonn, Germany)
12h30-13h00 ISII-3: "Brain somatic mutations in focal malformations of cortical development with epilepsy"
Stéphanie Baulac (Institut du Cerveau et de la Moelle Épinière, Paris, France)

13h00-14h00 Lunch break

14h00-15h00 Poster Viewing and Discussion

15h00-16h30 Invited Symposium III (ISIII): "Understanding disease through Big Data"
Chairs: Rosário Santos and Carolino Monteiro
15h00-15h30 ISIII-1: "Tumour transcriptomes reveal a prognostic alternative splicing signature in colorectal cancer"
Nuno Barbosa Morais (Instituto de Medicina Molecular, FMUL, Lisbon, Portugal)

15h30-16h00 ISIII-2: "Network based prioritization of genetically associated genes for 1225 human traits"
Pedro Beltrão (EMBL-EBI, Cambridge, UK)

16h00-16h30 ISIII-3: "Big data in healthcare in Rare genetic diseases"
Ignacio Medrano (Ramón y Cajal Hospital, Madrid, Spain)

16h30-16h45 Coffee-break

16h45-17h45 Invited Symposium IV (ISIV): "The importance of Small and Large: Single cells and whole Genomes"
Chairs: Sofia Dória and João Gonçalves
16h45-17h15 ISIV-1: "Single-cell sequencing and its importance for human genetics"
Malte Spielmann (Max Planck Institute for Molecular Genetics, Berlin, Germany)
17h15-17h45 ISIV-2: "Delineating the structure of chromosome rearrangements using multiple WGS technologies"
Anna Lindstrand (Karolinska Institute, Stockholm, Sweden)

17h45-19h05 Panel Discussion (PDII): "National and International Consortiums in Human Genetics"
Chairs: Carla Oliveira and Jorge Saraiva
17h45- 18h05 PDII-1: *"*The experience from the UK – Lessons from national projects: DECIPHER, DDD and 100,000 Genomes Project"
Lucy Raymond (Cambridge Institute for Medical Research, UK)
18h05-18h25 PDII-2: "GenomePT: From Gene Panels to WES, WGS and Population Genomics"
Manuel Santos (Institute of Biomedicine, University of Aveiro, Portugal)
18h25-18h45 PDII-3: "1 Million Genomes Project"
Astrid Vicente (Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal)
18h45-19h05 Open discussion with the audience

20h30 Conference Dinner

08h45-09h45 8 Selected Oral Presentations III (Clinical cases)
Chairs: Ana Berta Sousa and João Silva

09h45-10h45 BioEthics Debate (BE): "BIG Genomic DATA - Bigger scientific advantages means bigger ethical responsibilities"
Chairs: Heloísa Santos and André Pereira
BE-1: International genomic databases - Historical background. From Iceland to Africa.
Célia Ventura (Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal)
BE-2: Cross-border flow of human genetic data - The portuguese laws and norms. New law of National Health Service.
Cristina Caldeira (Universidade Nova de Lisboa, Lisboa, Portugal)
BE-3: National genomic databases – Ethical considerations including importance of transparency and other ethical standards. European Digital Single Market Big Data.
Carolino Monteiro (Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal)
Conclusions and general discussion
Heloísa Santos (President of SPGH's Committee of Ethics, Portugal)

10h45-11h30 Coffee-break

11h30-12h20 Keynote Lecture 2: "Functional characterization and therapeutic targeting of gene regulatory elements"
Nadav Ahituv (University of California San Francisco, California, USA)
Chairs: Isabel Carreira and President Elected of SPGH 2021

12h20-12h50 SPGH Award Lecture
Chairs: Carla Oliveira and João Gonçalves

12h50-13h15 SPGH Awards Ceremony
Chairs: Isabel Carreira and Carla Oliveira

13h15-13h30 Closing Session
Chairs: Isabel Carreira, Eunice Matoso, Rosário Pinto Leite

ORADORES CONVIDADOS

INVITED SPEAKERS

Thursday, November 14

Sérgio B. Sousa

Centro Hospitalar e Universitário de Coimbra-CHUC, Portugal

Sérgio B. Sousa, MD, PhD, is a medical geneticist at Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Portugal, with an especial interest in intelectual disability syndromes, dysmorphology and genetic skeletal disorders. Sérgio is the coordinator of the local skeletal dysplasias multidisciplinary team. This team has been reorganized and improved since 2015 and is integrated in the European Reference Network on Rare Bone Disorders ERN BOND. Sérgio trained in multiple centres across Europe, including the Centre de Référence des Maladies Osseuses Constitutionnelles, Hôpital Necker-Enfants Malades, Paris, and completed his PhD in 2014 at the UCL Institute of Child Health, London, focused on identifying novel genes for rare unsolved syndromes. Currently, Sérgio has also been involved in implementing a local multidisciplinary genomics interpretation team; is Board Member of the European Society of Human Genetics; and Assistant Professor at the University of Coimbra Medical School, involved in training medical students, future medical geneticist and other professionals.

Gaetan Lesca

Service de Cytogénétique
Centre de Biologie et de Pathologie Est
Groupement Hospitalier Est, France

Since 2008: MCU-PH (Assistant Professor) at the Université Claude Bernard Lyon 1, Faculté de Médecine Lyon Est
2007-2008: Associate doctor in the dans le Laboratoire de Génétique, Hôpital Edouard Herriot (Pr Calender)
2005- 2007: Research fellowship (jeune chercheur) INSERM U871, Lyon (Pr. Fabien Zoulim)
2001-2003: assistant professor (AHU): Laboratoire de Génétique Moléculaire, Hôp. E. Herriot, Lyon (Pr. Alain Calender)
2000–2001: Master 2 (DEA) génétique moléculaire des maladies du développement et de l'oncogénèse (Paris V)
1999–2000: Service National
1995–1999: Internship of Medicine, Lyon
1989-1995: Medical studies at the Faculty of Medicine of Grenoble

GRADUATIONS
2016: Habilitation à diriger les recherches (Université Claude Bernard – Lyon I)
2007: DU de pédagogie médicale (Pr. Guy Llorca, Lyon).
PHD in molecular biology (Université Ckaude Bernard Lyon I)
2001: DEA (Master 2) de génétique moléculaire des maladies du développement et de l'oncogénèse (Paris V)
Graduate in Medicine.
1999: Graduate in Medical Genetics
1998: Baccalauréat C

PUBLICATIONS
• 123 publications referenced in PubMed, among which 49 are related to epileptic disorders.
• Redaction of the chapter of the Encyclopédie Médico-Chirurgicale entitled "aspect génétique des epilepsies" (2018)

Francisco Sales

Epilepsy Unit, Neurology Department, Centro Hospitalar e Universitário de Coimbra CHUC, Coimbra, Portugal

Qualifications:
Medical degree in 1984; Neurologist since 1992; Neurophysiologist since 1994; Consultant of Neurophysiology since 1999
Main areas of interest:
Epilepsy, EEG and Clinical Neurophysiology
Awarding Institutions:
Faculdade de Medicina da Universidade de Coimbra
Serviço de Neurologia dos Hospitais da Universidade de Coimbra
Centro de Estudos Egas Moniz, Hospital de Santa Maria Lisboa
The Neurological Institute, Columbia Presbyterian Medical Center, New York
Current Career position:
Consultant of Neurophysiology at Epilepsy Unit, Neurology Department.
Centro Hospitalar e Universitário de Coimbra (CHUC).
Main current responsabilities:
Coordinator of the Epilepsy Reference Centre of CHUC
Coordinator of the Epilepsy Surgery Program CHUC
Past-President of the Portuguese League Against Epilepsy
Teaching and training activities:
Collaborated in teaching Neurology to medical students from the University of Coimbra
Supervised end of degree projects of students from Informatics Department of the University of Coimbra
Supervised end of degree projects of students from IEETA University of Aveiro
Collaborated in post graduate teaching in the Neurology at CHUC
Teacher / trainer of European Epilepsy Academy (EUREPA)
Coordinator of the training programme in EEG and Clinical Neurophysiology at CHUC
European Projects collaborations – ESBACE, NASH, EpiCARE, E_PILEPSY
Organized courses and national and international meetings on epileptology
Collaborated in international projects

Lucy Raymond

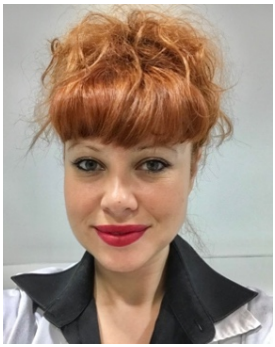
Cambridge Institute for Medical Research, United Kingdom

Lucy Raymond is Professor of Medical Genetics and Neurodevelopment at the University of Cambridge and Honorary Consultant in Medical Genetics at Cambridge University Hospital, Cambridge Biomedical Campus, UK. Her research interest is understanding the genetic basis of intellectual disability and has identified many disease causing genes over the years which have been rapidly translated into clinical service. She leads several collaborative efforts to identify rare disease genes including the 100,000 Genome Project and rapid diagnosis in NICU and PICU.

Miguel Seabra

Centro de Estudos de Doenças Crónicas - CEDOC, Nova Medical School, Lisbon, Portugal

Is a leading researcher in biomedical sciences (> 160 international peer-reviewed publications, h-index 61). His work involves molecular mechanisms of disease and novel therapies with a focus on retinal disease. He was the coordinator of a pioneering clinical trial of retinal gene therapy and a founder of Nightstar Therapeutics, a Nasdaq listed company. He is Full Professor at Universidade Nova de Lisboa-NOVA Medical School and a senior investigator at CEDOC-Chronic Diseases Research Centre. He had previous Faculty appointments at: University of Texas Southwestern Medical Center (1994-1997), Imperial College London (1997-2007); President of FCT (2012-2015); representative of H2020 ERAC strategic committee, co-responsible for the 2015 Portuguese RIS3 strategy; President of Science Europe (2014-2015).



Helena Gil Peña

AGC de Pediatría, Hospital Universitario Central de Asturias, Oviedo, Spain

Graduated in biology in 2004, obtained my PhD in 2009 at Universidad de Oviedo. I am related to many pediatric researches of hypocrecimiento and its molecular bases in the context of chronic kidney disease and hereditary diseases such as hypophosphatemic rickets, distal renal tubular acidosis or Gitelman syndrome among others. Principal coordinator since 2009 of the international RenalTube data base of rare diseases. Since 2015, carrying out diagnostic genetic tests of pediatric diseases at the Central University Hospital of Asturias, focusing on hereditary nephropathies, combining teaching tasks at the University of Oviedo and basic research with rat and mouse models at the Department's Research Laboratory of Paediatrics also from the University of Oviedo. Principal Investigator of several competitive Spanish research projects and author of almost forty publications in international journals.

Guillaume Canaud

Necker Hospital, Paris, France

Guillaume Canaud is a MD, PhD working in the Renal Division of Necker Hospital. He did his medical school in Montpellier and moved to Paris in 2002 to perform his Residency in Nephrology (2002 to 2007). He became Senior Resident in the Renal Division of Necker (Prof. Legendre) from 2007 to 2012. Concurrently, he spent four years from to 2008 to 2012 in the laboratory of Dr. Fabiola Terzi (INSERM U1151, Necker Hospital) to achieve his PhD degree in molecular and cellular biology. Then, he joined the Joseph Bonventre's Laboratory (Harvard Medical School, Boston, USA) from 2012 to 2014 to achieve a postdoc. He came back to Christophe Legendre's team with a Faculty position (Associate Professor) and built his own group of research dedicated to translational medicine. He obtained an European Research Council starting grant (2016) for his kidney research project and an ERC Proof of Concept Grant for his translational research (2017). Guillaume is now full professor at the Paris Descartes University and is working specifically on rare disorders involving the PIK3CA/AKT/mTOR pathway. Very recently, Guillaume and his group, identified and reported in Nature a very promising therapeutic for patients with a rare genetic disorder called PIK3CA-Related Overgrowth Syndrome. He published as a first or last author in top leading medical and scientific journals such as Nature, The New England Journal of Medicine, Nature Medicine, Journal of The American Society of Nephrology or Kidney International. He received several awards including the 2018 Prize Jean Lecocq of the French Academy of Sciences and the 2019 Paris Jean Hamburger Prize.

Friday, November 15

Gabriela Sousa

Portuguese Institute of Oncology (IPO, Coimbra, Portugal)

Diretor of Medical Oncology Department in Portuguese Institute of Oncology in Coimbra, she is dedicated in a clinical pratice to breast cancer and urologic cancers. Past president of Portuguese Society of Oncology (2015-2017) and now president of cientifique commitée. Co-editor of Portuguese Society of Oncology's cientific review.
Principal and co-investigator in some clinical trials (fase II – fase IV) and academic trials.
Member of ESMO and Investigations cooperative groups: EORTC; SOLTI; GPGU.
Coordinator of Familiar Risk Clinic breast and ovarian cancer since 2006.

Ana Berta Sousa

Hospital Santa Maria, Lisboa, Portugal

Ana Berta Sousa graduated in Medicine in 1995 and received her PhD in Genetics from the University of Cologne, Germany, in 2000. She trained in Medical Genetics at Hospital Santa Maria, Lisboa, and passed her exams in 2008. She stayed on at Hospital Santa Maria as a consultant and became Head of Genetics in 2013. From 2010 she is also a clinical geneticist at Hospital da Luz Lisboa. She is an invited professor at the Faculty of Medicine – University of Lisbon, where she teaches Immunology to undergraduates and is responsible for the Module on Familial Cancer Risk of the Oncobiology Master's Programme.

Antonio Capalbo

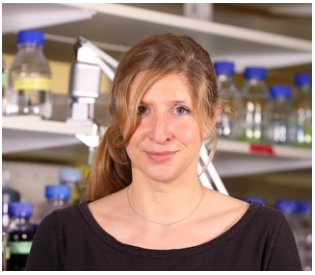
Scientific and Laboratory Director Igenomix, Rome, Italy

Dr. Capalbo received his Bachelor of Science degree in Biotechnology from University of Rome 'La Sapienza' and his Ph.D. magna cum laude in Human Genetics at the Catholic University of Sacred Heart of Rome in 2011. Since 2008 Dr. Capalbo has been working as clinical embryologist at GENERA, Reproductive Medicine in Rome. Since then, his research has focused on preimplantation genetic testing and on the development of novel molecular biology techniques to improve pregnancy and take-home baby rates in ART. He received several grants for innovative studies on these subjects. From 2012-2017 he was co-founder and Laboratory Director at GENETYX, largest PGT program in Italy. From 2017 he is working in the board of R&D directors at Igenomix and as Laboratory Director for Igenomix Italy. He is currently senior deputy of the ESHRE SIG in Reproductive Genetics and member of the genomics-working group of the Italian society of human genetics. He has published more than 70 peer-reviewed papers and book chapters and is currently reviewer for many journals in the field of reproductive biology and genetics.

Stefan Aretz

University of Bonn, Bonn, Germany

Stefan Aretz is a medical geneticist with a long-term interest in familial tumour syndromes working at the Institute of Human Genetics at the University Hospital Bonn in Germany. He is professor of genetics of familial tumour syndromes, speaker of the center for hereditary tumour syndromes in Bonn, member of the European Reference Network GENTURIS, head of the research group Familial Colorectal Cancer, and curator of the international InSiGHT APC mutation database. His main scientific interest is genetic and clinical research in gastrointestinal polyposis syndromes and hereditary non-polyposis colorectal cancer (HNPCC / Lynch syndrome), in particular the identification of novel genetic causes, the functional characterisation of unclear genetic variants, and the description of the mutational and phenotype spectrum. A few years ago, his team identified a novel rare recessive subtype, the MSH3-associated adenomatous polyposis.



Stéphanie Baulac

Institut du Cerveau et de la Moelle Épinière, Paris, France

The main objectives of my research are to unravel the molecular bases of inherited focal epilepsies and sporadic malformations of the cortical development, and to elucidate their underlying pathophysiological mechanisms. In 2013, we identified a novel gene for familial focal epilepsies, DEPDC5, a repressor of the mammalian target of rapamycin (mTOR) signaling pathway (Ishida et al. 2013).
My current research projects focus on focal epilepsies associated to focal cortical dysplasia (FCD), a malformation of cortical development that frequently causes intractable pediatric epilepsy. Brain somatic mutations in genes belonging to the mTOR-pathway have recently emerged as major actors in this pathology (Baldassari et al. 2019).
Our pioneer studies revealed the existence of second-hits somatic DEPDC5 mutation in resected brain tissues after epilepsy surgery in FCD patients (Baulac et al. 2015; Ribierre et al. 2018). Single cell studies demonstrated a clear link between neuropathology and genetic findings. Mice models of brain somatic mutations recapitulate most clinical and neuropathological features of FCD.

Stephanie Baulac holds a PhD in Neurogenetics from the University Paris Descartes (2001). She was trained at the Harvard medical School in Boston as a post-doctoral fellow. She obtained a tenure position at Inserm in 2005, and was promoted research director in 2013.
As a PI, she has obtained multiple grants among which the European Research Council (ERC consolidator-2016), and recently was awarded by the AXA Research Fund program.
She has received several prizes: Valerie Chamaillard Prize from the French Foundation for Research on Epilepsy (2014), Michael Fondation Prize (2019), Grand Prix Robert Debré (2019).

Nuno Barbosa Morais

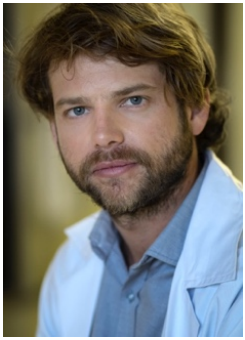
IMM, Lisbon, Portugal

Nuno Barbosa-Morais graduated in Physics Engineering at Instituto Superior Técnico in 2000. He did his PhD in Biomedical Sciences in 2007 at the Lisbon Medical School with Maria Carmo-Fonseca, with most of the associated research actually taking place at the University of Cambridge with Samuel Aparicio. His PhD work involved bioinformatics studies on the complexity of splicing and gene expression and his efforts include studies on the evolution of splicing factors and the RNA binding of splicing factors.Nuno Barbosa-Morais was a Research Associate in the Computational Biology Group from the University of Cambridge, led by Simon Tavaré and based at the CRUK Cambridge Research Institute, from 2006 to 2010 and a Senior Postdoctoral Fellow with Ben Blencowe at the University of Toronto from 2010 to 2013, being involved in the analysis of mRNA-seq data for the inference of tissue- and species-specific alternative splicing patterns. Nuno Barbosa-Morais was awarded a Postdoctoral Fellowship from the Canadian Institutes of Health Research and a Marie Curie International Outgoing Fellowship. From 2013 to 2015 he was an Honorary Senior Research Fellow at the Nuffield Department of Obstetrics and Gynaecology of the University of Oxford.He established the Disease Transcriptomics research group at Instituto de Medicina Molecular in 2015 as an EMBO Installation and an FCT Investigator Grantee.

Pedro Beltrão

Sanger Centre, Cambridge, United Kingdom

PhD in Biology, University of Aveiro (research conducted at EMBL-Heidelberg), 09/2007. Postdoctoral research at the University of California San Francisco. Group leader at EMBL-EBI since 2013.



Ignacio Medrano

Ramón y Cajal Hospital, Madrid, Spain

Neurologist at Ramón y Cajal Hospital for 10 years, Ignacio's curriculum includes several master's in health management, as well as being part of the Strategic Management Department of Ramón y Cajal Institute of Health Research. After graduating in 2014 from Singularity University (NASA, Silicon Valley), he founded two companies in artificial intelligence in healthcare: Savana (electronic medical record processing) and Mendelian (diagnosis and treatment of rare diseases). Ignacio was considered by the specialized press among the most influential people in health in 2016, for his work promoting systemic changes in the sector through IT and big data. In 2018 he was appointed the digital personality of the year in Healthcare. He has received the Princess of Girona Foundation Award - Company 2019 for "democratizing access to medical- scientific information of millions of patients through artificial intelligence".

Malte Spielmann

Max Planck Institute, Berlin, Germany

Malte Spielmann attended Medical School at the University of Witten/Herdecke in Germany followed by a research fellowship at Harvard Medical School in Boston. He completed his residency program in Medical Genetics at the Charité University Hospital in Berlin. From 2016 to 2018 he was a research fellow at the University of Washington in Seattle. Currently he is a group leader at the Max Planck Institute for Molecular Genetics in Berlin. His research focuses on the role of non-coding variants in human disease. His lab is using single cell and chromosome conformation capture technologies to study embryonic development and the onset of disease.

Anna Lindstrand

Karolinska Institute, Stockholm, Sweden

Anna Lindstrand is Associate Professor and specialist in Clinical Genetics. Head of the Clinical Genetics diagnostic laboratory at the Karolinska University Hospital and group leader for Rare Diseases research group at the Department of Molecular Medicine and Surgery at the Karolinska Institutet, in Sweden. Co-chair of Genomic Medicine Sweden - Rare Diseases. Area(s) of research: The study of rare genetic diseases, both clinically and at the molecular level to improve genetic diagnostics, increase knowledge about genotype-phenotype correlations and further understanding of disease biology. Especially interested structural genomic variation, how it forms and how it causes human genetic disease. Detailed characterization of chromosomal rearrangements is performed with a variety of methodologies and next generation sequencing platforms. Findings are functionally validated in induced pluripotent stem cells and zebrafish embryos.

Manuel Santos

iBIMED, University of Aveiro, Portugal

Manuel Santos is the coordinator of GenomePT and the founding director of the Aveiro Institute of Biomedicine – iBiMED. He did a degree in Biology at the University of Coimbra, a PhD in Biochemistry at the University of Kent-UK and was Wellcome Trust Fellow, EMBO Young Investigator and a founding director of the Medical Sciences Department of the University of Aveiro. He also coordinates the iBiMED Medical Microbiology and Genomics groups and is interested on understanding the evolution of the genetic code and the impact of protein biosynthesis errors on genome stability and evolution. He published over 130 papers over the last 30 years, including in Nature, PNAS, EMBO Journal, Genome Research, Genome Biology and Nucleic Acids Research, raised over 10 million euros of national and international funding to support his research and has a keen interest in teaching RNA Biology, Genetics and Genomics. He is currently involved in creating the conditions to implement Genome, Preventive and Personalized Medicine in Aveiro by setting up new MSC degrees in Management of Clinical Research, Medical Statistics and Genome Bioinformatics and boosting clinical collaborations with regional hospitals.

Astrid Vicente

INSA, Lisbon, Portugal

Astrid M. Vicente is a senior researcher in biomedical sciences and public health, and Head of the Department of Health Promotion and Non-Communicable Disease Prevention at the National Institute of Health Doutor Ricardo Jorge, in Lisbon, Portugal. Her research aims to understand the etiology and pathophysiological mechanisms underlying complex and highly prevalent non-communicable diseases, with a multidimensional perspective of non-communicable diseases, including biomedical but also epidemiological and social issues. Current research projects focus on the integration of multiple levels of information - biological, clinical, socio-demographic and environmental – using systems medicine methodologies to define models with predictive value for improved diagnosis, prognosis and drug therapy in clinical settings. This approach is expected to provide a broad perspective of gene-environment interactions in health and disease, and ultimately allow the progression towards translation of knowledge into personalised medicine tools for health promotion, disease prevention and better therapeutic strategies in public health and clinical settings. To develop these lines of research, she is involved with large research consortia and networks, established to foster the collaboration between experts from different fields and conduct large scale population analysis. Astrid M Vicente has contributed to the scientific literature in her research area, particularly in autism, other neuropsychiatric diseases like schizophrenia and bipolar disorder, cerebrovascular diseases (stroke) and autoimmune diseases (Systemic Lupus Erythematosus). She is an Invited Associate Professor at the Faculty of Sciences, University of Lisbon, regularly organizing and teaching graduate courses and workshops. She has served as elected President of the Portuguese Society for Human Genetics and received several national awards for her scientific contributions. She was recently appointed representative of the Portuguese Health Ministry for the International Consortium for Personalized Medicine (ICPerMed), of which she is currently elected Vice-chair, and the 1+M Genomes initiative, where she is part of the governance working group.

Célia Ventura

INSA, Lisbon, Portugal

Researcher in the genetic toxicology group of the Human Genetics Department, National Institute of Health Doutor Ricardo Jorge (INSA). Member of ToxOmics - Center for Toxicogenomics and Human Health, NOVA University of Lisbon (UNL). Presently, a research team member of three research projects, namely HBM4EU (H2020 grant 733032), INGESTnano (grant PTDC/SAU-PUB/29481/2017) and ToxApp4NanoCELFI (grant PTDC/SAU-PUB/32587/2017). Ethics adviser in the LungCard project (Horizon 2020 grant 734790) and member of the Ethics Committee of the National School of Public Health, UNL. Formerly, worked as a molecular genetics laboratory technician at INSA Department of Human Genetics, mainly in the field of coagulopathies (1993-2015). Graduation in Clinical Analysis and Public Health from the Lisbon School of Health Technology (1993), Master Degree in Bioethics from the Portuguese Catholic University with a dissertation on the use of biobanks for genetics research (2008), and PhD in Public Health from UNL (2019) with a thesis on the genotoxic and epigenotoxic effects of human exposure to nanofibres. Published a book and a book chapter on the ethics of biobanks for genetic research, and (co)authored several papers in international peer-reviewed journals. Invited speaker in several scientific meetings on bioethics, molecular biology of coagulopathies or genetic toxicology. Collaboration in the teaching of post-graduate courses, and MSc or PhD programmes.

Cristina Maria de Gouveia Caldeira

Universidade Nova de Lisboa, Lisboa, Portugal

Jurista, docente universitária e investigadora integrada da Fundação para a Ciência e Tecnologia, I.P, Portugal e da Universidade Nova de Lisboa. Professora convidada em instituições de ensino superior, nacionais e estrangeiras, e investigadora visitante na Universidade de Oxford, St Antony's College, com bolsa da Fundação Calouste Gulbenkian. Participa habitualmente em Júri de concursos para provimento dos cargos de direção, no setor público – DGRM, Ministério do Mar.Aviso n.º 2113/2019, publicado no D. R. 2.ª Série, n.º 26, de 6 de fevereiro de 2019 e publicitada na BEP com Código de Oferta OE201902/0274. Possui um pós-doutoramento na área da Propriedade Intelectual, intitulado: os direitos de autor como garantia da independência e dignidade dos criadores e intérpretes na era digital: uma perspetiva luso-brasileira, realizado na Universidade Nova de Lisboa. É doutorada em Direito, na Especialidade em Ciências Jurídicas e Políticas, pela Universidade Autónoma de Lisboa (UAL). Concretizou o programa doutoral em Ciência Política, na Especialidade de Políticas Públicas, no Instituto de Estudos Políticos da Universidade Católica Portuguesa, em Lisboa. Colabora com a Pontifícia Universidade Católica no Brasil/RS, em aulas e seminários e integra o projeto internacional Dignidade Humana, Direitos Humanos e Fundamentais e Proteção de Dados na Área da Saúde, num contexto de Regulação de Múltiplos Níveis, aprovado em 2018 pela CNPq Ministério da Ciência, Tecnologia, Inovações e Comunicações, Brasil. Colabora com o Laboratório de Bioética no Hospital de Clínicas do Rio Grande do Sul, Brasil, como investigadora na área de proteção de dados biomédicos. Como Adjunta do Ministério da Ciência, Tecnologia e Ensino Superior (2015-2018), com louvor (Despacho 1378/2018, de 8 de fevereiro), participou na elaboração de projetos de diplomas legais na área da proteção de dados, da ciência e da saúde e acompanhou a criação da Estratégia Europeia para a Inteligência Artificial.

Carolino Monteiro

Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal

Carolino Monteiro, doutorado em Genética Molecular, é especialista em Genética Humana pelo Ministério da Saúde e pela Ordem dos Biólogos, e foi-lhe atribuído o título de Clinical Laboratory Geneticist pelo European Board of Medical Genetics (CLG-EBMG).
Foi conselheiro do Conselho Nacional de Ética para as Ciências da Vida (CNECV) e é membro da Comissão de Ética para a Saúde do INSA Dr. Ricardo Jorge e do Conselho de Ética da ESTeSL-IPL.
Foi professor na Faculdade de Ciências Médicas UNL e no Instituto de Higiene e Medicina Tropical.
Orientou várias dissertações de mestrado e de doutoramento e publicou vários artigos científicos e capítulos de livro.
Atualmente é Professor Associado com Agregação na Faculdade de Farmácia da Universidade de Lisboa.

Heloísa G. Santos

President of SPGH's Committee of Ethics, Portugal

Consultant in Medical Genetics and Paediatrics. President of SPGH Bioethics Committee. Head of Genetics Unit of Paediatric Service, Hospital Santa Maria (1974- 1999). First Director of Medical Genetics Service of Hospital Santa Maria, Lisboa (1999 -2004). Consultant in Medical Genetics of Molecular Medicine Institute, Faculty Medicine, University Lisbon (2004-2007). Permanent Consultant in Medical Genetics of portuguese Directorate-General of Health, since 1996.University Assistant of Medical Genetics in Faculty of Medical Sciences of University of Lisbon (1977-1982). University Assistant of Medical Genetics of Faculty of Medicine University of Lisboa (1983-1991). PhD in Genetics (1991), Faculty of Medicine University Lisbon (FMUL). Invited Professor of Medical Genetics in FMUL (1991- 2004). Lecture in Bioethics in the Bioethics Centre of Faculty of Medicine University of Lisbon (from 2000). Member of UNESCO International Bioethics Committee (2002-2006). President of Bioethics Council of Portuguese Nacional Health Institute, INSA (2012- 2015); President of Bioethics Committee of Portuguese Paediatrics Society until 2016.National Genetics Award - 1991; Tuberous Sclerosis Association Award - 1994; INSA AWARD – 2017.Member of ESHG (from 1978), BSMG (from 1984), Portuguese Society of Paediatrics (from 1970), SPGH (Honorary), Tuberous Sclerosis Association (Honorary). European Society Human Genetics (ESHG) – member from 1978. Host and local President of the ESHG 30th Annual Meeting (Lisbon, 1997). Scientific Programme Committee Member (1997-1999). Portuguese Society Human Genetics- Founder member (1996), President in 1997 (first) and 2004. Honorary Member (2011), President of Bioethics Committee from 2010. Over 120 publications in Medical Genetics and Bioethics most in internacional journals and books, including "Genética para todos -de Mendel à Revolução Genómica do século XXI: a prática, a ética, as leis e a sociedade" (com André Dias Pereira, Gradiva, 2019). Research leader in several scientific projects.

Nadav Ahituv

UCSF, University of California San Francisco, USA

I am a human geneticist/genomicist that uses advanced computational and genomic tools to characterize how variation in gene regulatory elements leads to various phenotypes, in particular human disease. My lab uses numerous genomic technologies (ChIP-seq, ATAC-seq, Cut&Run, RNA-seq, Hi-C and single-cell technologies) to characterize gene regulatory elements. In order to functionally characterize these elements, we use zebrafish, mouse and cell culture functional assays. In addition, we have created and continue to develop technologies that can enable the massively parallel testing of thousands of sequences for gene regulatory activity. Finally, we are also using gene regulatory elements as therapeutic targets for various human diseases.

PALESTRAS

LECTURES

Brief ERNs introduction

Sérgio B. Sousa, Centro Hospitalar e Universitário de Coimbra-CHUC, Portugal

Thursday, 14th November – 11:45h

European Reference Networks (ERNs) are virtual networks involving healthcare providers across Europe. They aim to facilitate discussion on complex or rare diseases and conditions that require highly specialised treatment, and to concentrate knowledge and resources. The first 24 ERNs, targeting large thematic issues, were launched in 2017. In them, more than 900 health units from over 300 hospital centres and 150 patient association from 26 European countries participate. A brief overview of ERNs development will be presented.

The Clinical Patient Management System (CPMS) is a secure web-based application to support ERNs and help to gather multidisciplinary panel discussions and carry out e-consultations. It started working in 2018 and has been continuously optimised.

For all ERNs, the input of medical and laboratory geneticists is crucial. For the SPGH members, likely the more relevant networks are ERN-Ithaca, ERN-GENTURIS, MetabERN, ERN-BOND, ERN EpiCARE, EURO-NMD, among others.

In Portugal, there are 21 hospital centres participating in 16 ERNs. Focus will also be given to the required convergence between the Portuguese plan for national reference centres and the ERNs.

EpiCARE - ERN for rare and complex epilepsies: overall organization and what is in there for the geneticist

Gaëtan Lesca, Lyon University Hospital, France

Thursday, 14th November – 11:55h

Major advances have been performed in the identification of Mendelian causes of epilepsies, leading to the identification of more than 130 rare diseases. Diagnostic tools dramatically improved with the use of dedicated gene panels or exome sequencing, providing an accurate etiological diagnosis in many patients. Such advances are already paving the way to some targeted therapies, addressing the cause of disease. However, the increased size of genetic data often makes genetic assessment difficult and unravels a higher level of complexity in terms of phenotype variability and functional consequences. At the initiative of the European Commission the medical communities were recently asked to build-up European Reference Networks for rare diseases. This is a very challenging endeavor. If we succeed it, the benefits for the patients and for both fundamental and clinical research will be considerable. Success of the ERNs program highly depends upon the development of national networks in all EU countries. EpiCARE is the European Reference Network for Rare and Complex Epilepsies, bringing together 28 highly specialized healthcare centres, with expertise in rare an complex epilepsies in 13 EU countries and a number of affiliated partners. The coordination was transferred from London, GOSH (Pr Helen Cross) to the University Hospitals of Lyon in France (Pr Alexis Arzimanoglou) in 2019. The ERN EpiCARE aims at offering a coordinated approach for epilepsy diagnostics and treatment by utilizing e-tools and cross-country e-consultancy. The network functions through a series of vertical workpackages (WP), including between others laboratory diagnostics (WP2), and targeted therapies (WP7). Running through these WP are horizontal subnetworks to consolidate registries (WP12), guidelines (WP13), and education (WP14) of professionals and patients. Expert genetic labs and medical geneticists experts in epilepsy disorders are invited to play a significant role in WP2, whose objectives are to: i) refine phenotype genotype correlations, ii) optimize the genetic work-up to save resources when possible, and complete with functional validation if needed, iii) identify groups providing next generation sequencing and new gene identification, iv) identify expert groups able to provide genetic evaluation and diagnosis at the service of health care providers and researchers across Europe. The tasks in which genetic teams are currently involved are the WebEx sessions for interpretation of complex cases, the Open access publications on epilepsy phenotype per aetiology, and a steadily increasing number of research projects in the field.

EpiCARE: a European Reference Network for rare and complex epilepsies

Francisco Sales (Centro Hospitalar e Universitário de Coimbra CHUC, Portugal)

Thursday, 14th November – 12:20h

EpiCARE is a network of 28 centres in 13 European countries with expertise in the rare and complex epilepsies. The network was initially coordinated by Great Ormond Street Hospital for Children NHS Trust, London and led by Professor Helen Cross, and since March 2019, EpiCARE is being coordinated by the University Hospitals of Lyon (HCL), France, led by Professor Alexis Arzimanoglou, in close collaboration with Professor Cross.

The following are the main objectives of the EpiCARE network:

1. To improve accessibility of detailed diagnostics to individuals of all ages with rare and complex epilepsies across Europe, including clinical evaluation and investigation;
2. To develop treatment protocols and monitor standardized outcomes of rare and complex epilepsies;
3. To improve awareness and accessibility to protocols for physicians and individuals with rare and complex epilepsies across Europe for treatment;
4. To enhance educational activities and training opportunities across Europe by interchange across network;
5. To enhance opportunities for registries, and collaborative research for the benefit of individuals with rare and complex epilepsies across Europe.

In this talk I will address how EpiCARE is organized and the elevance of the different workpackages for the clinicians and research as well.

In the context of this meeting I will give some emphasis to workpackage 2 – Laboratory Diagnostics, due to the importance and the increasingly recognized genetic, immune and metabolic nature of some epilepsies.

Molecular Diagnosis of Neurometabolic Diseases Using SOPHiA Clinical Exome Solution

Sofia Isabel Gouveia (Hospital Clínico Universidad de Santiago de Compostela)

Thursday, 14th November – 13:15h

Inborn errors of metabolism (IEM) are rare genetic disorders characterized by diverse clinical and biochemical phenotypes. The complexity of signs and symptoms often impairs the prompt achievement of a reliable diagnosis. Next-generation sequencing (NGS) technologies are now more accessible and enable faster analysis of individual genomic data, thus improving the genetic diagnosis of IEM.

Our work describes the results obtained by using the SOPHiA Clinical Exome Solution (CES), an application that covers the coding regions (± 5bp of intronic regions) of 4,490 genes with known inherited disease-causing variants, including SNVs, Indels and CNVs.

A definitive genetic diagnosis was achieved for 32 out of the 86 patients (37%). In 14 of the diagnosed patients, trans or homozygous variants were found in genes with recessive inheritance pattern. In 18 patients the molecular defects were detected in genes with dominant inheritance pattern. The adoption of this molecular application in the IEM diagnostic workflow helps reduce the turnaround time, as well as the number of analysis and treatments required. Overall, this approach facilitates appropriate genetic counselling.

Implementing whole-genome sequencing in routine – consequences to the NHS.

Lucy Raymond, Cambridge Institute for Medical Research, UK

Thursday, 14th November – 14:15h

With growing evidence that Mendelian diseases present in the neonatal period, there is a need for rapid, systematic, and comprehensive genomic diagnoses in intensive care to assist with acute and long-term clinical decisions. We have performed trio whole genome sequence analysis (WGS) on a prospective cohort of families recruited from neonatal and paediatric intensive care units (NICU and PICU) at a single site in the UK. We developed a research pipeline in collaboration with the National Health Service (NHS) to deliver validated pertinent pathogenic findings from WGS within 2-3 weeks of recruitment. Of the 400 families analysed to date, 30% received a molecular genetic diagnosis. The phenotypic description of the child was a poor predictor of the gene identified in 90% of cases, arguing for non-selective/comprehensive testing in NICU/PICU. The diagnosis affected clinical management in >65% of cases (83% in neonates) including modification of treatments, initiating new specialist care pathways and/or informing palliative care decisions. A 2-3-week turnaround was sufficient to impact most clinical decision making. The use of WGS in intensively ill children is acceptable to parents and trio analysis facilitates diagnoses. Our trio and "gene agnostic" approach was highly effective in identifying an underlying genetic condition, with phenotypes and symptomatology being primarily used for data interpretation rather than gene selection. Scale up of this process will support NHS commissioning of WGS for first line management of intensively ill children as well as family and future genetic counselling.

From gene discovery to gene therapy in 20 years: the choroideremia case

Miguel Seabra, Centro de Estudos de Doenças Crónicas - CEDOC, Nova Medical School, Lisbon, Portugal

Thursday, 14th November – 14:50h

From disease mechanism to new treatments in X-linked hypophosphatemia

Helena Gil-Peña, Hospital Universitario Central de Asturias. Oviedo, Spain.
Thursday, 14th November – 15:20h

X-linked hypophosphatemic rickets (XLH) is a genetic disorder caused by mutations in the PHEX gene, resulting in excessive expression of the phosphaturic molecule fibroblast growth factor 23 (FGF23). Although it is the most frequent form of hereditary hypophosphatemia, XLH is a rare disease, which implies ignorance of some aspects of its pathophysiology, due to its low incidence. Thus, although it is well known that two of the most important characteristics associated with XLH are growth impairment and bone deformities, the mechanisms underlying these defects remain unclear. Through the RenalTube database, focused on collecting data from patients with some rare renal tubular disorders, it has been described the largest Spanish series of XLH patients, showing an affectation of growth that is not corrected even after being under treatment with phosphate and vitamin D analogs supplementation. In addition, growth hormone (GH) treatment, used in those cases with more accused growth retardation, results motive of discussion since it improves growth velocity but also seems to increase risk of bone deformities. Two recent studies (Fuente et al., Bone 2018; Fuente et al,. FASEB J. 2019), based on the murine model of XLH, the Hyp mouse, suggests, for the first time, that growth impairment and the long bone deformities in these animals may be related to marked alterations in the structure, dynamics and maturation of growth plate combined with osseous alterations and that treatments focused on blocking FGF23 or the inhibition of its signaling pathway, alone or combined with GH, may be the most effective therapies over growth plate abnormalities and bone structure in the young Hyp mice. These results point FGF23 blocking plus GH as a promising new therapy for growth retardation and bone deformities in children with XLH that must be profoundly investigated.

Targeted therapy in patients PIK3CA-related overgrowth syndrome

Guillaume Canaud, Necker Hospital, Paris, France

Thursday, 14th November – 15:50h

CLOVES syndrome (congenital lipomatous overgrowth, vascular malformations, epidermal naevi, scoliosis/skeletal and spinal syndrome) is a genetic disorder that results from somatic, mosaic gain-of-function mutations of the PIK3CA gene and belongs to the spectrum of PIK3CA-related overgrowth syndromes (PROS). This rare condition has no specific treatment and a poor survival rate. Here, we describe a postnatal mouse model of PROS/CLOVES that partially recapitulates the human disease, and demonstrate the efficacy of BYL719, an inhibitor of PIK3CA, in preventing and improving organ dysfunction. On the basis of these results, we used BYL719 to treat nineteen patients with PROS. The drug improved the disease symptoms in all patients. Previously intractable vascular tumours became smaller, congestive heart failure was improved, hemihypertrophy was reduced, and scoliosis was attenuated. The treatment was not associated with any substantial side effects. In conclusion, this study provides the first direct evidence supporting PIK3CA inhibition as a promising therapeutic strategy in patients with PROS.

Somatic testing of BRCA genes in tumours: implications for germline testing: The Oncologist perspective

Gabriela Sousa, Portuguese Institute of Oncology, IPO, Coimbra, Portugal
Friday, 15th November – 10:00h

The year 2019 marked the twenty fifth anniversary of the discovery of the breast cancer susceptibility gene, BRCA1. Since this discovery, our understanding of pathogenic BRCA variants and the associated increase in lifetime cancer risks has advanced significantly. International and nacional guidelines have been developed to help clinicians identify patients with an increased risk of pathogenic BRCA mutations, and genetic counselling for risk assessment is now a routine practice.
BRCA pathogenic mutation positive ovarian or breast cancers and more recently prostate and pancreatic cancer are susceptible to inhibitors of additional DNA damage repair pathways, such as PARP inhibitors or platinum based chomotherapy.

Metastatic cancer is a major cause of death and is associated with poor treatment efficacy. A better understanding of the characteristics of late-stage cancer is required to help adapt personalized treatments, reduce overtreatment and improve outcomes. In recent years, several large-scale whole-genome sequencing (WGS) analysis efforts have yielded valuable insights into the diversity of the molecular processes that drive different types of cancer and have fuelled the promises of genome-driven oncology care. Somatic tests identified 4-12% of germline mutations. The evidence show us that >50% of these patients would not have tested using clinical guidelines. Which genes and variants are important to consider when performing somatic NGS? A policy statament of the American College of Medical Genetics and Genomics define 59 medically actionable genes included BRCA 1 e BRCA2.

Important questions is about medical counselling for somatic NGS test. Current practice in many countries, included in Portugal requires face-to-face counselling with a qualified genetics counsellor both prior to, and following, BRCA testing. As demand for genetic testing increases, new approaches for delivering genetic counselling may be necessary.

Somatic testing of *BRCA* genes in tumours: implications for germline testing: The Geneticist perspective

Ana Berta Sousa, Hospital Santa Maria, Lisboa, Portugal

Friday, 15th November – 10:20h

Mosaicism in human blastocysts: incidence, prevalence and diagnostic capabilities in PGT-A cycles

Antonio Capalbo PhD, Laboratory director Igenomix, Italy

Friday, 15th November – 11:30h

Mosaicism is defined as the presence of two or more genotypically different cell lines in a given organism. Differently from uniform aneuploidies, which mostly derive from aberrant meiotic processes, embryo mosaicism mainly originates from mitotic segregation errors in post-zygotic developmental stages. Mosaicism has been detected in less than 2% of prenatal specimens (i.e. through amniocentesis and villocentesis). In sharp contrast, it has been reported in up to 73% of cleavage stage preimplantation embryos. Due to this alleged high incidence, mosaicism has recently attracted particular attention from the scientific community in relation to its impact on embryo viability and reproductive outcome in *in vitro* fertilization (IVF) cycles with preimplantation genetic testing for aneuploidies (PGT-A). The transition between array-based to NGS-based technologies has marked a radical change in this field, where the technically higher sensitivity toward chromosome copy number variations has theoretically provided improved investigative tools for mosaicism status in trophectoderm (TE) biopsies. The identification of profiles compatible with mosaicism in embryos tested for the presence of chromosomal aneuploidies has severe consequences on patient's treatment outcomes. Currently, the methodology for determining mosaicism is not highly specific and a definitive diagnosis is based on weak criteria, in which mosaicism is only a potential explanation. Indeed, the incidence of mosaicism in human blastocyst stage embryos has been extremely overestimated in the last years by interpreting as mosaic the physiological variability experienced during NGS chromosomal analysis. Despite its evident shortcomings, mosaicism diagnosis from TE biopsies has become a common practice in PGT-A treatments after the introduction of NGS based technologies.

Nonetheless, altered copy number profiles consistent with mosaicism for specific chromosomes cannot be completely ignored in the PGT-A diagnostic workflow at the current stage. This presentation will provide the basic theoretical knowledge of processes giving rise to embryo mosaicism, discussing latest studies on the subject and providing a logic framework for the management of embryo mosaicism in clinical contexts.

Somatic Mosaicism in Tumour Genetics

Stefan Aretz, University of Bonn, Germany

Friday, 15th November – 12:00h

Whereas malignant tumours represent a classic and well known paradigm of a mosaic disease, somatic (postzygotic) mosaicism is actually a ubiquitous phenomenon of dividing cells that is also observed in apparently normal tissue. The number of detectable mosaic events rises sharply after age 50 and is associated with a higher risk for developing sporadic, age-related cancer. By applying NGS technologies, somatic mutational mosaicism in genes underlying high-penetrant tumour predisposition syndromes is also more frequently observed than previously thought and its clinical importance is increasingly recognized. It is particularly relevant in sporadic cases of conditions characterised by a high de novo mutation rate such as familial adenomatous polyposis, neurofibromatosis, tuberous sclerosis, von Hippel-Lindau disease, and retinoblastoma.

Furthermore, somatic mosaic mutations are mandatory in genes where germline (constitutional) mutations are supposed to be lethal, in particular sporadic segmental overgrowth disorders such as Proteus, Sturge-Weber, CLOVES, Maffucci / Ollier, and Klippel-Trenaunay syndrome. However, low-level mosaicism detected in blood samples may also reflect mutations in circulating DNA from sporadic tumours. Likewise, it has been suggested that extended chemotherapy induce somatic TP53 mutations in haematopoietic cells, mimicking the presence of mosaic mutations. As a consequence, somatic mutational mosaicism presents a challenge for both molecular and clinical diagnostics and contributes to deviations from predicted genotype-phenotype correlations. In addition to mosaic index cases, subclinical or mild mosaicism can be found in parents of typically affected children with apparent de novo mutations. Hence, the confirmation of a mosaic mutation has major consequences for patient care and genetic counselling in families and thus, strategies to integrate screening for mosaicism in routine genetic diagnostics should be seriously considered.

Brain somatic mutations in focal malformations of cortical development with epilepsy

Stéphanie Baulac, Institut du Cerveau et de la Moelle Épinière, Paris

Friday, 15th November – 12:30h

Malformations of cortical development (MCD), in particular focal cortical dysplasia (FCD) or larger lesions such as hemimegalencephaly (HME), are major causes of severe pediatric refractory epilepsies subjected to neurosurgery. Neuropathological hallmarks of FCD type 2 and HMEs include cortical dyslamination and enlarged dysmorphic neurons and balloon cells.

Recently, there has been growing evidence that brain somatic variants play a major role in the etiology of these neurodevelopmental disorders. My talk will provide a comprehensive view of the occurrence of germline and somatic variants in a large cohort of patients with FCD and HME. We use ultra-deep sequencing to search for low-allele somatic variants in paired brain/blood patient samples, as well as in pools of microdissected dysmorphic neurons and balloon cells to elucidate the genetic cause. Our study unveils two distinct pathogenic mechanisms involving the non-mTOR-related gene SLC35A2 in FCD1, and mTOR-pathway in FCD2/HME, orienting towards targeted therapies. Our data also emphasize that a single hit in activators of the mTOR pathway or in MTOR itself is sufficient to cause the MCD, while two-hit mutations are necessary in repressors of the pathway (i.e. DEPDC5). We established a preclinical mouse model of brain somatic mutations combining in utero electroporation and CRISPR-Cas9 gene-editing to reproduce a focal and mosaic genetic hits. Mice with a low-level mosaic rate of crisperized or mutant neurons faithfully reproduced clinical and neuropathological phenotypes of focal epilepsy linked to FCD.

Tumour transcriptomes reveal a prognostic alternative splicing signature in colorectal cancer

Nuno Barbosa Morais, IMM, Lisbon, Portugal

Friday, 15th November – 15:00h

Colorectal carcinoma (CRC) is the fourth cause of cancer-related deaths worldwide. Dysregulation of alternative splicing (AS) is a molecular hallmark of cancer, having been associated with initiation and development of CRC. However, the global patterns of dysregulation of AS and its association to prognosis in CRC remain largely unexplored.

Clinically annotated tumour transcriptomes from The Cancer Genome Atlas (TCGA) were analysed in order to identify AS events with prognostic value in CRC. We revealed a novel gene expression-independent AS signature, with prognostic value additional to that assigned to pathological stage and age, dominated by three AS events in the mRNA complement of CELF2, a gene encoding for RNA-binding proteins and reportedly an onco-suppressor in CRC. Those events relate to the expression of three isoforms with alternative promoter usage and potentially distinct sub-cellular localization and functions in RNA processing, namely AS regulation, mRNA edition and translation inhibition. Analyses of CRC TCGA DNA methylation profiles revealed significant differences between the promoters of the involved isoforms consistent with their expression levels in matched patients. Our estimates, by digital cytometry, of immune cell composition of TCGA samples associate macrophage infiltration with worse prognosis. We also corroborated the prognostic value of alterations in CELF2 isoform expression using clinically annotated CRC samples from the local biobank. Further analyses in primary tumour-derived colon cancer cell lines suggest those alterations as markers of increased resistance to genotoxic therapy.

In summary, our analyses suggest that a switch in the relative expression of CELF2 isoforms represents a novel biomarker potentially usable in the prospective selection of patients for adjuvant therapy. We hypothesize that modifications in the dynamic balance between nuclear and cytoplasmic activities is the functional link between that switch and the CELF2 prognostic value, with cancer cells lacking the cytoplasmic isoform involved in translation inhibition associated with apoptosis signalling being able to evade apoptosis in response to genotoxic insult.

Network based prioritization of genetically associated genes for 1225 human traits

Pedro Beltrão, EMBL-EBI

Friday, 15th November – 15:30h

The number of genome wide association studies (GWAS) has grown over the past years with over 1000 human traits aggregated in the GWAS Catalog. However, connecting trait associated SNPs to causal genes remains a challenge. Based on the principle that genes associated with the same phenotype tend to be involved in the same cellular processes, network-based methods have been used to expand and prioritize trait associated genes. Here, we present and benchmark a strategy able to identify groups of highly connected genes relevant for a phenotype of interest, starting from GWAS candidate genes linked to SNPs by proximity. We combined network propagation with community detection via short random walks, to define gene modules, relevant for the different phenotypes evaluated. This approach enriches significantly for relevant drug targets and previously known disease associated genes. The network based trait association scores allows for trait-trait similarity analysis and can be further combined with patient specific mRNA/protein data for further prioritization. We focus on neurodegeneration diseases to illustrate the identification of shared aetiology and further integrate ALS SOD1 mutant patient mRNA/protein data to identify novel astrocyte candidate ALS linked genes.

Big data in heathcare in rare genetic diseases

Ignacio Hernandez Medrano, Ramón y Cajal Hospital, Madrid, Spain

Friday, 15th November – 16:00h

- Exponential technologies explode suddenly when an appropriate trigger appears.
- Digital health is exploding, thus a convergence of exponential technologies is happening.
- The biggest of all the exponential technologies is big data.
- Big data allows computers to learn patterns, thanks to a technique called machine learning, which disrupts classical statistics.
- Big data means finding correlations where human mind cannot do it, and allows the design of predictive algorithms.
- Predictive algorithms based on artificial intelligence are about to disrupt healthcare.
- The king of big data in Medicine is genome sequencing, an exponential technology, canalized by NGS, at very low price.
- Systems biology integrated with machine learning are alowing precision medicine.

Single-cell sequencing and its importance for human genetics

Malte Spielmann, Max Planck Institute, Berlin, Germany

Friday, 15th November – 16:45h

Embryonic development is an astonishing process. In mice most major internal and external organs develop within a short window of time, termed organogenesis. The key regulators of developmental defects can be studied during this critical window, but conventional approaches lack the throughput and resolution to obtain a global view of the molecular states and trajectories of a rapidly diversifying and expanding number of cell types.

We have recently developed a three-step combinatorial barcoding method to profile single-cell transcriptomes ('sci-RNA-seq3') without requiring physical isolation of each cell. We have used this new method to profile whole mouse embryos staged between 9.5 and 13.5 days of gestation with sci-RNA-seq3, and created a transcriptional atlas of mouse organogenesis at single cell resolution. We identify hundreds of expanding, contracting and transient cell types, many of which are only detected because of the depth of cellular coverage obtained here, and define the corresponding sets of cell type-specific marker genes, several of which we validate by whole mount in situ hybridization. We also delineate and annotate 56 single cell developmental trajectories of mouse organogenesis. We explore the dynamics of proliferation and gene expression within cell types over time, including focused analyses of the apical ectodermal ridge, limb mesenchyme and skeletal muscle.

This single cell atlas of the development of wild-type mice represents a first step towards understanding pleiotropic developmental disorders at the organismal scale. We are currently performing detailed single-cell investigations in mutant mice and patients' samples of subtle roles for genes and regulatory sequences involved in developmental defects.

Delineating the structure of chromosome rearrangements using multiple WGS technologies

Anna Lindstrand, Karolinska Institute, Stockholm, Sweden

Friday, 15th November – 17:15h

In recent years, technological advances have improved the turnaround time and cost-effectiveness of human genetic investigations in both diagnostics and research. However, most focus has been on single nucleotide variation (SNV). In contrast, the calling and interpretation of structural variants (SV) is still challenging and causal mutations often go unnoticed in genome studies. Further, the methods applied currently in genetic diagnostics have limitations in detection and resolution and in consequence causal variants may be missed or misinterpreted, resulting in sub-optimal clinical management.

Both balanced chromosomal rearrangements and copy number variants (CNVs) as detected by karyotyping or chromosomal microarray might benefit from further investigation by whole genome sequencing (WGS) to accurately resolve the structural rearrangement. We and others have shown that unexpected complexities are common findings in the breakpoints of karyotypically balanced chromosomal rearrangements. Such findings are of clinical importance, as they may be the cause of mendelian phenotypes in the rearrangement carrier.

Short read WGS allows for high resolution characterization of SVs, but problems remain for mapping breakpoints located in repetitive regions of the genome, which are known to be prone to rearrangements. In our study, we use multiple complementary WGS experiments to solve the structures of chromosomal rearrangements. In many cases, by delineating the derivative chromosomes we provide a molecular genetic explanation for the clinical symptoms observed in the carrier. Furthermore, we compare the performance, sensitivity and resolution of different WGS techniques in a clinical diagnostic laboratory set.

The experience from the UK – Lessons from national projects: DECIPHER, DDD and 100,000 Genomes Project

Lucy Raymond, Cambridge Institute for Medical Research, UK

Friday, 15th November – 17:45h

GenomePT: From Gene Panels to WES, WGS and Population Genomics.

Manuel Santos, iBIMED, University of Aveiro, Portugal

Friday, 15th November – 18:05h

GenomePT is the national research infrastructure for genome sequencing and analysis. It´s 11 nodes have invested over 7million euros over the last 5 years building genome sequencing and computing capacity and are now delivering high quality DNA and RNA sequencing data for both fundamental biomedical and biological research, helping the Portuguese Biomedical and Clinical communities to develop genomics and bioinformatics projects. However, Portugal remains one of the few European countries with little or no capacity to sequence Whole Human Genomes (WGS), Whole Human Exomes (WES) at competitive costs. Also, only a small number of research groups are engaged in Population Genomics projects, normally in collaboration with international partners.

Aging of the Portuguese population, increasing costs of health care and the urgent need to protect human genome data, while making it accessible to the research and clinical communities, demand a national action plan for WGS, WES and Population Genomics. The national consortium that represents Portugal in the European 1 Million+ Genomes initiative (MEGA) may provide a platform to prepare a white paper for the implementation of Population Genomics in Portugal, however it is important that the clinical and biomedical communities at large engage in this process. Additionally, human cohorts, biobanking, harmonized databases of clinical data, BIGDATA and Data Science in health, computing infrastructure and ethics need to be debated to ensure that the country puts in place the multiple components necessary for Population Genomics. The Society for Human Genetics can also play a role in mobilizing the research community, decision makers and funding agencies to this important endeavor. GenomePT is funded by the National Roadmap of Research Infrastrutures (Portuguese Foundation for Science and Technology) through project PINFRA/22184/2016, and European structural funds through project POCI-01-0145-FEDER-022184. iBiMED is supported by FCT through project UID/BIM/04501/2019.

1 Million Genomes project

Astrid Vicente, INSA, Lisbon, Portugal

Friday, 15th November – 18:25h

The recent progresses in DNA sequencing and digital technologies, as well as in bioinformatics, have provided a remarking opportunity to harness genomic knowledge to the benefit of patients and citizens. Individual genomic information will allow Personalised Medicine approaches for more accurate estimations of disease risk, earlier diagnosis and improved prognosis, and for more efficient therapeutic interventions. However, the challenge nowadays is still in the translation of research findings into clinical applications, and the potential of genomic medicine is still in its infancy. The areas that have benefited the most are rare diseases and cancer, and many projects all over the world, led by the UK 100,000 genomes, have been gathering genomic data to improve diagnosis and therapeutic decisions for these disorders. Multiple countries are also nowadays turning their focus on genomic data from the general population, aiming at better estimating common diseases risks based on polygenic risk scores. For any of these applications, large datasets with extensive clinical and genomic information are crucial. For these reason, a number of European countries have signed the "Towards access to at least 1 million sequenced genomes in the EU by 2022" Declaration, setting the stage for an European partnership for sharing genomes and clinical data across Europe. Sharing of BigData across countries with diverse legislation and genomic maturity levels brings enormous opportunity for Personalised Medicine, but also numerous challenges that need to be resolved: ELSI issues, standardization of sequences and clinical data, secure sharing technology, adoption by healthcare systems, stakeholders role including the private sector, and others. The 1+MGenome initiative is addressing these issues and aims to provide viable solutions across Europe, to make the Declaration a reality by 2022.

BIG GENOMIC DATA – Bigger scientific advantages means bigger ethical responsibilities

Heloísa G. Santos, Célia Ventura, Carolino Monteiro

Suturday, 16th November – 9:45h

Os Big Data são uma incontornável ferramenta da transformação digital da sociedade, denominada por K. Schwab, Presidente do Fórum Económico Mundial, de 4ª Revolução Industrial. Esta inclui as atividades de saúde. O termo pretende descrever conjuntos digitalizados de dados com grande volume ou complexidade nos quais os métodos de processamento convencional se mostram ineficazes para os interpretar. A medicina personalizada, genómica, nas suas 4 vertentes (personalizada, preditiva, preventiva e participativa) baseia-se na integração da atividade médica, curativa e preventiva (saúde pública), em três sistemas ciberfísicos - digitais, físicos e biológicos. Porque a informação genómica atual é complexa e as variações encontradas pouco claras, incertas ou de significado clínico duvidoso (Consent and confidenciality in Genomic Medicine, Report, BSGM, RCP, 2019) é indispensável a utilização de conhecimentos obtidos através de Big data genómico. Porém, estas megabases sendo modeladas, armazenadas, processadas, analisadas e utilizadas de forma não controlada poderão obviamente prejudicar direitos humanos, nomeadamente a dignidade individual dos dadores. Os detentores dos dados, poderosos grupos informáticos ou farmacêuticos, que os podem modelar e manipular de acordo com interesses científicos, mas também financeiros ou políticos, são também muitas vezes desconhecidos ou não-fiscalizáveis e, inclusive, a anonimização dos dados não é já uma garantia de que não possam vir a ser identificados. Pretende-se nesta MR, com a colaboração de todos, numa perspetiva bioética, lançar um olhar sobre esta imparável evolução. Será possível continuar a proteger a autonomia individual e a manter a autonomia, a confidencialidade e a privacidade dos dadores? Como poderemos garantir, a nível individual ou nacional, que informações "sensíveis" sobre algumas patologias e sobre outras características genómicas individuais não virão a ser distorcidas por falta de assegurado controle dentro ou fora do País? Uma "viagem" sobre os Big Data já existentes, a nível internacional e nacional, um melhor conhecimento sobre a legislação e as normas portuguesas e europeias sobre proteção de dados pessoais e Biobancos, irá permitir-nos a deteção das principais fragilidades existentes e uma reflexão sobre os desafios éticos destes novos tempos.

Normas de proteção de dados pessoais em big data genómico europeu, de acordo com a ética e a legislação portuguesa

Cristina Caldeira, Universidade Nova de Lisboa, Portugal

Saturday, 16th November – 09:45h

A nova era genómica, intrinsecamente ligada ao big data, é de suma importância para a melhoria da saúde dos indivíduos e da Humanidade, não obstante apresentar vulnerabilidades quer ao nível da identidade genética, quer ao nível da própria dignidade, que impõem um esforço de compatibilização entre os valores ético-jurídicos e o progresso da biotecnologia. O genoma humana não tem apenas um significado biológico, mas é também portador de uma dignidade antropológica que é protegida por séculos e séculos de civilização jurídica. A Convenção sobre os Direitos do Homem e a Biomedicina, protege o ser humano na sua dignidade e identidade e garante a toda a pessoa, sem discriminação, o respeito pela sua integridade e todos os outros direitos fundamentais, face às aplicações da biologia e da medicina.

O gozo desses direitos está associado à autonomia individual, à confidencialidade, à privacidade, bem como à proteção de dados pessoais, cujo tratamento no quadro do big data se submete às mesmas normas e princípios de proteção de dados pessoais constantes da legislação de proteção de dados pessoais, seja ela a Convenção do Conselho da Europa, seja a legislação europeia e nacional ou, para os Estados-membros da União Europeia o Regulamento (UE) 2016/679 relativo à proteção das pessoas singulares no que diz respeito ao tratamento de dados pessoais e à livre circulação desses dados, instrumento que permitirá a divulgação dos conhecimentos disponíveis na área da genómica e promoção do seu intercâmbio a nível nacional e internacional.

Esta breve reflexão constitui o ponto de partida para uma análise geral dos quadros jurídicos aplicáveis da União Europeia, do Conselho da Europa e da legislação portuguesa, apresentando ainda uma resenha jurisprudencial, sobre o sentido e alcance do direito à proteção de dados pessoais sensíveis e à privacidade, numa relação com outras liberdades e direitos fundamentais.

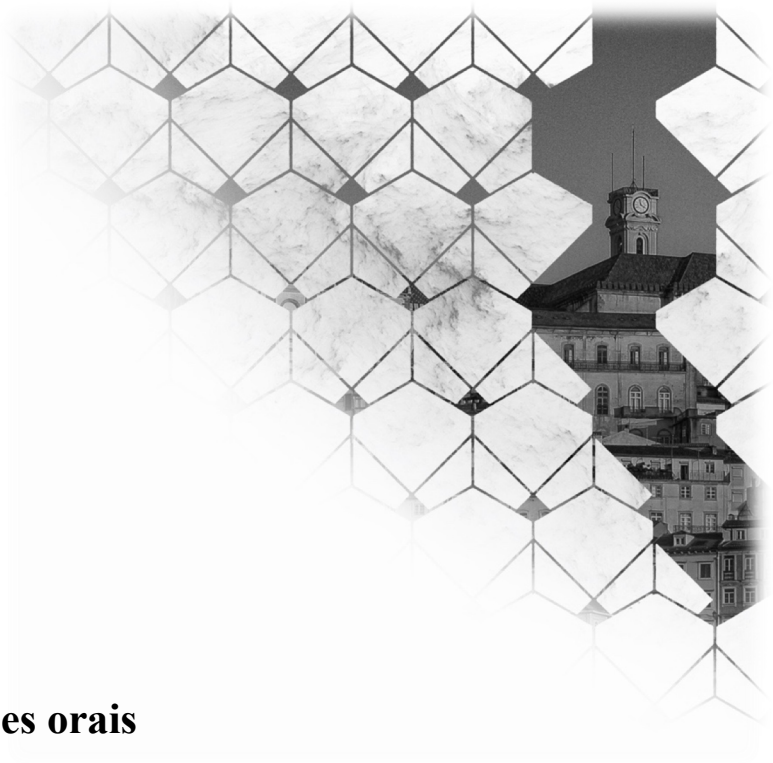
Palavras-chave: dignidade humana, genómica, dados de saúde, big data, proteção de dados pessoais.

Functional characterization and therapeutic targeting of gene regulatory elements

Nadav Ahituv, UCSF, University of California San Francisco

Saturday, 16th November – 11:30h

Nucleotide variation in gene regulatory elements is a major determinant of phenotypes including morphological diversity between species, human variation and human disease. Despite continual progress in the cataloging of these elements, little is known about the code and grammatical rules that govern their function. Deciphering the code and their grammatical rules will enable high-resolution mapping of regulatory elements, accurate interpretation of nucleotide variation within them and the design of sequences that can deliver molecules for therapeutic purposes. To this end, we are using massively parallel reporter assays (MPRAs) to simultaneously test the activity of thousands of gene regulatory elements in parallel. By designing MPRAs to learn regulatory grammar or to carry out saturation mutagenesis of every possible nucleotide change in disease causing gene regulatory elements, we are increasing our understanding of the phenotypic consequences of gene regulatory mutations. Regulatory elements can also serve as therapeutic targets. To highlight this role, we used CRISPR/Cas9 activation (CRISPRa) of regulatory elements to rescue a haploinsufficient disease (having ~50% dosage reduction due to having only one functional allele) in vivo. By targeting the Sim1 promoter or its 270kb distant hypothalamic enhancer, we were able to rescue the haploinsufficient obesity phenotype in Sim1 heterozygous mice, both using a transgenic and adeno-associated virus approach. Our results highlight how regulatory elements could be used as therapeutic targets to treat numerous altered gene dosage diseases.



Comunicações orais

Oral communications

OC1- The prognostic significance of E-cadherin in Gastric Cancer: an integrative approach based on patients' cohort and CRISPR-Cas9 engineered cell models

Carla Pereira[1,2], Marta Ferreira[1,2], Patrícia Oliveira[1,2], Fátima Carneiro[2,3], Gabriela M. Almeida[1,2,3], Carla Oliveira[1,2,3]

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E-cadherin/*CDH1* dysfunction is a well-established event in GC initiation and progression in nearly 80% of gastric cancers (GC), independently of histological type. While E-cadherin permanent loss is the trigger for diffuse GC (DGC), transient aberrant expression is common along progression in intestinal GC (IGC). DGC has poorer prognosis than IGC and it spreads to the peritoneum, while IGC metastasizes to distant organs. We hypothesize that the timing (initiation vs progression) and mode of E-cadherin loss of function (permanent vs persistent; complete loss vs aberrant) determine the GC pattern of tumour spreading and prognosis and therefore explored the underlying mechanisms. The pattern of E-cadherin expression was analyzed by immunohistochemistry and correlated with clinicopathological features and overall survival (OS) in 284 patients. Permanent (CRISPR-Cas9) and transient (RNAi) E-cadherin depleted cell models representative of DGC and IGC were established and characterized by RNA-sequencing and label-free quantitative proteomics profiling followed by bioinformatics analysis. GC presenting aberrant E-cadherin expression were more often IGC, more advanced, more often spread to distant organs, and displayed poorer prognosis than GC with complete E-cadherin loss or normal E-cadherin expression. Remarkably, GCs with absent/residual E-cadherin expression were more often DGC. Proteomics and transcriptomic profiling revealed that transient and permanent E-cadherin depletion in the DGC model dramatically impairs cell-cell (adherens, tight juntions and desmosomes), and cell-matrix adhesion. The same manipulations in the IGC model led to cadherin-switch and downregulation of adherens junction and cell motility proteins. Our study demonstrates that E-cadherin dysfunction is associated with poor prognosis. Our data supports the hypothesis that E-cadherin transient loss in DGC generates an acute phenotype of cell-cell and cell-matrix adhesion loss that persists and likely prevents spreading to distant sites; while transient or permanent E-cadherin loss in IGC likely triggers cell detachment and expression of alternative cadherins allowing spreading to distant organs.

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OC2- *CYP46A1*- gene therapy improves Machado-Joseph disease in mouse models

Clévio Nóbrega[1,2,3#], Liliana S. Mendonça[3#], Adriana Marcelo[1,2], Antonin Lamazière[4], Sandra Tomé[3], Gaëtan Déspres[4], Carlos Matos[3], Fatich Mechmet[1,2], Dominique Langui[5], Wilfred den Dunnen[6], Luís Pereira de Almeida[3,7*], Nathalie Cartier[8*] and Sandro Alves[9*]

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Aims/Context: Machado-Joseph Disease (MJD) is a neurodegenerative disease associated with extensive neuronal death. Defects in brain cholesterol metabolism may contribute to neurodegenerative diseases. Brain cholesterol is almost exclusively synthesized *in situ* and cannot cross the blood-brain-barrier. To maintain the cholesterol homeostasis, superfluous cholesterol is converted into 24S-hydroxycholesterol by the neuronal enzyme cholesterol 24-hydroxylase (*CYP46A1*). The present work evaluated i) whether *CYP46A1* levels are reduced in MJD, ii) if *CYP46A1* overexpression could improve MJD, and iii) the mechanisms behind the observed recovery. Methods: *CYP46A1* levels were evaluated in cerebellar extracts of MJD patients and in transgenic MJD mice cerebella. *CYP46A1* overexpression effect was assessed in two MJD mouse models. In the lentiviral-based mouse model, AAVrh10 encoding *CYP46A1* or GFP (control) were injected into the striatum of C57BL6/J mice, and 2 months post-injection the neuronal marker DARPP32 levels and mutant ataxin-3 (mutAtxn3) inclusions´ size and number were measured. Transgenic MJD mice were injected into the cerebellum with AAVrh10 encoding *CYP46A1* or GFP and motor performance was evaluated. Then mice´s cerebella were analyzed for mutAtxn3 inclusions, Purkinje cell numbers, and cerebellar atrophy. Moreover, *CYP46A1* potential activation of autophagy was evaluated in Neuro2A cells and *in vivo.* Results: Our data indicate that *CYP46A1* cerebellar levels are decreased by 46% in MJD patients and by 29% in MJD mice. *CYP46A1* overexpression reduced DARPP32 loss (48%), mutAtxn3 inclusion number by 59% and their size by 47%. Significant alleviation of motor behavior impairments correlated with mitigation of MJD-associated neuropathology, namely, reduction of Purkinje cell loss (34%) and of cerebellar atrophy (25.40% in lobule X) was observed. Finally, our work demonstrated that *CYP46A1* overexpression induces autophagy (LC3B-II increase and p62 decrease) both in *in vitro* and *in vivo* MJD models. Conclusions: Overall our results demonstrate that *CYP46A1* overexpression improves MJD-associated motor coordination and neuropathology through autophagy enhancement.

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OC3- The role of *CDH1* regulatory noncoding elements for E-cadherin expression

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Introduction: *CDH1* pathogenic germline variants cause Hereditary Diffuse Gastric Cancer (HDGC), in less than half of patients/families fulfilling clinical criteria. *CDH1*-negative cases often display germline *CDH1* monoallelic expression and somatic E-cadherin loss of function. We hypothesized that *CDH1*-negative HDGC may arise due to germline defects in *CDH1* regulatory regions. Therefore, we explored the *CDH1* regulatory network to find expression modifier sequences, controlling *CDH1* expression, that could potentially explain E-Cadherin loss of function phenotypes. Materials and Methods: Capture Hi-C (cHi-C) with a viewpoint in *CDH1* promoter was performed in 5 gastric cancer cell lines, either positive or negative for E-cadherin expression. Mouse embryo reporter assays were used to test a candidate regulatory region by cloning in LacZ-reporter, integration into ColA1 locus of mouse embryonic stem cells, and generation of transgenic mice. Empty-vector mice were used as control for ColA1-driven expression. Tissue-specific β-galactosidase expression was tested in dissected tissues: stomach, esophagus, duodenum, liver (endoderm), heart (ectoderm) and skin (mesoderm). Results: We found evidence that *CDH1* promotor interacts simultaneously with an intergenic region in the short arm of chromosome 2 and an intronic region within *CDH1* intron 2. These interactions were specific of *CDH1*-negative cell lines, highlighting a potential negative regulatory network. We so far tested the regulatory potential of the *CDH1* intronic region and found tissue-specific β-galactosidase expression in endodermal-derived tissues (stomach, esophagus and duodenum), where E-cadherin exerts a primordial function. Conclusion: We found a potential negative regulatory network in gastric cancer cell lines through cis and trans interactions of the CDH1 promoter, and evidence for its tissue-specific regulation in the stomach. These findings suggest a novel mechanism triggering E-cadherin loss of function, worth to be tested in HDGC patients negative for CDH1 germline coding variants.

Financial support: 1) Solve-RD project, grant agreement No 779257 (European Union's Horizon 2020 research and innovation programme); 2) FEDER/COMPETE,"POCI-01-0145-FEDER-030164"; 3) FCT Fellowship, "SFRH/BD/140796/2018"

OC4- Centrosome positioning and development of ciliopathies: role of the human centrosomal protein TBCCD1

Bruno Carmona[1,2,3], Carolina Camelo[2#], Mehraz M[4], Lemullois M[4], David C. Ferreira[1,2], Sofia Nolasco[3,5], Lince-Faria M[6], H. Susana Marinho[1,2], Mónica Bettencourt-Dias[6], Tassin AM[4], Koll F[4], Helena Soares[1,2,3]

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Aims/Context: Primary cilia are specialized microtubule-based signaling organelles that convey extracellular signaling and cellular polarity into a cellular response. Defects in primary cilia assembly/function cause severe diseases known as ciliopathies, typified by clinical manifestations, as infertility, obesity, brain problems, blindness and kidney cysts. Primary cilia assembly entails centrosome migration to the plasma membrane where a centriole docks, maturates into a basal body (BB), and assembles the cilia axoneme. The human centrosomal TBCCD1 is a critical factor in centrosome positioning previously identified by us. Our aim is to discover the mechanisms/signals required for the correct positioning of the centrosome during cilia assembly, and how these mechanisms, when compromised, are related to ciliopathies. Methods: The proximity-dependent identification (BioID) assay was used to screen for TBCCD1 interactors. Immunofluorescent and super resolution microscopy, as well as Western blot, were used to study the levels and cellular localization of the identified TBCCD1 interactors in human RPE1 cells overexpressing or depleted of TBCCD1. To study the impact of TBCCD1 knockdown in motile cilia the ciliate *Paramecium*, containing ~3,000 motile cilia, was used. Results: Our BioID screen for TBCCD1 interactors identified several well-known proteins encoded by ciliopathy genes, e.g. the centrosomal protein OFD1 involved in the Orofacio-Digital Syndrome. We show that TBCCD1 knockdown and overexpression in RPE1 cells affects OFD1 distribution. Super resolution microscopy shows TBCCD1 is localized at the distal region of the centrosome and that its depletion dramatically affects the centrosome subdistal protein CEP170, a component of cilia basal feet. In *Paramecium*, TBCCD1 knockdown causes abnormal BB-associated structures organization and anomalous BB positioning/anchoring defects. Conclusions: Our data support a role for TBCCD1 in the maintenance of centrosome structure and in BB anchoring at the cell membrane during ciliogenesis. TBCCD1 is emerging as a novel protein with a role in human ciliopathies.

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OC5- Altered expression of imprinted genes and epigenetic regulators in placental tissue from intrauterine growth restriction

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Intrauterine growth restriction (IUGR) is a fetal growth condition characterized by the inability of the fetus to achieve its growth potential, which is dependent on normal placental function and development. Abnormal imprinted gene expression has been associated with abnormal placental development and function. The expression of these genes is controlled by epigenetic modifications, such as DNA methylation, at Imprinting Control Regions (ICRs). DNA hydroxymethylation was recently described and arises from the conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the action of TET enzymes. The aim of this study was to evaluate the expression levels of imprinted genes and epigenetic regulators, DNA methylation at ICRs and global levels of DNA hydroxymethylation in placentas from IUGR pregnancies. Quantitative Real-Time PCR was performed in term placentas from 21 IUGR samples and 9 non-IUGR samples to evaluate the expression levels of seven imprinted genes (*PHLDA2, CDKN1C, KCNQ1, H19, IGF2, PEG10,* and *MEST*) and five epigenetic regulators (*DNMT1, DNMT3A, TET1, TET2* and *TET3*). Additionally, standard bisulfite genomic sequencing and Combined Bisulfite and Restriction Analysis (COBRA) were performed to evaluate ICR2 (or KvDMR1) methylation. Global 5-hydroxymethylcytosine (5-hmC) was measured using the ELISA-based assay. *CDKN1C, PHLDA2*, and *PEG10* expression were significantly upregulated in placentas from IUGR, which also showed concomitant KvDMR1 hypermethylation. 5-hmC was present in both groups of placental tissue, with no significant changes between the two groups (IUGR vs controls). Overexpression of *CDKN1C* and *PHLDA2* genes, concomitantly with KvDMR1 hypermethylation, are consistent with fetal growth restriction since these genes negatively regulate growth. The global DNA hydroxymethylation analysis confirmed the presence of 5-hmC in the placenta, although levels did not differ in fetal growth restriction placentas. Our results suggest an important role for epigenetic modifications and modifiers in the control of human fetal growth.

OC6- Implementing mainstreaming genetic testing of *BRCA1/2* for cancer patients in a Portuguese tertiary hospital: preliminary results

Mariana Soeiro e Sá[1], Juliette Dupont[1], Ana Berta Sousa[1]

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Context: "Mainstreaming Cancer Genetics" is a clinical research programme first created at the Royal Marsden NHS Foundation Trust to provide a faster and less costly pathway to bring *BRCA1/2* testing directly to cancer patients through oncology appointments. Our aim was to implement a similar programme in our hospital. Methods: We defined patient selection criteria and designed a protocol for patient referral, informed consent, sample collection, and genetic testing. We centralized the process through Genetics, thus making sure we have knowledge of all patients included and their results. We promoted educational sessions for oncologists, prepared brochures for patients explaining the principles of genetic testing and possible results, and produced standard letters and clinical reports to accompany laboratory test results. Statistical analysis was done after codification.
Results: Since September 2018, 106 patients were referred, of whom 94 were included and 12 were excluded due to not meeting criteria. The most frequent inclusion criteria was breast cancer (BC) <40 years (n=32), followed by ovarian cancer (n=18), and triple negative BC (n=15). Of 86 available results, we identified 9 (10.5%) pathogenic variants (8 germinal/1 somatic) and 6 variants of unknown significance (VUS) (5 germinal/1 somatic). All patients in whom a variant was identified had a genetics appointment (to provide counseling and/or try to clarify the significance of VUS) within a mean time of 17.9±15.8 days. The mean response time of genetic testing was 51.7±9.8 days. The mean time from patient referral to final results (sent to the oncologist) was 67.7±18.8 days. Conclusions: We significantly decreased our waiting time for urgent patients and lessened the burden of extra urgent appointments in our clinic, while providing patients with faster testing that might impact their treatment. In the future we will assess exactly what this impact was in patients and their families, the degree of satisfaction with the protocol (both from patients and oncologists) and the current classification of previously identified VUS

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OC7- The first genotype-phenotype study of European carriers of CDH1 germline mutations

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Introduction: Hereditary Diffuse Gastric Cancer-HDGC is caused by *CDH1* Pathogenic-P and Likely Pathogenic-LP germline variants, predisposing for diffuse gastric cancer-DGC and lobular breast cancer- LBC. *CDH1* variant carriers fulfilling HDGC criteria undergo intensive screening and/or preventive risk reduction gastrectomy/mastectomy while asymptomatic. We explored genotype-phenotype correlations to clarify the *CDH1*-associated disease spectrum and demonstrate the value of genetic-testing driven by phenotype and clinical-criteria. Materials and Methods: We collected/curated clinical-criteria and variant classification from 506 probands carrying coding and splice-site *CDH1* variants, from 10 EU Countries belonging to ERNGENTURIS. We registered 1361 phenotypes from 1302 family members. Results: 160/506 (31.6%) families fulfilled HDGC clinical criteria. While 87.5% of HDGC families carried P/LP actionable variants (96.4% truncating), only 13.3% (100% truncating) of those lacking criteria carried such variants (p=10-5). The preferential phenotype in LP/P variant carriers, independently of fulfilling HDGC criteria, was GC (23% GC, 39% DGC). In contrast, the preferential phenotype in VUS carriers (94.4% missense) was BC (59% BC, 2% LBC). Among families carrying P/LP variants, DGC was always the most prevalent phenotype in all age ranges. Among LP/P variants carriers, there was an excess of DGC<40, and LBC>40 years old (p=0.0003). In VUS and LB/B variant carriers, BC of undefined histotype was the major phenotype. No other relevant phenotypes were found in probands carrying LP/P variants and their relatives. Conclusion: This is the first genotype-phenotype and *CDH1* variant-driven study performed to date. It demonstrates that most carriers of truncating and clinically actionable *CDH1* variants fulfill HDGC clinical criteria, reinforcing their use in clinical practice. It establishes early onset DGC and LBC as the phenotypes associated with *CDH1* deficiency, and castoffs colorectal, ovarian and other GC and BC histotypes as part of the HDGC disease spectrum, supporting the need to expand clinical criteria to accommodate LBC.

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OC8- Management of Patients with Low-Penetrance Copy Number Variants – do they all need to see a Clinical Geneticist?

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Introduction: Since the introduction of Chromosomal Microarray as a first line test for children being investigated for neurodevelopment delay the number of patients referred to the Genetics Clinic with Low penetrance copy number variants (LP-CNVs) has increased dramatically. The aim of this study was to determine the outcome of clinical genetics assessment in children referred because of a LP-CNV with a view to developing best practice guidelines for referral to Genetics clinic. Methods: A retrospective analysis of the 10 most common LP-CNV referrals from 2016-2019. LP-CNVs were classified as inherited or de novo, not done or not available. In each case the LP-CNV was assessed in relation to the presenting phenotype as possibly causative, partially causative, not causative, incidental finding or carrier. Results: A total of 163 patients with LP-CNVs were included: 1q21.1q21.2 deletion -10; 1q21.1q21.2 duplication -23; 15q11.2 deletion -52; 15q13.2q13.3 duplication -10; 16p11.2 deletion -5; 16p11.2 duplication -8; 16p12.1 deletion -6; 16p13.11 deletion -14; 16p13.11 duplication -29; 22q11.21 duplication -10. Parental study was performed in 51 cases, not requested in 94 and not possible in 18 cases. The LP-CNV was considered to be a possible or partial cause in 128 (78.5%) and was not thought to be the cause in 27 cases (16.6%). In 18 cases (11%) a confirmed or suspected alternative diagnosis for the child's condition was made by the geneticist. Conclusion: Patients in which the LP-CNV was classified as possibly or partially causative had mild neurodevelopmental delay with or without a family history of mild learning difficulties. We propose that these patients could be managed by a ND Paediatrician and do not need referral to Clinical Genetics. The 18 children found have an additional genetic diagnosis all had various combinations of: moderate or severe neurodevelopmental delay, dysmorphic features, malformations, neurological or cutaneous signs. These clinical features should prompt referral to Clinical Genetics. These proposals would require specific training for Paediatricians, and good communication and teamwork between Paediatricians and Geneticists.

OC9- Genome-wide characterization of a cohort of Alzheimer's patients from Iberia: a focus on rare variants

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Aims/Context: To perform a genetic characterization of a cohort of late-onset Alzheimer's disease (LOAD) patients from Northern Portugal and Spain focusing on the spectrum of genome-wide rare variants (*MAF*). Methods: DNA was extracted from saliva and buccal swab samples and genotyped with Axiom Spain Biobank Array, which provides high coverage of whole-genome common and low frequency variation as well as Mendelian and functional alleles that are specific to Spanish. This analysis comprised 128 LOAD patients from Northern Portugal and from the Spanish autonomous community of Castile and León with a clinical diagnosis of AD. In addition, 59 controls (individuals over 65 years old with no signs of dementia or other brain disorders) from both regions were also analyzed. Rare variants in genes relevant for AD and with highly deleterious potential as assessed by CADD were prioritize for detailed annotation. Gene- based tests using SKAT-O and different models were performed to examine the aggregate effect of risk and protective variants. Results: In spite of the modest sample size, we detected a suggestive enrichment of rare variants in cases in several genes with functional links to AD. Overall, 16 AD genes harbored very rare pathogenic/likely pathogenic variants in our sample (cases and controls), thus making these variants much more frequent in our cohort than in control populations from GnomAD and ExAC. Conclusions: This study provides a characterization of genome-wide rare variation in AD in Castile and Léon and in Northern Portugal, the latter a region where dementing diseases are highly prevalent but still understudied. Even in a small sample of patients we were able to identify rare pathogenic variations and an excess of rare variants in cases in genes likely relevant to AD etiology, making this a valid approach to identify genes contributing to AD burden.

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OC10- miRNA expression profile of plasma-derived extracellular vesicles distinguishes Machado-Joseph Disease patients from controls

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Background: Machado–Joseph disease (MJD) is the most common dominantly-inherited ataxia worldwide. It is caused by a CAG over repetition in *ATXN3* gene, which translates into a mutated ataxin- 3 protein that accumulates in neurons, causing neuronal dysfunction and death. MJD leads to premature death and there is no therapy available. Potential therapeutic approaches have emerged, but the lack of large cohorts and biomarkers remain major barriers for the success of interventional studies. Extracellular vesicles (EVs) are enriched in specific small RNAs that are pointed out as promising biomarker candidates. Here, we established a Portuguese cohort of MJD patients that integrates the ESMI cohort and performed a transcriptomic analysis of EVs obtained from patients' plasma to identify potential MJD biomarkers. Methods: The study was approved by Ethics Committee of Faculty of Medicine, University of Coimbra. Informed consent was obtained from all participants. Participants were characterized using clinical and functional tests. EVs were isolated by size exclusion chromatography. EVs size was characterized by nanoparticle tracking analysis; plasma contaminants by ELISA and RNA profile by automated electrophoresis. Small RNAseq was performed with an Illumina NextSeq system. Results: 48 patients were enrolled (26 males; 49.8±13.6 years old). At baseline, mean score for Scale for the Assessment and Rating of Ataxia (SARA) was 13.6±10.5. Follow-up data revealed a decrease in the performance of 8-meter walking test, but no variations from baseline in SARA. Obtained EVs exhibited sizes <150 nm, low levels of plasma contaminants and enrichment in small RNAs. MJD-EVs contained lower RNA levels than controls. Principal component analysis of the top 50 variable miRNAs present in plasma-derived EVs shows a segregated miRNA expressing profile between controls and patients. Conclusion: A Portuguese cohort of MJD patients was characterized and new biomarkers candidates were explored. miRNA expressing profile of plasma-derived EVs revealed novel blood-based candidates that distinguishes patients from controls having thus potential to be used as biomarkers for MJD.

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OC11- Koolen-de Vries syndrome – National Case Series with clinical and molecular characterization

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Introduction: Koolen-de Vries Syndrome (KdVS) is a rare genetic condition, caused by a 17q21.31 microdeletion, or a pathogenic variant in *KANSL1* gene. The clinical picture includes developmental delay (DD)/intellectual disability (ID) with expressive language particularly impaired, dysmorphisms, neonatal hypotonia, and friendly behaviour. Aim: To characterize at the molecular and clinical levels all patients in Portugal diagnosed with KdVS. Methods: 15 patients with a 17q21.31 deletion were identified in Portuguese Genetics Laboratories and Clinical Genetics Departments. Data were collected retrospectively by means of a questionnaire. Results: The deletion was detected in all cases by array-CGH, ranging from 431,7 to 987,4 Kb, and encompassed partially or completely *CRHR1, SPPL2C, MAPT, STH,* and *KANSL1* genes. Three patients had a maternally inherited deletion, including two siblings whose mother has low-grade somatic mosaicism. All individuals (age 3 to 37 years-old) had DD/ID and dysmorphisms. Hypotonia was reported in 8/11, motor delay in 6/8, and language/speech delay in 13/15. Brain malformations were present in 7/9 patients, and 4/6 had EEG abnormalities. Nine patients were overfriendly, and 6/11 had a neuropsychological disorder. The most common facial dysmorphisms were bulbous nose (9/10), long face (7/11), and narrow/short palpebral fissures, large/prominent ears, and broad chin (4/10). Congenital heart defects were present in 4/7, renal/urogenital anomalies in 7/12, visual and hearing problems in 8/10, musculoskeletal abnormalities in 7/10, and ectodermal anomalies in 7/11. Daily functioning was partially evaluated in 4/6 adult patients: all lived with family members, participated in family life, and were independent in daily living activities; 3 could read; 2 of them completed high-school, worked, used public transportation, could write a simple letter and read time. Conclusions: This study adds information to the molecular - one deletion was longer than typically reported in the literature - and clinical profile, including assessment of functioning in adults, of KdVS. It also points to the importance of parental segregation and mosaicism analysis.

OC12- Pachydysostosis of the fibula in a case of Gardner Syndrome - a case report

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CONTEXT: Gardner Syndrome (GS) is a well-known variant of Familial Adenomatous Polyposis (FAP) caused by certain germline *APC* mutations. GS is characterised by prominent extra-colonic manifestations, namely skull and mandible osteomas, desmoid tumours, epidermoid cysts, fibromas on the scalp, shoulders, arms and back and dental abnormalities. Pachydysostosis of the fibula (PF) is a rare clinical entity characterised by unilateral bowing of the distal portion of the fibula and elongation of the entire bone, without affectation of the tibia. Unilateral bowing of the leg is relatively common, however, in most cases fibula and tibia are involved, being a clinical entity distinct from pachydysostosis. We describe a striking case of GS in which this life-saving diagnosis was unveiled by the multidisciplinary evaluation of skeletal findings: congenital PF and an osteoma. CASE REPORT: A 17-year-old male presented with a non-progressive bowing of the right leg detected at 18 months of age caused by a fibula malformation (later characterized as pachydysostosis) and a large exophytic osteoma of the left radius, noticed at the age of 15 years, without gastrointestinal symptoms. There was no relevant family history. Detailed clinical and radiological characterisation revealed multiple osteomas (of the left fibula, left ilium, metacarpals and mandible), skin lesions and dental abnormalities, raising the hypothesis of GS. This diagnosis was confirmed by genetic testing [c.4406_4409dup p.(Ala1471Serfs*17) *de novo* mutation in the *APC* gene] and endoscopic investigation, which identified the presence of multiple adenomatous polyps throughout the colon, ileum and stomach. CONCLUSION: This case report expands the known phenotypic spectrum of skeletal manifestations of GS: this patient has a congenital fibula malformation, not previously associated with FAP, but which is likely to have been its first manifestation in this patient. This clinical case also illustrates the challenges in the early diagnosis of patients with GS, especially without family history, and highlights the importance of a multidisciplinary team and the adequate study of rare skeletal abnormalities.

OC13- *DYRK1A*-related intellectual disability syndrome: report of seven Portuguese patients

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Introduction: *DYRK1A* heterozygous pathogenic variants have been recently shown to cause a syndromic form of intellectual disability (ID) with impaired speech development, features of autism spectrum disorder (ASD), microcephaly, and a recognizable facial gestalt that evolves with age. Most individuals present with foot anomalies, and about one third have short stature. We aim to contribute to further delineate the phenotype of this condition. Methods: Clinical data on all patients with *DYRK1A* pathogenic variants identified at Medical Genetics Departments in Portugal were retrospectively collected through a detailed clinical questionnaire. Results We describe seven unrelated patients, six females and one male, aged 3 to 21 years old, all representing simplex cases. Foetal growth restriction was present in 6/7, five of whom had birth length and head circumference at or below 2SD. Further growth evaluation showed microcephaly in 6/7 and short stature in 3/7. ID, ranging from mild to severe, and language impairment or absent speech were documented in all patients. ASD and/or stereotypic behaviours were present in 5/7. Three main facial features were consistently reported: deep-set eyes (5/7), large ears (5/7) and thin upper-lip (6/7). Foot anomalies and optic disc pallor were each present in three patients. One of the latter had optic nerve atrophy. Discussion/Conclusions: Our cohort illustrates the variable degree of severity of ID associated with *DYRK1A* pathogenic variants, which can include mild cases. Facial dysmorphisms, namely a characteristic appearance in older patients, microcephaly, and a typical neurobehavioral phenotype are all in accordance with the literature. Interestingly, optic disc pallor seems to be more frequent than previously reported and was related to optic nerve atrophy in one patient, highlighting the need for ophthalmological surveillance in the multidisciplinary management of this condition. Our study adds evidence to the existence of a consistent clinical phenotype of *DYRK1A*-related ID, hopefully contributing to increase awareness regarding the key features of this recognizable entity.

OC14- A *de novo* variant in myelin regulatory factor (*MYRF*) gene in an individual with cardiac urogenital syndrome

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Context: The myelin regulatory factor (*MYRF*) gene controls the expression of a transcription factor associated with myelination in the central nervous system. A new cardiac-urogenital syndrome (CUGS), highly overlapping the previously described PAGOD (pulmonary artery and lung hypoplasia, agonadism, omphalocele and diaphragmatic hernia) syndrome, was described in 2018 when 3 patients with *de novo* loss of function variants in *MYRF* were reported with congenital heart disease, genitourinary anomalies and pulmonary hypoplasia. Other phenotypic features include high hyperopia, nanophtalmos, encephalopathy and reversible myelin vacuolation. Case Report: We present a 17-year-old male with CUGS born at term to nonconsanguineous parents. Prenatal ultrasonography suggested genital ambiguity. Anthropometric parameters were adequate to gestational age. He had hypospadias, micropenis and bilateral cryptorchidism. No dysmorphic features were apparent. Hypoplastic left heart syndrome was diagnosed by echocardiogram. Hyperopia was diagnosed at 6 years of age. Brain MRI at 4 years old showed signs of delayed myelination. He had severe developmental delay since infancy and at 17 years old his IQ evaluation is 20. DiGeorge and Smith-Lemli-Opitz syndromes were excluded. aCGH was normal. Whole Exome Sequencing (WES) in trio at age 10 was normal. Revision of WES revealed a novel *de novo* heterozygous pathogenic variant c.2913dup p(Ala972Serfs*26) on *MYRF* which introduces a premature stop codon predicted to result in loss of function of the protein. Discussion: Truncating pathogenic variants in the *MYRF* gene account for CUGS and also for isolated high hyperopia and nanophtalmos. Ours is the 17th patient to be reported with CUGS and the oldest one. He encompasses the typical anomalies of CUGS and also hyperopia and delayed myelination. This is the first case of CUGS with reported signs of delayed myelination which may contribute to a severe intellectual impairment. Brain image and long term follow up of reported patients will further contribute to clarify the CUGS phenotype regarding developmental disorder spectrum. Sequencing of *MYRF* should be considered in patients with PAGOD syndrome.

OC15- *MED13L*-related intellectual disability - National Case Series

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Introduction: *MED13L*-related intellectual disability syndrome is characterized by moderate to severe intellectual disability (ID), speech delay, hypotonia, behavioural issues, autism spectrum disorder, and dysmorphic features. To date, at least 70 cases have been reported. Methods: Clinical and molecular characterization of all patients from 4 Portuguese medical genetics departments with the proposed diagnosis of MED13L syndrome was performed retrospectively through a questionnaire completed by each referring clinician. Results: Twelve individuals from 9 families were included in this study. Mean age at diagnosis was 13.6 years (range 5-42). Neurodevelopmental features included moderate to severe ID (12/12), variable speech delay (6/12) or absent speech (6/12), motor delay (8/12), muscular hypotonia (3/12), behavioural problems (6/12), autistic features (2/12), and epilepsy (3/12). Other findings comprised hand/foot anomalies (5/12), cleft palate (4/12), structural heart defects (2/6), ophthalmological issues (6/11), and cerebral MRI findings (3/8). Skin and hair anomalies (2/12) and chronic constipation (2/12) were also reported and are not yet documented in the literature. All cases were isolated, except for four affected brothers. Molecular diagnosis was achieved by, exome sequencing in 5, ID-NGS panel in 1, arrayCGH in 3 patients and Sanger sequencing to look for a familial variant in the remaining 3. Five patients had heterozygous MED13L pathogenic SNVs (1 nonsense, 3 frameshift, 1 intronic deletion affecting splicing) and 3 had CNVs (1 deletion, 1 gene tetrasomy, 1 intragenic duplication). Six simplex cases underwent segregation analysis and were all *de novo*. In the familial case, the four affected individuals had a missense variant classified as a VUS. Both parents were clinically unaffected, only the mother was tested (negative); paternal analysis and further functional tests are necessary to assess pathogenicity. Discussion: The clinical picture of MED13L-related ID is relatively nonspecific and heterogeneous making the genomic approach with arrayCGH and WES/WGS the best option for diagnosis.

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OC16- Syndromic Congenital Platelet Disorders: relevance of genetic diagnosis of 17 families followed in the Department of Clinical Haematology CHUP

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Introduction: Congenital Platelet Disorders (CPD) are heterogeneous, having as their main characterizing feature the variable expressivity of bleeding tendency due to thrombocytopenia and/or platelet dysfunction. The classification of CPD has been refined with the increasing number of patients studied, and bleeding tendency is no longer considered the only clinical presentation. In some forms, patients are likely to develop syndromic features, often more severe than the thrombocytopenia itself, that may manifest later in life. We present the profile of 17 index cases with syndromic CPD. Approach: CPD patients were evaluated clinically (bleeding tendency, associated diseases and family history) followed by laboratory tests to assess platelet number, morphology and function. Molecular analysis involved targeted Sanger sequencing of candidate genes or massive parallel sequencing for a panel of 121 genes. Results: We established the phenotypic and genetic diagnosis in 17 families with syndromic CPD: 3 Hermansky-Pudlack Syndrome (HPS), 7 MYH9-RD, 1 DIAPH1-RT, 1 RUSAT-2, 1 FLNA-RT, 1 Thrombocytopenia-Absent Radius Syndrome (TAR), 1 Grey Platelet Syndrome, 1 Wiscott-Aldrich Syndrome (WAS) and 1 DiGeorge Syndrome. Associated clinical manifestations included oculocutaneous albinism (HPS), kidney and cardiac alterations (MYH9-RD and DiGeorge syndrome, respectively), neurosensory deafness (MYH9-RD, FLNA-RD and RUSAT-2), skeletal malformations (TAR) and bone marrow aplasia and immunodeficiency (WAS). Besides the very rare HPS cases (published in 2019), several undocumented variants were identified. Conclusion: In CPD patients as a whole, genetic diagnosis is vital so as to avoid unnecessary medication. In the group of families presented here, the identification of the underlying genetic causes enabled referral to other specialities for anticipatory management of the often more severe clinical features associated with the respective syndromes. Of note is the fact that this study provided the differential diagnosis of the TAR case and in all other cases thrombocytopenia was the initial reason for referral.

OC17- Clinical and molecular characterization of neonatal inflammatory skin and bowel disease: the Portuguese panorama of a little-known syndrome

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AIMS: Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor important for cell proliferation and differentiation. The role of somatic *EGFR* mutations in some cancers is well known and led to the development of targeted therapies and improved survival. Germinative mutations are less well characterized. There are four patients described to date that developed severe neonatal skin and bowel inflammation. We aim to determine the current Portuguese prevalence and contribute to better portray the phenotype and prognosis of this syndrome. METHODS: We reviewed the literature and gathered data from the patients with germinative *EGFR* mutation described to date. We inquired all Portuguese medical genetics departments and collected phenotypic and molecular data from all cases with germinative *EGFR* mutation. RESULTS: There were 3 patients with homozygous c.1283G>A pathogenic variant in the *EGFR* gene in Portugal. The same variant was found in 3 patients in the literature. All our patients were female and descended from consanguineous parents. In the literature 2 patients were female, 2 were male, half of the total had a history of consanguinity. Pre-natal findings in both cohorts included polyhydramnios and intrauterine growth restriction; all patients were born pre-term with a median gestational age at birth of 30 weeks. The main clinical features in both groups were: severe ictiosis-like skin inflammation; alopecia; recurrent infections and sepsis; hypokalemia, hypomagnesemia and hypernatremia. No significant bowel inflammation was found in our patients. Median survival was 80 days and the most common cause of death was infection. CONCLUSION: By almost doubling the patients described we conclude that the main post-natal features of this syndrome are skin inflammation, recurrent infections, hypokalemia and hypomagnesemia. If a newborn presents with these symptoms, particularly with consanguineous parents, *EGFR* mutation should be considered. This is a potentially underdiagnosed entity and adequate molecular characterization might have a determinant role in predicting prognosis and for pre-natal counseling of future pregnancies

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OC18- Spondyloepiphyseal dysplasia, Stanescu type: the clinical and molecular overlap of a very rare type II collagenopathy

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Objectives/Context: Spondyloepiphyseal dysplasia, Stanescu type (SEDS), was first described in 1984. Until now, only 12 patients from four families have been reported, with some being first diagnosed with an unclassifiable spondyloepiphyseal dysplasia or progressive pseudorheumatoid dysplasia (PPD). SEDS was recently shown to be due to variants in COL2A1, classifying it as a type II collagenopathy. We aim to expand the spectrum of SEDS and to highlight the features that differentiate it from other overlapping skeletal dysplasias. Clinical case: We present an 8-year-old boy who was referred for genetic evaluation due to short trunk. Family history was unremarkable. Prenatal and neonatal periods were uneventful, and psychomotor development and growth were normal. He presented with limitation of cervical mobility due to C2-C3 fusion, and hand, foot, leg and thigh pain since 18 months of age. On physical examination, he had short trunk, stiffness and limited joint mobility, and waddling gait. Radiographs showed platyspondyly with anterior wedging and endplate irregularities, broad femoral necks with coxa valga, bulbous epiphyses of the short tubular bones, and large and flattened epiphyses of the long bones of the legs. Although the clinical picture suggested the diagnosis of PPD due to the absence of elongated femoral necks, a skeletal dysplasia multigenic NGS panel was performed and a heterozygous variant in COL2A1, c.620G>A, p.(Gly207Glu), was found. The diagnosis of SEDS was thus established. Conclusions: This case expands the clinical and mutational spectrum of SEDS. In fact, vertebral fusions were not previously described in SEDS. Moreover, this COL2A1 variant had not yet been associated with SEDS, but was interestingly reported in a family with other type II collagenopathy. The latter reinforces the importance of thorough phenotyping and clinical diagnosis in this group of disorders. SEDS clinically overlaps with PPD, thus multigenic skeletal dysplasia panels are a valuable resource. This case shows not all skeletal dysplasias present with short stature, thus any unexplained body disproportion warrants a comprehensive evaluation.

Poster Highlights

P1- Antisense transcription across the SCA37 locus and role in the disease

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Spinocerebellar ataxias (SCAs) are neurodegenerative diseases characterized by cerebellar atrophy and progressive motor impairment. In 2017, we established that SCA37 is caused by an $(ATTTC)_n$ insertion into an $(ATTTT)_n$ in a *DAB1 5'UTR* intron, resulting in RNA-mediated toxicity. Repeat expression in the *DAB1*-antisense strand, however, may contribute to SCA37 pathology as in other repeat diseases. In the *DAB1*-antisense strand, the $(GAAAT)_n$ is in the middle poly(A) of an AluJb element, close to a CpG island and a cluster of transcription factor binding sites (TFBS). To investigate bidirectional transcription in SCA37, we performed *in vivo* reporter assays in zebrafish embryos, using a Tol2 transposon system. After injecting the normal sequence containing $(AAAAT)_{14}$, CpG island and TFBS (FragT) upstream *EGFP*, we detected EGFP expression in zebrafish muscle and cerebellum. To map the position of the promoter(s), the FragT was divided in Frag1 (upstream of the repeat), Frag2 (AluJb) and Frag3 (downstream of the AluJb). Frag1 and Frag3 triggered EGFP expression in horizontal myosepta and muscle fibers, respectively. We detected the two transcription start sites by 5'RACE PCR, using the zebrafish transgenic lines. We, then, investigated interaction of FragT, 1, 2 and 3 with brain enhancers, cloning them upstream *EGFP* and a strong midbrain enhancer (*Z48*). The promoters in FragT, 1 and 3 enhanced EGFP expression in zebrafish midbrain, demonstrating interaction with Z48. To investigate whether the antisense repeat RNA is toxic, we injected $(AAAAU)_7$, $(AAAAU)_{139}$ and the insertion $(GAAAU)_{54}$ RNAs in zebrafish embryos and assessed lethality rate. No significant increase in the lethality rate of embryos injected with the $(GAAAU)_{54}$ RNA was observed compared with the other RNAs, 24 hr postfertilization. In summary, we identified two promoters that can be regulated by brain enhancers, in *DAB1*-antisense strand. Although the $(GAAAU)_{54}$ insertion is not toxic *in vivo*, the antisense $(AAAAT)_n$ is transcribed, suggesting that bidirectional transcription is involved in SCA37 pathology by an unknown mechanism.

P2- Modeling the neurogenetics of neurodevelopmental disorders - hints from brain organoids

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Background: Neurodevelopmental disorders (NDDs), such as autism and intellectual disability, affect more than 3% of children worldwide. Next generation sequencing has uncovered mutations at over 1000 loci, highlighting the extensive etiological variability of NDDs. This fact, combined with a heterogeneous clinical presentation, presents great challenges that range from delineating disease-associated molecular processes, to identifying effective therapies. 3D human brain organoids have revolutionized this field as they replicate key aspects of organogenesis. Brain organoids can be generated using patient's dental stem cells found in exfoliated teeth, presenting an opportunity for personalized approaches. In addition, organoids are amenable to gene editing which can be used to introduce rare mutations or test if their reversal restores neuronal function. Objectives: Our goal is to generate 3D brain organoids, derived non-invasively from human dental stem cells, to explore brain development in the context of specific gene mutations. Methods and Results: In collaboration with the Pediatric Hospital of Coimbra, we established the first biobank of dental stem cells to study NDDs in Portugal. Dental stem cells were analyzed using flow cytometry, qRT-PCR and immunocytochemistry and showed expression of Stro-1, Oct4 and Nestin. We differentiated dental stem cell-derived iPSC into brain organoids using the Lancaster et al. method. At 3 months, brain organoids expressed neuronal markers such as β3-tubulin and NeuN while at 6 months we identified subtype-specific neurons, such as dopaminergic, glutamatergic and GABAergic. We characterized specific time-points at which organoids show synaptic and neuronal network activity, using electrophysiology and calcium imaging, with reliable network function beyond 6 months of development. In parallel, we are performing gene editing via CRISPR/Cas9 to induce mutations in high risk genes. Conclusions: We show that dental stem cells are a suitable source to generate brain organoids and that 3D brain cultures are realistic models that will allow to understand the mechanisms behind NDDs and test for personalized therapeutic approaches.

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P3- Investigating the role of *CD44v6* in Gastric Cancer: development of exon v6 skipping models by CRISPR/Cas9

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Context/objective: Gastric cancer (GC) is the 5th most common cancer and the 3rd with the highest mortality. The standard of care for advanced disease is conventional chemotherapy with or without radiotherapy. We have shown that *de novo* expression of *CD44v6* is a poor prognosis factor in GC, but also a likely predictor of response to conventional chemotherapy. The cell adhesion molecule/gene *CD44* undergoes extensive alternative splicing, generating multiple isoforms, being *CD44v6 de novo* expression often associated with cancer aggressiveness. We aimed to develop exon v6 skipping models by CRISPR/Cas9 in CG cell lines, to explore the role of *CD44* variable exon v6 in response to therapy. Methods: We designed and established a pioneer strategy to produce the pleiotropy of *CD44* isoforms lacking specifically exon v6. We edited, by CRISPR/Cas9, two GC cell lines endogenously expressing *CD44v6* to specifically delete exon v6 from *CD44v6*-contaning isoforms, whilst maintaining the reading frame. v6-edited cell lines and corresponding *wild-type* controls were genotyped and characterized for *CD44* expression patterns and then treated with cisplatin and 5-Fluorouracil. Cell survival was evaluated in short-term treatments by PrestoBlue and Sulforhodamine B assays and long-term treatments by clonogenic assay. Results: We obtained 10 independent clones of homozygously edited GC cell lines lacking exon v6, while maintaining expression of the remaining portions of *CD44* variant isoforms. These were characterized and all sequenced transcripts mimicked exon v6 skipping where exon v5 was in-frame with v7 in the mRNA. Preliminary results from drug treatments indicate that there are no differences in survival of cell lines without exon v6 when compared to wild type cells. Discussion/conclusion: We successfully designed a strategy that mimics exon v6 skipping and generated two cell lines expressing *CD44* isoforms lacking exon v6. Although preliminary data is not conclusive regarding response to conventional therapy, these models are crucial to disclose the role of exon v6, that is responsible for binding to *c-MET* and *VEGFR-2* oncogenes in human aggressive cancers.

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P4- Using graph embedding methods to discover new autism spectrum disorder candidate genes

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Context: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by high phenotypic and genetic heterogeneity. It is hypothesized that several hundred genes are involved, however most ASD-associated mutations are characterized by low penetrance, which presents a challenge in identifying the relevant genetic determinants of ASD. This work aims to develop machine learning methods to identify novel ASD candidate genes and, through this contribute to a better understanding of the genetics of this complex disorder. Methods: We applied graph embedding methods to the discovery of ASD-related genes from gene-gene association networks obtained from protein-protein interaction (PPI) networks. We used a multi-step procedure, with a transductive learning step where gene representations (embeddings) are learned from the STRINGDB PPI, which are then used as features to train machine learning classifiers, using a dataset of ASD-associated and non-ASD-associated genes. For the first step we assessed three graph embedding methods (Deepwalk, LINE and GraRep), while for the second step we employed regularized logistic regression. Results: Embeddings from LINE yield the best results for discriminating between ASD and non-ASD genes, with a ROC AUC value of 0.88 for the full dataset, and 0.95 if we consider only high-confidence ASD-related genes. This compares favorably with other approaches from the literature (AUC 0.73 to 0.88) on similar datasets. Using the highest ranking genes we created a disease-specific network, from which we obtained communities enriched in pathways related to ASD. Conclusions: We developed a machine learning approach, based on graph embedding methods, to extract information from protein-protein networks and identify ASD candidate genes. Our approach outperforms other approaches reported in the literature. The resulting gene ranking was used to identify a set of pathways associated with ASD.

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P5- *VCAM1* modulation on endothelial cells – implications for vasculopathy in sickle cell anemia

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Sickle cell anemia (SCA) is a highly heterogeneous and multifactorial-like monogenic disease that arises from homozygosity for the c.20A>T mutation in the *HBB* gene. Vascular disease is systemic in SCA, with profound effects in organs like the brain, where stroke is the most severe end of the cerebral vasculopathy (CVA) spectrum. Endothelial dysfunction plays a major role in vasculopathy with several adhesion molecules, such as VCAM-1, being produced by the endothelium altered as a response to inflammatory cytokine (e.g., TNF-α) signalling. In previous association studies, we found positive associations between the presence of four specific *VCAM1* gene promoter haplotypes and i) high blood flow velocities in the median cerebral artery, and ii) a chronic hemolysis biochemical marker. In this study, we aimed to assess the functional role of those *VCAM1* promoter haplotypes in endothelial cell response following endothelial activation through TNF-α stimulation. After molecular cloning of 3 haplotype constructs, using a pGL4 promoterless vector, haplotype sequence was confirmed, by Sanger sequencing, prior to transfection. We used EAhy926, HUVEC and HBEC as different endothelial cell models, and performed transfection experiments for each construct, with and without TNF-α stimulation. Differences in promoter activity were assessed by luciferase reporter assay. All haplotypes showed differences in promoter activity, after TNF-α stimulation, in all cell models. Haplotype 1 showed decreased promoter activity, while haplotypes 7 and 8 showed increased activity after TNF-α stimulation, in all cell models. These results are consistent with lower *VCAM1* expression due to haplotype 1, and therefore a protective effect. Conversely, a higher expression due to haplotypes 7 and 8, suggests an increased vasculopathy risk, in a pro-inflammatory milieu. The association between specific haplotypes and endothelial cell response further enhances the modifier effect of *VCAM1* on endothelial dysfunction and its impact in SCA pathophysiology, as well as its potential role as a biomarker of SCA vasculopathy risk, severity and prognosis.

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P6- Contribution of rare cryptic deletions to severe spermatogenic impairment – insights from a large cohort of azoospermic men (GEMINI)

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Aims/Context: Azoospermia, the most severe form of male infertility, affects approximately 1% of men worldwide and in the great majority of the cases the aetiology of the disease remains unidentified. As an ongoing effort to characterize the genetic architecture of male infertility, the Genetics of Male Infertility Initiative (GEMINI) consortium funded by the NIH has now sequenced the whole exome of 927 well phenotyped NOA (non-obstructive azoospermia) cases from 6 countries and 84 men with normal sperm parameters, of which 375 are Portuguese (299 patients and 76 controls). Methods: CNV calling was successfully performed in 693 NOA and 76 normozoospermic controls using XHMM (eXome-Hidden Markov Model) and following functional annotation the most interesting deletions were validated by other methods. Results: Likely causal deletions were found in 12 patients (overlapping genes previously associated with male infertility and not present in controls) and putative causal deletions in 29 (overlapping genes important for spermatogenesis in mouse). Most of the genes deleted only in NOA were patient-specific (~60%). Conclusions: Although CNV calling from exome data has less power than from whole-genome it can contribute to the identification of genes affected in genetically heterogeneous diseases such as NOA.

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P7- Protein and Copy Number Evaluation in Head and Neck Cancer patients

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Context: Head and neck squamous cell carcinoma (HNSCC) is a cancer of the upper aero digestive tract that arises from several cumulative molecular and genomic alterations with aggressive clinical courses, poor prognosis and a high potential for metastization and relapse. The clinical use of a biomarker or set of biomarkers to predict the clinical outcomes of HNSCC patients could affect both the patient's prognosis and the type and duration of the treatment. Protein biomarker discovery and their correlation with genomic alterations for the early detection of metastasis or relapses is a crucial unmet need to improve the clinical outcome of these patients. Methods: Forty tumour samples from a previously studied cohort at the copy number variation (CNV) level, were analysed using information-dependent acquisition (IDA) of pooled samples for protein identification and SWATH-MS acquisition of each individual sample for protein quantification. A Mann-Whitney U test was implemented for the obtained proteins, taking into account the metastasis/relapse status of the patients and a classification model was built using the resulting proteins ($p<0.05$) and some clinical features. The protein levels were correlated with the corresponding genomic alterations. Results: The logistic regression model was constructed based on the clinical staging and three proteins related with stem cell population maintenance, fatty acid elongation and extracellular matrix composition, with 87.5% accuracy in predicting the outcome of the patients in what comes to the development of relapse or metastasis. The area under the ROC curve was 0.864, with a 95% CI of [0.766;0.962], rendering it a model with good class separation ability. Conclusions: We were able to identify a set of proteins that seem to have a good prediction ability in what comes to metastasis and relapse status of the patients and correlate them with the CNVs. This approach has the potential to identify biomarkers with diagnostic and prognostic value leading the way to a new era of personalized medicine, that may help with metastasis and relapse early detection and consequently improve the clinical outcome of these patients.

P8- The bigger the better? Evaluation of the value of large multi-gene panels in Portuguese cardiomyopathy genetic testing

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Introduction: Genetic testing of cardiomyopathies went through major changes in the last few years, from sequential Sanger sequencing of the most likely gene candidates, to multi-gene panels by NGS, with an ever increasing number of genes analyzed. Since only a few genes account for the majority of hereditary cardiomyopathies, the increase in the number of genes evaluated is largely accompanied by adding less relevant or penetrant genes to existing panels, which may translate in minor benefits in terms of diagnostic yield but a significant increase in the number of variants of uncertain significance (VUS). In order to access the pros and cons of larger gene panels, the results of different cardiomyopathy gene panels used in our laboratory were reviewed, taking into account current ACMG classification criteria. Methods: All results of different cardiomyopathy panels performed between 2011 and 2018 at Ipatimup Diagnostics were retrieved (n=1781 index cases). We calculated the diagnostic yield of each gene panel at the time they were used in the laboratory. Moreover, we compared the results before and after applying ACMG guidelines. Results: Before ACMG guidelines were adopted, a case was considered positive whenever a rare variant was identified. With the adoption of the ACMG guidelines, several variants previously considered relevant were classified as VUS, which lead to a drop in the diagnostic yield of the test (from 68% in 2011 to 37% in 2018). This drop is even increasing over time, as a result of the adoption of ever larger gene panels. Conclusions: The increase in the number of genes in cardiomyopathy gene panels does not necessarily mean an increase in the diagnostic yield of genetic tests. There is an increment in the number of variants detected, however most of them are VUS, some of which in genes of current limited value for cardiomyopathy genetic testing. Nevertheless, if we look at genetic testing as a tool to better understand a disease, the study of these variants (namely with functional assays and segregation studies) might help in the future to better understand some cases, which remain uncertain with the current available information.

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P9- Characterization of copy number variants (CNVs) identified by genetic testing of inherited retinal disorders

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Aims: Retinal dystrophies (RD) include a heterogeneous group of disorders that damage the photoreceptors in the retina causing visual impairment. Prompt genetic diagnosis of these disorders can assist in risk assessment measures, management of symptoms, and selection of the appropriate targeted treatment. To provide a comprehensive diagnosis, the genetic testing strategy needs to take into account sequence alterations as well as copy number variants (CNVs). The aim of this study was to evaluate the rates and characteristics of CNVs in a cohort of 2754 patients tested using a comprehensive RD panel. Methods: DNA from patients was sequenced by targeted OS-Seq using the Illumina NextSeq500 sequencing platform or the IDT xGEN Exome Research Panel using the Illumina NovaSeq platform. CNVs were detected by CNVkit and an in-house developed deletion caller. Results: CNVs in a total of 47 genes matching the patient's phenotype were reported as a primary finding in 128 out of 2754 (4.6%) cases. Of these, 91 (71.1%) were partial gene deletions, 17 (13.3%) whole gene deletions, four (3.1%) one exon deletions, and one (0.8%) partial exon deletion. In addition, ten (7.8%) partial gene duplications, three (2.3%) whole gene duplications, one (0.8%) whole gene gain and one (0.8%) partial gene gain (CN>3) were identified. The majority of CNVs (113, 88.3%) were either likely pathogenic or pathogenic while 15 (11.7%) were variants of uncertain significance. Of the likely pathogenic and pathogenic CNVs, 94 (73.4%) were diagnostic: 66 (70.2%) in autosomal recessive genes, 17 (18.1%) in autosomal dominant genes, and 11 (11.7%) in X-linked genes. The *USH2A* and *PRPF31* genes were enriched in CNVs compared to other genes. Notably, CNVs were identified also in genes in which CNVs are not commonly reported, e.g. *ABCA4* and *RPE65*. Conclusions: Overall, these results highlight the importance of a comprehensive genetic testing approach for the diagnosis of retinal dystrophies. We have identified CNVs ranging from one exon deletions to whole gene deletions in multiple genes. In addition, we have detected a relative high percentage of copy number duplications that warrant further investigation.

P10- Characteristics of a Portuguese cohort of Li-Fraumeni patients

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Context: Li-Fraumeni Syndrome (LFS) is an autosomal dominant condition caused by heterozygous germline pathogenic variants in *TP53* gene, which encodes a tumor suppressor protein. LFS is a cancer predisposition syndrome associated with the early development of cancers, such as sarcomas, brain tumors, leukemias, breast and adrenocortical carcinomas. Clinical diagnosis is usually based on classic or Chompret criteria. Patient testing and management is still challenging. Methods: Clinical and molecular characterization of all LFS cases observed at Familial Risk Clinic, IPO Lisboa. Patient and family's medical records were reviewed. Results: We identified 16 patients (4 males, 12 females) from 12 unrelated families, each with a different TP53 variant. Four families meet the classic criteria, 6 the Chompret criteria and 2 neither of these. From 2007 to 2016, affected patients were identified mostly by Sanger sequencing, while 7/12 cases were recently diagnosed through multigene testing for breast/ovarian cancer. Three patients were identified by predictive testing. We found 3 likely pathogenic and 9 pathogenic variants (2 hotspots and 1 Brazilian founder variant). As expected, the majority are missense (7/12) and 2/7 with known dominant-negative effect. Our patients had a first tumor at median age of 28 (1-59yrs) and were diagnosed with a median of 2 primary cancers (1-4). Childhood tumours were reported in 7/12 families, being diagnosed at median age of 8.5 (1-17yrs). One family had breast cancer as the only cancer diagnosis (6 cases). We also report a probable TP53 mosaic, in a 57yrs patient with fallopian tube endometrioid carcinoma. Discussion: TP53 pathogenic or likely pathogenic variants have been identified in individuals who don't meet classical criteria for LFS, mainly by multigene panel testing for breast cancer. This raises important questions: is LFS prevalence underestimated in the clinic? Should we go for reverse phenotyping in a condition where patient management is still not consensual? Additional data from this and other cohorts of LFS patients will be important for genotype-phenotype correlation, better-informed genetic counselling and patient management
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P11- ZTTK syndrome: first Portuguese case of a recently described syndrome?

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Introduction: *SON* gene is highly conserved in mammals and encodes a protein containing an arginine/serine rich domain and two RNA-binding motifs playing an important role in regulating multiple cellular processes such as cell cycle, chromatin remodeling and genome stability. Recently described *SON* heterozygous mutations, all predicted to result in loss of protein function, were associated with ZTTK syndrome (MIM #617140). The phenotype of the 28 described cases in the literature overlap with established spliceosomal disorders and this gene seems to be a critical gene in microdeletions encompassing 21q22.11. Case description: We present, to our knowledge, the first molecularly confirmed Portuguese case with ZTTK syndrome in a boy with global developmental delay, hypotonia, feeding difficulties, short stature, brain malformations, congenital heart defect and facial dysmorphisms. Our patient was first seen at the age of 2 years and fragile X syndrome mutation analysis and array-CGH were performed and had normal results. At the age of 4.5 years we reevaluated the patient and a next-generation sequencing panel of 6110 genes was performed. The *de novo* pathogenic *SON* (NM_138927.2) c.5753_5756del p.(Val118Glufs*87) variant in heterozygosity was identified, establishing the diagnosis. This 4-bp deletion seems to be a recurrent variant, which has been already reported in the literature in seven patients and was proven to be *de novo* in all described cases.

P12- Copy number variations analysis of NGS data in germline oncology testing

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Aims: Establish CNV detection using NGS data as part of diagnostic analysis for germline oncology genetic testing. Context: Genetic variation in human genome can range from large chromosomal abnormalities to single nucleotide variations (SNVs), including structural variations, copy number variations (CNVs), small indels and simple base alterations. CNVs comprise gains or losses of genomic material (duplications or deletions, respectively) that directly influence genetic dosage which have direct implications in inherited diseases. Copy number information can be obtained from NGS data, allowing detection of duplications and deletions of genomic regions in a single study. This study focus on patients with suspected hereditary cancer tested for different oncology gene panels, including CNVs analysis in a routine workflow. Methods: After software validation, CNVs analysis was performed on 902 clinical samples tested for oncology NGS panels. Copy number variations reported were confirmed by other methods (MLPA or qPCR) and the diagnostic yield was calculated. Results: A total of 902 patients were tested and 237 had relevant single nucleotide variants and 22 had gross deletions/duplications. From 22 CNVs reported 19 were deletions and 3 were duplications. The global diagnostic yield was 28.7%, 26.3% for SNVs and 2.4% for CNVs; these lines up or even slightly above to the referenced in literature (1.7%). Conclusions: CNVs detection through NGS data is an addition tool that allows accurate detection of large rearrangements and increases diagnostic yield by 2.4% which is relevant for clinical management and genetic counseling to patients and their relatives.

Posters

P13- Origin of *DAB1* $(ATTTC)_n$ insertion and repeat instability in spinocerebellar ataxia type 37

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Repeat expansions resulting from microsatellite instability cause a growing number of neuromuscular and neurodegenerative diseases. Repetitive sequences may drive pathogenicity, either by expansion above a given repeat length, or by insertion of abnormal tracts in nonpathogenic polymorphic repetitive regions, as is the case in spinocerebellar ataxia type 37 (SCA37). In 2017, we established that this neurodegenerative disease is caused by an $(ATTTC)_n$ insertion within an $(ATTTT)_n$ in a noncoding region of *DAB1*. After the discovery of this repeat insertion, three other similar noncoding repeats have been identified in *SMAD12*, *TNRC6A* and *RAPGEF2* in families with benign adult familial myoclonic epilepsy (BAFME) type 1, 6 and 7. Little is known about the molecular evolution of the complex pathogenic repeat insertions. Thus, we investigated in SCA37 the 1) pentanucleotide repeat structure in pathogenic and nonpathogenic alleles, 2) pentanucleotide somatic instability and mosaicism and 3) mutational mechanism that originated the $(ATTTC)_n$ insertion within an ancestral $(ATTTT)_n$. We observed that approximately 3% (44 out of 1293) of nonpathogenic $(ATTTT)_n$ alleles were interspersed by AT-rich motifs, contrarily to mutant alleles that were composed of pure $(ATTTT)_n$ and $(ATTTC)_n$ stretches. In pathogenic alleles of affected individuals, both $(ATTTT)_n$ and $(ATTTC)_n$ showed mosaicism in peripheral blood and reprogramed cells (iPSC). Haplotype studies showed the disease haplotype in two control chromosomes carrying an $(ATTTT)_{\sim 200}$. In conclusion, both ATTTT and ATTTC stretches of pathogenic alleles are unstable, haplotype studies in unaffected chromosomes suggested that the primary mutational mechanism, leading to the $(ATTTC)_n$ insertion, was likely one or more T>C substitutions in a large $(ATTTT)_n$ allele. Then, the $(ATTTC)_n$ expanded in size, originating a deleterious allele in *DAB1*. This is likely the mutational mechanism for the three similar $(TTTCA)_n$ insertions responsible for BAFME. Because $(ATTTT)_n$ tracts are frequent in the human genome, many loci could be at risk for this mutational process.

P14- A multiplex DNA methylation SNaPshot assay for age prediction using blood samples: a replication study

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Background: Many age-dependent DNA methylation markers have been identified in various tissues and body fluids, using methodologies such as bisulfite pyrosequencing, massive parallel sequencing or SNaPshot analysis. In a previous study [1], five CpG sites located in *ELOVL2, FHL2, KLF14, C1orf132/MIR29B2C* and *TRIM59* genes, were tested for age prediction purposes in blood, saliva and buccal swab samples using a multiplex methylation SNaPshot assay, showing accurate age prediction in blood. Aim: To replicate the multiplex methylation SNaPshot assay of Jung et al. [1] in blood samples of Portuguese healthy individuals. Methods: Fifty nine blood samples (37 females, 22 males; aged 1-94 years-old) were evaluated using the SNaPshot method, which consisted of multiplex PCR followed by a multiplex SBE (single-base extension) reaction. The specific primers were those previously described [1]. Linear regression models were used to analyze relationships between methylation levels and chronological age using IBM SPSS software v.24. Results: Among the five markers, the CpG site in the *ELOVL2* showed the strongest correlation between DNA methylation and age ($R = 0.951$, $p = 3.58e-29$), following by *FHL2* ($R = 0.946$, $p = 1.49e-29$), *C1orf132/MIR29B2C* ($R = -0.924$, $p = 1.67e-25$) and *TRIM59* ($R = 0.910$, $p = 2.04e-23$). *KLF14* showed the lowest age correlation value ($R = 0.791$, $p = 1.57e-13$). The final age prediction model, using simultaneously the 5 CpG sites, showed a high correlation coefficient ($R = 0.982$), highly significant ($p = 3.63e-34$), explaining 96.4% of age variation. A strong correlation between predicted and chronological ages was observed (Spearman correlation coefficient, $r = 0.971$), with a mean absolute deviation (MAD) between predicted and chronological ages of 4.27 years. Conclusions: The multiplex methylation SNaPshot assay revealed high prediction accuracy in blood, showing to be useful in forensic analysis for age estimation.

References: 1. Jung SE et al. DNA methylation of the ELOVL2, FHL2, KLF14, C1orf132/MIR29B2C, and TRIM59 genes for age prediction from blood, saliva, and buccal swab samples. Forensic Sci Int Genet. 2019; 38:1-8

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P15- Exploring the association between Y-chromosome lineages and *G6PD* African mutations in Portuguese patients

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Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency results from mutations on *G6PD* gene on chromosome Xq28. In Portuguese patients, the two most common mutations are *G6PD* A- (202G>A) (63.4%) and *G6PD* Betica (968T>C) (14.1%) observed in the context of the common sub-Saharan African A (376G) haplotype. Y chromosome lineages were previously identified in Portugal, being the typical Western European haplogroup R1b-M269 the most common (~60%), following by haplogroups E-M35 (~12 %) and J-M304 (~10%). Aim: To address the association between Y-chromosome haplogroups and *G6PD* African mutations to determine whether there is signs of African male lineages assimilation in Portugal. Methods: DNA samples from 34 male *G6PD* deficient Portuguese patients, without known black ancestry, with mutations *G6PD* A-, and *G6PD* Betica, were analysed for Y-chromosome haplogroup identification using binary markers. The internal variation of the male lineages was evaluated by analysis of 7 Y-STRs. Results: Y genotyping of the *G6PD* patients revealed 6 larger haplogroups (18 R1b-M269; 7 E1b1b1- M35; 6 J-M304; 1 I-M170; 1 L-M22; 1 K*-M9), all previously reported in the general Portuguese population. No common sub-Saharan African haplogroups were found. The multidimensional scaling (MDS) plot based on a pairwise RST matrix from seven Y-STR loci, distributed 14 populations in three main groups comprising European, Middle East and North African populations. The *G6PD* group was strong clustered with Europeans, and a sub-Saharan African population is clearly separated from all other populations. The E1b1b1a-M78 sub-haplogroup (total frequency 11.8%), which is widely distributed in northern and eastern Africa, Europe, and western Asia, but was also found in sub-Saharan Africa (1 in 883 samples), was the most common within E1b1b1-M35 (4 in 7 samples). The MJ network of E-M78 showed 2 samples identified with Spanish haplogroups and 2 isolated haplogroups, one of these at one mutational step from the sub-Saharan African haplogroup. Conclusions: Male lineages from Portuguese *G6PD* deficient patients do not show signals of Sub-Saharan African ancestry, except for one E-M78 haplogroup.

P16- Genetic association with HbF levels in beta-thalassemia carriers using multi-locus models

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Introduction: Genetic association studies showed that HbF levels in sickle cell anemia (SCA) and β- thalassemia (β-thal) is under the influence of single nucleotide polymorphisms (SNPs) at three major quantitative trait loci (QTL): *BCL11A* on chromosome 2p16, *HBS1L-MYB* intergenic region (HMIP) on chromosome 6q23 and *HBG2-XmnI* on chromosome 11p15. Combining SNPs into genetic risk scores (GRS) or using multiple regression models joining several SNPs can help to explain a larger amount of the variability in HbF levels. Aims: i) to replicate the association with HbF for representative genetic variants in the three HbF QTLs in a Portuguese sample of β-thal carriers; and ii) to test different genetic multi-locus models to account for the variability in HbF levels. Materials and Methods: A total of 79 Portuguese β-thal carriers (39 males, 40 females), aged 2 to 70 years old, were genotyped for three HbF markers *BCL11A* (rs1427407), *HMIP* (rs66650371) and *HBG2-XmnI* (rs7482144). The two SNPs rs1427407 and rs7482144 were genotyped by PCR-RFLP using HpyCH4III and XmnI, respectively. SNP HMIP rs66650371 (a 3 bp deletion), was genotyped by PCR gel electrophoresis as described elsewhere. Linear regression models were used to test for genetic association with log transformed HbF levels. Statistical analysis was performed with PLINK and IBM-SPSS version 24. Results: The minor alleles of individual SNPs rs1427407 (T) (0.165), rs66650371 (del) (0.247) and rs7482144 (T) (0.196), were significantly associated with increased levels of HbF (P = 0.029, P = 0.002 and P = 0.0004, respectively), explaining about 6%, 12% and 15% of HbF levels variability, respectively. In a multiple linear regression approach the three SNPs accounted for ~32% of HbF levels variability (P = 2e-6). A risk-allele score by summing the number of minor alleles of the three QTL into a single genetic variable explained ~30% of HbF levels variability (P = 1.39e-7). Conclusion: We replicated previously known significant association with HbF levels of individual SNPs in Portuguese β-thal carriers. Two different multi-locus models combining the three major HbF QTL can explain about 30% of variability in HbF levels.

P17- Cis-regulatory similarities of the human and zebrafishpancreas identify diseases related enhancers

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The pancreas is a central organ for human diseases that have a dramatic societal burden, such as pancreatic cancer and diabetes. Non-coding cis-regulatory elements (CREs) of DNA control gene expression, being required for proper pancreas function. It has been shown that most disease- associated alleles are non-coding, many overlapping with CREs, suggesting that alterations in these regulatory sequences can contribute to human pancreatic diseases by impairing gene expression. However, functional testing of CREs in vivo is not fully explored. In this work we use Assay for Transposase-Accessible Chromatin (ATAC-seq), Chromatin Immunoprecipitation (ChIP-seq) and HiChIP-seq to identify CREs active in the adult zebrafish pancreas and their target genes. Using this data, we searched for human functional equivalent CREs, identifying disease-associated sequences across species. We found a zebrafish ptf1a distal developmental enhancer, which deletion generates pancreatic agenesis, as its human counterpart. Additionally, we identified a novel human pancreatic enhancer of the tumor suppressor gene *ARID1A*, which zebrafish deletion impairs gene transcription, conferring a potential tumor suppressor role to this non-coding sequence. This work explores the zebrafish pancreas regulome, identifying CREs important for pancreas development, function and homeostasis.

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P18- c-Src/Fyn role in Huntington's Disease progression

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Huntington's Disease (HD) is an autosomal dominant progressive neurodegenerative disorder affecting the striatum and later the cortex, with no effective neuroprotective therapies. Mutant huntingtin (mHTT), the main HD proteinaceous hallmark, participates in reactive oxygen species (ROS) formation, mitochondrial dysfunction and modified N-methyl-D-aspartate receptors (NMDAR) activity. Importantly, c-Src and Fyn, two ubiquitous members of the Src Kinase Family (SKF), are enriched in striatal neurons and implicated in brain neuronal development, transmission, synaptic regulation of NMDAR activity and mitochondrial function, and are activated by ROS. These observations favor a common inter-player between mHTT and HD-related neuronal dysfunction, suggesting a relevant role for c-Src/Fyn-regulated pathways in HD pathogenesis. In this study, we analyzed c-Src/Fyn levels in different HD models, namely in human postmortem caudate brain samples, brain tissue and primary neurons derived from YAC128 transgenic mice and STHdhQ111/ Q111, as well as the influence of autophagy on c-Src/Fyn regulation, using Western Blotting and immunocytochemistry. We also investigated the role of these kinases on NMDAR regulation in HD context, using calcium probes and immunocytochemistry. Our data showed consistent decreased c-Src/Fyn levels and activation in all models tested, when compared to the respective controls, along with augmented Fyn degradation by autophagy in HD. Moreover, primary striatal neurons from YAC128 mice evidenced decreased c-Src/Fyn levels in distal neurites and postsynaptic density, as well as diminished PSD-95 levels and puncta, suggesting altered synapse morphology and number in HD neurons. Concordantly, decreased c-Src/Fyn in YAC128 mice was accompanied by decreased Tyr1472 phosphorylation of GluN2B-composed NMDAR and by decreased NMDAR-mediated intracellular Ca^{2+} levels. We demonstrate c-Src/Fyn tyrosine kinases involvement in HD pathogenesis. Further studies should be performed to better understand the impact of Src/Fyn modulation on neuronal function in HD; however, this work supports that c-Src/Fyn-related pathways may constitute novel potential targets for HD.

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P19- Age-at-Onset variation in Val30Met Familial Amyloid Polyneuropathy: The genetic landscape of *TTR*

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Aims/Contex: Val30Met in transthyretin (*TTR*) gene is causative for familial amyloid polyneuropathy (FAP). Substantial phenotypic heterogeneity has been described in Val30Met patients, including in its age-at-onset (AO) between clusters, families, and among generations. Other variants at the *TTR locus*, outside the disease-causing variant, could play a regulatory role in its expression level and modify disease expressivity. We aim at identifying genetic variants at the *TTR locus* and interpret how they contribute to genetic modification in TTR-FAP. Methods: We analyzed DNA samples of 330 Val30Met carriers (299 patients, 31 aged-asymptomatic carriers) from 120 families currently under follow-up. A generalized estimating equation analysis (GEE) was used to take into account non-independency of AO between relatives. An intensive *in silico* analysis was performed in order to understand a possible regulation of gene expression. Results: We found 11 rare variants in the promoter, coding and intron/exon boundaries of the *TTR* gene associated with the onset of symptoms before and after age 40 years, namely 2 novel ones and a tandem CA-dinucleotide repeat. The seven Val30Met/Val30Met homozygous do not carry any of the variants identified in this study, including the common ones. *In silico* analysis disclosed significant alterations in the mechanism of splicing, transcription factors and miRNAs binding. Conclusion: Variants within the promoter region may modify disease expressivity and variants in the 3'UTR can impact the efficacy of novel therapeutic interventions. Importantly, the putative mechanisms of regulation of gene expression within the *TTR* gene deserve to be better explored, in order to be used in the future as potential therapeutical targets.

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P20- Signal Transduction Pathways Regulating the Alternative Splicing of Tumor-Related RAC1b

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Introduction: In colon cancer distinct genetic subtypes have been described, one of which involves overexpression of RAC1b, a variant generated by alternative splicing. Aberrant splicing is known to occur in cancer and can be caused by mutation in a gene or splicing factor but also represents a dynamic response to oncogene-induced cellular signaling and in this case it may be pharmacologically targeted. Here we explore how signaling pathways are involved in the deregulation of alternative RAC1b splicing in colorectal tumor cells. Materials and Methods: HT29 colorectal cells represent serrated colorectal tumors with *BRAF* gene mutation V600E in one allele and RAC1b overexpression. Cells were transfected with shRNA vectors directed against target candidate protein kinase transcripts and their effects on RAC1b levels analyzed 24h later by Western Blot and qRTPCR. Treatment with kinase inhibitors or anti-inflammatory drugs was performed 24h prior to cell lysis. Results and Discussion: Two kinases, SRPK1 and GSK3β, were found required to sustain RAC1b levels and both were shown to act upon the phosphorylation of splicing factor SRSF1, which binds to and promotes the inclusion of the alternative exon in RAC1b. SRPK1 knockdown or pharmacological inhibition reduced SRSF1 phosphorylation decreasing its nuclear translocation and concomitantly RAC1b splicing. The same regulatory pathway was also found to be controlled by GSK3β. Interestingly, GSK3β phosphorylation was identified to serve as target for the anti-inflammatory drug ibuprofen, decreasing SRPK1 nuclear translocation and inhibiting RAC1b overexpression. Together, our results demonstrate that oncogenic signal transduction pathways deregulate alternative splicing and this may be drug revertable.

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P21- The interplay between KIAA0753 and TBCCD1 in the control of ciliogenesis and cell cytoskeleton architecture

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Aims/Context: Cilia are slender protuberances found in eukaryotic cells, consisting of a microtubule (MT)-based ciliary axoneme, which can confer motility and sensory functions to the cells. These organelles have a basal body, which can be derived from the centrosome and nucleates the ciliary axoneme. Centriolar satellites are protein granules located around the centrosome that play an essential role in centrosome and primary cilium assembly. Mutations in genes encoding centrosome and/or centriolar satellite components lead to various human disorders, such as ciliopathies. Previous work from our group characterized the interactome of a new centrosomal TBCC domain-containing human protein (TBCCD1). Included among the identified proteins were several well-known proteins encoded by ciliopathy genes, e.g., centrosomal and centriolar satellites protein KIAA0753 (also known as OFIP and Moonraker). *KIAA0753* is mutated in several ciliopathic syndromes as, e.g., Joubert syndrome. Methods: Immunofluorescent microscopy and Western blot were used to study the levels and cellular localization of components of centriolar satellites, cytoskeleton and cilia, in human RPE1 cells overexpressing TBCCD1. Similarly, RPE1 cells depleted of either TBCCD1 or KIAA0753 were analyzed. Results: In RPE-1 cells, both the knockdown and overexpression of TBCCD1 affect the levels of KIAA0753, as well as its localization around the centrosome. This suggests that TBCCD1 plays a role in the recruitment of KIAA0753 to the centrosome. Regarding the levels of KIAA0753, we show that its depletion affects centriole connection with consequences on the organization of the MT network. This may compromise cell polarity, cell migration, and cilia assembly. Furthermore, we show that KIAA0753 localizes in the basal body and along the axoneme of cilia, which points to a role in ciliogenesis. Conclusions: Our results support a new function for KIAA0753 in centrosome structure maintenance, as well as a new functional interaction between TBCCD1 and KIAA0753, suggesting a new pathway in ciliopathy development.

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P22- *APOE* allele frequency in late onset Alzheimer's disease patients from Iberia

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Aims/Context: Alzheimer's Disease (AD) is a progressive neurodegenerative disease associated with cognitive decline. It is one of the most severe brain disorders affecting the elderly population, being secondary to the increase of life expectancy. Although multi-factorial, the primarily genetic risk factor for late-onset AD is the *Apolipoprotein E* (*APOE*) ε4 allele. The *APOE* gene encodes a 299 amino acid protein that plays a key role in the transport and metabolism of plasma cholesterol and triglycerides, as well as in injury repair in the brain. *APOE* isoforms differ in the amino acids 112 and 158, which affect its structure, influencing its capability to bind to lipids and receptors, leading to the onset of AD. Due to the preponderant role in AD pathology, screening the *APOE* gene can facilitate AD diagnostics. Methods: DNA was extracted from saliva samples from 95 patients with clinical diagnostic of AD from Iberia (Northern Portugal and Castile and León). After whole genome amplification, we sequenced the *APOE* locus by Sanger sequencing to analyze SNPs rs429358 and rs7412, therefore assessing *APOE* alleles in our cohort of AD patients. Results: We observed 34% of individuals carrying the risk *APOE* ε4 allele, whereas the more common ε3 allele was present in 59% of the patients. The frequency of ε2 allele (associated to a decreased disease risk) was estimated in our sample set with a frequency of 7%. Conclusions: This work is a preliminary study about the frequency distribution of the *APOE* polymorphic ε2, ε3 and ε4 alleles in a cohort of late-onset patients of AD from Northern Iberian regions. The genetic characterization of *APOE* provides a forecast on the landscape of AD risk in these regions based on the haplotype data obtained from *APOE* alleles at SNPs rs429358 and rs7412.

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P23- Sperm DNA damage, active mitochondria and spermatic parameters: influence of the lifestyle, body mass index and age.

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Sperm DNA damage and an altered mitochondrial activity have been related to male infertility. Several common lifestyle (LS) factors, obesity and advanced paternal age may affect sperm quality, although the consequences are not unanimous in the literature. The aim of this study was to evaluate the impact of LS factors (smoking, alcohol intake and exposure to harmful occupational factors), body mass index (BMI) and men's age on sperm (spz) DNA damage, active mitochondria (AM) and spermatic parameters (SP). A total of 149 men (22-52 years old) undergoing infertility investigation collected a sample for routine semen analysis and were asked to complete an anonymous questionnaire about their LS. In 26 individuals, it was evaluated the sperm DNA integrity by Alkaline Comet and TUNEL assays, and the percentage of spz with AM (MitoTrackerTM Red FM dye). Based on the absence or presence of one/more risk factors associated with the LS, two groups of men were formed: with risks (R) and without risks (NR) associated. DNA integrity, spz with AM and SP were compared between individuals R and NR. The SP were also compared between normal weight (NW), overweight (OW) and obese (O) men. The same comparison was made exclusively in individuals NR. We also evaluated the presence of correlations (r) between BMI and DNA damage and spz with AM in individuals R and NR; and between men's age and DNA damage, spz with AM and SP. Individuals NR tended ($p>0.05$) to have sperm samples with less DNA damage (69.8AU vs 73.2AU), less spz with fragmented DNA (4.6% vs 5.3%), more spz with AM (70.3% vs 66.5%) and better SP than the individuals R. In individuals R the higher the BMI, the more DNA damage the sperm samples presented ($r=0.717$, $p=0.030$), although in individuals NR it was only observed a trend ($r=0.661$, $p=0.053$). Individuals O presented worse SP than individuals NW or OW ($p>0.05$), with statistical significance only in individuals NR. Regardless of the LS, the older the men, the more DNA damage the semen samples presented ($r=0.523$, $p<0.01$). Given the results, it is urgent to sensitize the population to adopt a healthy lifestyle and to warn about the decline in semen quality with age.

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P24- Zebrafish: an interesting model to study CDKL5 deficiency disorder

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CDKL5 deficiency disorder is a rare X-linked condition that results in early onset of impaired motor and cognitive skills such as motor rigidity, stereotypical hand movements and deficient language acquisition as well as recurrent seizures. It is caused by mutations in the cyclin-dependent kinase-like 5 (*CDKL5*) gene, which encodes a serine/threonine kinase involved in important neuronal processes such as cell signaling and neuron morphogenesis. CDKL5 is responsible for its autophosphorylation as well as the phosphorylation of its substrates including AMPH, MECP2, MAP1S and ARHGEF2. Although CDKL5 deficiency is a very severe condition, the mechanisms involved in its onset are not clearly understood and existing mouse models do not fully mimic the human phenotype. Thus, the use of alternative models represents a powerful tool to further study CDKL5 deficiency disorder. Zebrafish has been shown to be a suitable biomedical model and shares many physiological processes with human; therefore our objective was to validate the use of zebrafish as a model to study CDKL5 deficiency disorder. Through a comparative *in silico* analysis we confirmed that gene structure of zebrafish *cdkl5* and Cdkl5 substrates appear to be conserved when compared to their human orthologs. The corresponding proteins also show a degree of sequence conservation, particularly Cdkl5 catalytic domains required for phosphorylation. Zebrafish larvae were exposed to PTZ, a seizure inducing drug, total RNA was extracted and qPCR was carried out to investigate expression levels of neuronal marker genes. The results show a downregulation of *mecp2* and an upregulation of *bdnf* after PTZ treatment. Immunohistochemistry of zebrafish brain sections following treatment with PTZ showed a clearly alteration of cdkl5 expression, mostly in telencephalon, comparing with the control. We are presently conducting experiments in zebrafish with morpholinos to suppress Cdkl5 expression in order to investigate the resulting morphological, behavior and molecular changes. In conclusion, our results contribute to validate the use of zebrafish as a suitable model for the study of CDKL5 deficiency disorder mechanisms.

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P25- A BRCA2 intronic variant of uncertain significance alters pre-mRNA splicing

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Context/Aim: Heterozygous germline mutations in the BRCA2 gene are associated with an increased risk for developing breast and ovarian cancers, whereas biallelic pathogenic germline variants in BRCA2 cause Fanconi Anemia. However, a large fraction of genetic alterations found in the BRCA2 gene are classified as variants of uncertain significance (VOUS), precluding an appropriate clinical approach to patients and relatives. The aim of this study was to characterize the splicing pattern and stability of BRCA2 mRNAs in cells from two sisters with cancer that carry a biallelic intronic variant in the BRCA2 gene classified as VOUS. Analysis of DNA crosslink sensitivity was negative for Fanconi Anemia but suggested the presence of genomic instability. Methodology and results: The variant studied localizes in the intron downstream of exon 8, BRCA2: c.681+5G>C. Using a high precision quantitative RT-PCR assay, based on the digital droplet PCR technique (ddPCR), we show that the great majority of BRCA2 mRNAs produced in the patient cells exclude exon 8. The exclusion of exon 8 results in frameshift and generation of a premature stop codon (PTC) that is expected to drive mRNA to Nonsense Mediated Decay (NMD). We developed an assay based on ddPCR to confirm that BRCA2 transcripts in the patient' PBMCs (peripheral blood mononucleated cells) were indeed targets of NMD. We further found a newly identified exclusion of exon 7 in cells from control donors as well as in the cells from the patients harboring the variant. Conjugated skipping of exon 8 and exon 7 abrogates the frameshift, leading to the expression of an internally truncated BRCA2 protein. Conclusion: Our results suggest that in normal cells the exclusion of exon 7 commits the mRNA to degradation by NMD, whereas in the patients' cells it prevents degradation of the mis spliced mRNA resulting in expression of an abnormal BRCA2 protein. We are currently studying DNA repair function in the patient cells.

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P26- Exploring responsibility about reproduction in the accounts of individuals at risk for and affected by late onset neurological diseases

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People with a family history of hereditary disease commonly face a number of difficult questions about reproduction. Such decisions involve weighing the risk of passing on a genetic condition to offspring alongside responsibilities to self and others, including those not yet born. This paper reports accounts from people at-risk for and affected by the incurable, late-onset neurological diseases, familial amyloid polyneuropathy (FAP) ATTRVal30Met, Machado-Joseph disease and Huntington disease, about their reproductive decisions. We draw on individual and family semi-structured interviews with participants recruited through the respective national patients' associations (n=36, 19 women; mean age: 39.4y, range 17-68). Data were analysed thematically, which revealed that participants drew on various - sometimes ambivalent and competing - relations to their awareness of genetic risk and their wish or desire for children. Findings highlighted that the elimination of genetic risk was perceived as responsible behaviour by some participants; while for others responsibility entailed accepting risks because they prioritized values such as parenthood, family relationships and the value of life, above any question of genetic disease. Some participants shared accounts that were fraught with ambivalence, repentance and guilt, especially when children were born before participants knew of their or their partner's risk. Hope in the future advances of science was often emphasized by those participants with children at-risk. We discuss the findings in the context of the participants' negotiations between reproductive risks and their sense of responsibility to self and others, and how the accumulated multigenerational experience with the disease influence participants' decisions about reproduction and their continued efforts to present themselves as responsible persons and appear responsible to others. We conclude that 'genetic responsibility' is present not only in the accounts of those who chose not to have children, but also in those who knew their situation and chose to have at-risk children.

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P27- 2q31.1 microdeletion syndrome: mapping the clinical phenotype

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Context: Microdeletions of 2q31.1 region are rare. The clinical phenotype is variable, including intellectual disability (ID), facial dysmorphism and limb defects of varying severity. Less frequently the brain, eyes, heart, and urogenital system may also be affected. Haploinsufficiency of the HOXD gene cluster has been linked to limb anomalies, however the etiology of ID remains unclear. We describe three new cases with 2q31.1 microdeletion, aiming to contribute to the genomic mapping of clinical features for this rare syndrome. Methods: Case 1- 9yo boy, mild ID. Bitemporal narrowing, small palpebral fissures, strabismus, prominent columella, thin upper lip, retrognathia, cupped ears with thickened helices and lobes; 3rd finger camptodactyly, 5th finger clinodactyly; 2nd/3rd toes complete syndactyly; cryptorchidism. Case 2- 6yo girl, mild ID and ADHD. Small palpebral fissures, retrognathia, dysplastic helices; broad hallux, sandal gap and 2nd/3rd toe syndactyly. Case 3- 9yo girl, mild ID. Microcephaly; triangular face, narrow forehead, underfolded helices, misaligned teeth, high palate; 5th finger clinodactily; 2nd/3rd toes partial sindactily. DNA samples were studied by aCGH (180K CGX-HD). Available parents were studied by FISH analysis. Results/discussion: 2q31.1 microdeletion was identified in all cases: 1-(173550859_176967147)x1; 2-(173120478_176272245)x1 dn; 3-(174436582_175704751)x1. Deletions of HOXD genes result in hand/foot anomalies. Although all our patients have limb defects, only case 1 encompasses HOXD genes, reinforcing the hypothesis that deletion of upstream regulatory elements may also cause limb anomalies. *DLX1/2, RAPGEF4* and *CHN1*, all playing a role in brain development, were suggested as candidates for ID etiology. Only *CHN1* is included in the minimal overlap deleted region in our cases, supporting its key contribution to ID in these patients. *CHN1* participates in the pruning of dendritic arbors. Disruption of these events during circuit maturation and refinement may lead to brain dysfunction and neurological disease. Our cases, with breakpoints defined by aCGH, contribute to the genomic mapping of clinical features for this rare syndrome.

P28- Reproductive Options and Familial Amyloid Polyneuropathy

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Introduction Familial Amyloid Polyneuropathy (FAP), Portuguese type, is a late onset neurodegenerative disease with high penetrance and impressive morbidity. Prenatal diagnosis (PND) and preimplantation genetic diagnosis (PIGD) are currently available as reproductive options (RO), the later since 2001. Methods Between January 2018 and July 2019, a representative cohort of FAP subjects followed at FAP cardiology consultation of our hospital, aged between 18 and 55 years, were requested to complete an anonymous questionnaire about their RO. The aim of this study is to determine the current knowledge about RO, analyze their choices, information sources and the impact of genetic counseling on their decisions. Results A total of 126 subjects volunteered to answer the questionnaire: 40% were females; 66% had less than 12 years of schooling education; 62% were married and 53% claimed to have RO information. Thirty-nine percent had medical genetics consultation (MGC), being the FAP neurology consultation the main information source (42%), followed by MGC (18%). In general, the prevalence of PND and PIGD use was 6% and 13% with a successful pregnancy of 63% and 38%, respectively. Nine percent chose not to have children due to fear of transmission. In MGC group (49/126), the prevalence of PND and PIGD use was 6% and 14%, 22% chose not undergo reproductive planning and 12% were unaware of RO. In those without MGC (77/126), the prevalence of PND and PIGD use was 7% and 10%, 27% chose not undergo reproductive planning and 29% were unaware of RO. In those with offspring born after PIGD availability (44/126), 41% have had MGC, 68% did not use reproductive techniques, the main reasons being choice and RO unawareness. In those that planned to have children (25/126), 52% had MGC, 92% elected PIGD for reproductive family planning since most (88%) didn't want to terminate a pregnancy; 17% reported the waiting time as the reason for not choosing PIGD. Conclusions This study highlights the importance of medical genetics in the multidisciplinary approach of this disease. A greater investment should be made in reproductive counseling and in facilitating access to RO.

P29- Clinical, radiological and molecular findings in four cases of X-linked spondylo-epiphyseal dysplasia tarda

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Introduction: X-linked spondylo-epiphyseal dysplasia tarda (SEDT) is an X-linked recessive, late-onset, progressive skeletal disorder characterized by mild-to-moderate short-trunk short stature. X-linked SEDT is caused by heterozygous mutations in TRAPPC2. We report on four cases of SEDT, highlighting its clinical, radiological and molecular data. Methods: Retrospective analysis of clinical records of four patients with SEDT followed at our Medical Genetics Department. Results: All patients are adults (26, 33, 35 and 42 years). Three had a family history compatible with an X-linked mode of transmission. Their symptoms began during the middle to end of the first decade of life. One of the patients had moderate hearing loss, requiring hearing aids, since the age of 6 whilst the others had no significant extra-skeletal manifestations. Physical examinations showed short neck and trunk, barrel-shaped chest, dorsal kyphosis, and lumbar hyperlordosis; one of the patients also had pes planus and reported significant shoulder pain. X-rays were similar in all patients, and showed signs of epiphyseal dysplasia, platyspondyly with characteristic hump-shaped deformity of vertebral bodies, narrow disc spaces, dorsal hyperkyphosis and lumbar hyperlordosis, short femoral necks, coxa vara and signs of arthritis, namely in the hip and spine. The diagnosis of X-linked SEDT was clinically established in the four patients, and was confirmed by molecular analyses in three (Sanger sequencing or skeletal dysplasia NGS panel). These three patients presented two different pathogenic variants in TRAPPC2, both frameshift. Conclusions: We report on four patients with X-linked SEDT. Despite the importance of molecular confirmation, the diagnosis is mainly based on clinical characteristics with X-rays providing the key diagnostic clues. One of the patients also had moderate hearing loss, which is probably unrelated to the disorder. Although short trunk, barrel-shaped chest and lumbar hyperlordosis are present in these patients, breathing difficulties do not seem to occur. The diagnosis of this condition is important for surveillance of joint pain and scoliosis, and for genetic counseling.

P30- Mutational spectrum of the PKHD1 gene in autosomal recessive polycystic kidney disease

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Contex and aims: Autosomal recessive polycystic kidney disease (ARPKD, OMIM 263200) is a rare disease caused by mutations in PKHD1, a large 67 exons gene that codifies a protein expressed in kidney epithelium and in the bile duct. Although genetic confirmation is the only certain diagnosis method for ARPKD, the size of the gene and the fact that mutations can be dispersed all along the entire gene, makes Sanger sequencing difficult and expensive. We propose Next Generation Sequencing (NGS) as a facilitator for establishing a mutational spectrum of PKHD1 in a cohort of ARPKD Spanish patients. Methods: 30 patients with ARPKD diagnostic based on the presence of multiple cysts in both kidneys and the absence of a family history of polycystic kidney disease (characteristic of the adult dominant disease), were analyzed. The absence of cysts was confirmed in both parents. DNA was obtained by salting-out method and submitted to NGS through Ion Torrent PGM technology as described by Gómez J (Pediatr Res,2016). Candidate variants were confirmed by Sanger sequencing. Results: Overall coverage of PKHD1 coding sequence, plus at least 5 intronic flanking nucleotides, was greater than 95%. Three changes were found accounted for 40% of the pathogenic variants: c.9689delA (p.D3230fs), c.5895dupA (p.L1966fs), and c.107 G>A (p.T36M). Two pathogenic variants were found in 20 patients, one in 6 patients and in only 4 cases no candidate variants were identified. While patients with two variants showed a typical early onset. Conclusions: Our study shows how PKHD1 NGS facilitates the rapid and cost-effective genetically screening of large cohorts of ARPKD patients. We also identify three Spanish recurrent pathogenic variants. Our results suggest that in cases without pathogenic variants, might be other de novo or hypomorphic variants at dominant genes, or even other genes associated with the disease.

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P31- Liquid Biopsy in CRC patients – when no tumor sample is available

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Introduction: Liquid biopsy (LB) has been emerging as a useful tool in the management of colorectal cancer (CRC) patients, both at diagnosis and follow-up. Its major advantages include being a minimally invasive methodology and the possibility of better assessing the molecular heterogeneity and dynamics of the disease. Currently, expanded tumor RAS analysis is mandatory for patients with metastatic CRC being considered for EGFR-targeted monoclonal antibodies. For patients with no molecular analysis of primary tumor, LB may be the best choice. Methods: Six CRC patients', stage IIA to IV were studied. Tumor molecular results were only available for two patients. Peripheral blood samples (5-7 ml) in EDTA tubes were processed for plasma collection within one hour. Cell-free DNA (cfDNA) was extracted from 2.5 -3.5 ml of plasma using MagMax cfDNA isolation Kit. Molecular profile was performed with Oncomine™ Colon cfDNA Assay. Ion Chef™ System and Ion 530™ Kit-Chef were used for template preparation. A 50pM library pool containing six samples were applied in Ion 530™ Chip and sequenced on the Ion Gene Studio S5 Plus platform (Thermo Fisher Scientific). For variant analysis, Torrent Variant Caller plugin, Torrent Suite™ Software v5.2.1 and the Ion Reporter online tool were used (Thermo Fisher Scientific). Results: Plasma cfDNA concentration varied from 0.6 to 15.7 ng/µl, the higher value being detected in a patient with multiple metastasis. Using a cfDNA input ranging between 8 to 31 ng, limits of detection (LoD) from 1.25 to 0.1% were achieved. KRAS actionable mutations were detected in five patients including the two patients with tumor molecular study (concordant results). Five patients had driver mutations in three or more genes, the most frequently mutated being KRAS, PIK3CA and APC. Driver mutations in seven genes were identified in the LB of a patient with evidence of mismatch repair (MMR) system deficiency in tumor biopsy. Conclusion: in patients with high stage CRC, with no primary biopsy of tumor tissue available, LB using NGS approach may evaluate KRAS mutational status and identify other eventually actionable or prognosis-associated somatic variants.

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P32- Diagnosis of congenital fibrinogen deficiencies – a center´s experience

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Aim/Context:Congenital fibrinogen deficiencies are rare disorders, classified as quantitative (hypofibrinogenemia/afibrinogenemia, with partial/complete absence of fibrinogen) or qualitative (dysfibrinogenemia and hypodysfibrinogenemia, with normal or reduced fibrinogen levels and/or abnormal functional activity). Clinically, quantitative deficiencies are associated with hemorrhagic events, while dysfibrinogenemias are mainly asymptomatic, or can present with bleeding, thrombosis or both. Fibrinogen is a hexameric protein consisting of three pairs of polypeptide chains (Aa, Bb and g), encoded by FGA, FGB and FGG genes, respectively. Variants in these genes have been reported, both in homo- or heterozygosity. Our aim was to identify the molecular diagnosis of patients with fibrinogen anomalies and/or hemorrhagic diathesis of unknown cause by next generation sequencing(NGS). Methods: We have analyzed 17 unrelated patients and 5 relatives. The molecular diagnosis was done using a custom panel for NGS (43 genes). Library preparation and sequencing was done using IonS5 (TFS) protocol. Results:We have identified 13 different variants (pathogenic and potentially pathogenic) in FGA (5), FGB (2) and FGG (6) genes, including six new variants in FGB (Glu240Lys and Arg196Cys) and FGG (Asp63Val, Trp360*, Glu422del and Tyr27Cys). The patients were diagnosed as hypofibrinogenemia (n=7), hypodisfibrinogenemia (n=4) and dysfibrinogenemia (n=4). The patients diagnosed with afibrinogenemia (n=2) were homozygous for 2 variants in FGA (complete deletion and Arg181*) and had severe hemorrhagic manifestations. Five presented with thrombosis: one was carrier for FGA Cys64Tyr, associated with both thrombosis and bleeding. In total, 11 patients were asymptomatic. Conclusions:We have identified 6 new variants in the coding region of the fibrinogen cluster, together with high phenotypic variability. A phenotype-genotype correlation was observed in the quantitative deficiencies. In dysfibrinogenemias, it is still a challenge. We add another example of the valuable tool that NGS represents to clinical practice, allowing for a faster and broader diagnosis of fibrinogen hereditary anomalies.

P33- Circulating Cell-free DNA levels as a potential biomarker in cancer – a preliminary study

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Context: Cell-free DNA (cfDNA) is extracellular DNA present in plasma of both healthy individuals and patients with benign lesions, inflammatory diseases, tissue trauma and cancer. The release of DNA into blood might rise to higher levels in cancer patients as a consequence of the necrotic and apoptotic processes typical of tumor cells. These increased cfDNA levels in cancer patients suggest a clinical relevance for the diagnosis and prognosis of those patients, as well as for disease monitoring. The aim of this preliminary study was to evaluate the ability to isolate cfDNA from plasma and then compare cfDNA levels of different patients with head and neck cancer in different stages of the disease, before and after treatment. Methods: cfDNA was isolated from 2 mL of plasma from 12 samples using a kit (QIAamp Circulating Nucleic Acid kit; Qiagen Manchester Ltd, Manchester, UK) adapted from the manufacturer's instructions. Results: Four of the patients (stages I to IV) were only analyzed before the treatment and four other patients were analyzed also after the treatment (surgery or chemotherapy/radiotherapy). When evaluating pre-treatment samples, no large variations in cfDNA were observed. Comparing pre and after treatment cfDNA levels we observed an increase after treatment. Tumor staging and BMI of the patients were correlated with these results. Conclusions: This preliminary study allowed us to see the differences in the cfDNA levels in cancer patients and evaluate the clinical relevance of those levels for the diagnosis, prognosis and follow-up of patients.

P34- MLPA and array CGH evaluation in Oral Squamous Cell Carcinoma

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Introduction: Head and Neck cancer affects seven anatomical locations; amongst them, in the oral cavity, squamous cell carcinoma is the most common (90%) histological type. Oral squamous cell carcinoma (OSCC) affects different oral regions, most frequently the floor of the mouth and the tongue. Due to their proximity with structures with vital importance and with lymph nodes, this cancer still has a high mortality rate. As for genetic imbalances, alterations in copy number occurring in gene-dosage sensitive genes can have an important carcinogenic role in this disease, since associations between some imbalances and OSCC, such as in *TP53*, *RB1*, *EGFR* and *FHIT*, have already been reported. Aims: Identification of genetic imbalances that can be relevant as biomarkers, by characterizing copy number alterations (CNAs) in a cohort of squamous cell carcinomas, from the tongue and the floor of the mouth. Methodologies: Two different techniques, Multiplex Ligation-dependent Probe Amplification (MLPA) and microarray-based Comparative Genome Hybridization (aCGH) were performed in a 62 samples cohort. For MLPA a specific probe panel was used to evaluate CNAs. Data analysis was performed by Excel and by R (version 3.5.2). Results: The type of genetic alterations with higher frequency in our cohort were gains. MLPA most common imbalances were seen in chromosomes 3, 8 and 11, particularly in the 11q13.3 region, which presented high number of gains in *FADD*, *FGF4*, *CCND1* and *CTTN* genes. In aCGH, the most frequent abnormalities were seen in 8p23.1, 8p11.22, 6p25.3, 15q11.2, 5q13.2 and 11q11 regions. Common alterations to both locations detected in high frequency were observed in 8p23.1, 8p11.22, 8q24.3 and 3q29 regions. Conclusion: We were able to find that 11q13.3 is a hotspot region in this cancer, showing high rates of gains by both techniques. MLPA and aCGH also showed that chromosomes 3, 8 and 11 were the most commonly altered. Correlation between the detected genetic imbalances and clinicopathological features is crucial to propose novel biomarkers with prognostic value.

P35- Design and validation of a mathematical model and a restriction-TP-PCR assay for assessing *FMR1* Allelic Score

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Introduction: Fragile X mental retardation-1 (*FMR1*) gene has been the target of several studies, besides Fragile-X syndrome, particularly the implication of premutation alleles in female fertility. In premutations, an AGG interspersions number below 2 was recently correlated with a diminished ovarian reserve. To the best of our knowledge, the AGG profile has never been studied in normal-range alleles. Our aim is to determine the AGG pattern and simultaneously analyze the *FMR1* gene sub-genotypes; however, the standard TP-PCR technique does not adequately discriminate between alleles with similar AGG patterns and may fail to detect pure CGG alleles. Methods: A restriction-TP-PCR assay was developed to further analyze the presence of pure alleles in 131 female control samples. This new assay consists of TP-PCR analysis after digestion of gDNA with *MnlI* (3'…GGAG(N)6…5'). This endonuclease cleaves the AGG sequence and thus only pure or the most 5' pure region is amplifiable. Furthermore, a mathematical model was designed to combine the *FMR1* sub-genotype, allele size and the AGG number and pattern, producing the *allelic* score. Results: Routine TP-PCR allowed determination of the AGG interspersion pattern in 88.5% of the alleles, and the remaining 15 samples were additionally tested using the new assay. Overall, 5% of the alleles showed no AGGs: belonging to the *normal-low* (n=5) and *low-low* (n=4) sub-genotypes. No statistically significant difference in the distribution of the *FMR1* sub-genotypes was observed. Conclusion: We describe a new methodology to accurately determine the AGG number and pattern, and identification of pure alleles. The allelic complexity allowed the categorization of samples in two statistically significant different groups: equivalent and dissimilar. The first is enriched with samples belonging to the *normal-normal* sub-genotype while those with the *normal-low* sub-genotype are more prevalent in the dissimilar group. Interestingly, the latter has been associated with ovarian dysfunction. Our results clearly indicate the importance of the AGG interspersion pattern in *FMR1* and validate the mathematical model as a tool to assess female fertility.

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P36- From copy number alterations to genomic correlations in oral cavity cancer – Cohort of patients from Brazil

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Introduction: Oral squamous cell carcinoma (OSCC) presents a high incidence and mortality worldwide. The progress of whole-genome technologies has opened new opportunities to explore cancer-associated biomarkers with diagnostic and therapeutic applications. This study aimed to perform a genome-wide characterization of OSCC patients and to identify the most common altered chromosomes and genes related to cancer development. Methods: The genomic characterization of 22 tumor tissue samples with oral cavity cancer diagnosis from a cohort of Brazil was performed using array comparative genomic hybridization technique. A fraction of alteration (number of altered base pairs over total base pairs) was calculated for every chromosomic arm, which was then used to determine the Spearman correlation coefficient, in order to find concomitant alterations. Results: We detected imbalances in almost all chromosomes; however, it was possible to verify that the most common losses and gains were observed in specific chromosomal regions. The most frequent gains were observed at 14q32, 11q21, 3q29, 17q12, 4q34, 3q24, 7q11, 8q11, 4q13, 8q11, 1p36, 11p15, 11q24, 19q13, 8q24, 20q11, 22q11, 7q21, 9q22, 15q11, 3q25, 3q28, 15q15, 20p12, 4p16, 11q11, 11q12, 11q14, 11q23 and 3q26, and the most frequent losses were observed at 8p23, 10q11, 6p25, 8p11, 11p15, 12p13, 6p21, 4q13, 11q11, 3p26, 5p15, 9p24, 5q11, 15q11. Several genes with a possible association with OSCC diagnosis and prognosis were highlighted, namely *ADAM3A* (8p11), *CDKN1C* (11p15), *MAML2* (11q21), *ASIC2* and *CCL2* (17q12). Regarding the Spearman correlation coefficient, we observed a strong correlation between 3p and 8q, i.e. in our cohort we simultaneously verify 3p loss and 8q gain. Additionally, a strong correlation between gains in 5p and 7p and in 5p and 12p was also observed. Regarding copy number losses, a strong correlation was observed between 3p and 9p. Conclusions: These results are in agreement with other cohort of patients studied in our lab, showing the importance of genomic evaluation to identify possible diagnostic and prognostic biomarkers. The correlation between molecular and clinic-pathological data is vital.

P37- Genomic characterization of chronic lymphocytic leukaemia and multiple myeloma patients: aCGH contribution

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Context: The characteristic variable clinical behaviour of haematological malignancies reflects the tumour biological heterogeneity and ultimately, the tumour genomic abnormalities. In chronic lymphocytic leukaemia (CLL) and multiple myeloma (MM) the heterogeneous genomic landscape has long been reported. Conventional Cytogenetics (CC) and Interphase Fluorescence in Situ Hybridization (i-FISH) became common clinical practice in the detection of cytogenetic alterations. However, both techniques have limitations: CC implies division cells while i-FISH is a targeted technique. Array Comparative Genomic Hybridization (aCGH) detects copy number variations at a genome-wide level, allowing clinically relevant abnormalities detection, otherwise missed. Therefore, the goal of this work was to characterize the genome of CLL and MM patients, by aCGH and to compare the suitability of aCGH and i-FISH (results from the clinical practice) in the detection of genomic abnormalities. Methods: A total of 19 CLL patients, 1 monoclonal B lymphocytosis, 15 MM and 1 monoclonal gammopathy of undetermined significance were analysed by aCGH. Mononuclear cells of CLL patients and plasma cells from MM patients were respectively isolated from peripheral blood and bone marrow samples, followed by genomic DNA extraction. Results: In patients clinical routine, i-FISH studied a few number of regions previously associated to some prognostic value. For its part, aCGH allowed a whole genome research and the identification of new disease-associated chromosomal regions. The most frequent genetic alterations found in CLL patients' samples were 14q and 13q deletions (40% and 35%) and trisomy 12 (30%), whereas in MM patients' samples the most common genomic abnormalities were 13q deletions (63%), 1q gains (44%) and trisomies 9 and 7 (38%), already described in literature. Conclusions: Whole genome level characterization of tumour samples was performed by aCGH, detecting alterations in regions other than the ones evaluated by standard method i-FISH. Nevertheless, i-FISH detects low expression mosaicisms and balanced rearrangements, denoting the convenience of applying both techniques at the clinical level.

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P38- Intratumor genetic and epigenetic heterogeneity in oral cancer

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Background: Cancer presents intertumoral and intratumoral heterogeneity, since tumors comprise subpopulations of different cells either within a primary tumor or between tumors of different tissues. The diagnostic and management of cancer patients is difficult due to the presence of tumor heterogeneity, which could be originated by subpopulations of distinct cells with nonrecurring mutations and genomic alterations as well as by clonal evolution and positive selective pressure from therapeutics. The molecular heterogeneity of oral cancer seems to hamper the development and efficacy of target treatments. This study aimed to perform a genetic and methylation characterization of oral tumor samples and to evaluate the presence of intratumor heterogeneity. Methods: Tumor and non-tumor tissue samples from 9 patients diagnosed with oral cavity squamous cell carcinoma were analyzed using Methylation Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA). From each patient 4 different biopsy tissue sites -two from distinct regions of tumor and two from distinct regions of non-tumor -were analyzed. Results: From the 9 patients, a total of 36 samples were analyzed (tumor and non-tumor), being *WT1* gene methylated in 18 samples. The second most common methylated gene was *GATA5* in 8 samples. We identify few copy number alterations, being the most frequent gain observed at *GSTP1* gene and the most frequent loss observed at *CDKN2A* gene. We found intratumor heterogeneity in different genes of 6 patients from the 9 analyzed. Conclusion: Our results highlight the challenges and difficulties to identify a comprehensive molecular profile of oral carcinoma with single site biopsy, which could have implications for precision medicine and clinical patient's management and follow-up.

P39- Molecular diagnosis of haemophilia A: four novel variants identified in five patients

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Aims/Context: Haemophilia A (HMA) is an X-linked bleeding disorder caused by reduced levels of the coagulation factor VIII (FVIII) due to alterations in the *F8* gene. Decreased levels of FVIII activity leads to a loss of clotting activity and to bleeding (predominantly into joins, muscles and inner organs). The severity of HMA ranges from mild (5-30% activity) to moderate (2-5% activity) to severe (<1% activity). During the last five years, we have found four novel variants identified in five index patients with no family history of HMA. Three frameshift variants were detected in patients presenting severe HMA and one missense variant was identified in two unrelated patients with a mild phenotype. Methods: Analysis of the *F8* gene was performed in five index patients using PCR followed by Sanger sequencing, after *F8* IVS22 and IVS1 inversions being excluded in severe HMA cases. Bioinformatics analysis was performed with several pathogenicity prediction tools (Alamut Visual, VarSome, VEP and Human Splicing Finder). Results and Conclusions: In the three patients with severe HMA, three different novel *F8* variants were identified: c.1060_1061delCT, p.(Leu354Thrfs*5), c.4804delC, p.(Gln602Lysfs*19) and c.3561dupT, p.(Pro1188Serfs*10). All these variants create a frameshift, leading to a premature termination codon and presumably resulting in non-functional truncated proteins, confirming the patient's phenotypes. The novel *F8* missense variant c.5836G>T, p.(Asp1946Tyr) was identified in two unrelated patients, both with mild HMA. The Asp1946 is a highly conserved amino acid in the FVIII protein. Additionally, physicochemical properties between Asp and Tyr are significantly different, and *in silico* analysis classified it as pathogenic due to the amino acid substitution. Normal mRNA splicing process can also be disturbed due to the creation of a new donor splice site. RNA studies and other functional assays are essential in order to establish this variant clinical significance. Identification of novel pathogenic *F8* variants in HMA patients allows genotype-phenotype correlations, appropriate genetic counseling and new knowledge about the molecular bases of this pathology.

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P40- Presumed *TP53* mosaicism: variants detected using a NGS hereditary cancer multigene panel

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Aims/Context: NGS multigene panels are routinely used to identify germline pathogenic variants in cancer susceptibility genes. In addition, NGS allows the identification of low-level mosaicism events that may not be detectable by conventional Sanger sequencing. We describe two cases of presumed *TP53* mosaic variants detected by NGS on blood-derived DNA, and confirmed by ARMS-PCR and Sanger sequencing. Case 1: female, 87 years old, colon cancer at 83 and metachronous breast cancer at 86, no history of familial cancer. Case 2: female, 75 years old, ovarian cancer at 71, local relapse at 74. Methods: NGS using TruSight® Cancer Sequencing Panel and TruSight® Rapid Capture kit (Illumina) and paired-end sequencing on MiSeq® platform (Illumina). Bioinformatic analysis with MiSeq Reporter, Enrichment, VariantStudio, VEP, Alamut Visual, VarAFT, VarSome and IGV. ARMS-PCR and Sanger sequencing were used to confirm the *TP53* variants. Results and Conclusions: Two cases of presumed *TP53* mosaic variants were studied. Case 1: the missense alteration *TP53*: c.764T>G, p.(Ile255Ser) was detected with a variant allele frequency (VAF) of 26% (39/150 reads). This variant is described in ClinVar as a somatic alteration, classified as likely pathogenic. It is not reported in gnomAD and VarSome software classified it as a variant of uncertain significance. Case 2: missense variant *TP53*: c.524G>A, p.(Arg175His) detected with a VAF of 15% (10/58 reads). This variant is described as pathogenic in HGMD Professional, LOVD and ClinVar, in association with Li-Fraumeni syndrome. These two cases seem to represent *TP53* mosaicism, supported by: i) VAF lower than 30%, ii) detection at the sensitivity limit of Sanger sequencing and iii) confirmation by ARMS-PCR. Confirming this hypothesis by studying tumor and other tissue samples and offspring analysis (underway in both cases), is essential for disease diagnosis, assessing recurrence risk and genetic counseling. The hypothesis of acquired aberrant clonal expansion limited to the hematologic compartment, versus a germline variant should be considered in similar cases, and confirmatory methodologies are mandatory.

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P41- Genome-wide association in a cohort of Alzheimer's patients from Iberia: an exploratory analysis with common variants

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Aims/Context: The polygenic form of Alzheimer's disease (AD) is complex and late-onset (LOAD, after 65 years old) with heritability ranging from 25% to 80%. APOE ε4 remains the strongest genetic risk factor for AD. Almost 40 other genetic variants have been identified, although current findings account for only 31% of LOAD heritability. Currently, known AD markers cannot explain the majority of genetic variance and are not helpful for predicting or diagnosing the disease (aside from APOE ε4). We aimed at performing an exploratory association analysis in a cohort of LOAD patients from Northern Portugal and Spain. Methods: DNA was extracted from saliva and buccal swab samples and genotyped with Axiom Spain Biobank Array. This analysis comprised 128 LOAD patients from North Portugal (n=55) and from the Spanish autonomous community of Castile and León (n=73) with a clinical diagnosis of AD. In addition, 59 controls (individuals over 65 years old with no signs of dementia) from both regions were also analyzed. Using PLINK we implemented a genotypic additive model including 4 principal components, sex and age as covariates. Results: Excluding known APOE risk variants, SNPs were selected for p-values below 10-4 to evaluate the performance of the model, taking into account the small sample size (which prevents us from drawing significant results at the genome-wide correction threshold, 5.0e-08). We found nominal significance for other variants previously associated with AD or in relevant molecular pathways for the disease. Namely, rs3773341 (p-value=6.98e-06) and rs2046210 (p-value=6.5e-06), the most significant variants assuming logistic regression, are within the makorin ring finger protein 2 (MKRN2, which is involved in neurogenesis) and near the estrogen receptor (ESR1), respectively. Conclusions: These results suggest that our model is being correctly implemented. Increasing the sample size with more patients and controls from Iberia will likely yield significant results.

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P42- Novel TP-PCR based detection of repeats within *AFF2* gene (FRAXE) and accurate homozygous identification

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Context: Fragile XE syndrome (FRAXE) is a form of mild to moderate intellectual disability associated with learning deficits, hyperactivity and autistic behaviour. FRAXE, with an estimated frequency of 1/50000, is a trinucleotide repeat disease mostly caused by a GCC expansion in the *AFF2* gene. The broad and unspecific spectrum of FRAXE clinical presentation makes molecular testing essential for a definitive diagnosis. Routinely, the sizing of the *AFF2* GCC repetitive region includes PCR-based and Southern blot (SB) analyses, the latter being a very time-consuming methodology. For that reason, SB is being replaced by alternative approaches such as triplet-repeat primed PCR (TP-PCR). To the best of our knowledge, this method has never been applied to the diagnosis of FRAXE. Methods: A novel TP-PCR was developed using a primer binding upstream the repeat and a $(GCC)_5$ primer with a tail (F+R) that also binds to a second region within *AFF2*. The assay was optimized resorting to samples with known allele sizes and validated using DNA samples from 500 unrelated females with a previous uninformative routine PCR testing result. Results: Firstly, the assay correctly sized 100% of the alleles in 475 samples with a normal-range GCC genotype. In the remaining 25 samples originally genotyped as homoallelic, our assay determined 19 with alleles within the normal range, four intermediate alleles and two premutations. Among the first group of 19, four had been incorrectly genotyped due to a SNP near the repetitive region (validated by Sanger sequencing). To verify the correct repeat length in the discrepant samples, they were additionally analysed by SB and the presence of the expanded alleles was confirmed. Conclusions: We describe a simple, accurate and specific tool that can be used in the molecular diagnosis of FRAXE. The assay unambiguously identified homoallelic samples often obviating the need of a second, usually time-consuming technique and representing an attractive alternative for diagnostic laboratories. Furthermore, in six samples this assay correctly identified a putatively pathogenic and unstable allele which had escaped detection with the previously performed PCR.

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P43- Secondary findings identified in broad-scale sequencing in a Portuguese 915 patient cohort

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Context: Controversy concerning the active search for secondary findings (SF) remains since broad massive parallel sequencing emerged in clinical practice. The American College of Medical Genetics (ACMG) recommends the return of SF classified as known or expected pathogenic variants in a set of 59 genes. In this study we review our department's experience in addressing SF. Methods: We included all consecutive results from broad NGS–4813 or 6110 genes panels–or whole exome sequencing concluded between January 2015 and August 2019. The studies were performed by CGC Genetics (860) or within the In2Genome project (55). SF were reported based on the 2013,2016 and 2017 ACMG recommendations. Evaluation of family medical records and segregation studies were performed whenever possible. Results: A SF was reported in 11 out of the 915 analyzed cases (1.2%). The eleven unique SF variants identified were associated with: cancer predisposition–BRCA1, BRCA2 (2), MSH2; cardiomyopathy– MYBPC3 (2), DSC2, KCNQ1, GLA; and familial hypercholesterolemia–APOB, LDLR. None of the index cases had clinical phenotype related to the SF. In two families, relatives already had the clinical diagnosis, one with molecular confirmation (BRCA1) and one without (MSH2). Three additional families had some clinical manifestations within the spectrum of the SF (DSC2, KCNQ1, LDLR). In the six remaining families with a SF, there were no affected relatives. In 3 additional cases, the variant VHL [c.154G>T p.(Glu52Ter)] was found: in 2015 it was reported as SF and now reclassified as a VUS. Discussion: The 1.2% of SF identified in our cohort is consistent with the literature. SF reporting allows the diagnosis and improves the health-care in families that did not meet the clinical criteria for routine diagnostic genetic testing. The absence of affected relatives in 6/11 families may be explained by the fact that some disorders have oligo/polygenic inheritance or that reported variants may turn out to be non- pathogenic. This was the case of the recurrent nonsense VHL variant, which will be discussed in detail. Controversial questions and other examples illustrating our clinical experience and will be presented.

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P44- Gut microbiota variation in obese patients submitted to bariatric surgery

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Introduction: Obesity is a chronic non-communicable disease associated with co-morbidities and premature death. Bariatric surgery is the most successful approach. New insights in the pathophysiology of obesity revealed a role for gut microbiota both in the susceptibility to the disease and response to therapy. Methods: Prospective, observational clinical study enrolled 24 patients, 4 class II obesity (Body mass index, BMI 35.0–39.9kg/m2) and 20 class III (BMI >40kg/m2). Age ranged from 23 to 64 (average 42.4 years, sd-10.14); 11 were submitted to gastric bypass and 13 to sleeve gastrectomy. Clinical, analytical parameters and gut microbiota were evaluated before (T0) and 6 months (T6) after surgery. V4 hypervariable region of 16S rRNA was sequenced by NGS approach in MiSeq (Illumina) using "MiSeq Reagent Nano Kit version 2(500 Cycles). Contigs assembly, quality control and taxonomic classification of NGS data were performed with mothur and ARBSilva database. Results: Differences between T0 and T6 were significant for BMI (44.27/sd-6.33 vs. 33.6/sd-5.56;p<0.0001), waist circumference (123.96/sd.13.9 vs 101,85/sd.13.21;p<0,0001), insulin resistance (HOMA-IR/Cpeptide of 1.75/sd-1.5 vs 1.01/sd-0.27;p=0.02), C-reactive protein (1.13/sd-0.9 vs 0.41/sd.0.49;p<0.005), triglycerides (130.1/sd-31.5 vs 94,83/sd-29.14;p<0.01), uric acid (6.3/sd-1.33 vs 5.2/sd-1.3;p<0.001) and systolic blood pressure (146.8/sd-12.9 vs 127.8/sd-14.5;p<0.0001) (paired t-studenttest). The average %excess weight loss (%EWL) at T6 was 57.43/sd-15.74 (95%CI 51.14-63.72), with no significant difference between bypass and sleeve (p>0.05). Microbiota analysis revealed a high interindividual variability and a shift in phila representation after surgery, with increase in the rate (Verrucomicrobia+Proteobacteria) vs (Bacteroidetes+Firmicutes), particularly for patients undergoing bypass (6.9/sd-2.3 vs 19.04/sd-8.93;p. Conclusions: Both gastric bypass and sleeve gastrectomy are associated with metabolic and gut microbiota variations.

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P45- Osteoporosis: Gene interaction between haptoglobin and *HFE* polymorphisms

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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 stimulate 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. 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. 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 appear 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.

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P46- Cytogenetic analyses of CLL patients

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Introduction: Chronic lymphocytic leukemia (CLL) is a biologically heterogeneous disease with a highly variable clinical course ranging from indolent to very aggressive. The international prognostic score (CLL-IPI) in order to identify distinct risk groups of CLL patients, integrates clinical variables and also genetic and biological features (del(17p) and/or mutations of the TP53 gene and the somatic hypermutation status of the IGHV gene). The presence of an increased number of cytogenetic abnormalities detected by chromosome banding analyses (CBA) has been associated with more aggressive clinical outcomes, namely the presence of a complex karyotype (CK). We present a series of 55 CLL patients analyzed by CBA and FISH for del(17p). Material and Methods: Bone marrow or peripheral blood samples from 55 CLL patients at diagnosis and before the initiation of any treatment, were cultured with DSP30 and Interleukin 2. CBA was performed with GTL banding. The presence of three or more aberrations was classified as a complex karyotype, whereas High-CK was defined by the presence of five or more. In all cases, FISH analyses with the ON TP53/17 (Leica) probe was performed. Results: A successful karyotype was obtained in 52 of the 55 samples. Thirty-seven out of 52 patients presented an abnormal karyotype (71%), with 16 (43%) classified as CK, and 11 of these (69%) exhibiting High-CK. Deletion of 17p (*TP53*) was observed in 4 out of 55 patients (7%), 3 of which had a CK. Discussion and Conclusions: Routine cell stimulation protocols for CBA in samples of CLL patients allowed to overcome the difficulty in obtaining sufficient metaphases of the malignant clone that rendered a low detection rate of chromosome abnormalities. Recent studies suggest that High-CK may be a predictive marker, independently of the presence of *TP53* aberrations. In our series, karyotype result was obtained in 95% of the samples, with CK in over 40% of the cases. A significant proportion of CK (69%) showed a High-CK. Interestingly, only one case with High-CK exhibited del(17p) detected by FISH. Therefore, CBA before treatment initiation may be useful in the clinical practice.

P47- Rare autosomal dominant hereditary hemochromatosis associated with *SLC40A1* gene: ferroportin disease or type 4 hereditary hemochromatosis?

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Ferroportin (FPN1), encoded by the *SLC40A1* gene, is the unique cellular iron exporter identified in mammals. FPN1 transfers iron from the intestine and macrophages into the bloodstream. This function is negatively regulated by hepcidin. Mutations in *SLC40A1* may affect FPN1 function, originating distinct autosomal dominant diseases: (i) the Ferroportin Disease (FD), due to loss-of-function mutations, is characterized by decreased iron export from enterocytes and severely affected iron transfer in macrophages, giving rise to a marked iron accumulation in spleen and liver; and (ii) the Type 4 Hereditary Hemochromatosis (HH), resulting from gain-of-function mutations conferring resistance to hepcidin-mediated FPN1 degradation and consequently high cellular iron export. In this study, 335 individuals suspected of having non-classic HH were enrolled. Six genes related with iron metabolism were analysed by SSCP, dHPLC or NGS. The latter used *TruSeq* or *Nextera XT* libraries and a *MiSeq* platform (*Illumina*). Genetic variants found were validated by Sanger sequencing. Predictive consequences at protein level were evaluated using *Polyphen-2* and *SIFT* softwares. From all patients analysed, three *SLC40A1* pathogenic variants were detected in heterozygosity in three women: two missense, c.238G>A, p.Gly80Ser and c.610G>A, p.Gly204Ser; and one deletion, c.485_487delTTG; p.Val162del. These variants had been reported in public databases, but they were not known to be present in the Portuguese population. The p.Gly80Ser and the p.Val162del are FPN1 loss-of-function mutations and were found associated with hyperferritinemia and low transferrin saturation (FD). In contrast, the p.Gly204Ser induced a gain of FPN1 function with a full iron export capacity giving the patient a type 4-HH phenotype, which includes iron overload, hyperferritinemia and high transferrin saturation. Detailed clinical evaluation of the suspected patients are useful to unravel the effect of different mutations in FPN1 function, expression and regulation.

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P48- Evaluation of mathematical indices as tools for distinguishing β-thalassemia trait from iron deficiency anemia in Portuguese females with microcytic anemia

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Microcytic anemia is a common condition frequently caused by iron deficiency anemia (IDA) or β-thalassemia trait (BTT). Some mathematical indices have been described as fast and inexpensive tools for distinguishing these two conditions. This approach is very useful in mass screening programs especially in countries with limited resources. This study aimed to evaluate the diagnostic performance of 13 distinct indices: RBC, England&Fraser, Mentzer, Srivastava, Shine&Lal, RDW, Ricerca, Jayabose (RDWI), Green&King (G&K), MDHL, MCHD, Sirdah and Ensani. We investigated 102 adult Portuguese female, presenting anemia (Hb<12g/dL) and microcytosis (MCV<80fL). The *HBB* gene was screened for pathogenic variants by ARMS or PCR following Sanger sequencing. The iron status was evaluated by standard approaches. IDA was considered when ferritin<12µg/L and/or transferrin saturation<15%. Two groups were generated: 51 BTT (with one *HBB* variant: c.92+1G>A; c.92+6T>C; c.92+110G>A or c.1188C>T) and 51 IDA, being assured that no individual had simultaneously the two conditions. To determine the performance of the indices, sensitivity, specificity, Youden index (YI) and receiver operating characteristic (ROC) curves were calculated. Due to the high values of AUC (Area Under the *Curve)* from ROC analysis, a cutoff of 0.70 for the YI was established in order to determine the best formulas. We find that the 3 best performing indices to differentiate the 2 groups were RBC (YI=0.71; AUC=0.902), RDWI (YI=0.84; AUC=0.973) and G&K (YI=0.82; AUC=0.972). Our results suggest a similarity with other Mediterranean countries such as Spain and Greece, where G&K and RDWI also performed above our set cutoff. The same is observed in Brazil probably due to its Portuguese ancestry. We conclude that aiming to diagnosis the condition underlying a microcytic anemia in a female population, there is value in using this method to recognize the individuals suspected of BTT and forward them for HbA2 measurement or *HBB* molecular test. In the future, a robust group of male patients should be added to the analysis in order to extrapolate which of these indices would best apply to the whole adult Portuguese population.

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P49- Chromosomal abnormalities in a cohort of 1341 patients with infertility

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About 7% of the couples worldwide suffer from infertility. There are two forms of male or female infertility: primary or secondary. It is a heterogeneous pathology with a complex etiology that includes environmental and genetic factors. Genetic defects account for 50% of the cases and can be of the following categories: chromosome aberrations, DNA copy number variants, single-gene disorders, complex conditions and epigenetic disorders. The aim of this study is to identify the prevalence of chromosome abnormalities in a cohort of 1341 patients with primary or secondary infertility and correlate the reproductive history with the type of abnormality. We studied 1341 patients distributed as: 293 couples with secondary infertility, 65 couples with primary infertility, 544 males with primary sterility and 81 females with primary infertility. Chromosome analysis was performed on blood lymphocytes, using GTG high resolution banding. Complementary molecular studies were performed when needed. The study identified 91 chromosomal abnormalities, 62 were aneuploidies of the sex chromosomes, 24 in the females and 38 in the males. Out of this 31 were mosaics and 8 involved a structural aberration of the sex chromosomes. Autossomal balance rearrangements were observed in 17 patients most associated with secondary infertility. Reciprocal translocations involving sex chromosomes were found only in two infertile male and one female. Our data shows an increased prevalence of chromosome abnormalities along the sub-groups: couples with primary infertility (1,5%), couples with secondary infertility (4,4%), males with identified sterility (9,19%) and female with identified sterility (16,04%). Between couples with secondary infertility the most prevalent cause were balance structural aberrations of the autossomes, mostly affecting the female. When the cause was identified on the male, usually involving spermatogenesis defects, the majority of cases revealed numerical aberrations, reciprocal translocations or men with a 46,XX karyotype. This study supports the usefulness of cytogenetic studies in couples with reproductive failure and the good relation cost-benefit of the karyotype.

P50- *CYP1B1* mutational screening in a Portuguese cohort of congenital glaucoma patients

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Purpose: To determine the prevalence and spectrum of *CYP1B1* mutations causing primary congenital glaucoma in a Portuguese population followed at the Ophthalmology or Medical Genetics Unit of the Coimbra Hospital University Center. Methods: The *CYP1B1* coding regions and intron/exon boundaries were screened by Sanger sequencing in 41 unrelated Portuguese patients with congenital glaucoma. Results: Twelve disease-causing mutations were present in 70.7% (29/41) of the patients. The mutations found corresponded to 5 frameshifts (A179RfsX18, R355HfsX69, T404SfsX30, D449MfsX8 andS464FfsX14), 5 missenses (L378Q, E387K, P437L, I471N and K477E), 1 nonsense (R444X) and 1 non-frameshift (R468_S476dup). All mutations segregated with the disease phenotype, consistent with an inherited autosomal recessive form with complete penetrance. The most frequently mutated allele was the A179RfsX18 frameshift (17/58, 29.3%), being the only mutation found in the exon 2. All other mutations were located at the 5' end of the exon 3. In terms of allele frequencies, the A179RfsX18 mutation was followed by E387K (10/58, 17.2%), R355HfsX69 (9/58, 15.5%), T404SfsX30 (8/58, 13.8%), R468_S476dup (4/58, 6.9%), S464FfsX14 (3/58, 5.2%) and P437L (2/58, 3.4%). L378Q, R444X, D449MfsX8, I471N and K477E mutations were the least frequent in the studied patients (1/58, 1.7%). Both I471N and K477E are not yet associated with primary congenital glaucoma, are not described in the Genome Aggregation Database (gnomAD) and are predicted as deleterious according to PolyPhen-2 and SIFT. Conclusions: *CYP1B1* is responsible for the disease in almost three-quarters of the patients analysed. These results reinforce the importance of *CYP1B1* screening in PCG patients and at-risk relatives as a first-line genetic test instead of extended studies.

P51- Case Report of Parental Transmission of Koolen-De Vries Syndrome

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Koolen-De Vries Syndrome (KDVS) is a rare disorder caused by haploinsufficiency of *KANSL1* gene, either by heterozygous mutation of *KANSL1* or microdeletion on chromosome 17q21.31. Major clinical features include delayed psychomotor development, hypotonia and characteristic facial features. To this day, all individuals reported with KDVS were identified as having the syndrome because of a *de novo* microdeletion/mutation event. Although parent-to-offspring transmission of the syndrome is thought to take place in an autosomal dominant manner, no KDVS individual has been reported to have children of his/her own. In this case report, we present two novel patients with KDVS, in which the microdeletion pattern associated with this syndrome was vertically transmitted, from mother to son. To our knowledge, this is the first case report of a parental transmission of KDVS. Patients were tested using array-based comparative genomic hybridization (array CGH), performed on an Affymetrix platform, Cytoscan 750K. Data analysis was performed on ChAS Software, Affymetrix (NCBI_hg19 reference). Array CGH results revealed an interstitial microdeletion of 477 Kb and 503 Kb at 17q21.31 in proband and his mother, respectively. Diagnosis of both patients was concluded as Koolen-De Vries syndrome. To our knowledge, this is the first case report of a parental transmission of KDVS.

P52- Phenotypic characteristics of two patients with mandibulofacial dysostosis - Guion Almeida type

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Background: Mandibulofacial dysostosis - Guion Almeida type (MD-GA – MIM#610536) is an autosomal dominant malformation syndrome comprising craniofacial anomalies, microcephaly, abnormalities of the ears and hearing, intellectual impairment and in some cases extracranial malformations and/or short stature. It is caused by heterozygous pathogenic variants or deletions in EFTUD2 gene. With this work we propose to review the phenotypic features of patients with MD-GA and their developmental milestones. Methods: We reviewed the clinical and developmental data of two female patients with MD-GA followed up at Centro Hospitalar Universitário do Porto. Their development was compared with the Haizea-Llevant development table. Results: Both patients had a confirmed molecular diagnosis of MD-GA given by multigene panel, with patient (P) 1 presenting an intronic missense variant on a splicing site and P2 a stop gain missense. Both variants are novel and occurred de novo. Both patients presented typical features of MD-GA such as microcephaly, prominent metopic ridge, upslanted palpebral fissures, prominent nose, micrognathia, and conductive hearing impairment. Only P1 presents the "squared-off" ear lobule typical of MD-GA. Both patients presented a delayed development with P1 acquiring independent walking at age 20 months old (mo), first words at 21 mo, and sentences at 72 mo. P2 acquired independent walking at age 36 mo, first words at 24 mo, day continence at 48 mo. At the age of 6 years P2 does not say sentences or shows continence at night. Conclusion: Our patients have some features typical of MD-GA but most of their features are non-specific. The use of multigene panel was an important strategy to reach a diagnosis, allowing specific genetic counseling for parents and other relatives. In our study we describe the development of our patients, which can be a useful reference to compare with other patients.

P53- Disruption of *SHH* gene due to an apparently balanced *de novo* t(7;17)(q36;q23) causes holoprosencephaly

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Introduction: Holoprosencephaly (HPE) is a structural anomaly of the brain that results from failure of cleavage of the forebrain vesicle to form the cerebral hemispheres. It is associated with malformations of midfacial structures, developmental delay (DD), seizures and pituitary dysfunction, of variable intra and interfamilial severity. HPE is genetically heterogeneous: a chromosomal abnormality is present in ≈25- 50%; a recognizable syndrome in ≈18-25%; and nonsyndromic monogenic HPE in the remainder. Case Report: The patient is the third child of non-consanguineous parents, with irrelevant family history. Pregnancy was uneventful, with normal prenatal ultrasound scans. Amniocentesis was done due to advanced maternal age. Fetal karyotype was 46,XY,t(7;17)(q36;q23)dn; DNA microarray showed no genomic imbalances, and uniparental disomy of chromosome 7 was excluded. After a term caesarean delivery, a male baby was born with microcephaly, bilateral anophthalmia, facial dysmorphism, dysgenesis of corpus callosum and micropenis. Observation of the newborn was suggestive of HPE, most likely caused by disruption of *SHH* gene, which is located in the 7q36.3 region. Peripheral blood karyotype confirmed the prenatal result. Subsequent fluorescence in situ hybridization (FISH) studies, using DNA probes specific for the 7q36.3 (RP11-749O9), which includes *SHH* gene, 7q36.1 (RP11- 163I18) and 17q24.3 (RP11-1150F22) regions, showed FISH signals for RP11-749O9 probe in normal chromosome 7, in der(7) and in der(17). These results indicate that the breakpoint in chromosome 7 is located in 7q36.3 [chr7:155.547.952-155.714.378 (hg19)], which is compatible with disruption of *SHH* gene. Brain magnetic resonance imaging revealed middle interhemispheric variant HPE and dysgenesis of corpus callosum. The patient is now 8 months-old and has moderate DD, pyriform aperture stenosis, feeding difficulties and central diabetes insipidus. Conclusion: This case illustrates the need to correlate phenotype in patients with apparently balanced *de novo* rearrangements with genes located in breakpoint regions, as the pathogenic mechanism can be the disruption of a specific gene located in such regions.

P54- *RERE* gene related disorder - a glimpse of a new neurodevelopmental syndrome

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Introduction: The *RERE* gene is located in the proximal 1p36 critical region and positively regulates retinoic acid signalling in multiple tissues during embryonic development. RERE-related disorder is characterized by mild to severe developmental delay (DD) and intellectual disability (ID), behavioral issues including autism spectrum disorder, hypotonia, epilepsy, variable coexisting structural anomalies (of the eyes, heart, genitourinary tract and other systems), and neurosensorial hearing loss. It is caused by *de novo* pathogenic variants in *RERE* gene and follows an autosomal dominant pattern of inheritance. To our knowledge, at least 19 affected individuals have been reported in the literature. Clinical Report: We describe a 12-year-old boy, with irrelevant family history, and a 13-year-old girl, who has two maternal first cousins once-removed and a paternal first cousin with ID of unknown origin. Both had DD with language impairment and later ID, which is mild to moderate in the boy, and moderate to severe in the girl. The boy also has generalized hypotonia with muscle weakness, myopathic gait, attention deficit disorder, epilepsy, hydronephrosis and gastroesophageal reflux. The girl was diagnosed with epilepsy (now asymptomatic without medication), pulmonary fibrosis, tracheal stenosis, myopia and hypercholesterolemia. Both patients have minor dysmorphic features. Whole exome sequencing (WES) was performed after extensive investigation, revealing the presence of a *de novo* heterozygous likely pathogenic missense variant in RERE in both cases. Discussion: Proximal 1p36 deletions including *RERE* gene share some characteristics with this disorder, suggesting that haploinsufficiency of RERE may cause at least some of the phenotypic features associated with this CNV. Because individuals with RERE-related disorder can present with a range of clinical phenotypes, and the clinical features are often not specific enough to point at a specific diagnosis, most affected individuals are likely to be diagnosed using NGS panels or WES/WGS. Due to its rarity, ID panels may not include this gene, highlighting the advantages of WES/WGS in the diagnosis of syndromic ID.

P55- A novel *RAD21* variant in family with mild Cornelia de Lange syndrome phenotype

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Context: Cornelia de Lange syndrome (CdLS) is an autosomal dominant or X-linked developmental disorder characterized by facial dysmorphism, hirsutism, intellectual disability, growth retardation, upper limb and multiorgan anomalies. The clinical features vary widely among affected individuals. Only eight intragenic *RAD21* pathogenic variants and five 8q24.1 deletions encompassing *RAD21* have been identified so far to give rise to CdLS type 4. Results: We report a 12 years old boy with microcephaly, facial dysmorphisms with synophrys, thick highly arched eyebrows, long eyelashes, right palpebral ptosis, depressed nasal bridge, short nose, anteverted nares and long philtrum; hirsutism, atrophic left kidney and claw toe deformity of the left feet with metatarsophalangeal dislocation. Cognitive profile assessment showed a borderline IQ (75). He also presents an attention deficit hyperactivity disorder predominantly inattentive, treated with methylphenidate. His mother showed microcephaly, similar facial dysmorphisms, borderline IQ, hirsutism and brachydactyly. Previous genetic studies, including two NGS panels for mitochondrial genes and microcephaly were normal. Exome sequencing revealed heterozygosity for a novel *RAD21* variant, c.1858A>T (p.Ile620Phe), in exon 14. This variant is not reported in population (gnomAD) or disease (ClinVar and HGMD) databases and bioinformatic analysis predicted the variant as disease-causing. Familial segregation analysis revealed that the variant was inherited from the mother. Conclusion: The patient presented with clinical features previously associated to CdLS type 4 as well as an anomaly of the feet that had not been reported before in CdLS patients. This is the fourth familial case with familial transmission of *RAD21* pathogenic variants and highlights the phenotypic variability of Cornelia de Lange syndrome and also the relevance of molecular diagnosis and genetic counseling to probands and their families.

P56- A complex phenotype of a Multiple Synostoses Syndrome

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Clinical case report: We report a 3 years old girl, with craniofacial dysmorphisms, bilateral humero-ulnar synostosis, bilateral symphalangism and mild development delay (Griffiths DQ 51). She perform a cerebral magnetic resonance imaging with a normal result. She has a positive family history of Multiple Synostoses Syndrome, with maternal grandmother, mother and brother affected, with molecular confirmation. The screening of the familial pathogenic variant confirmed that she inherited the missense variant, c.122T>G (p.Leu30Gln) in the exon 1 of the *NOG* gene in heterozygosity. However, since the development delay could not be explained by the Multiple Synostoses Syndrome, a chromosomal microarray analysis was performed. This study identified a 4Mb deletion on chromosome 22q13.31q13.33 diagnosing Phelan-McDermid Syndrome explaining the observed development delay. Since the deletion may result of a balanced translocation in one of the parents, testing for a balanced chromosome rearrangement in the parents is in course. Conclusion: The finding of different symptoms between patients affected with a known genetic syndrome or within the same affected family may suggest phenotypic variability. However, the possibility that the child is affected simultaneously with two different genetic disorders should be raised, in particular when the clinical features do not fit with the expected clinical spectrum.

P57- Lateral meningocele syndrome presenting as neonatal hypotonia and dysmorphic features

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Introduction: Lateral meningocele syndrome (LMS), also known as Lehman syndrome (MIM # 130720), is a rare disorder, with only 14 patients reported in the literature, half of which with molecular diagnoses that consist of heterozygous *NOTCH3* mutations affecting exon 33. LMS should be suspected in patients with multiple lateral spinal meningoceles, distinctive craniofacial appearance, musculoskeletal involvement with hypotonia and joint hyperextensibility, and developmental delay usually with no intellectual disability, with or without congenital malformations and hearing loss.
Clinical Case: a 9 month-old girl was referred to our outpatient clinic for neonatal hypotonia and low weight. She was the only child of a healthy non-consanguineous couple, and there was no family history of congenital anomalies or developmental delay. She had macrocephaly, tall forehead, highly arched eyebrows with medial thinning, depressed nasal bridge, thin upper lip vermillion and downturned corners of the mouth. Brain MRI showed enlarged ventricles. Initial investigations included metabolic study, 15q11.2 MS-MLPA, array-CGH and *DMPK* molecular testing, all normal. Whole exome sequencing (WES) identified a recurrent de novo heterozygous pathogenic variant in *NOTCH3* - c.6692dup (p.Ala2233Glyfs*9) - providing the diagnosis of LMS. Complete CNS MRI showed multiple lateral mielomeningoceles and ectopic amygdalae. Evaluation by Cardiology, ENT, and Orthopaedics showed no additional findings, and Ophthalmology appointment was scheduled. Throughout follow-up, she overcame the initial developmental delay. Prenatal diagnosis was performed in a subsequent pregnancy, revealing an unaffected foetus.
Discussion: Comparing with previous reports, our patient had a milder presentation, attributed to the absence of congenital malformations as well as to fully preserved hearing. The multiple lateral mielomeningoceles, core feature of LMS, were initially unsuspected. In this case, WES established the diagnosis, enabling a better understanding of prognosis and improving patient management and followup. Molecular diagnosis also allowed specific genetic counselling and prenatal diagnosis.

P58- Novel *YY1* pathogenic variant: expanding the phenotype of Gabriele-de Vries Syndrome

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Background: Gabriele-de Vries is a recently described syndrome caused by *YY1* gene dysfunction. Clinical manifestations include developmental delay/intellectual disability, intrauterine growth restriction, feeding problems, and variable functional and morphologic abnormalities of the face, brain, eye, heart, kidney, genitals and skeleton. All pathogenic variants described so far are *de novo* and found in heterozygosity. Case report: We present a 10 years-old boy, first child of non-consanguineous healthy parents. The pregnancy resulted from *in vitro* fertilization and at 34 weeks' gestation intrauterine growth restriction and a single umbilical artery were detected, leading to caesarean section at 38 weeks. Somatometry at birth was below 5th percentile and Apgar score was 8/10. Admission to the neonatal unit was required due to feeding difficulties. Additional clinical features included neonatal teeth, a left duplex kidney, left inguinal hernia and bilateral iris coloboma. Cranioencephalic MRI at 2 years of age was normal. Cardiac MRI at the age 9 revealed left ventricular noncompaction with normal systolic function. At present, his weight is below P5 and stature between P5-10 (under growth hormone therapy) and he presents mild intellectual disability, attention deficit hyperactivity disorder, retractable testicles, micropenis and abdominal hypopigmented lesions. Etiologic investigation by aCGH revealed a 16q23.1 duplication inherited from his healthy mother, and molecular studies of *SOX2* gene, Cat-eye, Papillorenal and CHARGE Syndromes were normal. Whole exome sequencing trio analysis revealed a *de novo* and novel heterozygous variant in *YY1* gene (c.137_138insT; p.(Glu46Aspfs*102)) classified as pathogenic. Conclusions: YY1 is a zinc-finger transcription factor with critical roles in development. To date, only 11 patients with pathogenic variants in *YY1* and 13 patients with deletions involving *YY1* gene have been reported. The phenotype of our patient is consistent with that of Gabriele-de Vries Syndrome. Iris coloboma and left ventricular noncompaction were not previously reported, thus enlarging the phenotypic spectrum of this syndrome.

P59- Newborn whit a derivative chromosome X and ambiguous genitalia

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Translocations involving the short arms of the X and Y in human chromosomes are uncommon. One of the primary functions of the X and Y chromosomes is gender phenotype determination. Here we report a newborn female with ambiguous genitalia and abnormal X chromosome. Karyotype was performed using the standard methods and Fluorescence in situ hybridization (FISH) directed for the SRY gene was used for confirmation of the clinical and cytogenetic suspicion. Chromosomal microarray analysis (CMA) was performed using CytoScan HD (Affimetrix®) to identified gains/loses on the der(X) chromosome. The analyse revealed one abnormal X chromosome in a female karyotype. Considering the ambiguous genitália clinical information the abnormal X was considered to be compatible with a translocation X/Y. This was confirmed by the presence of signal for the SRY using FISH. CMA allowed to clarify a loss of 12.34 Mb at Xp22.33p22.2 and a gain of 7.41 Mb at Yp11.31p11.2 (ISCN = arr[GRCh37] Xp22.33p22.2(2703632_15050955)x1,Yp11.31p11.2(2650140_10059230)x1). The X deleted region includes several OMIM morbid genes, including CLCN4. Mutations in CLCN4 are associated with intellectual disability and impaired language development, and heterozygous females can be as severely affected as male. The gain on the Y encompasses nine OMIM genes, including the SRY gene, involved in the sexual male development. This additional information can be of great value for the child development. Translocations of segments of Y chromosome containing SRY are described in sexual reversion and true hermafroditism cases, which could explain the reason for referral for the newborn. Nevertheless, translocations between the X/Y chromosomes in females are expected to have a skewed inativation pattern in favour of the abnormal X and X-inativation studies could prove this likelihood. If a normal developmental of the child is observed over time this will be likely due to the preferable inativation of the abnormal X. Presently the child is about 1-year-old and she presents normal uterus, ovarian, and external genitalia, with absence of male gonads. No other clinical features have been identified.

P60- STAG1 haploinsufficiency: an emerging phenotype

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Context: Cohesinophaties are rare neurodevelopmental disorders characterized by distinctive facial dysmorphism, growth retardation, developmental delay/intellectual disability (DD/ID), and limb abnormalities. They originate from a dysfunction in the cohesin pathway, which enables chromosome segregation and regulates transcription. So far eight genes have been identified belonging to this pathway, including STAG1. We report a patient with a STAG1 intragenic deletion, thus contributing to reinforce the association between STAG1 haploinsufficiency and the cohesinopathy syndromic ID spectrum. Patients/Methods: A 4-year-old boy was referred to our medical genetics department due to mild DD. Physical examination showed minor dysmorphism, namely arched and sparse eyebrows, downslanting palpebral fissures, wide nose with short columella, and thick lips. Family history was unremarkable. DNA samples were studied by array-CGH (180K CGK-HD, Perkin Elmer). Results/Conclusion: Array-CGH analysis detected a 206.24 Kb intragenic deletion, involving exons 2 to 12 of STAG1 – arr[GRCh37]3q22.3(136184662_136390897)x1. The mother presented a normal aCGH profile. The father was not available. Two different cohorts including a total of twenty individuals with syndromic ID and putative STAG1 haploinsufficiency were recently reported. Nine patients presented CNVs involving STAG1, including small intragenic deletions. All patients had mild to moderate DD/ID and common facial features. There was no clear phenotypic difference between patients with gene deletions and those with single nucleotide variants. Transmission of STAG1 variants was reported. Our patient shares common characteristics with these patients, reinforcing STAG1 as a new cohesinopathy gene that acts via a loss-of-function mechanism.

P61- Usher syndrome and Nebulin-associated myopathy due to a unique double molecular defect unravelled by homozygosity mapping

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Recent progresses in sequencing technologies, and the large amount of generated data, demand the development of guidelines and several tools in order to simplify data interpretation. One such tool is homozygosity mapping, which has proved invaluable in the identification of variants, especially in consanguineous families affected with recessive Mendalian disorders. More challenging is the diagnosis of conditions with a complex clinical presentation. In fact, new genome-wide technologies revealed a significant number of patients affected by disorders caused by different combinations of pathogenic variants in more than one locus. Herein, we report the case of a 21-year-old male patient, with consanguineous parents, presenting a clinical phenotype resembling Usher Syndrome (ocular and hearing impairment). Additionally, the patient presented skeletal muscle involvement, usually not observed in Usher patients. Autosome homozygosity mapping, based on exome sequencing (ES) data, allowed the identification of two large homozygosity regions (chromosomes 2 and 11). After ES data filtering, a homozygous missense variant was identified in each region, one in *NEB* and other in *MYO7A* genes. Both variants have been previously reported in ClinVar and classified as VOUS and pathogenic, respectively. This report represents the first molecular diagnosis of two Mendelian diseases unravelled by ES data homozygosity mapping, in a patient with a clinical diagnosis of Usher syndrome and unrelated muscle complaints. The paucity of there being more such cases, unduly said to represent clinical heterogeneity, highlights the importance of a thorough clinical and molecular investigation, as this will provide upfront information for more adequate patient management and follow-up.

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P62- A novel splicing *FOXP1* variant found in a patient with syndromic intellectual disability through the GenoinVar end-to-end solution

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Introduction: Clinical complex cases with genetic heterogeneity and/or unspecific phenotype benefit from whole exome sequencing (WES) analysis. We report such a case solved whitin the In2Genome project. Methods**:** GenoinVar - an end-to-end solution for the discovery of causal variants - was developed in In2Genome. GenoinVar involves Illumina WES and the IDT capture procedure, with above specifications metrics. Sequencing data is processed by an in-house pipeline, and an encrypted database of annotated variants is created. Candidate variants are prioritized in ExomeLoupe, a user-friendly software for variant selection and interpretation. ExomeLoupe interacts directly with the encrypted database enabling users to securely store, analyze and share genetic data in compliance with the GDPR. Results: We report a Portuguese 4-year-old male patient, born to healthy consanguineous parents (r=1/128), presenting global developmental delay (with autistic features and severe speech delay), unilateral cryptorchidism, intermittent strabismus, relative macrocephaly and dysmorphic features. WES was performed in the proband and candidate variants prioritized, through GenoinVar. The heterozygous variant c.1530+1G>A in *FOXP1* was identified using ExomeLoupe with the filters: global developmental delay (HPO), MAF<0.01 (gnomAD) and deleterious according to predictive tools. The splicing *FOXP1* variant was neither observed in the in-house Portuguese control individuals nor in public databases. We confirmed this result by Sanger sequencing and excluded its presence in both unaffected parents, implying a *de novo* mutation. Discussion: *FOXP1*-related intellectual disability syndrome is a recently delineated condition with wide clinical spectrum in which the patient phenotype integrates well. As in this example, most cases have been identified by WES without previous specific clinical recognition, reinforcing the importance of this test in this group of disorders. GenoinVar has shown to be a user-friendly successful solution, as well demonstrated in this example.

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P63- *PPP2R5D*-related neurodevelopmental disorder - a case report

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Context: *PPP2R5D*-related neurodevelopmental disorder is characterized by mild to severe neurodevelopmental delay. The phenotypic spectrum is characterized by pronounced hypotonia with delay in gross motor skills, severe speech impairment with a wide range of disabilities and important macrocephaly. Ataxia and autism spectrum disorder are often reported. Seizures and ophthalmologic abnormalities are present in fewer than half of individuals. Additional anomalies include skeletal, endocrine, and cardiac malformations, each linked to few individuals. At the time no more than 50 individuals have been reported. Case report: A 1-month-old girl, first child of healthy and non-consanguineous parents with no relevant family history, was referred to our Clinical Genetics consultation due prenatal macrocephaly and minor dysmorphisms. Her father has a macrocephaly (+ 2SD) and the diagnosis of Familial Macrocephaly was proposed. Two years later, the girl presented important macrocephaly (+ 4SD) and a global development delay. An extensive etiological investigation was performed, that included chromosome microarray followed by sequencing and multiplex ligation-dependent probe amplification of *PTEN* and *NSD1*. At the age of 14 she has moderate intellectual disability with severe speech delay, ocular torticollis, important macrocephaly (+ 6SD), minor dysmorphisms, toe walking and intentional tremor. At that time, it was performed an exome sequencing, which found a heterozygous de novo pathogenic variant in *PPP2R5D* gene [c.592G>A p.(Glu198Lys)] that yielded the diagnosis. Conclusion: This case report makes us aware of a condition recently associated with global development delay/intellectual disability and some consistent phenotypic aspects. Also, it reinforces the importance of a genetic consultation follow-up in patients with no molecular diagnosis, underlining the power of the new diagnostic tools in the Clinical Genetics field. At the time, it is difficult to establish a specific follow-up plan, since the number of affected individuals is limited and there is no description about the disease natural history.

P64- A case of a novel BCL11A variant associated with Dias-Logan syndrome

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Introduction: Dias-Logan syndrome (MIM #617101) is a novel autosomal dominant disorder caused by *de novo BCL11A* mutations. BCL11A protein is a transcriptional repressor of fetal hemoglobin and a transcription factor associated with the BAF SWI/SNF chromatin remodeling complex. The protein is highly expressed in fetal human brain, where it has significant roles (migration control of neurons, axon branching and dendrite outgrowth). Dias-Logan syndrome is characterized by global developmental delay, cognitive dysfunction, behavioral features, joint laxity, strabismus, microcephaly and significantly elevated fetal hemoglobin without recognizable dysmorphic features. Clinical description: We report a girl who is the second child of a non-consanguineous healthy couple and single case in the family. She was born prematurely at 35 weeks of pregnancy after labor induction due to oligohydramnios detected at 27 weeks of gestation and fetal echodoppler with inverted flow. She has moderate developmental delay, prenatal microcephaly [head circumference at birth and at the age of three years and five months below the 1st centile (-3SD and -2.8SD, respectively)], congenital heart defect (atrial septal defect and congenital stenosis of the pulmonary artery branches), cerebellar vermis hypoplasia, joint hypermobility, facial dysmorphisms and persistence of fetal hemoglobin (16.3%). Results and discussion: A next-generation panel of 6110 genes was performed and identified the *de novo* likely pathogenic variant *BCL11A* (NM_022893.3) c.1847dup p.(Leu617Profs*18). This frameshift variant has not been previously described in the literature and is predicted to introduce a premature STOP codon. Eleven of the 15 patients reported so far in the literature harbor frameshift or nonsense mutations. The remaining four patients harbor missense mutations and for three of them *in vitro* functional assays have been performed and results were consistent with a loss of function. To our knowledge, this is the first report of a Portuguese patient diagnosed with Dias-Logan syndrome and it will help delineate the mutational and clinical spectrum of this novel and rare syndrome.

P65- Discordant dichorionic diamniotic (DCDA) twins: clinical and cytogenetic characterization of a foetus with structural chromosomal aberrations

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Introduction: Different structural chromosomal aberrations involving more than two chromosomes in the same individual are extremely rare. In these cases, particularly in prenatal diagnosis settings, a good ultrasound description is very important since carriers may display various phenotypes, ranging from normal to a foetus with different congenital abnormalities, depending on the size of deletion/duplication resulting from the chromosomal rearrangement and the chromosomes involved. These rearrangements may be *de novo* or familial. The minimum presumed risk of phenotypic abnormality for *de novo* multiple chromosome rearrangements identified prenatally may be estimated as the additive risk of the presumed number of identifiable chromosome breakpoints. Methods: A 35-year-old multiparous woman with a dichorionic diamniotic (DCDA) twin pregnancy referred to our Centre at 19 weeks' gestation for prenatal cytogenetic studies; foetus 1 presented with severe hydrocephalus and growth restriction and foetus 2 with positive biochemical screening. Conventional GTL-banding karyotyping was performed on metaphase mitotic cells obtained from amniotic fluid according to standard procedures. ArrayCGH was requested. Results: Foetus 1 revealed a 46,XY,t(3;6)(p13;p23),del(7)(q31.2q32.3) karyotype and foetus 2 was 46,XY. ArrayCGH confirmed del(7q). Parent's karyotypes were normal. Selective feticide was successfully performed on the hydropic foetus. Discussion: The authors present the cytogenetics and clinical findings of a *de novo* balanced chromosomal translocation associated with a *de novo* interstitial deletion and compare them with previous studies presenting with similar chromosomal breakpoints and genes mapped to that region.

P66- Non Invasive Prenatal Testing (NIPT): four years' experience, interesting cases and challenges

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Background
In the last years, NIPT has become a widespread method of screening for common autosomal (13, 18, 21) and sex chromosome aneuploidies. It is based in the massive parallel sequencing of cell-free DNA (cfDNA) from maternal plasma, which contains a mixture of small maternal and placental DNA fragments. Although NIPT has been shown to be highly accurate for the detection of these aneuploidies, a small percentage of women have low confidence or nonreportable results. Methods: A revision of the cases received during the last 4 years is presented for evaluation of interesting and challenging data. The test was performed on maternal whole blood samples, collected in Streck tubes and received up to 5 days after collection. cfDNA was extracted and analyzed by whole genome sequencing (NextSeq_Illumina). Results: During these 4 years, several interesting cases came to our attention, with either low confidence or nonreportable results. Main reasons for low confidence results were low fetal fraction, high maternal weight and borderline results to the cutoff value (mainly for sex chromosomes). Presence of vanishing twins and maternal chromosomal alteration were also identified. Among the unreportable results, the main reason for this finding was a "no call", meaning the analysis software was not able to give a result. Conclusion: There are several reported biological causes for these low confidences or nonreportable results that ultimately can lead to false positive and false negative results. Those include confined placental mosaicism, vanishing twins, maternal incidental findings and low fetal fraction. So far, to our knowledge in these 4 years' experience, no false negative results were reported, and false positive results were mainly related with borderline values for sex chromosomes. Professional societies recommend offering NIPT to women at increased risk of fetal chromosome aneuploidies, as part of a contingent screening or even for all women. The knowledge of outcomes, when other diagnostic methods are performed, will increase not only the knowledge about doubtful profiles, but also reinforce the validation of this screening test.

P67- Nonsyndromic juvenile myelomonocytic leukemia with a somatic PTPN11 mutation in a 4-year-old boy

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Background: Juvenile myelomonocytic leukemia (JMML) is an aggressive pediatric myelodysplastic syndrome/myeloproliferative disorder characterized by malignant transformation in the hematopoietic stem cell compartment with proliferation of differentiated progeny. The only curative therapy is hematopoietic stem cell transplant. Mutations in NF1, NRAS, KRAS, PTPN11 and CBL ("Ras pathway") currently allow for a molecular diagnosis in 85% of patients. We present a 4-year-old boy with acute myeloid leukemia (with monosomy 7) with features suggestive of primary juvenile myelomonocytic leukemia in which whole exome sequencing was performed. Method: Whole exome sequencing was performed by capture of target regions using oligonucleotide probes (V6, Agilent Technologies) and subsequent next generation sequencing (NextSeq, Illumina). Alignment and variant calling was performed using the BWA and GATK, respectively. Variants with MAF<1% were filtered and processed with bioinformatic analysis tools to assess its pathogenicity and potential to explain the clinical phenotype. Results: The NM_002834.3:c.227A>G p.(Glu76Gly) likely pathogenic variant was detected in heterozygosity in the PTPN11 gene in DNA from peripheral blood. This variant has been previously reported as a germline variant in patients with Noonan syndrome but also as a somatic mutation in colon cancer and acute myeloid leukemia. After transplant, DNA from a novel blood sample and buccal swab tested negative for the detected variant. Additionally, the variant was also not present in DNA from the parents. Conclusions: We report a JMML case with a proven somatic variant in PTPN11. The absence of the variant in a new blood sample after treatment supports that the detected variant is somatic, further reinforced by the absence of the variant in a buccal swab sample and in DNA from the parents Additionally, our case illustrates the importance of obtaining germline tissue. This is of critical importance to further clarify the origin of the variant and treatment options as in several cases, patients have inherited syndromes that predispose to the development of JMML.

P68- X-chromosome terminal deletion including the FMR1 gene in a family with fragile X-syndrome and a full mutation.

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Background: Most cases of fragile X syndrome result from an expansion of CGG repeats in the FMR1 gene; deletions and point mutations of FMR1 are much less common. Terminal deletions on the X chromosome in female patients have already been described as a promising explanation for POF, especially when involving the Xq28 region. Case Report: In this study we report one case of an asymptomatic female referred due to family history of three brothers with clinical diagnosis of Fragile X-syndrome. Previous molecular study by msTP-PCR for FMR1 gene, performed in the mother (also asymptomatic), revealed the presence of a normal and a full mutation allele, most probably the cause of the segregation of this condition in the affected brothers. msTP-PCR for FMR1 gene was performed and revealed the presence of an intermediate allele with 51 (± 3) CGG repeats in the analyzed region of the FMR1 gene and no amplification was obtained for methylated alleles, suggesting the presence of a deletion involving at least the promoter region of the FMR1 gene. To confirm this, deletion/duplication analysis was performed by MLPA analysis (ME029- B3, MRC Holland) and a hemizygous deletion that includes at least the FMR1 gene and other four downstream genes in the X chromosome terminal region (AFF2, IDS, MTM1 and FLNA) was detected. Considering the overall obtained results in this family, the detected deletion was most likely resultant from instability of the full mutation allele transmitted by the mother. Deletions of different sizes in the Xq27.3-Xq28 region have already been described in the literature including not only the FMR1 gene but extending to the terminal region of chromosome X, leading to a wide variability of phenotypes, not only because of the deleted region, but also because of a skewed X inactivation. In this case, despite the hemizygosity of FMR1 gene, she does not present fragile X syndrome features, since the normal Xchromosome is not subject to methylation. However, it is not possible to exclude that she may develop symptoms of premature ovarian failure (POF).

P69- Array-CGH in prenatal diagnosis – cohort of a public laboratory in the Centre of Portugal

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Context: Array comparative genomic hybridization (array-CGH) is a useful approach to detect submicroscopic imbalances, known as copy number variants (CNVs). It was shown that in prenatal cases with a normal karyotype, the array-CGH revealed additional relevant information in ~6% of the prenatal cases with ultrasound abnormalities and in ~1.7% of cases with indications for invasive prenatal diagnosis (PND). According to these results, array-CGH is recommended to be performed as the first tier genetic test when fetal ultrasound abnormalities are detected. Methods: A cohort of 822 prenatal cases was studied by array-CGH, due to different clinical indications (such as ultrasound abnormalities, increased nuchal translucency, positive biochemical screening). Array-CGH was performed using Agilent 4x180K or 8x60K platform. Results: 724 prenatal cases were studied by array-CGH as the remaining showed a positive result to rapid aneuploidy detection (RAD). Regarding the CNVs detected, pathogenic CNVs were identified in 7.6% of studied cases and in 12% of samples at least one likely pathogenic CNV or a variant of uncertain significance (VOUS) was observed. 12.7% of pathogenic CNVs revealed a deletion or duplication of the 16p11.2 region, being the most frequently altered region detected. The presence of ultrasound abnormalities was the most common clinical indication among the cases with pathogenic CNVs (65.5%). Array-CGH also allowed identifying mosaicism in 1.1% of studied cases. Conclusions: These results show the fundamental role of array-CGH in PND to detect clinical relevant CNVs that would not be found using conventional cytogenetic methodologies, more quickly, allowing to increase the diagnostic yield and decrease the turnaround time.

P70- Familial 18q23 deletion: the same alteration with different phenotype - a case report

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We present a 18q23 terminal deletion identified in an 1 year old boy with cardiac anomalies, pulmonary stenosis moderated, short neck, facial dysmorphisms and feet malformations. Array-based comparative genomic hybridization (aCGH), using a 180K Agilent oligonucleotide microarray revealed a 18q terminal deletion of about 2.8 Mb including 2 genes reported in OMIM Morbid Map (*CTDP1* and *TXNL4A*). Cytogenetic analysis with GTG-banding was carried out in the proband and in his parents and revealed normal results. The deletion wasn't found in the kariotypes of proband and parents, since it was below of the karyotype resolution limit. Fluorescence *in Situ* hybridization (*FISH*) analysis using the subtelomeric probe for 18q23, confirmed the deletion in the proband and showed that the father carried the same alteration as the child. Since the father has no clinical signs, array-CGH analysis was performed and the same identical deletion was observed. The phenotype associated with a 18q terminal deletion has been well described and can include mental retardation and development delay, hypotonia, short stature, congenital aural atresia, abnormal genitalia, facial dysmorphisms, foot malformations and delayed myelination. The incidence of these clinical features in different patients with 18q deletion is variable. In the present study, father and son have the same deletion, although they are phenotypically different. The proband has many of the typical clinical features associated with the 18q deletion syndrome and the father is not clinically recognizable as having a 18q-syndrome phenotype. It was previously reported a child with hypotonia and other features associated with 18q-syndrome at the age of 2, but all of them were absent at the age of 4. In conclusion, our case report demonstrates that genotype/phenotype correlation studies are very difficult to establish on the 18q- syndrome and also shows the dilemma that exists in the categorization of patients with 18q deletions.

P71- The challenges of pseudogenes in genetic diagnosis by Next Generation Sequencing

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Background
Targeted next-generation sequencing (NGS) enables rapid identification of genetic variation in a large subset of genes with high confidence. However, since current methodology lack the sensitivity to distinguish reads that come from homologous parts of the genome, it is a challenge to work with genes with paralogues or pseudogenes. We present three case studies, with variants in *HYDIN* and *PMS2* genes, known to have pseudogenes. Methods: Next generation sequencing (Illumina) of genomic DNA was performed upon oligonucleotide-based target capture (Agilent Technologies). Alignment and base calling were performed with BWA and GATK, respectively. Results: For two different cases, NGS was performed and revealed two variants in exon 11 and 13 of the *HYDIN* gene with an allele frequency of 44.5% and 42.4%, respectively. Blast analysis was performed to identify possible mismatches and to design specific primers to confirm the result obtained in the NGS analysis; the mismatches identified were present in the NGS data with a heterozygosity of nearly 50% as well. Sanger sequencing was performed and confirmed the presence of the variants in homozygosity for both cases. Segregation analysis confirmed the heterozygosity in both parents. A similar approach was used in a third case regarding the *PMS2* gene. In the NGS data analysis, a variant with an allele frequency of 29% was detected. Specific Sanger sequencing was performed confirming the presence of the variant in heterozygosity. Conclusions: Due to high sequence similarity, sequenced reads arising from a pseudogene may be misaligned to the functional gene and result in false positive variant calls or false negative results. Furthermore, variants in regions of homology to a pseudogene are difficult to validate with Sanger sequencing. Here we show that with NGS technology one should be cautious interpreting variants in genes with pseudogenes/homology. Given the limitations of this methodology, an integrated data analysis and the use of other strategies should be addressed to reliable detect the presence and zygosity of variants identified in regions with high homology.

P72- Non-Invasive Prenatal Test Implementation in a public laboratory in the Centre of Portugal

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Objectives/Background: Non-invasive prenatal testing (NIPT) has been widely used to detect common fetal chromosome aneuploidies (T13, T18 and T21) and has expanded to sex chromosome aneuploidies. In this work we review the performance of the implementation of a NIPT test from blood cfDNA at our center. Methods: Retrospective analysis of NIPT Clarigo (Agilent Technologies), using MiSeq platform (Illumina), in pregnancies with moderate risk for common fetal chromosome aneuploidies. Maternal blood samples were collected from February 2019 to September 2019 in Coimbra University Hospital Center (CHUC). Control samples, in which the laboratory was aware of the aneuploidy status were not included. Results: From received NIPT samples, 2% had positive NIPT result, (80% T21 and 20% T13), 90,5% were negative and 7,5% were inconclusive. NIPT positive results were validated by invasive test in 60% of the cases, one mother decided to continue pregnancy and in one case there was a spontaneous fetal loss (T13). There weren´t known false-negative results (sensitivity = 100%). Of the 7,5% inconclusive cases, 42% were due to hemolysis; 21% to low fetal fraction; 16% to possible vanishing twin and 21% to an unknown cause. A new blood sample was required in 50% of the inconclusive cases with conclusive result in 87,5% of the resampled cases. NIPT was also performed in pregnancies with Body Mass index greater than 30 (22% of the cases) with conclusive result in 98% of them. A relevant incident finding on maternal X chromosome was identified in 2,4% cases (2% with a dup Xp; 0,4% with a maternal del Xp, confirmed by Array-CGH, with possible pathogenic impact on a male fetus and 0,4% with a turner mosaic). Conclusion: Our NIPT results, based on NGS, showed good performance for detecting T13, T18, and T21. Nevertheless, the number of samples is still very small for an accurate assessment of performance of screening. Our results demonstrate that targeted NIPT using MiSeq platform could be an alternative for centers with low NIPT sample flow. The implementation of the NIPT can contribute to reduction in the number of invasive exams and eventual associated fetal losses.

P73- Del 8p in Chronic Lymphocytic Leukemia: a case report

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Context and Objectives: In the last few years, several studies using appropriate stimulation of chronic lymphocytic leukemia (CLL) cells have enhanced the performance of conventional karyotyping, detecting additional chromosomal alterations of potential prognostic significance. Chromosomal abnormalities in CLL are detected in up to 80% of patients, they have a known prognostic value and play an important role in CLL pathogenesis and evolution, determining patient's outcome and therapeutic strategies. According to guidelines, in general practice, Fluorescence in situ Hybridization (FISH) should always be performed, identifying the most common chromosomal rearrangements (trisomy of chromosome 12 and deletions in loci located on chromosomes 13q14, 11q22-q23 and 17p13) in peripheral blood lymphocytes and conventional cytogenetic not generally indicated. Methods: The authors present a case of an 83-year old woman with CLL and previous cytogenetic study (FISH and karyotype) of trisomy 12 in 2015. FISH panel and cytogenetic analysis were performed after 4 years. FISH and conventional cytogenetics were performed according to the protocols established in the laboratory. Results and Conclusion: FISH results detected trisomy 12 in 53% of cell, with a similar incidence from the previous analysis. Conventional cytogenetic reveal the karyotype: 46,X,del(8)(p11),+12[7]/46,XX[11]. The del 8 appears in this analysis. It has not yet been determined whether del 8p plays any role in driving drug resistance, however, it was already reported to predict an increased risk for disease progression in the setting treatment. Also, a recent large study identified del 8p as recurrent event significantly associated with 17p and CLL. The present case highlights the importance of conventional cytogenetics in the study of CLL patients. FISH could not detect the del8p, which were very important to explain the partial response to the therapy and uncover the patient adverse prognostic.

P74- Third case of Cantú Syndrome associated to a novel KCNJ8 variant

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Case report: Here we report a case of a 20-month-old female referred to our clinic for prenatal polyhydramnios and facial dysmorphic features. At clinical observation it was noted coarse facies, low frontal hairline and generalized hypertrichosis predominantly on both arms. Cardiac evaluation showed dysplastic aortic valve. A possible diagnosis of lysosomal storage disease was suspected but an extensive metabolic evaluation excluded this hypothesis. Clinical reevaluation at 8 yrs, showed short stature (< 5th percentile), joint hyperlaxity, pectus carinatum, astigmatism and hyperopia, as well as learning difficulties. Facial features became more evident, resembling Cantú syndrome. Sanger gene sequencing of *ABCC9* gene was normal. After reevaluation at 14 yrs, cardiac evaluation revealed hypertrophic cardiomyopathy and pulmonary hypertension. By that age, she developed panhypopituitarism (growth hormone deficit, hypogonadotropic hypogonadism, hypothyroidism, hypocortisolism) and relapsing polychondritis. Clinical exome was performed which did not reveal pathogenic variants, however targeted reanalysis of *KCNJ8* gene showed a novel variant (p.E331K) *de novo* predicted to be pathogenic. Discussion: Cantú syndrome is a rare autosomal dominant disorder characterized by hypertrichosis, a distinctive facial appearance, heart defects and several other abnormalities caused by mutations in *ABCC9* and *KCNJ8* genes. More than 30 patients with pathogenic variants on *ABCC9* have been previously reported. Pathogenic variants in *KCNJ8* gene, which encodes a member of the inward rectifier potassium channel family, were only previously reported in two patients. Both of them shared phenotypical characteristics with our patient like hypertrichosis, coarse facies, cardiomegaly. Endocrinological abnormalities were not previous reported. Conclusion: To our knowledge, this is the third case report of Cantú syndrome associated to *KCNJ8* gene, reinforcing its contribution to the phenotype.

P75- A new truncating mutation is associated with the second inherited case of *MED13*-related neurodevelopment disorder.

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Context: The known genetic causes of development delay (DD) and intellectual disability (ID) are remarkably heterogenous, dispersed through more than 700 genes, being each individual pathogenic variation extremely rare. Large-scale DNA sequencing and data-sharing have been crucial to the discovery of new ID/DD associated genes. This approach led recently to the establishment of a new DD/ID phenotype associated with likely pathogenic variants in *MED13* gene. Case report: We report three cases of mild to severe DD/ID in one family of Portuguese descendant. The index patient was a first son of non-consanguineous parents presenting severe DD associated to severe neurosensorial hearing loss, hypotonia, focal epilepsy, obesity, chronic sleep disturbances, self-aggressive behavior, stereotypy and particular craniofacial features, namely: brachycephaly, narrow face, peri-orbital fullness, full nasal tip, flat filtrum and thin upper lip. Brain MRI revealed minor abnormalities, namely a thin corpus callosum most likely related to a visible myelination delay. His mother and his maternal grandfather also exhibited a mild ID with similar facies. A 6110 genes NGS panel in the index patient identified an heterozygosity for a novel frameshift variant, c.807_808insA p.(Leu279Thrfs*25), in *MED13* gene. This variant was not reported in the literature nor in gnomAD or other databases and in silico analysis points out to likely pathogenicity. Further familial studies revealed the heterozygosity for this variant in the mother and the maternal grandfather, supporting the correlation between the *MED13* variant and the familial DD/ID phenotype. Conclusion: To our knowledge, this is a novel *MED13* variant associated with a neurodevelopment disorder, the 4th frameshift variant and the second inherited case described. This report endorses *MED13* as a new DD/ID related gene, confirming that *MED13* pathological variants are associated with neurodevelopment disorders, spanning from severe DD/ID to autism spectrum disorder and attention deficit hyperactivity disorder. Moreover, it highlights the importance of the Mediator complex regulation on the etiology of neurodevelopment disorders.

P76- Do we really need to know fetal gender for array-CGH in prenatal diagnosis?

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Array Comparative Genomic Hybridization (aCGH) is currently recommended for all fetus with major structural anomalies after obstetric ultrasound. The guidelines also recommend rapid aneuploidy detection (RAD) to be performed before array-CGH analysis, as it not only excludes a common aneuploidy but also indicates fetal gender, for appropriate array-CGH control hybridization. We retrospectively reviewed the 822 prenatal samples received in our laboratory, for 180K oligonucleotide array-CGH analysis, consisting of amniotic fluids (AF-498), chorionic villus (CVS-219), fibroblasts (90) and cordocentesis (15). In CVS, RAD gave a positive result in 28.7% of the samples, with 37.1% positive for trisomy 21. In AF, only 3.4% of samples revealed a RAD positive result, 47% of which for trisomy 21. All the 724 samples with a normal result for RAD proceeded to aCGH analysis with a sex matched commercial control. We observed 25 imbalances on sex chromosomes, 19 on the X chromosome, 4 on the Y chromosome, a triple X and a XYY, both in miscarriage products whose gender was determined by visual examination. Considering the Y chromosome imbalances, 3 were duplications - one paternal, 2 whose origin was not determined- and the other was an Y rearrangement previously observed by conventional cytogenetics. On the X chromosome we observed 16 imbalances on the short arm – 6 deletions (3 maternal, 2 paternal, 1 unknown) and 10 duplications (6 maternal, 4 paternal) – and 3 imbalances on Xq – 1 *de novo* deletion, 1 paternal duplication and 1 paternal triplication. Only 2 maternal deletions on Xq28 were considered to be pathogenic in male fetus, and one proceeded to pregnancy interruption. Taking into account our results we consider that: in CVS RAD should be performed before aCGH, as it gives a high positive rate for aneuploidies; in AF, RAD should be performed before array-CGH only when ultrasound anomalies are indicative of an aneuploidy. When an aneuploidy is not suspected or was excluded, fetal gender can be given by ecographic evaluation or if not determined, samples can by blindly hybridized with a control, as we can always infer copy number changes on sexual chromosomes.

P77- Germline *ABL1* variant identified in a Nepalese girl with congenital heart defects and skeletal malformations syndrome: a case report

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Germline variants in *ABL1* gene were recently identified in six patients with the previously unreported congenital heart defects and skeletal malformations syndrome. This is an autosomal dominant disorder characterized by congenital atrial and ventricular septal defects, with aortic root dilation in adulthood; and variable skeletal defects such as pectus excavatum, scoliosis, and finger contractures. Here, we report the case of a four-year-old girl referred to our clinic for multiple congenital anomalies, namely atrial and ventricular septal defects and pulmonary valve stenosis, bifid uvula, craniosynostosis, umbilical hernia, and finger contractures, resembling Loeys-Dietz syndrome, a hereditary connective tissue disorder (HCTD). At observation, it was noted borderline short stature and dysmorphic craniofacial features such as synophris, small nose, small deep-set eyes, micrognathia and microcephaly. Clinical exome sequencing revealed a pathogenic heterozygous variant in *ABL1* gene, c.1066G>A (p.(Ala356Thr)). The A356T variant was not observed in large population cohorts, and it has been previously reported as a *de novo* variant in an individual with features of congenital heart defects and skeletal malformations syndrome. The A356T variant leads to an amino acid substitution, which is likely to impact secondary protein structure. Functional studies indicate that the A356T variant is associated with increased tyrosine phosphorylation. The literature review showed that clinical features were similar to all other patients with *ABL* variants; our case is the first presenting with craniosynostosis. Parental genetic testing is ongoing to confirm the *de novo* origin of the mutation. This report further broadens the phenotypic spectrum of germline *ABL1* variants associated syndrome, adding craniosynostosis as a further disease-associated feature. However, we cannot completely exclude the possibility of this feature not being related to the main diagnosis. We suggest clinical and genetic reassessment of other cases with overlapping characteristics of HCTD since most next generation sequencing panels for HCTD often do not include the *ABL1* gene.

P78- The mildest known phenotype in a patient with *GFM1* biallelic variant: case report and literature review

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Introduction: In the last years, the evolution of next-generation sequencing, such as whole-genome or whole-exome sequencing (WES), revealed a growing number of metabolic disorders underlying intellectual disability (ID) syndromes, namely with milder or atypical phenotypes otherwise difficult to identify. Combined oxidative phosphorylation deficiency-1 due to biallelic pathogenic variants in the gene encoding the mitochondrial elongation factor G1 (*GFM1*) is an ultra-rare early onset progressive hepatoencephalopathy, associated with recurrent vomiting and metabolic decompensation episodes, persistent severe lactic acidemia and multiple respiratory chain deficiency, often fatal in infancy or childhood. We report on an adult patient, likely the oldest patient alive with this diagnosis. Clinical report: As part of an ID cohort study, In2Genome project, WES was performed on a 21-year-old female patient. This patient was born from a healthy couple with remote consanguinity. Gestation and delivery were uneventful. At birth, hypotonia was noticed that evolved to global psychomotor developmental delay reported since the second semester of life. The first time that the patient was referred to the Metabolic Unit, at 3y10m, she presented ataxic walking with inability to run, dysmetria, limited speech, failure to thrive and postnatal short stature. Later, she further developed seizures (onset at 5y) with abnormal EEG, pigmentary retinopathy (without significant vision defect), severe ID and osteoporosis. She had persistent increased serum lactate but further extend metabolic tests were normal, namely cerebral spinal fluid lactate, liver tests and respiratory chain studies in muscle biopsy. WES identified a known likely pathogenic homozygous missense variant in *GFM1* gene: c.2011C>T; p (Arg671Cys). Discussion: This specific variant is likely hypomorphic and has been reported previously in 3 cases, one also in homozygous state and two in compound heterozygosity. One of these patients, despite being a child at the time of report, also had a stable condition without decompensation episodes and with expected long-term survival.

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P79- Cardiospondylocarpofacial syndrome as a distinct hereditary connective tissue disorder: novel missense variant in MAP3K7 in two unrelated patients

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Introduction: Cardiospondylocarpofacial syndrome (CSCFS, ORPHA: 3238) firstly delineated by Sousa et al. (2010) is an autosomal dominant multisystemic condition to date reported in few cases, even after the causative has been identified (Le Goff et al., 2016): MAP3K7 that encodes TGF-β-activated kinase 1, an important regulator of p38 mitogen-activated protein kinase signaling pathway. We describe two patients from unrelated families (7th and 8th reported) with the same novel missense variant and expand the phenotypic spectrum.
Clinical report: A 12-year-old Portuguese male presented at birth with hypotonia, bilateral inguinal hernia, cryptorchidism, facial dysmorphisms (myopathic-like facies, puffy eyes, ptosis, hypertelorism, downslanting palpebral fissures) and hands with loose and wrinkled skin resembling cutis laxa. Subsequently, he had motor and speech delay and at 4 years a bilateral conductive hearing loss was diagnosed. The cardiac follow-up revealed a myxomatous mitral and tricuspid valves. At last examination, he had normal intellect and growth, pectus excavatum, flat foot, significant joint laxity, and maintained the skin, hands and facial features. Retrospective X-rays analysis revealed cervical vertebral fusions. Trio WES identified a de novo heterozygous missense variant in MAP3K7 gene, c.629G>A (p.Cys210Ser), absent in control databases and only reported in one case from DDD project at Decipher database. This patient is an 8-year-old British patient with short stature, dysmorphic features, mitral insufficiency, conductive hearing loss, carpal fusion and also connective tissue features: joint laxity, soft velvety skin, hands with redundant skin, flat foot and pectus excavatum.
Discussion: CSCF is likely underdiagnosed. WES unveiled this diagnosis in both our patients who presented significant connective tissue features, not highlighted in the initial patients described. Underlying molecular mechanisms will also be discussed, CSCF is caused by loss-of-function heterozygous MAP3K7 non-recurrent missense variants or in-frame deletions, while frontometaphyseal dysplasia type 2 is caused mainly by a recurrent gain-of-function MAP3K7 frameshift variant.

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P80- A new case of Snijders Blok-Campeau Syndrome

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Introduction: Multiple members of the chromodomain helicase DNA-binding (CHD) protein family have been implicated in the aetiology of a significant number of neurodevelopmental disorders. Recently, heterozygous pathogenic variants in *CHD3* have been found to cause a syndromic form of intellectual disability with a characteristic facial appearance, impaired speech and macrocephaly, known as Snijders Blok-Campeau Syndrome (SBCS). We describe a patient with a *CHD3* pathogenic variant, aiming to contribute to the phenotypic characterization of this recently described condition. Case report: We report a 30-month-old girl, first child of a healthy non consanguineous couple, with no relevant family history. Pregnancy and delivery were uneventful. Neonatal period was marked by floppiness, with preserved reflexes. Growth progressed on the 50th-75th centile. Evaluation at 30 months documented significant global developmental delay (all areas bellow 2SD, namely gross motor and language). Oromotor problems were remarkable. Physical examination revealed height and weight between the 50th and 75th centiles and head circumference in the 97th centile. Facial features included prominent forehead, deep-set eyes, periorbital fullness, narrow and downslanting palpebral fissures, and a prominent chin. Echocardiogram and cranial MRI were normal, as was arrayCGH. Whole exome sequencing identified an heterozygous pathogenic variant in *CHD3* gene (c.3130C>T p.(Arg1044Trp). Parental testing is ongoing. Discussion/Conclusions: The variant in *CDH3* gene identified in this patient is located on the surface of the Helicase C-terminal domain of CHD3 protein, and has been previously reported in five patients with SBCS. The facial features, initially suggestive of a Sotos-like syndrome, are very consistent with the cohort of 35 patients from the DDD study described by Snijders Blok et al. in 2018. This report of a new patient with SBCS raises awareness and contributes to the phenotypic characterization of this newly recognized condition, highlighting that it should be considered in the differential diagnosis of the overgrowth syndromes.

P81- Novel *POGZ* gene truncating variant: a case report of syndromic intellectual disability

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Introduction: Numerous novel candidate genes associated with developmental disorders are being identified based on the application of large-scale cohort based whole exome sequencing. Among them, stands *POGZ* - pogo transposable element with zinc finger domain, implicated in the normal mitotic progression, as a regulator of chromatin remodeling and proper chromosome segregation. Initially associated with isolated neurodevelopmental disorders, like autism spectrum disorder and intellectual disability, it has more recently been associated to syndromic intellectual disability phenotypes. Case report: We report a case of a ten-year-old girl, first child to non-consanguineous and healthy parents, that later gave birth to two healthy sisters. She presented prenatally with augmented nuchal translucency and the diagnosis of Tetralogy of Fallot. Global developmental delay was evident in early infancy, with more prominent speech impairment, currently associated with attention deficit hyperactivity disorder and mild conductive hearing loss. Karyotype and array comparative genomic hybridization were normal. Distinct facial dysmorphic features became gradually apparent during her childhood, in addition to thoracic asymmetry and one cafe-au-lait spot on her right knee. Whole exome sequencing identified a novel heterozygous frameshift variant c.3843_3846del (p.Lys1281Asnfs*28) in *POGZ* gene, subsequently validated by Sanger sequencing. Parental testing revealed it to be a de novo variant. Discussion: We present a novel truncating variant in *POGZ* gene associated with a syndromic phenotype, here including unusual and remarkable cardiac involvement, apart from various facial features similar to those depicted in literature, increasing the number of presently described similar cases. The presented clinical case supports the relevance of a wide molecular study approach and reinforces that truncating variants in *POGZ* may lead to an identifiable syndromic intellectual disability.

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P82- Co-occurrence of pathogenic mutations in patients at-risk for Hereditary Breast and Ovarian Cancer: two cases diagnosed using a multi-gene panel

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Introduction: Breast Cancer (BC) is the second most common cancer worldwide, and the most frequent cancer in women. Ovarian cancer (OC), is the fourth common cause of female cancer death in the developed world. About 10-30% of BC and OC show a familial aggregation but it is estimated that only 5-10% of BC and 20% of OC are hereditary. The main genes involved in hereditary breast and ovarian cancer (HBOC) are BRCA1 and BRCA2. Multiple recent studies have shown that a percentage of high-risk individuals have germline pathogenic mutations in cancer risk genes other than BRCA1 and BRCA2, such as ATM, BRIP1, CDH1, CHEK2, NBN, PALB2, RAD51C, RAD51D, PTEN, TP53, EPCAM, STK11.
In this study we report two cases with co-occurrence of pathogenic mutations in breast and ovarian high-risk cancer genes. Methods: We performed NGS analysis of 18 genes: BRCA1, BRCA2, ATM, BRIP1, CDH1, CHEK2, NBN, PALB2, RAD51C, RAD51D, PTEN, TP53, EPCAM, STK11, MLH1, MSH2, MSH6 e PMS2. Also, MLPA for BRCA1, BRCA2, EPCAM genes and BRCA2 c.156_157insAlu was performed. Results: We identified 2 probands with co-occurrence of pathogenic mutations. One proband had co-occurrence of pathogenic variants in BRCA1 and RAD51C genes. This proband had triple negative breast cancer at the age of 48 years old and no familial history. The other proband had co-occurrence of pathogenic variants in PALB2 and CHEK2. This proband had breast cancer at the age of 23-year-old and colorectal cancer at 55 and positive family history for breast cancer. Discussion: The use of a multigene panel is nowadays considered the best strategy to improve detection of pathogenic mutations in high-risk HBOC genes. Furthermore, this strategy enables the identification of rare situations as co-occurrence of actionable pathogenic mutations in patients. The identification of individuals with multiple clinically actionable mutations have important implications for probands and their family members. Although higher risk and aggravation of the phenotype in those patients should be expected and a specific surveillance plan implemented, further investigations is needed to better understand the implications of having multiple pathogenic mutations.

P83- PIK3CA-related overgrowth spectrum: case report

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Introduction: PIK3CA-related overgrowth spectrum (PROS) encompasses a range of complex progressive conditions associated with asymmetric overgrowth caused by mosaic postzygotic activating variants in PIK3CA gene, which encodes for the 110-kD catalytic alpha subunit of PIK3. These variants lead to increased downstream catalytic activity, promoting cell survival and proliferation through AKT and mTOR signaling, important pathways also in cancer development. Clinical Report: We report a three-year-old boy, the only child of healthy non-consanguineous parents. During pregnancy, pleural effusion with spontaneous resolution occurred. At birth, he had extensive capillary malformation in the trunk and limbs and striking large hands and feet. In the first six months of life, all growth parameters climbed in the growth charts; at 30-months-old, he had weight at 77th, height at 66th and head circumference at 97th centile. Segmental overgrowth has been affecting mainly limbs soft tissues and he further developed extensive epidermal nevi. Heart and abdominal ultrasounds were normal. Brain MRI showed focal left temporal cortical dysplasia type II, mild ventriculomegaly and a slight descent of the cerebellar tonsils without Chiari malformation. Psychomotor development has been normal. WES-based virtual panel of 12 genes from the PI3K/AKT/mTOR pathway identified the heterozygous pathogenic variant c.2176G>A p.(Glu726Lys) in PIK3CA gene at the mosaic state in DNA directly extracted from affected skin. Discussion: We report a new patient with a known PIK3CA variant, previously described in patients with Megalencephaly-Capillary Malformation (MCAP) syndrome and curiously also as somatic mutation in different types of cancer. This patient's phenotype integrates well within PROS, to date fitting better the MCAP description but evolving with components of Congenital Lipomatous Overgrowth, Vascular Malformations, Epidermal Nevi, Scoliosis/Skeletal and Spinal (CLOVES) syndrome. Due to the progression of this group of conditions and often poor long-term prognosis, the efficacy of recently described targeted therapies such as PIK3CA inhibitors constitute a real hope for these patients.

P84- A new case with a splicing *PIEZO2* mutation causing distal arthrogryposis with distal muscle weakness, scoliosis and proprioception defects

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Introduction: Homozygous or compound heterozygous loss-of-function mutations in the piezo-type mechanosensitive ion channel component 2 (*PIEZO2*) gene were identified in patients with sensory ataxia and proprioception defects together with arthrogryposis, myopathy, scoliosis and progressive respiratory failure. The PIEZO2 protein functions as a nonselective cation channel, is expressed in sensory endings of proprioceptors innervating muscle spindles, Golgi tendon organs and Merkel cells. To date only 17 patients were described with this phenotype. Clinical Report: We report a 23-year-old girl, first child of healthy and non consanguineous parents, that was born with hypotonia, distal laxity, club feet, contractures/arthrogryposis, and feeding difficulties. She was referred to our unit due to a previous diagnosis of a congenital hypomyelinating neuropathy without molecular confirmation, intellectual disability, short stature, mild restrictive pulmonary disfunction, sleep disturbance, dysmorphic features, an asymmetric involvement of shoulder and trunk muscles, progressive severe kyphoscoliosis and mild mitral insufficiency. She is able to walk eyes open, with a wide-based, unsteady gait and without support. She performed an extensive etiological investigation that was inconclusive, and, after this, we included this patient in I2G Project for whole exome sequencing (WES) that identified a previously reported pathogenic splice site homozygous variant (c.5083-1G>A) in *PIEZO2* gene. Discussion: The observed symptoms of sensory ataxia and proprioception defect with muscle weakness, distal arthrogryposis and progressive scoliosis represent core characteristics of a recently delineated condition caused by biallelic loss-of-function *PIEZO2* mutations. As in this case, all patients have been identified by WES without previous clinical recognition, highlighting the importance of this approach in this group of disorders and suggesting that this condition is likely underdiagnosed.

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P85- A Prenatal Case of a fetus with Inverted Duplication and Terminal Deletion 5p

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Introduction: Inverted duplication and deletion of 5p is a complex chromosomal rearrangement (CCR) rarely described in the literature. Phenotypic features of the carriers are variable, depending on the size of the duplicated and deleted segments. Clinical diagnosis of trisomy 5p is difficult, due to the broad spectrum of manifestations. Total duplications of 5p arm or a small segment between 5p10-5p13.3 usually determine a more severe phenotype, the latter being proposed as the critical region for trisomy 5p. Trisomy of segments distal to 5p13 mostly causes mild features. A 35-year-old pregnant woman was referred for prenatal diagnosis due to fetal cystic hygroma and increased nuchal translucency. Amniocentesis was performed at 14 weeks of gestation. Methods: Routine aneuploidy screening was performed by Multiplex-PCR, and amniotic fluid sample cultured and harvest followed in situ protocol. Parental karyotypes were analysed by conventional cytogenetics. FISH study for 5p15.2 region and array-CGH ISCA 8x60K (Oxford Gene Technology, UK) were performed. Results: Aneuploidy screening revealed absence of aneuploidies in a female fetus. Fetal karyotype showed additional material at the terminal region of chromosome 5p, suggestive of a duplication. Two co-localized signals in the short arm of the abnormal chr. 5 were observed by FISH, indicating duplication of the cri-du-chat critical region (5p15.2) and that the abnormality was an inverted duplication of 5p arm. Parental karyotypes were normal. The fetal karyotype was described as 46,XX,add(5).ish dup(5)(p15.2p15.2)(D5S23++,D5S721++)dn. Array CGH study showed a deletion of 1.64 Mb in 5p15.33 region and a contiguous duplication of 42.8 Mb at 5p15.33p12. Discussion: We present a prenatal case of a *de novo* inverted duplication and terminal deletion of chromosome 5p initially detected by conventional cytogenetic study, confirmed by FISH and fully characterized by array CGH analysis. Fetal ultrasound examination at 18 weeks showed increased nuchal translucency, large edema of the neck, hyperchogenic bowel and short long bones. Pregnancy was terminated at 19 weeks of gestation. Fetal autopsy is in progress. This case demonstrates the value of the combination of several cytogenetic techniques, mainly the usefulness of array CGH analysis for the characterization of CCR, such as inv dup del, which are rarely observed in prenatal context.

P86- Novel mutation in addition to functional *TMPRSS6* gene polymorphisms originate an IRIDA-like phenotype in an African child

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Iron-refractory iron deficiency anemia (IRIDA) is a rare autosomal recessive anemia often unresponsive to oral iron intake and partially responsive to parenteral iron treatment. The disease originates from mutations in *TMPRSS6* gene, encoding Matriptase 2, a transmembrane serine protease that plays an essential role in down-regulating hepcidin. Once *TMPRSS6* is mutated, the corresponding protein is absent or inactive at the hepatocyte membrane leading to uncontrolled high levels of hepcidin and impaired iron absorption. This study aimed to investigate a 4-year-old boy of sub-Saharan ancestry (Mozambique/Angola), presenting with microcytic hypochromic anemia, low transferrin saturation, normal ferritin, and having a partial response to intravenous iron treatment. He is a -α3.7-thalassemia carrier. *TMPRSS6* was screened for variants by Next-Generation Sequencing using Nextera XT libraries in a *MiSeq* platform (*Illumina*). Genetic variants found were validated by Sanger sequencing. *In silico* analyses were performed in HSF, SIFT, Poly-Phen2 and Missense3D softwares. A novel missense mutation (c.871G>A) was found in heterozygosity, in *TMPRSS6* exon 8. *In silico* analysis indicates the conserved amino acid change (G291S) may be damaging to the protein stability. Due to its location in the CUB1 domain, it may also affect the enzyme activation and substrate recognition. Additionally, 3 SNPs previously associated with a greater risk of developing iron deficiency anemia (K253E, V736A and Y739Y) were also identified in *TMPRSS6*. Although IRIDA is known as an autosomal recessive disease, we conclude that, in this case, the result of a digenic inheritance of the novel damaging mutation (c.871G>A; G291S) and the 3 common modulating SNPs in the same gene and a co-inheritance of the α-thalassemia *HBA* deletion may lead to an IRIDA-like phenotype. Further functional studies of the mutated protein as well as family studies should be conducted.

P87- A complex intrachromosomal 4q rearrangement: a case report

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Introduction: Deletions or duplications on the long arm of chromosome 4 are a rare event. Clinical manifestations depend on the amount of the genetic material detected or duplicated and vary from healthy to several degrees of intellectual disability and multiple congenital anomalies. 4q deletion has been associated with craniofacial dysmorphism, micrognathia and heart defects. Growth deficiency, microcephaly, facial dysmorphism, hypotonia, difficulties feeding has been related with 4q duplication. The authors present a case of a newborn with a complex intrachromosomal rearrangement on the long arm of chromosome 4

Clinical report: New born with respiratory distress syndrome, hypotonia, peculiar facies and feeding difficulties has attended a genetic consultation. Cytogenetic analysis revealed a *de novo* translocation between the long arm of chromosome 4 and the short arm of chromosome 20. Array Comparative Genomic Hybridization (aCGH) detected a deletion of 9 Mbp on 4q24 region and a gain of four copies of 1Mbp on 4q23 region and three copies of 1.7 Mbp on 4q22.3 region.

Discussion: In the present case, conventional cytogenetics detected a translocation between chromosomes 4 and 20. The array clarify a complex rearrangement in 4q24 region involved in translocation that comprise a loss of 9 Mbp and a total gain of 9.1 Mbp. The newborn has several dysmorphias consistent with 4q deletion and duplication syndrome. An isolated 4q duplication or 4q deletion are very rare structural anomalies with a heterogeneous spectrum of clinical manifestations. To our knowledge this is the second case of a duplication deletion involving chromosome 4q.

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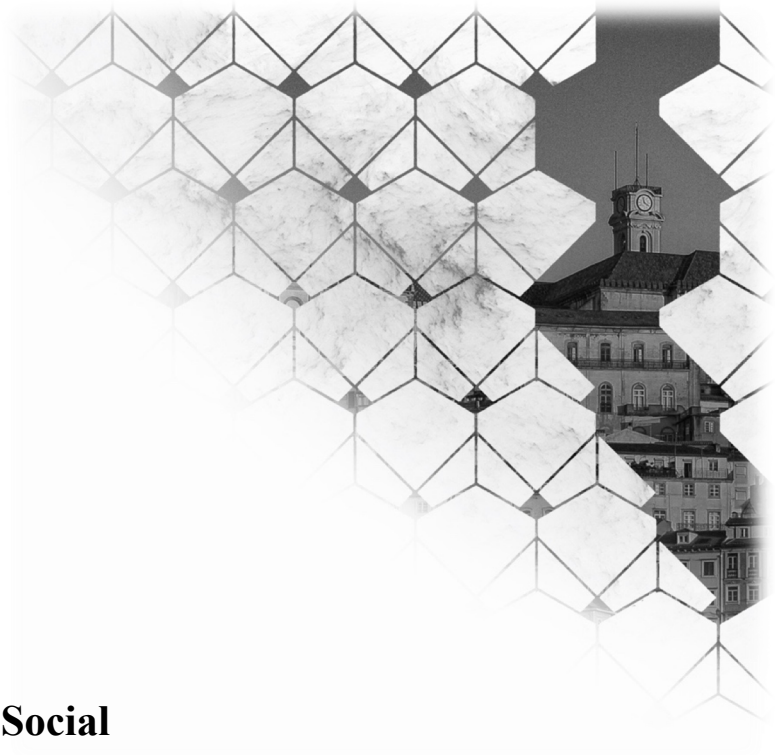

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