21ª REUNIÃO ANUAL

16 -18 NOV 2017

GENÉTICA DA MEDICINA PERSONALIZADA:

CANCRO E DOENÇAS RARAS

SOCIEDADE PORTUGUESA DE GENÉTICA HUMANA

SPGH

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21.ª Reunião Anual

Sociedade Portuguesa de Genética Humana

16–18 Novembro 2017

Capuchos, Almada

21.ª Reunião Anual da Sociedade Portuguesa de Genética Humana

Dear Colleagues,

It is our great pleasure to welcome you to the 21st Annual Meeting of the Portuguese Society of Human Genetics (SPGH), in Lisbon.

We are immensely grateful to all our sponsors for making this meeting possible again, and to all the members of the Local Organizing Committee and the Scientific Committee, whom we wish to address a special acknowledgement.

In a moment when Personalized Medicine is an emerging approach for disease diagnosis, treatment and prevention, providing extraordinary opportunities to improve public health, the SPGH Organizing Committee and the Scientific Committee made their best effort to organize an appealing program with lectures delivered by leading national and international scientists, who will present exceptional findings in the Human Genetics of Personalized Medicine field. We are looking forward to listening to our outstanding invited senior lecturers, as well as the young researchers who will present their works that have been selected among the submitted abstracts—a highly stimulating and exciting meeting, we presume. We received a remarkable number of abstract submissions, which demonstrates how important Human Genetics has become, nowadays.

Although our program seems a bit packed, we hope you can still have some spare time to visit the Caparica beaches and the nearby city of Lisbon.

We expect an enjoyable and productive meeting.

Welcome to Lisbon!

The Organizing Committee,

Luísa Romão
Ana Sousa
Rosário Pinto Leite

21.ª Reunião Anual da Sociedade Portuguesa de Genética Humana

Capuchos, Almada
16–18 November 2017

Caros Colegas,

É com enorme prazer que vos damos as boas vindas à 21.ª Reunião Anual da Sociedade Portuguesa de Genética Humana (SPGH), em Lisboa.

Estamos imensamente gratos a todos os nossos patrocinadores por tornarem esta reunião possível novamente, bem como a todos os membros da Comissão de Organização Local e da Comissão Científica, a quem queremos endereçar um agradecimento especial.

Numa altura em que a Medicina Personalizada emerge como uma nova abordagem ao diagnóstico, tratamento e prevenção de doenças, contribuindo para ganhos em saúde pública, a Comissão Organizadora e a Comissão Científica da SPGH delinearam um programa focado na Genética Humana da Medicina Personalizada. Este é constituído por palestras proferidas por notáveis cientistas nacionais e estrangeiros, assim como por jovens cientistas que apresentarão os seus trabalhos selecionados de entre os resumos submetidos — prevemos uma reunião extremamente estimulante e interessante. A quantidade de resumos recebida foi excecional, o que demonstra a importância da Genética Humana, atualmente.

Apesar de o nosso programa parecer um pouco denso, esperamos que consigam ter algum tempo disponível para visitar as praias da Caparica e a cidade de Lisboa, aqui ao lado.

Esperamos uma reunião agradável e produtiva.

Bem-vindos a Lisboa!

A Comissão Organizadora,

Luísa Romão
Ana Sousa
Rosário Pinto Leite

Comissão Organizadora
Organizing Committee

Luísa Romão

Ana Sousa

Rosário Pinto Leite

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Thursday, November 16		
9:30	Registration Opening	
10:00	Cytogenetics and Molecular Genetics Club Hildeberto Correia Sílvia Serafim João Gonçalves Patrícia Theisen Luís Vieira Catarina Silva [Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Lisboa, Portugal]	Medical Genetics and Clinical Dysmorphology Club Patrícia Dias Vera Silva Lopes Catarina Machado André Travessa Mariana Sá [Serviço de Genética Médica, Centro Hospitalar Lisboa Norte (CHLN), Hospital de Santa Maria (HSM), Lisboa, Portugal]
14:00	Opening and Welcome Direção da SPGH Conselho Diretivo do INSA	
14:15	Personalized Medicine for Cystic Fibrosis Margarida Amaral, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal *Chair: João Lavinha*	
15:00	DYNAMICS OF GENOME ARCHITECTURE *Chairs: Joana Barbosa de Melo; Sérgio Bernardo Sousa* Interpretation of Structural Variations in a Regulatory Context Stefan Mundlos, Max Planck Institute for Molecular Genetics, Berlin, Germany Spectrum of Structural Genomic Abnormalities in Subjects Carrying Putative Disease-Associated Chromosome Rearrangements and their Pathogenic Implications Dezso David, INSA, Lisboa, Portugal Chromatin Reorganization Events in Endothelial Cells Associated with Pulmonary Arterial Hypertension Armando Reyes Palomares, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany	
16:30	Coffee Break / Poster Viewing	

17:00 Selected Oral Communications I (1-6)
Chairs: Susana Fernandes; Sofia Dória

18:00 Genetic Counselling and Personalized Medicine in Rare disorders
Corporate Panel Discussion (Shire)
Moderator: Maria do Céu Machado, Presidente do Infarmed, Lisboa, Portugal

Fabry's disease: phenotypic heterogeneity and therapeutic implications
Patrício Aguiar, Centro de Referência em Doenças Metabólicas, CHLN-HSM, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

Fabry's disease: issues in genetic counseling
Oana Moldovan, Serviço de Genética Médica, CHLN-HSM, Lisboa, Portugal

Genetic Counselling: the psychological dimensions in the individual and family. A reflection from the clinic.
Alexandra Leonardo, Serviço de Genética Médica, CHLN-HSM, Lisboa, Portugal

18:45 SPGH Assembly

Friday, November 17	
8:45	Selected Oral Communications II (7-12) *Chairs: Carla Oliveira; João Fernando Silva*
9:45	Nusinersen (Spinraza™): The First FDA/EMA-Approved Treatment for SMA Adrian Krainer, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA *Chair: Luísa Romão*
10:30	Coffee Break / Poster Viewing
11:00	CANCER GENETICS *Chairs: Paulo Matos; Peter Jordan*
	Networks of Alternative Splicing Regulation in Cancer Juan Valcarcel, Centre for Genomic Regulation, The Barcelona Institute of Science and Technology, Barcelona, Spain
11:30	RNA Binding Proteins in Cancer Progression Fatima Gebauer, Centre for Genomic Regulation, The Barcelona Institute of Science and Technology, Barcelona, Spain
12:00	Selected Oral Communications III (13-18) *Chairs: Rosário Pinto Leite; Ana Medeira*
13:00	Lunch
14:15	TOWARDS PERSONALIZED MEDICINE IN ONCOLOGY *Chairs: Manuel Teixeira; Juliette Dupont*
	Classification of Mutations in the Exonuclease Domain of POLE Claire Palles, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK
	Targeting Cancers using Individual Systems Medicine Krister Wennerberg, Institute for Molecular Medicine Finland, Nordic EMBL Partnership for Molecular Medicine, University of Helsinki, Helsinki, Finland
15:15	MECHANISM-GUIDED THERAPIES FOR GENETIC DISORDERS *Chairs: Ana Sousa; Glória Isidro*
	Translational Medicine in Familial Hypercholesterolemiam—from Phenotype to Genotype to Treatment Mafalda Bourbon, INSA, Lisboa, Portugal

	Development of RNA Based Approaches to Exploit Alternative Therapies for Lysosomal Storage Diseases Sandra Alves, INSA, Porto, Portugal
16:15	Coffee Break / Poster Viewing
16:45	Genetic Counselling and Personalized Medicine in Cancer Corporate Panel Discussion (Astrazeneca) Moderator: Ana Berta Sousa, Serviço de Genética Médica, CHLN-HSM, Lisboa, Portugal
	Mainstreaming Cancer Genetics Angela George, Cancer Genetics Unit, The Royal Marsden, London, UK
	Commentary: The Portuguese Situation and Where to Go from Here Manuel Teixeira, Serviço de Genética, Instituto Português de Oncologia do Porto Francisco Gentil, Porto, Portugal
17:30	Panel Discussion "Challenges in Personalized Medicine" Moderator: Astrid Vicente, INSA, Lisboa, Portugal
	Further Knowledge towards Improved Precision João Lavinha, INSA, Lisboa, Portuga
	Genetic Tests and Personalized Medicine in Hospital Settings Luisa Mota Vieira, Hospital do Divino Espírito Santo, Ponta Delgada, Portugal
	ICT and Data-driven Medicine: Challenges and Solutions Tiago Guerreiro, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
	Personalized Medicine Today: Who Knows What? Luciana Costa, INSA, Lisboa, Portugal
	Personalized Medicine in the R & I Agenda for Health, Clinical and Translational Research Marta Abrantes, Fundação para a Ciência e a Tecnologia, Lisboa, Portugal
19:00	City Tour
21:00	Conference Dinner

Saturday, November 18	
9:00	Selected Clinical Cases for Oral Communication (1-10) *Chairs: Lina Ramos; Isabel Cordeiro*
10:00	Bioethics Debate Medicina Personalizada na Era Genómica Personalized Medicine in the Era of Genomics Moderador: Heloísa Santos, Presidente Comissão Bioética SPGH, Lisboa, Portugal Considerações Éticas e Científicas da Medicina Personalizada Scientific and Ethical Issues in Personalized Medicine Célia Ventura, INSA, Lisboa, Portugal A medicina Personalizada Face à Legislação Portuguesa Portuguese Legal Issues in Personalized Medicine André Pereira, Faculdade de Direito da Universidade de Coimbra, Coimbra, Portugal
11:00	Coffee Break / Poster Viewing
11:30	Genome Editing with Programmable Nucleases Dana Carroll, University of Utah School of Medicine, Salt Lake City, UT, USA *Chair: Rosário Santos*
12:15	Basic and Clinical Research Awards Ceremony
12:25	SPGH Award Conference
12:55	Closing Session Conselho Diretivo do INSA Direção da SPGH

Oradores Convidados

Invited Speakers

21.ª Reunião Anual da Sociedade Portuguesa de Genética Humana

Margarida Amaral

Faculty of Sciences, University of Lisbon

Lisbon, Portugal

mdamaral@ciencias.ulisboa.pt

Margarida Amaral is Full Professor of Biochemistry/ Molecular Biology at the Faculty of Sciences, University of Lisboa (Portugal) and Coordinator of BioISI - Biosystems & Integrative Sciences Institute. MDA is alumna of EMBL-European Molecular Biology Laboratory (2008-10; 2016) and IGC - Gulbenkian Institute of Science alumna (1983-1993). Research: Molecular and cellular mechanisms of the genetic disease Cystic Fibrosis. My lab has its major focus on the molecular and cellular mechanisms of biogenesis, traffic and degradation of normal and mutant protein CFTR, which when mutated causes the genetic disease Cystic Fibrosis (CF). To understand CF mechanisms globally we use transcriptomics, proteomics and functional genomics (functional siRNA screens). Our results translate into the clinic for better CF diagnosis, prognosis and personalized therapies. Author of 116 international publications (average citations per article: 17.93); Researcher ID: E-5748-2012. Awards & Honours: member of EMBO - European Molecular Biology Laboratory; member of the Portuguese Academy of Sciences (2014); Pfizer Award for Basic Biomedical Research (2013); Annual Award of European Cystic Fibrosis Society (2010). Other: Elected member of the Board of ECFS-European Cystic Fibrosis Society and associate editor of J Cystic Fibrosis (Elsevier) and Scientific Reports (Nature Group). Former SAB member of the Cystic Fibrosis Trust (UK) and of the German Cystic Fibrosis Foundation

https://ciencias.ulisboa.pt/en/perfil/msamaral

http://bioisi.campus.ciencias.ulisboa.pt/

Stefan Mundlos

Development & Disease Group Max Planck Institute

Berlin, Germany

stefan.mundlos@charite.de

Stefan Mundlos was born in Marburg an der Lahn, Germany, in 1958. He studied Medicine at the Universities of Marburg and Göttingen and got his approval (“Approbation”) as physician in 1985. Following his PhD and a clinical training and Board Certification in Pediatrics, he spent a year as a research fellow in Melbourne, Australia, followed by a postdoc period at the Harvard Medical School in Boston, USA. Back in Germany, he received his habilitation in 1997 and the Board Certification in Human Genetics in 1998. In 2000, he was appointed director of the Institute for Medical Genetics and Human Genetics at the Charité – Universitätsmedizin Berlin and head of the research group “Development & Disease” at the Max Planck Institute for Molecular Genetics in Berlin. He is particularly interested in malformations and diseases in the context of development, growth, and aging of muscles and bones. In his work, Mundlos combines research on human hereditary diseases with studies on fundamental gene functions in vitro, in cell culture and animal models (in vivo). In recent years, his interest has become more focused on gene regulation and the role of the non-coding regions of the genome. In 2016, he has been honored with ESHG Award for his fundamental work on the identification and characterization of disease genes and disease-causing mechanisms of gene regulation.

Dezso David

Instituto Nacional de Saúde Doutor Ricardo Jorge

Lisbon, Portugal

dezso.david@insa.min-saude.pt

Dezso David is the PI of the Genomic Disorders Research Group at the Department of Human Genetics, National Health Institute Doutor Ricardo Jorge, Lisbon, Portugal. He has several decades of experience in molecular basis of human genetic and genomic disorders, manly chromosomal rearrangements such as translocations and inversions. He has about 30 scientific publications, including in journals like Nature Genetics and European Journal of Human Genetics, and participates in multiple international collaborations. Presently, he and his group propose introduction of large-insert Whole Genome Sequencing (liWGS) for detection of structural chromosomal rearrangements (SCR), identification of candidate genes for SCR-associated disorders and mapping of the human morbid genome. This genomic approach will bridge sequence-based Next-Gen Cytogenetics and personalised or precise medicine, which will catalyse a dramatic advancement in clinical diagnostics and in deep phenotyping.

Armando Reyes-Palomares

European Molecular Biology Laboratory (EMBL)

Heidelberg, Germany

armando.reyes@embl.de

Armando Reyes-Palomares works as postdoctoral fellow at the European Molecular Biology Laboratory (EMBL) in Heidelberg. Armando is originally from Spain, studied Biology and finished his PhD on network biology models for studying rare diseases by integrating biomedical ontologies at the University of Malaga. During his PhD studies, he worked as visiting scientist at the Folkhälsan Institute of Genetics Biomedicum Helsinki and The Max Delbrück Center for Molecular Medicine in Berlin with Miguel Andrade. After completing his PhD, he worked as postdoc in the Centre of Biomedical Research in Rare Diseases studying the phenotype-genotype relationships among a heterogeneous population of patients suffering of genomic syndromes with rare structural variants. In 2015, he moved to Heidelberg where he joined to Judith Zaugg's group to study how chromatin architecture pervades gene regulation and disease conditions at the Structural and Computational Biology Unit, his current postdoctoral fellowship has been partially granted by the Fundacion Ramon Areces.

Maria do Céu Machado

Infarmed

Lisbon, Portugal

Maria do Céu Machado graduated in Medicine at Lisbon University Medical School and completed her trainee in Pediatrics at Hospital Pediatrico D Estefânia (Lisbon, Portugal). She obtained her PhD at Lisbon NOVA Medical School. Currently, she is president of the Board of Infarmed - National Authority of Medicine and Health Products. She is full Professor of Pediatrics at Lisbon University and Member of the National Ethics Council for Life Sciences. She was the Director of the Department of Pediatrics of the Hospital de Santa Maria and Professor of Pediatrics at NOVA Medical School and at Lisbon University Medical School. She was also Vice-President of the National Health Council and of the European Federation of the Academies of Medicine, and Clinical Director of University Hospital de Santa Maria. Between 2006 and 2011 she was the High Commissioner for Health. She was honored as Grand Officer of the Order of Merit in 2010 and received the Gold Medal of the Ministry of Health in 2012.

Adrian Krainer

Cold Spring Harbor Laboratory

Cold Spring Harbor, NY, USA

krainer@cshl.edu

Born in Uruguay, Adrian Krainer arrived in the United States of America in 1977 to enroll as an undergraduate student at Columbia University—the same year Rich Roberts and Phil Sharp discovered RNA splicing, which would earn them the Nobel Prize in 1993. Four years later, he began his graduate studies in biochemistry at Harvard University. In 1984, he published an efficient “cell-free” system that was and is still used to work out the rules and steps involved in splicing and its regulation. This work caught the eye of Rich Roberts, at Cold Spring Harbor Laboratory (CSHL), who was trying to recruit young talents to the institute. Krainer finished his Ph. D. in 1986 and, soon after, became the first member of the CSH Fellows Program, in which Ph. D. or M. D. fellows tackle independent research projects before taking faculty positions—he was appointed assistant professor in 1989 and became full professor in 1994. His group studies the mechanisms of RNA splicing, ways they go awry in disease, and the means by which faulty splicing can be corrected. Particularly, he focused on correcting defective splicing in spinal muscular atrophy (SMA), leading to the first treatment for this motor-neuron disease. Krainer is actively involved in organizations like Fight SMA and CureSMA, and also interacts with the SMA Foundation, and the Muscular Dystrophy Association, all of which have raised funds to support several of his research projects over the years. He often accompanied SMA-affected families to speak with legislators to advocate for increasing federal funding for SMA research and coordination among clinical centers. He has published ~200 research papers, such as last year’s “Antisense oligonucleotide-directed inhibition of nonsense-mediated mRNA decay”, in Nature Biotechnology. Among the multiple awards with which he has been distinguished is the 2017 Inventor of the Year Award (New York Intellectual Property Law Association) in recognition of the contribution of his work towards society as a whole.

Juan Valcárcel Juárez

Centre for Genomic Regulation (CRG)

Barcelona, Spain

juan.valcarcel@crg.eu

Juan Valcárcel obtained his PhD in 1990 at Department of Biochemistry and Molecular Biology, Autónoma University of Madrid (Spain). After his PhD (1991-1995), he went to the University of Massachusetts (USA) as a Postdoctoral research associate in Michael Green's laboratory. In 1996, he joined the European Molecular Biology Laboratory in Heidelberg as a group leader. In 2002, he moved to Centre for Genomic Regulation (CRG) in Barcelona (Spain), as a group leader and ICREA research professor. Since 2012, he is the Coordinator of the Gene Regulation, Stem Cells and Cancer Programme and since 2017 Associate Director of the Centre for Genomic Regulation, Barcelona (Spain). The main research interest of his group is on how pre-mRNA are spliced and how this process can be regulated, with a focus on molecular mechanisms of splice site recognition, alternative splicing and cancer, and alternative splicing and stem cell biology. He is author of 113 publications in scientific journals, such as Nature, Science Cell and Molecular Cell. One of his lab's most recent work (Hernández et al., 2016), published in RNA Biology, shows that splicing regulatory factor RBM10 has tumor suppressor properties in lung adenocarcinomas. He is a member of EMBO and, in 2016, was elected President of the International RNA Society for the 2017-2018 period.

Fátima Gebauer

Centre for Genomic Regulation (CRG)

Barcelona, Spain

fatima.gebauer@crg.eu

Fátima Gebauer obtained her PhD in 1990 in Biological Sciences at Department of Biochemistry and Molecular Biology, Autónoma University of Madrid (Spain). After her PhD, she went to Worcester Foundation for Biomedical Research Massachusetts (USA) as a Postdoctoral research associate in Joel D. Richter's laboratory (1992-1996). In 1996, she joined Matthias W. Hentze's laboratory in European Molecular Biology Laboratory (EMBL-Germany) as a Postdoctoral Research Associate. In 2001, she became Staff Scientist at EMBL. Since 2002, she is a Group leader at the Centre for Genomic Regulation (CRG) in Barcelona (Spain). As group leader of the Regulation of Protein Synthesis group, she is interested in the regulation of mRNA translation by RNA-binding proteins (RBPs) and by elongation of the mRNA poly(A) tail. She wishes to understand the molecular mechanisms of translational control and the RNA networks that are established to maintain cell homeostasis. In addition, her group is interested on how de-regulation of RBP function leads to disease, in particular to cancer. She is co-author of more than 30 publications in scientific journals, such as Cell, Science and Nature Communications. One of her lab's most recent work (Wurth et al., 2016), published on Cancer Cell, demonstrates that UNR/CSDE1 protein drives a post-transcriptional program to promote melanoma invasion and metastasis. This year, she was elected EMBO member that recognizes her as outstanding life scientist.

Claire Palles

Gastrointestinal Cancer Genetics, Institute of Cancer and Genomic Sciences, University of Birmingham

Birmingham, United Kingdom

c.palles@bham.ac.uk

Claire undertook her PhD studies on variants in the Insulin like growth factor (IGF) pathway and their contribution to breast cancer risk at the London School of Hygiene and Tropical Medicine and in the Breakthrough Breast Cancer Centre, Institute of Cancer Research. In 2010 she joined Professor Ian Tomlinson's group in Oxford as a post doctoral researcher. Her key achievements during her time as a post doc were: identifying the first genetic variants associated with risk of Barrett's oesophagus at genome wide significance, identifying common genetic variants associated with toxicity to 5-fluoropyrimidine based therapy and identifying germline mutations in the exonuclease domains of POLE and POLD1 in patients with multiple bowel polyps. In 2015 Claire was appointed as a junior group leader in the Oxford Centre for Cancer gene research working on inherited risk factors for gastrointestinal cancers and their precursors. Her desire to establish an independent research group received further support following her appointment as a Birmingham Fellow in the autumn of 2017. To date Claire has authored over 30 scientific papers in high impact journals and reviews grants for CRUK and MRC as well as acting as an expert reviewer for journals.

Krister Wennerberg

Institute for Molecular Medicine Finland (FIMM), University of Helsinki

Helsinki, Finland

krister.wennerberg@helsinki.fi

Krister Wennerberg received his Ph.D. in biochemistry from Uppsala University, Sweden, and had his postdoctoral training at the University of North Carolina at Chapel Hill, USA. Following his postdoc, he worked as an R&D scientist at Cytoskeleton, Inc., and later as an assay development group leader at Southern Research Institute in Birmingham, AL, USA. In 2010, he joined the Institute for Molecular Medicine Finland (FIMM) as group leader of the Cancer Chemical Systems Medicine research group, focused in the study of cancer and related phenotypes using a chemical systems biology approach. Since August 2017, he also holds a professorship at the Biotech Research & Innovation Centre (BRIC), University of Copenhagen, Denmark.

Mafalda Bourbon

Instituto Nacional de Saúde Doutor Ricardo Jorge

Lisbon, Portugal

mafalda.bourbon@insa.min-saude.pt

Mafalda Bourbon received her Ph.D. in Clinical Sciences from Imperial College, Faculty of Medicine, London, UK, in collaboration with Instituto Nacional de Saúde Doutor Ricardo Jorge (INSARJ), Lisbon, Portugal. Since early her research work has focused on the study of the molecular and genetic factors related to Familial Hypercholesterolemia. In 1999, while still doing her Ph.D., Mafalda Bourbon has become Coordinator of the Portuguese Familial Hypercholesterolemia Study that has emerged as an attempt to improve diagnosis and patient management in Portugal. In 2005, she became group leader of the Cardiovascular Investigation Group, R&D Unit of the Department of Health Promotion and Prevention of non-Communicable Diseases, INSARJ, Portugal, and Coordinator of the Diagnosis Laboratory Unit of the same department in 2011. Her efforts in the area of the dyslipidemias earned her dozens of publications in relevant international journals and two prizes: 1º PRIZE “Familial hypercholesterolemia in Portugal” Award Amélia da Silva Mello for Health Sciences (Lisbon, 2006), and 1º PRIZE “Familial hypercholesterolaemia: an opportunity for preventive medicine” Pfizer Award in Clinical Research (Lisbon, 2009).

Sandra Alves

Instituto Nacional de Saúde Doutor Ricardo Jorge, Centro de Saúde Pública Dr. Gonçalves Ferreira

Oporto, Portugal

sandra.alves@insa.min-saude.pt

Sandra Alves was born in Portugal, in 1973. In 2002, she completed her PhD in Biology at the Faculty of Sciences of the University of Porto. Following her PhD, she was granted a postdoctoral fellowship at the Institute of Molecular Pathology and Immunology of the University of Porto (Ipatimup). In 2004 Sandra Alves became Auxiliary Researcher in the Department of Human Genetics in the Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA), Porto and Head of the Lysosomal Storage Diseases Research Group since 2009. Her research focus is lysosomal storage diseases (LSD), mainly mucolipidoses (ML) and mucopolysaccharidoses (MPS), where the aim is to develop RNA based therapeutic approaches to address such pathologies. The correction of splicing mutations and the decrease of accumulated substrates are some of examples of aims that drive the development of such approaches. Recently, she was awarded the best oral communication in the 12th International Symposium of the Portuguese Society of Metabolic Diseases (2016) for her work on “Development of RNA based approaches to exploit alternative therapies for Lysosomal Storage Diseases”.

Ana Berta Sousa

Hospital de Santa Maria | Hospital da Luz

Lisbon, Portugal

anabertasousa@gmail.com

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.

Astrid Moura Vicente

Instituto Nacional de Saúde Dr. Ricardo Jorge

Lisbon, Portugal

astrid.vicente@insa.min-saude.pt

Astrid Vicente is currently a Principal Investigator and Coordinator of the Department for Health Promotion and Non-Communicable Disease Prevention at INSA, the Group Leader of the Biomedical and Translational Research Group at the Biosystems and Integrative Sciences Institute (BioISI) and Invited Associated Professor at the Faculty of Sciences of the University of Lisbon. She holds a PhD in Molecular Biology from the University of Coimbra, with a thesis on the Neurogenetics of Schizophrenia developed at the Clarke Institute of Psychiatry in Toronto, Canada. She was awarded two postdoctoral fellowships, to investigate the Genetic of Psychosis (at Neurosciences Center of Coimbra) and the Genetics of Autoimmune Disorders (at Instituto Gulbenkian de Ciência, IGC). She has since held several research positions at IGC, INSA and at the Center for Biodiversity of Functional and Integrative Genomics (BioFIG). During her career she has mainly focused on the genetic basis of complex disorders, namely autism and stroke, and currently uses a systems biology strategy to identify interactions between genes, environment, lifestyle and demographics in health and disease. She was awarded with the PFIZER Prize for Clinical Investigation (2005) and two Honorable Mentions for Bial Prize (2006) and Amélia da Silva de Mello Prize for Health Sciences (2007) for her work in autism. In 2006 she was also awarded with the APIFARMA Prize for Mobility. From 2011 to 2012 she was elected president of the Portuguese Society for Human Genetics (SPGH). Her most recent publications are: *Rannikmäe K. et al. COL4A2 is associated with lacunar ischemic stroke and deep ICH: Meta-analyses among 21,500 cases and 40,600 controls. Neurol. 89(17), 1829–1839 (2017)*; *Weiner DJet al. Polygenic transmission disequilibrium confirms that common and rare variation act additively to create risk for autism spectrum disorders Nat Genet. 49(7):978-985 (2017)*; *Conceição, I. C. et al. Definition of a putative pathological region in PARK2 associated with autism spectrum disorder through in silico analysis of its functional structure. Psychiatr. Genet. 27, (2017)*.

Heloísa G. Santos

Comissão Bioética SPGH (Presidente) | Serviço Genética Médica Hospital Santa Maria, Lisboa | Faculdade Medicina Universidade de Lisboa (FMUL).

heloisa.santos@mail.telepac.pt

Consultant in Clinical Genetics and Paediatrics. President of SPGH Bioethics Committee. Head of Genetics Unit of Paediatric Service, Hospital Santa Maria (1974–1999). Training with CO Carter in medical genetics and cytogenetics in Clinical Genetics Unit, Institute of Child Health, London (1978). First Director of Medical Genetics Service of Hospital Santa Maria, Lisboa (1999–2004). GENOMED—Consultant in Medical Genetics of Institute Molecular Medicine of 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 m–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. Over 120 publications in Medical Genetics and Bioethics most in international journals and books. Research leader in several scientific projects.

Célia Ventura

Instituto Nacional de Saúde Dr. Ricardo Jorge

Lisbon, Portugal

celia.ventura@insa.min-saude.pt

Presently, she works in the R&D Unit of the Department of Human Genetics of the National Institute of Health Doutor Ricardo Jorge (INSA) and is a member of the Center for Toxicogenomics and Human Health (ToxOmics) of NOVA University of Lisbon (UNL). She is also ethics adviser in European research projects. Formerly, she was a laboratory technician at the Molecular Genetics Unit of INSA, mainly in the molecular diagnosis of coagulopathies (1993-2015). She graduated in Clinical Analysis and Public Health (1993), had a Master's Degree in Bioethics (summa cum laudae) from the Portuguese Catholic University (2006-2008) on the use of biobanks for genetics research. She is undertaking a PhD in Public Health at the National School of Public Health of the UNL on the field of the toxicogenomics of nanoparticles. She published a book and a book chapter on the ethics of biobanks for genetic research, and is an author or co-author of several peer-reviewed articles. She was an invited speaker in 16 lectures on bioethics or on the molecular biology of coagulopathies and has teaching experience in post-graduate courses and PhD programmes.

Dana Carroll

Department of Biochemistry, University of Utah School of Medicine

Utah, UT, USA

dana@biochem.utah.edu

Dana Carroll is Distinguished Professor of Biochemistry at the University of Utah School of Medicine, where he has been on the faculty since 1975. He received his B.A. degree from Swarthmore College and a Ph.D. from the University of California, Berkeley. He did postdoctoral research with John Paul in Glasgow, Scotland, and Donald Brown in Baltimore, USA. He served as Co-chair and Chair of the Department of Biochemistry between 1985 and 2009. He is considered a pioneer in the development and applications of genome editing with programmable nucleases. His research group was the first to show that zinc-finger nucleases stimulate targeted mutagenesis and gene replacement in living cells and whole organisms. He received the Novitski Prize from the Genetics Society of America in 2012, was elected a Fellow of the American Association for the Advancement of Science in 2013, and was awarded the H.A. Sober Lectureship by the American Society for Biochemistry and Molecular Biology in 2014. He is a member of the American Academy of Arts and Sciences and the US National Academy of Sciences. He continues to pursue research with each of the current genome editing technologies – ZFNs, TALENs, and CRISPR-Cas.

Palestras

Lectures

21.ª Reunião Anual da Sociedade Portuguesa de Genética Humana

Personalized Medicine for Cystic Fibrosis

Margarida D. Amaral

University of Lisboa, Faculty of Sciences, BioISI—Biosystems & Integrative Sciences Institute, Portugal

Cystic Fibrosis (CF), the most common genetic life-shortening disease, affecting ~34,000 in Europe, is caused by mutations in the CF transmembrane conductance regulator (CFTR), a chloride/bicarbonate channel expressed at the plasma membrane of epithelial cells. Defective CFTR thus originates impaired ion transport causing severe epithelial dehydration, namely in the airways 1,2. Although one single mutation (F508del) occurs in ~85% of CF patients worldwide, to date >2,000 CFTR variants were reported, most presumed to be CF-causing. Despite major advances in symptomatic treatments which enabled most CF patients to live into adulthood, many patients still die prematurely from respiratory insufficiency (mean age at death in EU ~25 yrs). CFTR modulator therapies targeting each mutation defect constitute the much ambitioned alternative and have had some recent success 1-3. However, these new drugs are restricted to a few mutations, still leaving many patients with rare mutations without therapy. Indeed, the >1,000 CFTR mutations for which there are less than 5 patients worldwide still pose considerable challenges to drug development. CFTR mutations have thus been grouped by their cellular/functional defect, in the expectation that mutations in the same class/theraptype can be treated by the same drug. We are now determining whether rare CFTR mutations in the same class respond to the new CFTR modulator drugs by pre-assessing them directly on patient's tissues namely intestinal organoids 4,5 or polarized primary cultures of nasal cells 5. This is the way forward to extend these drugs to more CF patients, namely to those with ultra-rare ("orphan") mutations.

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Structural Variations in the Light of the 3D Genome

Stefan Mundlos

Max Planck Institute for Molecular Genetics, Berlin, Germany, Charité, Universitätsmedizin Berlin, Germany

Recent studies have shown that the genome shows a specific three-dimensional organization in the nucleus which has a major influence on gene regulation. These studies have shown that mammalian genomes are organized in distinctly folded chromatin modules, called topologically associated domains (TADs) that are separated from each other by boundary regions. TADs subdivide the genome into discrete genomic units that restrict the possible contacts enhancers can establish with their target genes. We use a CRISPR/Cas9 based strategy to investigate the effect of human disease-associated structural variations in vivo in mice. We show that deletions can result in the fusion of TADs and the re-wiring of enhancer-promoter contacts. At the EPHA4 locus, for example, deletion of parts of the TAD including the boundary result in the activation of the nearby Pax3 gene by Epha4 enhancers and a limb malformation. Furthermore, we analyzed overlapping duplications at the SOX9 locus, that are associated either with a limb malformation (Cooks syndrome), sex reversal, or no abnormality. We show that large duplications spanning a TAD boundary result in the formation of a novel TAD, or neo-TAD. This formation of neo-TADs explains the divergent phenotypes of overlapping duplications at the SOX9 locus. Further, we demonstrate that the increased copy number of cis-regulatory elements is functionally isolated within the neo-TAD and does not affect gene expression of neighboring genes. Moreover, the pathogenicity of duplications depends on the genes and the cis-regulatory information that are incorporated within the neo-TAD. Our results show that duplications including TAD boundary elements can result in the formation of novel genomic units that are functionally and spatially separated from their genomic neighbors. Besides shedding light onto genome biology, our findings provide a framework for interpreting the pathogenic effect of duplications, which are frequently detected in patients with congenital malformations, intellectual disability and cancer.

Spectrum of structural genomic abnormalities in subjects carrying disease-associated chromosome rearrangements and their pathogenic implications

Dezso David

Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal

Structural chromosomal rearrangements (SCRs) have long been recognized as a major source of human developmental anomalies, including, among others, congenital anomalies, and neurodevelopmental, intellectual and cognitive disabilities. Indeed, causal relationship between congenital anomalies and related SCRs are expected to occur in up to 40% of the affected subjects. Approaches used for detection of such SCRs evolved significantly from classical and molecular cytogenetic technologies, such as FISH and microarrays, to whole genome sequencing (WGS) with high physical and low sequence coverage, also known as large-insert WGS. The spectrum of SCRs, at DNA sequence-level resolution, in subjects carrying disease-associated SCRs, and the emerging pathogenic mechanisms will be presented. Like classical haploinsufficiency due to point mutations, disruption of the coding regions or genomic elements controlling quantitative expression of a dosage-sensitive gene will lead to a haploinsufficient phenotype. Position effect is a complex pathogenic mechanism of these SCR-associated disorders, resulting from disruption of native gene-specific and adoption of alien long-range cis-acting control elements. Haploinsufficiency or position effect on the same gene may lead to dissimilar clinical phenotypes. Certain balanced translocations can yield clinical phenotypes that are similar to microdeletion syndromes caused by hemizygosity of a major causal gene locus or of a variable number of contiguous genes. In such cases, haploinsufficiency of the causal gene with or without position effect on the contiguous genes can be considered as possible pathogenic mechanisms. Occasionally, gene fusions occur through SCRs that may lead to fusion transcripts. Although formation of such transcripts is a fundamental pathogenic mechanism behind different forms of cancer, apparently this is insignificant in SCR-associated disorders, mainly because many of such transcripts are non-functional and therefore non-pathogenic. There is no direct correlation between complexity of chromosomal rearrangements and severity of clinical phenotypes. Such genomic approach allows personalized medicine-based care and necessarily has to be accompanied by deep clinical phenotyping. The emerging picture from these SCRs data highlights the extent to which the human genome can be affected by these rearrangements, its tremendous plasticity, and the intricacy of pathogenic mechanisms leading to SCRs-associated disorders.

Chromatin Reorganization Events in Endothelial Cells associated with Pulmonary Arterial Hypertension

Armando Reyes-Palomares[1], Fabian Grubert[2], Mingxia Gu[3], Ivan Berest[1], Michael P Snyder[2], Marlene Rabinovitch[3], Judith B Zaugg[1]

[1]European Molecular Biology Laboratory, SCB Unit, Heidelberg, Germany, [2]Stanford University School of Medicine, Department of Genetics, Stanford, CA, [3]Stanford University School of Medicine, Department of Pediatrics, Stanford, CA

Pulmonary arterial hypertension is a low prevalent disease characterized by high-blood pressure in lung arteries. Here we aim to study the epigenetic and gene expression changes in primary pulmonary arterial endothelial cells from PAH patients and controls. This study was performed taking into account the stratification of patients according to their disease causes, idiopathic or hereditary (BMPR2 mutation). Our multi-omics' approach consisted on the integration of diverse molecular phenotypes such as histone modifications (H3K27ac), gene expression (RNA-Seq) and long-range chromatin interactions (RAD21 and CTCF). The multi-level integration allowed us to uncover disease-specific chromatin regulatory domains that can be entirely up-/down-regulated by a specific transcription factors. We used these chromatin modules as the basis for building a comprehensive-gene regulatory network to understand the molecular mechanism underlying hypertension injury and vascular remodelling in endothelium. Our results suggest specific-epigenetic changes related to transcription factor activities and chromatin reorganization events that are sensitive for hypertension injury. These findings will be useful for getting a deeper insight of the genetic causes and the development of intervention therapies.

Nusinersen (SpinrazaTM): First FDA/EMA-Approved Treatment for SMA

Adrian R. Krainer

Cold Spring Harbor Laboratory, NY, USA

Spinal muscular atrophy (SMA) is a motor-neuron disease, caused by mutations in SMN1. Patients retain one or more copies of the nearly identical SMN2 gene, which mainly expresses mRNA lacking exon 7, coding for an unstable protein isoform. The small amount of full-length mRNA and protein expressed from SMN2 only partially compensates for the loss of SMN1. Together with Ionis Pharmaceuticals, we developed nusinersen, an antisense oligonucleotide (ASO) that efficiently promotes exon 7 inclusion and restores SMN protein levels. Nusinersen hybridizes to intron 7 of the SMN2 pre-mRNA, preventing binding of the splicing repressors hnRNPA1/A2 to a bipartite intronic splicing silencer, ISS-N1; this in turn facilitates binding of U1 snRNP to the intron 7 5' splice site, resulting in enhanced exon 7 inclusion. Clinical trials of nusinersen in SMA patients began five years ago; based on the results of two phase-3 trials in infants with the most severe form of SMA, and in children with an intermediate form of SMA, respectively, nusinersen was recently approved by the FDA and EMA for all SMA types.

We are continuing to explore aspects of SMA pathogenesis and treatment, using ASO therapy in SMA mouse models. We found that SMA is not motor-neuron cell-autonomous in the mouse models, such that correcting SMN2 splicing in peripheral tissues exclusively is necessary and sufficient for full phenotypic rescue. We are also exploring prenatal ASO treatment, as it is likely that early intervention can have the greatest clinical benefit.

Forty years after the discovery of RNA splicing, nusinersen exemplifies a successful path from basic studies of cellular mechanisms to an effective treatment for a devastating disease.

Networks of alternative splicing regulation in cancer

Juan Valcárcel[1-3]

[1]Centre de Regulació Genòmica (CRG), [2]Universitat Pompeu Fabra (UPF), [3]ICREA, Barcelona, Spain.

Alternative splicing of mRNA precursors allows regulation of gene function and physiology in higher eukaryotes, from the speciation of vampire bats to the selection of eatable quinoa seeds in South America. Alterations in alternative splicing can impact every hallmark of cancer and provide biomarkers of prognostic value. For example, activation of alternative splice sites in the oncogene BRAF confers resistance to vemurafenib and facilitate the frequent relapse of melanoma tumors. Splicing alterations can be caused by cancer-associated mutations in splicing regulatory sequences or by other genetic alterations, including mutations or changes in expression levels of splicing factors. Splicing factor mutations are particularly common in hematological tumors, including myelodysplastic syndromes and chronic lymphocytic leukemia. While advantageous for cancer progression, splicing alterations appear to make cancer cells particularly sensitive to splicing inhibitory drugs. These and other observations suggest that mis-regulation of alternative splicing networks contributes to tumor progression and at the same time can confer vulnerability to cancer cells.
I will summarize our recent efforts to systematically reveal splicing regulatory circuits altered in cancer cells and the potential of this knowledge to design novel anti-cancer therapies. These include methods for saturation mutagenesis of alternative exons, genome-wide identification of regulatory factors and reconstruction of splicing regulatory networks via profiling of alternative splicing after systematic knock down of spliceosomal components. Our results reveal highly dense regulatory content of alternative exon sequences and extensive regulatory potential of core splicing factors. They also uncover circuits of cell cycle and apoptosis control by spliceosomal components, reveal detailed molecular mechanisms of versatile splicing modulation by anti-tumor drugs and by modified antisense oligonucleotides, and provide insights into the impact of signaling pathways important for cancer cell proliferation on alternative splicing.

RNA binding proteins in cancer progression

Fátima Gebauer

Centre for Genomic Regulation, The Barcelona Institute of Science and Technology, Barcelona, Spain

RNA binding proteins (RBPs) are gaining attention in the oncology field for their potential to regulate essentially every hallmark of tumor development. My talk will focus on the RNA binding protein UNR/ CSDE1, a protein conserved from Drosophila to humans for which we found a selective role in metastasis. I will explain how we got to suspect a role for this protein in tumour progression, and how we identified relevant downstream targets and mechanism of action. I will also explain our current efforts to identify new RBPs involved in cancer, with a focus on metastasis.

Classification of mutations in the exonuclease domain of POLE

Claire Palles

Gastrointestinal Cancer Genetics, Institute of Cancer and Genomic Sciences, University of Birmingham, United Kingdom

Variant effect prediction methods are not generally gene specific. Many variants in disease-associated genes are classified as variants of unknown significance by these methods. Germline mutations in the exonuclease domain of POLE are known to cause proof-reading associated polyposis. Somatic mutations in this domain are also found in ~1% of CRCs and 8% of endometrial cancers and patients with such mutations have a good prognosis and may benefit from immunotherapy.
We have screened ~3000 patients with CRC or polyposis to identify additional POLE EDM germline variants. Data from co-segregation analysis, tumour sequencing, fluctuation assays in S.pombe and in vitro biochemistry assays are being generated and will be used in machine learning approaches to improve variant classification for this gene. In order to ensure patents with germline or somatic mutations in POLE receive appropriate cancer screening or treatment it is imperative that mutations are classified as either pathogenic or not.

Targeting Cancers using Individual Systems Medicine

Krister Wennerberg

Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Finland

The concept of targeting cancers strictly based on their genetic alterations has gained massive attention in recent years. However, cancer precision medicine clinical trials attempting to broadly link oncogenic alterations with targeted treatments have yet to show significant clinical impact. In an attempt to address these current shortcomings in genomic cancer precision medicine, we have at the Institute for Molecular Medicine Finland (FIMM), University of Helsinki, established an Individualized Systems Medicine platform where we study primary cancer samples by combining comprehensive functional chemosensitivity profiling, deep molecular and genetic profiling and clinical information with the aims to allow us understand the linkages between the molecular profile of a cancer and drug sensitivities and to allow for rapid and efficient identification of precision therapies for current and future patients. In having profiled more than 200 acute myeloid leukemia and other myeloid neoplasm cases, we have learnt that the vast majority of selective drug responses are not directly linked to individual genetic alterations and that selective cancer cell killing effects are rarely seen with single agents. Our profiling has on the other hand allowed us to identify new potential targeted uses for approved and investigational drugs such as the VEGFR inhibitor axitinib, which unexpectedly targets a drug resistant gatekeeper mutation of the BCR-ABL oncoprotein. Furthermore, the compiled information allows us to search for more complex biomarkers that may predict clinical drug responses in an unbiased manner. I will present our approaches, some of our findings as well as some of the challenges that lie ahead.

Translational medicine in Familial hypercholesterolaemia – from phenotype to genotype to treatment

Mafalda Bourbon

Departamento de Promoção da Saúde e Prevenção de Doenças não Transmissiveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal

Familial Hypercholesterolaemia (FH), is a monogenic autosomal dominant disorder associated with an increased risk of premature coronary artery disease due to life long exposure to high levels of plasma LDL-cholesterol.
The correct determination of the pathway affected in hyperlipidaemic patients adds additional information for counseling and treatment of clinical FH patients. Determination of LDL receptor activity allows the classification of patients in null allele carriers and defective allele carriers. Null allele carriers are more affected than patients with a missense variant or other type of variant that retains some residual LDL receptor activity (defective allele). This knowledge allows for a better cardiovascular risk stratification; patients with null allele mutations should be more aggressively treated since they will have a poorer response to all therapies that are based of the increment of the LDL receptor protein. For the paediatric group the finding of a null allele mutation in a child can make the difference for the clinician to decide to start treatment earlier, due to the knowledge that the child will have a life long burden of very high LDL-cholesterol and the sooner it is treated the better prognosis will have.
It is still controversial if FH should be defined at the genotype or phenotype level, although evidence is building up towards the definition of disease at the genotype level. We have described several cases from the Portuguese FH Study that adds evidence for this discussion; in the XXI century patients should be treated based on their genotypes and not phenotypes since several phenocopies of FH are known at the present time and the treatment varies according to the pathway affected (gene) for best patient prognosis.

Development of RNA based approaches to exploit alternative therapies for Lysosomal Storage Diseases.

Sandra Alves

Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal

Lysosomal Storage Disorders (LSDs) are a group of rare inherited metabolic diseases caused by the malfunction of the lysosomal system, resulting in the accumulation of undegraded substrates inside the lysosomes and leading to severe and progressive pathology.
Treatment strategies such as substrate reduction and enzyme-replacement therapy, among others, are available for some LSDs, yet still with some limitations.
In recent years, the RNA molecule became one of the most promising targets for therapeutic intervention and currently, a large number of RNA-based therapies are being investigated at the basic research level and in late-stage clinical trials, as also some of them are already approved for treatment (e.g. Duchenne muscular dystrophy; Familial hypercholesterolemia).
RNA-based approaches constitute a potential alternative or an adjuvant therapeutic strategy for many diseases; either acting at pre-mRNA levels (by splicing modulation/correction using antisense oligonucleotides or U1snRNAs vectors) or at mRNA levels (e.g. using small interfering RNA (siRNA) and antisense oligonucleotides).
Currently we are developing some of these therapeutic approaches for LSDs. Two main research lines are ongoing; one involves the use of antisense U1 snRNAs and antisense oligonucleotides to overcome the effect of the LSDs causing mutations c.234+1G>A in Mucopolysaccharidosis type IIIC and c.3503_3504delTC in Mucolipidosis type II respectively, and the other is based on the use of RNA interference (RNAi) technology to promote efficient substrate reduction therapy for a subset of LSDs called Mucopolysaccharidoses.

Considerações éticas e científicas sobre a medicina personalizada

Célia Ventura[1], Heloísa Santos[2]

[1]Instituto Nacional de Saúde Doutor Ricardo Jorge, Departamento de Genética Humana, Unidade de Investigação e Desenvolvimento
[2]Médica Geneticista, Presidente da Comissão de Bioética da SPGH

Os avanços biotecnológicos dos últimos anos, particularmente ao nível da sequenciação genómica, têm criado expectativas sobre os benefícios da implementação de um novo modelo de apoio médico adaptado a cada indivíduo. Esta medicina personalizada ambiciona maximizar os benefícios para a saúde pelo conhecimento da respetiva predisposição individual para a doença e escolha das medidas preventivas e de promoção da saúde que melhor se lhe adaptem, incluindo modificações do ambiente e estilo de vida. Este conceito é também relevante quando existir doença já instalada, pela escolha da terapêutica medicamentosa que apresente melhor eficácia ou menores reações adversas. A farmacogenómica tem já grandes implicações na oncologia, mas também noutras doenças genéticas, principalmente as de causa metabólica. Uma estratégia intermédia seguida em oncologia é a estratificação dos tumores em subtipos, baseados nos marcadores genéticos tissulares, para prever com maior rigor a sua evolução e as respostas a cada medicamento. Propostas mais arrojadas de medicina preventiva personalizada são o rastreio genético alargado no período pré-natal e a sequenciação do genoma do recém-nascido, tendo este último como objetivo usar esta informação ao longo da vida. Contudo, além de ainda não existir uma total segurança sobre a utilidade clínica de algumas variações genéticas, as conclusões nem sempre são válidas para todos os grupos populacionais, o que cria inaceitáveis assimetrias respeitantes a minorias étnicas ou etnias menos frequentes nos países desenvolvidos. A ausência de investigação básica nestas populações, ou o desinteresse da indústria farmacêutica em comercializar fármacos dirigidos a "genótipos órfãos", põem em causa a justiça distributiva e a equidade no acesso à saúde. Além disso, possibilita a discriminação negativa de alguns subgrupos populacionais com maior risco detetável de morbilidade ou mortalidade de causa genética. As decisões sobre alocação de recursos da saúde são também postas em causa pelo elevado custo associado à medicina personalizada que, inevitavelmente, deslocará verbas de outras áreas, a nível nacional e internacional. A generalização da partilha das bases de dados pessoais e dos biobancos criados para investigação biomédica, bem como dos resultados científicos obtidos, tão necessária para potenciar a translação do conhecimento básico na prática clínica, coloca também questões éticas relevantes sobre o direito da confidencialidade desta informação e a privacidade dos participantes. Por outro lado, a medicina personalizada implica a partilha da informação genómica pessoal dentro do sistema de saúde, o que provavelmente só será possível pela sua inclusão no processo clínico eletrónico do doente. Desta forma, a confidencialidade e privacidade no contexto clínico são igualmente postas em causa. Outras questões éticas associadas à implementação na clínica da sequenciação do genoma são também aplicáveis à medicina personalizada (consentimento informado, achados acidentais, direito a não saber, etc.). A primeira recomendação para o sucesso da medicina personalizada é a imprescindível maior literacia sobre a correta utilização da informação genómica por parte dos profissionais de saúde, mas também da população, que terá uma maior

responsabilização sobre a sua própria saúde. Este último facto tornou-se evidente na realização dos testes genéticos de venda direta ao consumidor. Finalmente, esta mudança de paradigma irá trazer profundas alterações a conceitos individuais com consequências psicológicas ainda não avaliáveis, mas também ao planeamento estratégico do próprio modelo de sistema de saúde. Este terá de concordar com a implementação da medicina personalizada para adequadamente responder aos múltiplos achados clínicos dela decorrentes. Assim, esta modificação conceptual exige também a urgente educação dos próprios políticos e legisladores e deve ser acompanhada de ampla discussão pública. Será aconselhável iniciar o debate sobre esta nova realidade e abstermo-nos de usar, de forma indiscriminada e por decisão individual, orientações de países com prováveis visões éticas e socioprofissionais distintas.

Genome Editing with Programmable Nucleases

Dana Carroll

Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT, USA

The history of genome editing with targeted nucleases is relatively brief, but the advances have been stunning, providing powerful tools for intentional genetic manipulations. This talk will review some of that history, including ZFNs, TALENs and CRISPR. Remarkably, all these reagents do is make a targeted double-strand break in genomic DNA. Everything that follows – including mutagenesis by NHEJ, sequence replacement by HDR, and more complex outcomes – depends on cellular activities and our ability to bend those to our advantage. A specific potential application to human therapy will be described, and some of the societal issues raised by the technology will be discussed.

Comunicações Orais

Oral Communications

21.ª Reunião Anual da Sociedade Portuguesa de Genética Humana

Session I	Thursday, 16, 17:00	OC1–OC6
Session II	Friday, 17, 8:45	OC7–OC12
Session III	Friday, 17, 12:00	OC13–OC18

OC1 | Clinical Genetics

KBG syndrome: clinical and molecular findings in 13 patients

Travessa A.M.[1]; Machado C.[1]; Soeiro-e-Sá M.[1]; Dias P.1; Moldovan O.[1]; Dupont J.[1]; Medeira A.[1]; Santos H.[1]; Sousa A.B.[1]

[1]Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal.

Introduction: KBG syndrome is a genetic entity characterized by facial dysmorphism, macrodontia, skeletal anomalies, and intellectual disability (ID)/developmental delay (DD). KBG syndrome is caused by heterozygous mutations in ANKRD11 gene. Results from the DDD study found that mutations in ANKRD11 account for 1% of undiagnosed ID/DD, suggesting KBG syndrome is under-recognized. We present our Department's experience with this syndrome. We aim to expand its phenotypic and mutational spectrum.
Methods: We report 13 individuals with KBG syndrome. Clinical and molecular characterization was performed by revising each patient's medical records.
Results: Mean age at diagnosis of index patients was 9 years (range 3-19). The diagnosis of KBG syndrome was clinically suspected in 12/13 patients. In these patients, neurodevelopmental features included variable ID/DD (12/12), attention-deficit/hyperactivity disorder (4/12), epilepsy (2/12), and autism (1/12). Medical problems included deafness (8/12), short stature (6/12), delayed bone age (3/8), heart defects (3/12), cryptorchidism (1/5), palate anomalies (1/12), and multiple episodes of otitis (2/12). They all presented typical KBG syndrome dysmorphism, including triangular face, synophrys/thick eyebrows, almond-shaped palpebral fissures, macrodontia, thin upper lips, and hand anomalies. Eight novel ANKRD11 pathogenic variants (7 frameshift and one missense) were found. Apart from one family with 5 affected members, all cases were non familial. No correlation between genotype and phenotype was apparent. The remaining patient presented an intragenic ANKRD11 deletion identified by array-CGH. He had ID/DD, IUGR, hydrocephalus, microcephaly and short stature, but no typical KBG syndrome dysmorphism.
Discussion: In all but one patient, KBG syndrome was suspected by gestalt combined with the evaluation of specific dysmorphic features. All patients had ID/DD and minor hand anomalies, namely brachydactyly and 5th finger clinodactyly. Another common problems were deafness and short stature. No major organ malformations were reported. The great majority of KBG syndrome cases were due to frameshift variants, supporting the haploinsufficiency hypothesis of ANKRD11 causality. Interestingly, the patient with an intragenic ANKRD11 deletion did not present the typical KBG syndrome gestalt. In this case, RNA studies would be important to understand the effect of this variant on protein expression.

OC2 | Clinical Genetics

Identification of new familial dyslipidaemia causative genes

Graça R. [1], Rossi N. [1], Alves AC. [1, 2], Medeiros A. [1], Zimon M. [3], Rausch T. [4], Benes V. [4], Pepperkok R. [3, 5], Bourbon M. [1, 2]

[1]Unidade de I&D, Grupo de Investigação Cardiovascular, Departamento de Promoção da Saúde e Prevenção de Doenças Não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal 2 BioISI-Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal. 3 Cell Biology and Cell Biophysics Unit, EMBL Heidelberg, Heidelberg, Germany. 4 Genomic Core Facility, EMBL Heidelberg, Heidelberg, Germany. 5 Advanced Light Microscopy Facility, EMBL Heidelberg, Heidelberg, Germany.

Introduction: Familial Hypercholesterolemia (FH), one of the most frequent dyslipidaemias, leads to premature atherosclerosis and coronary heart disease. Clinically, FH is characterized by high plasma concentrations of total and low-density lipoprotein (LDL) cholesterol from birth. Since 1999 the FH Portuguese study sequenced over 725 probands for LDLR, APOB and PCSK9 genes. However, only 40% of the patients carry a putative pathogenic mutation. The remaining include individuals with polygenic forms of dyslipidaemia or mutations in genes not yet associated with FH. The design of a custom cost-effective target sequencing panel for FH diagnosis is a fundamental step for a correct patients' classification and personalized therapy.
Methods: Candidate genes were retrieved from publicly available genome wide association studies (GWAS) and protein-protein interaction screen of dyslipidaemias causative genes (STRING). Functional characterization was performed for some of the hits to assess their involvement in lipid metabolism. We evaluated the impact of gene knock down on the uptake of fluorescently-labelled LDL and free cholesterol cellular content using automated microscopy on cultured cells. Targets enrichment for sequencing was based on Haloplex system (Agilent) Tier 1 design, leading to a capacity of sequencing 112 genes (exons, first 50 bp of introns and UTRs). 188 FH-negative patients from the Portuguese FH study were sequenced at EMBL genomic facility in Heidelberg according to our custom panel. Variants calling, and annotation pipeline was based on BWA, FreeBayes and VEP tools. Low frequency variants (MAF < 1%) were prioritized for Sanger cascade screening. Primers were designed with NCBI primer design tool and SNPcheck.
Results and discussion: Five patients carried known pathogenic LDLR variants and were removed from analysis. We identified 484 rare variants: 459 missense, 4 synonymous and 21 indels (8 in frame). Four novel variants have been identified in APOB: p.Asp2398Glu, p.Ser4135Asn, p.Leu2726Val, p.Val325Ile, which need further functional characterization. 125 patients and 165 variants spread over 50 genes were prioritized for cascade screening including: 4 frameshift indels, 1 inframe deletion, 2 start loss, 6 stop gained, 21 splicing, and 131 missense variants. The next step is to analyse the Sanger sequencing data and verify the co segregation of the variants with the dyslipidaemia phenotype within the families under study.

OC3 | Clinical Genetics

HUWE1 missense mutations and X-linked intellectual disability: report on 5 families and literature review

Ribeiro S. M.[1]; Sá J.[1]; Beleza A.[1]; Reis C.[1]; Ramos F.[1]; Louro P.[1]; Maia S.[1]; Williams H.[2]; Harakalova M.[3]; Haaften G.V.[3]; Saraiva J.M.[1,4]; Sousa S.B.[1,5]; Venâncio M.[1,5]

[1] Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; 2. GOSgene, Genetics and Genomic Medicine Programme, UCL GOS Institute of Child Health, London, England; 3. Department of Medical Genetics, Centre for Molecular Medicine, University Medical Centre Utrecht, Utrecht, the Netherlands; 4. University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra, Portugal; 5. University Clinic of Genetics, Faculty of Medicine, University of Coimbra, Portugal

Introduction: X-linked intellectual disability (XLID), which constitutes 10% of cases of ID in boys, includes more than 100 genes and 200 phenotypes identified to date. Most of these phenotypes are not specific and next-generation sequencing (NGS) techniques revealed to be crucial for their accurate diagnosis. The HECT, UBA and WWE domain-containing protein 1 (HUWE1) is a HECT family ubiquitin ligase with growing links to syndromic XLID. It regulates neural progenitor proliferation, differentiation and migration in early development of the nervous system. HUWE1 missense variants have been described as causative in a limited number of XLID families but associated with a wide phenotypic spectrum of ID severity and other features.
Methods: Retrospective analysis of medical records of all cases with HUWE1 point mutations identified in our department from 2009 to 2017. These were detected through untargeted NGS approaches in ID cases, using either whole-exome, broad commercial 4813 gene panel or X-chromosome exome sequencing. Segregation studies were done in all families as well as the observation of the patients' mothers and their X-inactivation pattern analysis. Clinical and molecular characterization of these patients and detailed literature comparison was performed, including reassessment of each variant´s pathogenicity.
Results and Discussion: We report 8 affected male members from 5 unrelated families, each with a distinct HUWE1 missense variant identified. One variant had already been reported segregating in a XLID family and was considered likely pathogenic. Four variants were novel: 3 were considered as of uncertain significance and 1 as benign due to its presence on the unaffected brother (case excluded for the phenotypic analysis). All variants were inherited and X-inactivation patterns were normal in 3/5 heterozygous mothers. The clinical findings in the present study are in accordance with the literature. All male patients had significant global development delay / ID with very limited speech. Four had behavioral anomalies. Facial dysmorphic features were present in all patients but not considered consistent or recognizable. Features not previously reported include a multicentric ganglioneurocytoma and 2 hemangiomas in one patient and a linear hypopigmented nevus in another. Our results reinforce the likely pathogenic role of HUWE1-missense variants in XLID but also the difficulty in interpreting novel variants in this gene, especially in single cases.

OC4 | Clinical Genetics

Whole exome sequencing in patients with undiagnosed genetic conditions: a 4 year experience

Catarina Machado, Juliette Dupont, Oana Moldovan, Patrícia Dias, Mariana Soeiro e Sá, André Travessa, Ana Berta Sousa

Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal

Introduction: Whole-exome sequencing (WES) is increasingly being used in clinical practice to diagnose rare Mendelian disorders. The diagnostic yield of WES averages from 20-25% in solo WES to 30-40% using a trio approach. Notwithstanding considerable interpretation challenges, it is recognized that WES is the best option to investigate patients with severe but unspecific features, combinations of features overlapping multiple recognizable syndromes, atypical presentations, disorders associated with significant genetic heterogeneity, or conditions without a known causal gene.

Methods: To determine the clinical utility of WES in our practice and compare our results with the literature, we performed a retrospective review of 85 patients in whom WES was performed between 2013 and 2017 (69 trio and 16 solo). All patients had negative previous more or less extensive workup. The patients were distributed in four groups: 1. Non-syndromic moderate to severe developmental delay (DD)/intellectual disability (ID) with or without autism spectrum disorder (ASD) (4/85); 2. Syndromic DD/ID (69/85); 3. Multiple congenital anomalies (6/85); and 4. Specific diagnosis suspected (6/85). Clinical data were extracted from medical records. Written informed consent for WES was obtained from patients or legal guardian.

Results: The diagnostic rate overall was 41% (35/85), 43% (30/69) for the trio-approach and 31% (5/16) for solo. 23 (66%) of the patients with a diagnosis had an autosomal dominant de novo mutation, 11 (31%) had an autosomal recessive condition and 1 (3%) had a de novo X-linked variant. The highest rate of diagnosis was observed among patients in groups 1 (3/4) and 2 (28/69, 41%). In groups 3 and 4 diagnostic rates were 1/6 and 2/6, respectively. Within group 2, the presence of neurological features 22/58 (38%), short stature 6/14 (42%), and microcephaly 11/20 (55%) were associated with the highest diagnostic yield. 15 (18%) variants of unknown significance were identified, including 2 in genes concordant with the patient's clinical features and 2 in new candidate genes that possibly explain the phenotype. In one case the diagnosis had a dramatic impact on medical management, and in two cases it changed the empiric recurrence risk.

Discussion: Our results are concordant with the literature and illustrate the value of WES, particularly trio, in providing a diagnosis for patients with rare genetic disorders, with the added advantage of novel gene discovery. Accurate diagnosis benefits patients and their families by ending the invasive, time consuming and costly diagnostic odyssey, and allowing more specific management and proper genetic counseling. This methodology should be considered earlier in the clinical investigation of challenging patients.

OC5 | Cytogenetics and Genomics

The Eye Genotype-Phenotype Database (EyeG2P)

Carvalho AL.[1]; Lampe AK.[2]; Black GC.[3]; Hall HD.[4]; Thormann A.[5]; Firth HV.[6]; Venâncio MM.[7]; FitzPatrick DR.[8]

[1]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; University Clinic of Genetics, Faculty of Medicine, University of Coimbra, Portugal; MRC Human Genetics Unit, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom, [2]South East of Scotland Clinical Genetics Service, Western General Hospital, Edinburgh, United Kingdom, [3]Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre, St Mary's Hospital, Manchester, United Kingdom, [4]MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom; Princess Alexandra Eye Pavilion, NHS Lothian, Edinburgh, United Kingdom, [5]European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge, United Kingdom, [6]Wellcome Trust Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, United Kingdom, [7]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; University Clinic of Genetics, Faculty of Medicine, University of Coimbra, Portugal; MRC Human Genetics Unit, [8]MRC Human Genetics Unit, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom; South East of Scotland Clinical Genetics Service, Western General Hospital, Edinburgh, United Kingdom

Introduction: In recent years, next generation sequencing (NGS) technology has strikingly increased the capacity to identify the underlying genetic cause of Mendelian diseases, including genetic diseases of the eye (GDE). owever, these tests generate lots of data that need interpretation which constitute a crucial challenge for the lab and clinical geneticist. For this it is essential to know the phenotypes associated with each genotype. There are some tools to help this analysis. For example, as part of Deciphering Developmental Disorders (DDD) project, the Developmental Disorders Genotype-Phenotype Database (DDG2P) facilitates the clinical feedback of likely causal variants. Methods: We created the Eye Genotype-Phenotype Database (EyeG2P) with a list of genes reported to be associated with eye disorders. For this selection, we analyzed predesigned NGS panels, a database of inherited retinal diseases (RetNet), scientific publication indexed in PubMed and published books. For each gene, we characterized the gene-disease attributes, such as disease confidence (the level of certainty that the gene causes the phenotype), allelic status associated with disease and mutation consequence. We also disclose the most relevant scientific publications, the corresponding phenotypes (according to the Human Phenotype Ontology) and which organs are involved (the organ specificity). Results and discussion: 635 genes were selected, which are associated with 794 phenotypes. The syndromic phenotypes are more common and 8% of the genes may have a syndromic and non-syndromic association. The most frequent affected organs are the retina and the brain. Regarding disease confidence, confirmed eye genes constitute the majority (70%). The most common allelic requirement and mutation consequence are biallelic (60%) and loss of function (71%), respectively. The high number of genes and phenotypes associated with ocular pathologies are in agreement with the genetic heterogeneity of most GED and with the frequent ocular involvement in the syndromic disorders. Regarding the most affected organs, our results are expected considering the embryonic origin of the various eye tissues and the groups of pathologies often associated with ocular manifestations, such as, for example, ciliopathies. This database is very useful, for diagnostic laboratories and clinicians, as it allows link genotype to eye genetic disorders with mutation mechanism to enable rule-based reporting of variant.

OC6 | Other: Oncogenetics and Public Services

The impact of genetic counseling, screening and multidisciplinary care in well being of families with hereditary diffuse gastric cancer syndrome (HDGC)

Garrido L[1,2], Leal R[3,4], Costa S[2,6], Magalhães A[2,6], Guimarães R[1,2], Ferro L[1,2], Gullo I[3,4,7,8], Pinheiro H[3,4], Sousa S[4], Carvalho B[5], Neto AP[5], Capela L[5], Gil C[6,9], Teixeira C[6,9], Fareleira A[6,10], Devezas V[6,10], Barbosa J[6,10,11], Baptista M[6,9,10,11], Fougo JL[2,6], Costa Maia J[6], Fernandes S[5], Carneiro F[3,4,7,8], Castedo S[1,3,4,5,12*], Oliveira C[3,4,8,*] *Joint last authors

[1]Oncogenetic Consultation, Breast Center, Centro Hospitalar São João (CHSJ), Porto, Portugal, [2]High Risk Consultation of Breast Tumors, Breast Center, CHSJ, Porto, Portugal, [3]Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Porto, Portugal, [4]Institute of Molecular Pathology and Immunology at the University of Porto (Ipatimup), Porto, Portuga, [5]Genetics Department of Pathology, Faculty of Medicine of the University of Porto (FMUP), [6]General Surgery Service, CHSJ, Porto, Portugal, [7]Department of Pathology, CHSJ, Porto, Portugal, [8]Department of Pathology, FMUP, Porto, Portugal, [9]High Risk Consultation of Digestive Tumors, General Surgery Service, CHSJ, Porto, Portugal, [10]Department of General Surgery, FMUP, Porto, Portugal, [11]Esophageal and Gastric Surgery Unit, General Surgery Service, CHSJ, Porto, Portugal, [12]Medical Genetic Service, CHSJ, Porto, Portugal

CDH1 germline mutations predispose to Hereditary Diffuse Gastric Cancer (HDGC) syndrome and increase the risk for early onset diffuse gastric cancer (DGC) in both genders and lobular breast cancer (LBC) in females. This is an actionable diagnosis, as prophylactic gastrectomy and breast MRI may, respectively, prevent gastric cancer and screen for early breast cancer. Alternatively, laborious, expensive and low disease pick-up rate screening measures (annual gastroscopy/multiple biopsies) is offered to carriers refusing gastric surgery and to all 1st/2nd degree relatives from CDH1 negative HDGC families. We aim at understanding the clinical and socio-economical impact of counseling, pre-symptomatic genetic testing and genetic diagnosis in HDGC families. Between 2005 and 2017we identified 7 apparently unrelated HDGC families carrying the c.1901C>T (p.Ala634Val) CDH1 mutation. These were referred for genetic counseling due to DGC or LBC at early age or relevant family history. A positive genetic test triggered integration in the adequate care pathway. Potential carriers referred by probands or by primary health care services were also counselled and tested. Hospital-based patient registries were used to infer the socio-economical impact of the care pathway. 108 individuals from 7 CDH1 positive HDGC families were called in and another 32 members were followed in other health care institutions. From 140 individuals, 65 (46.4%) tested negative and were discharged from preventive clinical follow-up; 69 (49.3%) tested positive and 6 have pending studies. 6/7 index cases died; 44/69 CDH1 carriers (63.8%: 22M:22F) enrolled in high risk assessment consultation, and 21 of these (8M:13F) accepted prophylactic gastrectomy; 16/44 carriers (9M:7F) engaged in endoscopy surveillance; 1/44 carriers refused information about carrier status and was diagnosed with DGC 18 months after genetic screening; the remaining 6 carriers have not decided yet. 7/22 females accepted risk reduction mastectomy, 12/22 are under MRI surveillance, and 3 have not decided yet.Hospital-based integrative and multidisciplinary care pathways allow better clinical care for carriers and reassurance and discharging for non-carriers, saving health care services' resources and improving patient well being. Adoption of similar strategies in other cancer-associated syndromes will greatly benefit the National Health Care System, the society and contribute for uniformization of care in Europe.

OC7 | Cancer Genetics

Mapping of cis-regulatory variants helps dissecting the risk mechanism for breast cancer associated 5q14.1 locus

Xavier J.M.[1,2,3]; Magno R.[2,3]; Almeida B.P.[1,2,3,4]; Dunning M.[5]; Jacinta-Fernandes A.[1,2,3]; Russell R.[5]; Samarajiwa S.[6]; O'Reilly M.[5]; Rosli N.[2,3]; Nobrega C.[1,2,3]; Barbosa-Morais N.[4]; Caldas C.[5,6]; Ponder B.[A.5,6]; Maia A.T.[1,2,3]

[1]Department of Biomedical Sciences and Medicine (DCBM), University of Algarve, Campus de Gambelas, Faro, Portugal; [2]Centre for Biomedical Research (CBMR), University of Algarve, Campus of Gambelas, Faro, Portugal; [3]Algarve Biomedical Center, Campus Gambelas, Faro, Portugal; [4]Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal; [5]Cambridge Institute—CRUK/University of Cambridge, Cambridge, United Kingdom; [6]Medical Research Council (MRC) Cancer Unit, University of Cambridge, Hutchison/MRC Research Centre, Cambridge, United Kingdom

Genome-wide association studies (GWAS) have identified a significant number of new loci associated with increased risk of developing breast cancer. Follow-up functional studies of these loci, including previous evidence from our work, have demonstrated that cis-acting regulatory variants (rSNPs) are strongly involved in BC risk. rSNPs can generate differential allelic expression (DAE), which can be easily detected by comparing the relative expression of the two alleles in a heterozygous individual. Therefore we hypothesize that mapping the cis-regulatory loci in normal breast tissue, using DAE analysis, is the most efficient way to map the causal variants of risk. We have performed whole-genome DAE quantification of 64 normal breast tissue samples using Illumina Exon510S-Duo arrays, followed by DAE mapping analysis. We found that 76.1% of all expressed genes displayed DAE, indicating that cis-regulation is a widespread mechanism in breast tissue. For one-third of these genes we identified common cis-variants possibly affecting gene expression (10% FDR significance). Overlapping of the cis-regulatory variants map with published BC GWAS revealed 9 loci for which the rSNPs were also associated with BC risk (r2>0.8). An in-depth bioinformatics and functional analysis of the BC risk locus 5q14.1, identified two functional rSNPs located in the shared promotor of RPS23 and ATP6AP1L genes and three candidates rSNPs with potential to be affecting ATG10 splice variants levels, including a non-coding isoform. Moreover, the DAE mapping results strongly associate the rSNPs affecting ATG10 with BC risk, and evidence from differential expression analyses in breast tumours supports a tumorigenic effect of ATG10, which is less expressed in tumours compared to adjacent normal tissues. Overall, our results suggest that variants in ATG10 associated with an increased BC risk, act by down-regulating expression of ATG10 through regulation of splice variants expression. This study reveals that DAE mapping is a powerful method to identify variants with direct impact in complex regulatory landscapes, such as that of the 5q14.1 risk locus.

OC8 | Cancer Genetics

Annotator: a novel custom tool for genomic variants annotation and classification

Lemos D. [1,2]; São José C. [1,2,3]; Oliveira P. [1,2]; Carvalho J. [1,2]; Oliveira C. [1,2,4]

[1]i3S—Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; [2]IPATIMUP—Institute of Molecular Pathology and Immunology, University of Porto, Rua Dr. Roberto Frias s/n, 4200–465 Porto, Portugal; [3]ICBAS-Instituto Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal; [4]Department of Pathology and Oncology, Medicine Faculty of the University of Porto—Alameda Prof. Hernâni Monteiro, 4200–319 Porto, Portugal

Genome sequencing produces large amounts of data that enable the discovery of genomic variants. Using appropriate bioinformatics tools and public databases which aggregate clinical information, it is possible to understand the functional impact of such variants on phenotypic traits. Several bioinformatics tools already exist, however with limitations such as, the license costs and the limited choice of public databases consulted. Therefore, our aim was to implement a free tool that aggregates information collected from user-defined public databases, allowing the custom annotation of genomic variants and their classification in terms of pathogenicity. We implemented a tool, Annotator, which annotates and classifies genomic variants using data from 5 renowned public databases (Uniprot, OMIM, ClinVar, dbSNP, Pubmed). Annotator can also integrate keywords, defined according to sample characteristics, for advanced data mining, in order to refine collected data and variant classification. As validation, Annotator was used to analyze and classify a set of 151 genomic variants, detected in 52 probands with Familial Intestinal Gastric Cancer. These genomic variants had already been analyzed/classified using a commercial software. The comparison of the results obtained with both analyses showed that Annotator was able to add relevant information for 7/42 somatic variants and 4/24 germline variants of unknown significance for the commercial software. In conclusion, Annotator is a valid tool for an accurate annotation and efficient classification of genomic variants derived from sequencing experiments.

OC9 | Cancer Genetics

Clinical use of Liquid Biopsies in Cancer Precision Medicine

Cardoso J.[1], Pereira A.[2], Rego de Sousa M.J.[2], Germano de Sousa J.[2], Germano de Sousa[2]

[1]Ophiomics - Precision Medicine, Lisboa, Portugal, 2. Centro de Medicina Laboratorial Germano de Sousa, Lisboa, Portugal

The clinical management of cancer patients still relies on the assessment of the primary tumor characteristics at the time of diagnosis. However, it is now clear that cancer cells are very heterogeneous and adaptable. Thus it is of most importance to assess tumor cells in real time in order for physicians to intervene and customize the cancer treatment when it is most beneficial for the patient. This is the key idea to personalized medicine in oncology and is based in the analysis of specific biomarkers that can predict drug response. It is a rapidly progressing area, based on innovative translational medicine strategies such as the usage of circulating biomarkers (e.g. circulating tumor DNA (ctDNA) or cells (CTCs)). The assessment of ctDNA or CTCs may enhance treatment options in real-time for example by focusing on DNA mutations at the basis of chemotherapy resistance or on the few cells that are the direct precursors of distant metastasis, which are likely inherently different than the primary tumor. But the assessment of such biomarkers poses many technical challenges due to their rarity.

We implemented laboratory and bioinformatics workflows that allow us to provide personalized medicine services to clinicians. Our main approach is based on the extraction, quantification and mutational analysis of ctDNA or ctRNA using Ion Torrent Next Generation Sequencing (NGS) and digital PCR (dPCR) technologies. Mutation data is used to infer the pharmacogenomics profile of the tumor available targeted therapies and clinical trials, providing the oncologist with the information relevant for the management of the disease.

Using the above described strategy we have in the last two years analyzed dozens of patients affected by different tumor types, sent from distinct public and private hospitals. The quantification of ctDNA, the first outcome of our analysis, allows for real-time monitoring of tumor load and response to therapy (e.g. pancreatic/ovarian cancer) while the analysis of the mutational landscape through NGS or dPCR allows for detection of potentially targetable mutations (e.g. TP53 druggable pathogenic mutation in ovarian cancer).

We outline the overall laboratory and bioinformatics pipeline, discuss technical challenges and exemplify the clinical use of this approach in the clinical setting with examples from our own practice.

OC10 | Cancer Genetics

miRNA-mediated cis-regulation in breast cancer susceptibility

Jacinta-Fernandes A.[1,2,3]; Esteves F.[1,2,3]; Magno R.[2,3]; Xavier J.[2,3] and Maia A.-T.[1,2,3]

[1]Department of Biomedical Sciences and Medicine (DCBM), University of Algarve, Campus de Gambelas, Faro, Portugal, [2]The Functional Genomics of Cancer Laboratory, Centre for Biomedical Research, University of Algarve, Campus de Gambelas, Faro, Portugal, [3]Algarve Biomedical Center (ABC), University of Algarve, Campus de Gambelas, Faro, Portugal

Breast cancer (BC) is a complex and largely polygenic disease. To date, several common low-risk variants associated with BC risk were identified through Genome-Wide Association Studies (GWAS). These variants are single nucleotide polymorphisms (SNPs), mostly located in non-coding regions, and for a few a cis-regulatory role has been validated via functional studies. Still, the latter studies have been biased towards the effect on transcription factor binding, overlooking other possible regulatory mechanisms, such as microRNA (miRNA) post-transcriptional regulation. In this mechanism, SNP alleles can either destroy or create new target sites, if located at the seed region of miRNAs or the target sequence of regulated genes, as well as regulate the levels of expression of the miRNAs themselves.

In this study, we evaluated the contribution of allele-specific miRNA regulation to BC risk. We screened the effect of 223 published GWAS-significant SNPs (and their proxies) on differential miRNA-regulation. We filtered these SNPs based on 1) location in miRNA genes and/or miRNA target sequences, and 2) previous evidence of differential allelic expression (DAE), a hallmark of cis-regulatory variation. Selected SNPs were then screened using the TargetScan algorithm, modified to analyse sequences carrying SNP alleles. Finally, results were shortlisted for miRNAs with evidence of expression in breast tissue or BC.

Interestingly, none of the SNPs mapped to miRNA genes, thus suggesting that miRNA biogenesis and creation/destruction of miRNA-mRNA binding via seed sequence alterations are mechanisms not likely to be involved in BC risk. Of the SNPs located in 3' UTRs, we found 37 out of 3911 (tag&proxy) that were predicted to alter the miRNA-mRNA binding stability in 16 genes. The top result was found for the specific binding of miR-21-3p to the G allele of rs6884232, located in the 3'UTR of ATG10. Functional studies were carried using a dual-luciferase system, with constructs carrying either the A or the G allele, and in combination with miRNA mimics and inhibitors, but no allele-specific differences in luciferase activity were observed. Nevertheless, from our predictions we found rs4245739 in MDM4 and rs11540855 in ABHD8, already functionally validated to cause allele-specific miRNA binding.

To our knowledge, this is the first study looking into the global role of miRNA regulation in BC risk, further improved by the integration of DAE data from normal breast samples.

OC11 | Cancer Genetics

IRES-dependent translation of shorter p53 isoforms is affected by mutations in p53

Lacerda R.[1, 2], Neves A. R.[1, 2], Maruo M. [3], Romão L.[1, 2], Matsuda M.[4], Candeias M. M. [1, 3]

[1]Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal, [2]Center for Biodiversity, Functional and Integrative Genomics, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, [3]Molecular and RNA Cancer Unit, Department of Anatomy and Developmental Biology, Graduate School of Medicine Kyoto University, Kyoto, Japan, [4]Laboratory of Bioimaging and Cell Signaling, Graduate School of Biostudies Kyoto University, Kyoto, Japan

Full-length p53 (FLp53) is a tumour suppressor protein that has been considered a master regulator of many cellular functions. Several isoforms have been described for p53 so far and some of the functions of shorter p53 isoforms have been elucidated and they are different from and complement FLp53 activity. p53 is the most commonly mutated gene in cancer and depending on its mutation status p53 may act as a tumour suppressor or a proto-oncogene. Recently, we have shown that the most common p53 cancer mutants express a larger number and higher levels of shorter p53 protein isoforms that are translated from the mutated FLp53 mRNA (Candeias et al. EMBO R. 2016). Also, we found that cells expressing these shorter p53 isoforms exhibit mutant p53 'gain-of-function' cancer phenotypes, such as enhanced cell survival, proliferation, invasion and adhesion, altered mammary tissue architecture and invasive cell structures. Here, we found that some of these mutations affect the function of an Internal Ribosome Entry Site (IRES) in p53 mRNA. We investigated which mutations influence - by altering IRES structure and function - IRES-dependent translation of shorter p53 isoforms and to what extent this may lead to the onset or progression of some types of tumours.

OC12 | Molecular Genetics

Mutation analysis of the PAH gene in phenylketonuria patients from Rio de Janeiro, Southeast Brazil

Vieira Neto E.[1,2]; Laranjeira F.[3]; Quelhas D.[3]; Seabra A.[3]; Mineiro N.[3]; Carvalho L.M.[4]; Lacerda L.[3]; Ribeiro M.G.[2]

[1]Agência Nacional de Saúde Suplementar, Brasil, [2]Instituto de Puericultura e Pediatria Martagão Gesteira, Universidade Federal do Rio de Janeiro, Brasil, [3]Centro de Genética Médica Jacinto de Magalhães, Centro Hospitalar do Porto, Portugal, [4]Instituto de Diabetes e Endocrinologia Luiz Capriglione, Rio de Janeiro, Brasil

Introduction: Phenylketonuria (PKU) is an autosomal recessive disease resulting from more than 600 mutations in the PAH gene. Most of the patients are compound heterozygotes, and the combination of mutations is a major factor in determining the phenotypic differences found among them. The mutational spectrum of PKU in the state of Rio de Janeiro is unknown so far. Methods: This study included 102 PKU patients from Rio de Janeiro. The project was approved by the National Research Ethics Commission of Brazil. Informed consent was obtained from patients and parents of minors. Dried blood spot samples were collected from patients in Rio de Janeiro and sent by air transport to Portugal. Genomic DNA was extracted from samples. The 13 exons and exon-intron junctions of the PAH gene were amplified by PCR and sequenced. Results: It was possible to detect 195 (95.6%) of the possibly mutant alleles. Nine (8.8%) patients were homozygous and 86 (84.3%) compound heterozygous. The spectrum included 36 causative mutations, of which one has not been described hitherto - p.G312C, found in a pair of siblings. Missense, nonsense, and splicing variants corresponded to 64.6%, 3.1% and 22.6% of the mutant alleles, respectively. Inframe (5.6%) and frameshift (4.1%) small deletions comprised the remainder. The most frequent mutations were: p.V388M (12.7%), p.R261Q (11.8%), IVS10-11G>A (10.3%), IVS2+5G>C (6.4%), p.R252W (5.4%), p.S349P (5.4%), p.I65T (4.4%), and p.T323del (4.4%). Discussion: Rio de Janeiro's population has a tri-hybrid contribution of Africans, Amerindians, and Europeans, mainly of Portuguese ancestry. Apparently most of the PAH gene causative mutations are of Portuguese origin. Five of the most frequent mutations in our study (44.6% of the alleles) are also common in South and Southeast Brazil and in Portugal: p.V388M, p.R261Q, IVS10-11G>A, p.R252W, and p.I65T. Two frequent mutations, p.S349P (5.4%) and p.T323del (4.4%), associated with haplotypes 4.3 and 1.7/1.8, respectively, not previously described in Brazil, have been observed in rare patients from South Portugal. The mutation IVS2+5G>C (6.4%), associated with haplotype 5.9, has been reported in other Southeast Brazilian states. This mutation has not been reported in Portugal or Spain, but is frequent in Middle East and Eastern European populations. The PAH mutational spectrum found in Rio de Janeiro's PKU population reflects the state history, with genetic drift and founder effect playing important roles.

OC13 | Molecular Genetics

Development of an antisense-mediated exon skipping therapeutic strategy to correct a frequent causing mutation in Mucolipidosis II

Matos L[1], Vilela R[1,2], Coutinho MF[1], Gaspar P[3], Prata MJ[2,4], Alves S[1]

[1]Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal; [2]Biology department, Faculty of Sciences, University of Porto, Portugal; [3]Newborn Screening, Metabolism and Genetics Unit, Department of Human Genetics, INSA, Porto, Portugal; [4]i3S – Health research and innovation institute, University of Porto, Portugal

Lysosomal Storage Disorders (LSDs) are a group of rare inherited diseases caused by the malfunction of the lysosomal system, resulting in the accumulation of undegraded substrates inside the lysosomes and leading to severe and progressive pathology. Among them is Mucolipidosis II (ML II), one of most severe LSDs, which is caused by the total or near total deficiency of the GlcNAc-phosphotransferase, a key enzyme for the correct trafficking of lysosomal hydrolases to the lysosome. GlcNAc-phosphotransferase is a multimeric enzyme and is encoded by two genes: GNPTAB and GNPTG. One of the most frequent ML II causal mutations is a dinucleotide deletion on exon 19 of the GNPTAB gene that disrupts the reading frame and prevents the production of an active GlcNAc-phosphotransferase, which in turn impairs the proper targeting of lysosomal enzymes. Despite broad understanding of the molecular causes behind this and other LSDs, the same progress has not been observed in the development of new therapies, with current treatments still mostly symptomatic and presenting several limitations. Therefore, alternative options should be investigated in order to provide patients and families with better healthcare and more promising therapies. One possibility is the modulation of splicing by antisense oligonucleotides (AOs) with the purpose of altering the splicing pattern, the mature mRNA and ultimately the final protein product. Acknowledging this, the present study intends to design and develop a RNA-based therapeutic agent through the use of AOs capable of inducing the skipping of exon 19 of the GNPTAB gene and consequently circumvent the effects of the most common ML II causal mutation. The approach is presently ongoing and different 2'O-Methyl AOs were designed and synthesized to target the GNPTAB exon 19 and promote its skipping. We have already succeeded in inducing the skipping of exon 19 in control and ML II patient fibroblasts. At biochemical level, 48 hours following transfection, enzyme activity suffered a small increase in patients fibroblasts for all enzymes tested, even if the results are still much lower than the observed for healthy controls. In conclusion, this work constitutes a proof of principle for correcting in vitro the defects caused by a frequent ML II mutation with antisense therapy.

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OC14 | Molecular Genetics

ENDOTHELIAL FACTORS AND STROKE RISK IN PEDIATRIC SICKLE CELL ANEMIA PATIENTS: INSIGHTS FROM VCAM1 AND ITGA4 VARIANTS

Silva M.[1], Vargas S.[1], Coelho A.[1], Mendonça J.[1], Vieira L.[1,2], Kjöllerström P.[3], Maia R.[3], Silva R.[4], Dias A.[5], Ferreira T.[5], Morais A.[6], Mota Soares I.[7], Lavinha J.[1,8] and Faustino P.[1,9]

[1]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa; [2]ToxOmics, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa; [3]Unidade de Hematologia, Hospital de Dona Estefânia, CHLC, Lisboa; [4]Unidade de Neuropediatria, Hospital de Dona Estefânia, CHLC, Lisboa; [5]Departamento de Pediatria, Núcleo de Hematologia, Hospital Prof. Doutor Fernando da Fonseca, Amadora; [6]Departamento de Pediatria, Hospital de Santa Maria, CHLN, Lisboa; [7]Departamento de Pediatria, Hospital Garcia de Orta, Almada; [8]BioISI, Faculdade de Ciências, Universidade de Lisboa, Lisboa; [9]ISAMB, Faculdade de Medicina, Universidade de Lisboa, Lisboa; Portugal.

Sickle cell anemia (SCA) arises from homozygosity for the mutation c.20A>T in the HBB gene which originates hemoglobin S (HbS). In hypoxic conditions, HbS polymerizes inside erythrocytes deforming them and ultimately leading to hemolysis and vaso-occlusion. SCA shows a multifactorial-like behaviour with a high heterogeneity of clinical features, with stroke being the most severe of them. This heterogeneity may arise from underlying genetic modifiers, namely those affecting vascular adhesion/endothelial dysfunction. These include genes encoding the VCAM-1 molecule and its ligand VLA-4 (ITGA4 or integrin α4), increasingly studied due to their expression in activated human endothelium and leucocytes/stress reticulocytes, respectively. The aim of this study was to identify putative genetic modulators of stroke risk by analyzing 70 pediatric SCA patients, grouped according to their degree of cerebral vasculopathy. Molecular analysis was performed using Next-Generation Sequencing (NGS) and Sanger Sequencing. R software was used for statistical analyses and association studies. In silico studies were performed using PHASE, TFbind, PROMO and Human Splicing Finder software tools. We identified six different VCAM1 promoter variants and seven haplotypes. The VCAM1 promoter rs1409419_T allele was associated with stroke events (p=0.008; O.R.= 4.33; C.I.95% =1.391-14.257), while one VCAM1 promoter haplotype was found to be protective of stroke (p=0.011; O.R.=0.22; C.I.95% =0.048-0.784). On the ITGA4 gene, forty variants were found, six of them novel. All patients presented with at least one variant in this gene. We observed co-inheritance of specific sets of ITGA4 variants indicating the presence of haplotypes not previously described. Additionally the presence of specific variants seems to result in a predisposition for either high reticulocyte count, elevated lactate dehydrogenase, raised bilirubin levels or increased transcranial Doppler velocity values. Our results reinforce the role of endothelial molecules and blood cell interaction in SCA severity. The association between specific VCAM1, as well as ITGA4, variants with certain cerebral vasculopathy predictors, further enhances their putative modulating effect on pediatric stroke severity and prognosis. These findings provide additional clues on the SCA pathophysiology and uncover features of both genes that may prove to be crucial as potential therapeutic targets.

OC15 | Molecular Genetics

Behind the curtain: unveiling DIS3L2 role in NMD and human cancer

da Costa P.J. [1,2]; Saramago M. [3]; Viegas S.C. [3]; Arraiano C [3]; Romão L. [1,2]

[1]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal, [2]Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, [3]Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade Nova de Lisboa, Oeiras, Portugal

Nonsense-mediated mRNA decay (NMD) is a surveillance mechanism that targets and degrades mRNAs carrying premature translation-termination codons (PTCs), preventing the production of truncated proteins potentially harmful for the cells. In addition to this, several studies have shown that NMD regulates the levels of many physiological mRNAs that encode full-length proteins. Nevertheless, NMD is inhibited in tumor microenvironment and (de)regulates oncogenes and tumor suppressors in several types of cancer. In humans, the mRNA degradation pathways involve exonuclease proteins, such as the DIS3-like protein family (DIS3, DIS3L1 and DIS3L2); however, it is not known whether these proteins are involved in NMD. In order to unveil the role of DIS3L2 in NMD, we performed its knockdown, by RNA interference, in HeLa cells and measured, by RT-qPCR, the mRNA levels and half-lives of various natural NMD targets. Our results show that some NMD targets are highly stabilized in DIS3L2-depleted cells. In addition, mRNA half-life analysis indicates that these NMD targets are in fact direct DIS3L2 substrates. By performing DIS3L2, TUT4 and TUT7 triple knockdown, we also observed that DIS3L2-mediated decay depends on the terminal uridylyl transferases (TUTases) Zcchc6/11 (TUT7/4) activity. Among the NMD targets regulated by DIS3L2, we highlight GADD45A. GADD45A is involved in cell cycle arrest, DNA damage response and apoptotic process. Furthermore, GADD45A deregulation is associated with several types of cancer, such as, esophageal, lung, bladder and pancreatic. Together, our findings establish the role of DIS3L2 and uridylation in NMD and in the regulation of oncogenes and tumor suppressor gene expression. These results might be highly relevant for the advance in diagnosis, prognosis and treatment of many human cancers.

OC16 | Neurogenetics

Role of RNA binding proteins in Polyglutamine diseases

Tomé S.[1]; Marcelo A.[2,3,4]; Ferreira M.[1]; Koppenol R.[2]; Santos M.[2]; Nunes C.[2,3]; Pereira de Almeida L.[1,5]; Nóbrega C.[1,2,3,4]

[1]Center for Neuroscience and Cell Biology (CNC), University of Coimbra; [2]Center for Biomedical Research (CBMR), University of Algarve; [3]Department of Biomedical Science and Medicine (DCBM), University of Algarve; [4]Algarve Biomedical Center (ABC), University of Algarve and Hospital Center of Algarve; [5]Faculty of Pharmacy, University of Coimbra

The Polyglutamine (polyQ) diseases are neurodegenerative disorders, caused by an abnormal expansion of CAG trinucleotide repeats in the causative genes, resulting in an expanded polyglutamine tract in the translated proteins. Machado-Joseph disease (MJD) and spinocerebellar ataxia type 2 (SCA2) are two types of these diseases whose mutated protein is ataxin-3 and ataxin-2, respectively. This mutation results into a severe phenotype mainly characterized by progressive ataxia in both cases, and currently there are no effective therapies to delay or stop disease progression. Although several molecular processes, such as protein aggregation, proteolytic cleavage, mitochondrial dysfunction, have been proposed, there is still some controversy regarding the mechanisms that trigger the pathogenesis of these disorders. Stress granules (SGs) are cytoplasmic structures that assemble under stress conditions in eukaryotic cell, recruiting several components to its formation, including the ataxin-2 protein. The SGs assembly process is regulated by several RNA-binding proteins (RBPs). RBPs are widely expressed in the brain being involved in several biological activities. They are also part of neuronal RNA granules, which are particles that contain in its structure several translation molecules and are responsible for mRNA transport. Therefore, RBPs have an important function in modulating mRNA transport and translation levels, suggesting them as a possible target for neurodegenerative disorders. Previously, we have shown that an RBP modulation might be used as therapeutic strategy for polyQ diseases. In the present work we aim to investigate the role of RBPs in polyglutamine diseases, specifically SCA2 and MJD. We observed that by increasing the expression of a specific RBP, we were able to reduce mutant ataxin-2 and ataxin-3 levels as well as reduce global protein translation levels. Moreover, we found that the RBP overexpression significantly decreases the aggregation of mutant ataxin-3 in a cellular model of MJD. Importantly, our in vivo experiments revealed that overexpression of the RBP significantly reduces the number of mutant ataxin-3 aggregates as well as the loss of neuronal markers in the striatum of a lentiviral mouse model of MJD. Overall, this study suggests that RBPs could be a potential target for the treatment of polyglutamine disorders.

OC17 | Neurogenetics

Exploring genetic risk factors for Alzheimer's and Parkinson's diseases as modifiers of age-at-onset in familial amyloid polyneuropathy (TTR-FAP Val30Met) patients

Abreu-Silva J.[1,2]; Santos D.[1,3]; Coelho T.[4]; Alves-Ferreira M.[1,3]; Conceição Pereira M.[1,3]; Sequeiros J.[1,3,5]; Alonso I.[1,3,5]; Sousa A.[1,3]; Lemos C.[1,3]

[1]UnIGENe, IBMC—Instituto de Biologia Molecular e Celular, i3S—Instituto de Investigação e Inovação, Universidade do Porto, Portugal; [2]FMUP—Faculdade de Medicina da Universidade do Porto, Portugal; [3]ICBAS—Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal; [4]UCA, Centro Hospitalar do Porto (CHP), Portugal; [5]CGPP, IBMC—Instituto de Biologia Molecular e Celular, i3S—Instituto de Investigação e Inovação, Universidade do Porto, Portugal

Introduction: Familial amyloid polyneuropathy (FAP) is an autosomal dominant systemic amyloidosis, rare worldwide but with the largest TTR-FAP Val30Met cluster in Portugal. As an increasing number of late-onset cases (≥50y) and aged-asymptomatic carriers have been ascertained, a wider variation in age-at-onset (AO) [19-82y] was uncovered. Genetic anticipation was found to be a true biological phenomenon. However, many factors and mechanisms that influence TTR-FAP phenotypic variability remain unknown. APOE Ɛ4 has been associated with late-onset familial and sporadic Alzheimer's disease, whereas Ɛ2 allele seems to have a protective effect. GBA variants have been established as the commonest genetic risk factor for Parkinson's disease. Methods: A retrospective family-based study was conducted in 319 patients and relatives (120 families of Portuguese ancestry) to evaluate whether functionally-significant APOE variants have a modifier effect in mean AO, and to explore APOE-GBA interactions among APOE Ɛ2 and Ɛ4 carriers using bioinformatics tools. Generalized estimating equations (GEE) were used to account for correlated AO within families. Results and Discussion: Mean AO was 39y, higher in females (40.71y vs. 37.35y in men), as previously described. Sequencing of APOE exonic and flanking intronic regions revealed the presence of 16 variants, 11 of which were statistically significant associated with an earlier-onset, corresponding to a decrease of approximately 4 to 13 years in mean AO. Moreover, differences in mean AO were not found to be significantly associated with the presence of an APOE Ɛ2 allele nor a Ɛ4 allele. A novel non-synonymous variant was identified in APOE exon 4, predicted as probably damaging by PROVEAN, SIFT and PolyPhen-2 scores and hypothesized to influence the lipid-binding properties and/or result in a potential electrostatic domain interaction with C-terminal residues. A total of six GBA variants (one novel non-coding) were detected, and a strong synergistic interaction between APOE-GBA was observed in the MDR analysis (p=0.015). Rare APOE variants may act as potential genetic modifiers of AO variation, leading to an earlier-onset of TTR-FAP symptoms. The contribution of rare variants to the increase of disease susceptibility is a matter of considerable debate. These results can open new perspectives into TTR-FAP patients' stratification and provide further insight into the mechanisms underlying common neurodegenerative disorders.

OC18 | Neurogenetics

Regulatory RNA genes are targeted by Copy Number Variation in Autism Spectrum Disorder

Marques A.R.[1,2]; Martiniano H.[1,2,3]; Santos J.X.[1,2]; Vilela J.[1,2]; Asif M.[1,2]; Oliveira G.[4,5]; Romão L.[2,6]; Vicente A.M.[1,2,7]

[1]Departamento de Promoção da Saúde e Doenças não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [2]BioISI—Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; [3]Departamento de Informática, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; [4]Unidade de Neurodesenvolvimento e Autismo (UNDA), Serviço do Centro de Desenvolvimento da Criança, Centro de Investigação e Formação Clínica, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; [5]Institute for Biomedical Imaging and Life Sciences, Faculty of Medicine, Universidade de Coimbra, Coimbra, Portugal; [6]Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; [7]Instituto Gulbenkian de Ciência, Oeiras, Portugal

Introduction: Autism Spectrum Disorder (ASD) is a highly heterogeneous neurodevelopmental disorder with an unclear etiology. Genetic factors are estimated to account for 50 to 80% of the familial ASD risk, but most of the genetic determinants are still not known and a role for epigenetic factors is likely. The involvement of noncoding RNAs in ASD is, so far, insufficiently explored. MicroRNA (miRNA) and long noncoding RNA (lncRNA) are regulatory molecules, abundantly expressed in the brain, that play an important role during early stages of neural development. In this work we sought to address their potential role as ASD candidates, by identifying Copy Number Variants (CNVs) targeting miRNA and lncRNA genes in a large cohort of ASD patients, and examined their target genes and biological pathways.

Methods: We compared the frequency of miRNA and lncRNA genes targeted by CNVs in a cohort of 2446 ASD subjects and 9649 control subjects with the same ancestries. Genetic data from ASD patients was obtained from the Autism Genome Project (AGP) and the control group from the Database of Genomic Variant (DGV). Cases and controls were genotyped using the same platform. AGP data was transformed to hg19 annotation followed by functional annotation using the most recent dataset from MIRBASE and LINCPEDIA. Statistical analysis was performed using Fisher's exact test followed by Bonferroni correction (p-value<0.05).

Results: We found 9 miRNAs exclusively targeted by CNVs in ASD subjects and 7 miRNAs more frequently targeted by CNVs in ASD subjects, when compared to controls. Two miRNA were previously associated with ASD in serum miRNA profiling studies. Interestingly, we identified 5 novel miRNAs that were previously described to be involved in schizophrenia, a disorder that presents some phenotypic overlap with ASD. Putative targets of these 16 miRNAs were enriched for ASD risk genes described in SFARI database. Gene enrichment analysis indicates that these genes are involved in neurodevelopmental processes, which is consistent with literature. In addition, we also found 102 novel lncRNAs more frequently targeted by CNVs in ASD.

Discussion: These results support our hypothesis that genetic variants targeting noncoding regulatory RNAs are involved in ASD pathophysiology. This innovative approach may eventually allow the identification of novel biological processes, biomarkers and drug targets for ASD, which can contribute to a better diagnosis and treatment.

Casos Clínicos

Clinical Cases

21.ª Reunião Anual da Sociedade Portuguesa de Genética Humana

Session I	Saturday, 18, 9:00	CC1–CC10

CC1 | Clinical Genetics

Severe familial exudative vitreoretinopathy caused by a novel homozygous variant in TSPAN12 gene

Carvalho AL.[1]; Paiva C.[2]; Monteiro M.[3]; Venâncio M.[4]; Saraiva JM.[5]

[1]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; University Clinic of Genetics, Faculty of Medicine, University of Coimbra, Portugal [2]Ophthalmology Unit, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal [3]Ophthalmology Unit, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal [4]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; University Clinic of Genetics, Faculty of Medicine, University of Coimbra, Portugal 5. Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra, Portugal

Introduction: Familial exudative vitreoretinopathy (FEVR) is a rare inherited disorder of a developmental anomaly of the retinal vasculature that impairs vision and causes retinal detachment. This condition is genetically heterogeneous with various patterns of inheritance, depending on the type of variant and the affected gene.
Cinical Report: We report one family with multiple individuals with severe, bilateral visual impairment from infancy. All affected individuals are born to consanguineous parents belonging to one large Iberian Romani family. Clinical evaluation was performed on two affected sisters from this family (both had clinical presentation of FEVR), and also their healthy parents and brother. Whole exome sequencing identified a novel homozygous missense variant in TSPAN12 gene, c.175T>G (p.Y59D), that segregated with the ocular disease in the family. Ocular observation of heterozygous individuals was normal, with any evidence of subclinical symptoms.
Discussion: The TSPAN12 gene was previously reported to cause autosomal dominant and less frequently autosomal recessive FEVR, usually associated with a more severe phenotype. FEVR phenotype has been associated with an allelic dosage, with even eye manifestations in heterozygous family members with autosomal recessive pattern. This is the second family reported with normal eye evaluation in heterozygous and severely affected homozygous.

CC2 | Clinical Genetics

Bain type syndromic X-linked neurodevelopment disorder - clinical report of a new patient

Soares A.R.[1], Fortuna A.M.[1,2], Soares G.[1]

[1]Department of Medical Genetics, Centro de Genética Médica Dr. Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal [2]Unit for Multidisciplinary Research in Biomedicine, Instituto de Ciências Biomédicas Abel Salazar/Universidade do Porto, Porto, Portugal

Introduction: Exome study sequencing has been a useful technique for genetic study of patients with neurodevelopmental disorders and nonspecific dysmorphisms. From the beginning of its use in clinical practice, many genes have been discovered to be associated with disease. HNRNPH2 gene has been recently described in the literature as being associated with syndromic intellectual disability. Six females with de novo missense variants in this gene have been reported by Bain and collaborators. With this work, the authors aim to present a female patient whose clinical exome allowed us to identify a new case of this rare disease.

Clinical report: A two years-old girl, only child of nonconsanguineous healthy parents, was referred to our consultation due to developmental delay and hypotonia. In the family history, her only maternal aunt has epilepsy and psychosocial problems. During pregnancy, late fetal growth restriction and threatened preterm labor were diagnosed. She was born at 39 gestational weeks with adequate somatometry and no perinatal complications. She evolved in percentile 5 for weight and length and percentile 25 for head circumference, revealed mild global developmental delay with hypotonia and had recurrent upper air tract and gastrointestinal infections. On physical examination, she presented with mild facial dysmorphisms, short neck, anteriorly placed anus, bilateral 2nd toe clinodactyly and generalized hypotonia with postural kyphosis. Studies performed with normal results included: brain MRI, metabolic study, karyotype, Fragile-X molecular study and array-CGH. Clinical exome revealed the c.616C>T(p.Arg206Trp) variant in heterozygosity in the HNRNPH2 gene. This variant has already been described in the literature and occurred de novo in this patient. A second pregnancy was ongoing at this time, and it was possible to offer a prenatal diagnosis for this disease, which revealed an unaffected female fetus.

Discussion: This case highlights how clinical exome can be a fast diagnostic tool for patients with nonspecific neurodevelopmental disorders of unknown etiology, mainly by allowing an answer to parents' anxiety and the possibility for prenatal diagnosis. It is important to remember that a careful pre and post test genetic counselling is essential for this type of study.

CC3 | Clinical Genetics

Clinical and radiological characterization of EXTL3- related skeletal phenotype

Sousa S.B.[1,2,3], Hall C.M[4], Dias P.[5], Medeira A.[5], Mendoza-Londono R.[6], Davies E.G.[3,7], Oud M.M.[8], Tuijnenburg P.[9,10], Hempel M.[11], Stabej P.[3], Lessel D.[11], Arts H.[8,12], Kuijpers T.[9]

[1]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; [2]Faculty of Medicine, University of Coimbra, Portugal; [3]GOSgene, Great Ormond Street Hospital - Institute of Child Health, London, UK; [4]Emerita, Department of Radiology, Great Ormond Street Hospital, London, UK; [5]Serviço de Genética, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal; [6]Division of Clinical and Metabolic Genetics, Department of Paediatrics, The Hospital for Sick Children and University of Toronto, Toronto, Canada; [7]Department of Immunology, Great Ormond Street Hospital, London, UK [8]Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Centre, Nijmegen, the Netherlands [9]Department of Experimental Immunology, Academic Medical Centre, Amsterdam, the Netherlands; [10]Department of Pediatric Hematology, Immunology, and Infectious disease, Academic Medical Centre, Amsterdam, the Netherlands [11]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany [12]Department of Pathology and Molecular Medicine, McMaster University Medical Centre, Hamilton, Canada

Biallelic EXTL3 mutations were recently described as causative of a complex phenotype characterized by specific spondyloepimetaphyseal dysplasia often associated with variable degrees of immune deficiency, liver cysts and developmental delay. This gene encodes for a member of the exostosin family of glycosyltransferases involved in the biosynthesis of the glycosaminoglycans. Recently, three independent studies have described altogether 14 patients, including 9 individuals from 5 unrelated families from our study. Altogether six different hypomorphic missense mutations, as some are recurrent, have been reported. Our presentation focuses on the skeletal phenotype, the only feature present in all patients, and the results of one of above mentioned studies in which we participated. We will describe in detail the natural history and long-term clinical and radiological progression of three affected individuals from a Portuguese family, two of whom are adults, homozygous for the p.P461L variant, one of the recurrent mutations. These findings will further be compared to other reported cases and the main skeletal features and potential complications will be summarized. In more detail, most patients develop a severe kyphoscoliosis at the thoracolumbar junction associated with moderate to severe platyspondyly, with wide intervertebral spaces and vertebral bodies with some posterior constriction, more evident in the early years. Cervical vertebral malformations, including hypoplastic odontoid peg, instability, and spinal cord compression; were reported in 8 patients. Pectus excavatum and rib deformity are common. The pelvic radiographs show coxa valga, small capital femoral epiphyses and variable degrees of sloping acetabula with shallow lateral notches. Patients often have limited elbow mobility, with dislocated and dysplastic radial heads on the radiographs. The fifth middle phalanges are frequently short or absent and there is a delay in carpal bone ossification up to the age of six years. On the opposite end of the EXTL3-phenotypic spectrum, we will briefly describe a Turkish family with two affected brothers in which the clinical picture is mainly an epileptic encephalopathy associated with immunodeficiency and minor skeletal problems. In contrast to the skeletal findings, not all patients developed immunodeficiency (9/14) nor seizures (6/14), not even the ones bearing the identical missense mutation.

CC4 | Clinical Genetics

Osteogenesis imperfecta type VI: report of two cases due to a novel homozygous SERPINF1 mutation

Travessa A.M.[1]; Dias P.[1]; Aza-Carmona M.[2]; Moldovan O.[1]; Godinho F.[3]; Almeida S.[4]; Duarte C.[4]; Cassiano-Neves M.[5]; Heath K.E.[2]; Sousa A.B.[1]

[1]Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal; [2]Institute of Medical and Molecular Genetics (INGEMM), IdiPAZ and Skeletal Dysplasia Multidisciplinary Unit, Hospital Universitario La Paz, UAM, Madrid, Spain and CIBERER, ISCIII, Madrid, Spain; [3]Serviço de Reumatologia, Hospital Garcia de Orta, Almada, Portugal; [4]Serviço de Medicina Física e de Reabilitação, Hospital Garcia de Orta, Almada, Portugal; [5]Unidade de Ortopedia da Criança e do Adolescente, Hospital CUF Descobertas, Portugal.

Introduction: Osteogenesis imperfecta (OI) is a skeletal dysplasia characterized by recurrent fragile fractures and deformities of the bones. The majority of OI cases are due to dominant mutations in collagen type I genes (COL1A1 and COL1A2). However, mutations in several other genes, codifying for proteins involved in the intracellular and extracellular processing of collagen type I and the regulation of bone formation, account for about 15% of OI cases, mostly with autosomal recessive inheritance. In 2002, Glorieux et al. recognized a special type of OI, which they designated OI type VI, characterized by the accumulation of osteoid due to a mineralization defect. The latter was shown to be caused by biallelic mutations in SERPINF1, coding for pigmented epithelium-derived factor, a protein that interacts with RANKL receptor increasing osteoclast activity. We report two independent cases of OI type VI and detail their clinical and radiological features. Clinical Report: The patients are a 13-year-old girl and a 24-year-old man. There is no acknowledged consanguinity but both families originate from the same region. The first fractures occurred at the age of 2 and 1 year, respectively. They both sustained more than 100 fractures and were submitted to multiple orthopedic surgeries, including scoliosis correction surgery in the girl. No relevant extra-skeletal problems were present. Both patients lost ambulation during childhood, and the girl is currently on opioids for chronic pain. Both were treated with bisphosphonates, suspended in the girl due to thrombocytosis. On observation, they have disproportionate short stature, rhizomelic limb shortening, severe curvature of the spine, long bone deformities, white sclera and normal teeth. Radiological evaluation showed signs of previous fractures, thin and deformed long bones, metaphyseal flaring, and severe vertebral compression fractures. The man also presented popcorn calcifications. A customized NGS skeletal dysplasia panel (SKELETAL.SEQV6) identified a novel homozygous pathogenic mutation c.815_818del (p.Met272Ilefs*8) in SERPINF1 in both patients. Discussion: We describe the first cases of OI type VI in Portugal underscoring the importance of gene panel approaches in the molecular diagnosis of OI. This result bears clinical relevance because a poor response to bisphosphonates, and a possibly good response to denosumab, is described in OI type VI. It is hoped that in the future therapy can be tailored according to the specific gene defect causing OI.

CC5 | Clinical Genetics

Camurati-Engelmann disease: a case report highlighting the clinical and imagiological features

Travessa A.M.[1]; Dias P.[1]; Aza-Carmona M.[2]; Oliveira-Freixo J.[3]; Heath K.E.[2]; Sousa A.B.[1]

[1]Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal; [2]Institute of Molecular & Medical Genetics (INGEMM), IdiPAZ and Skeletal Dysplasia Multidisciplinary Unit, Hospital Universitario La Paz, UAM, Madrid, Spain and CIBERER, ISCIII, Madrid, Spain; [3]Serviço de Pediatria, Hospital de São Bernardo, Centro Hospital de Setúbal, Setúbal, Portugal.

Camurati-Engelmann disease (CED) is a rare skeletal dysplasia characterized by progressive increase of bone density primarily affecting the diaphyses of the long bones and the skull base, leading to pain, a waddling gait, muscle weakness, anemia and neurological deficits. CED is due to heterozygous mutations in TGFB1, which codes for transforming growth factor β-1 (TGFβ-1). We present a case of CED with asymmetric lower limb findings. A 16-year-old girl was referred for genetic evaluation with suspected CED. Family history was unknown. Pregnancy, delivery and neonatal period were uneventful. She was submitted to surgery to correct an anorectal vestibular fistula at 2y. At 3y she had a distal right tibia fracture as a result of a fall. She was submitted to further surgeries to correct a mitral valve cleft at 8y, and lower limb dysmetria at 11y. At 15y, she presented with right leg pain and edema. On observation at 16y, she had normal somatometry, lower limb dysmetria, normal craniofacial features and no apparent neurological deficits pertaining to the cranial nerves. Cognition was normal. X-rays showed hyperostosis of the cranial vault, skull base and facial bones, long bones, right tibia and fibula. Right tibia biopsy showed partially necrosed bone trabeculae, and right leg CT scan demonstrated diaphyseal cortical thickening of tibia and fibula due to endosteal and periosteal deposition, and thinning medullary cavity. Bone scintigraphy showed mild to moderate hyperfixation in the left femoral, tibial and peroneal diaphyses and intense hyperfixation in the right tibial diaphysis. A customized NGS skeletal dysplasia panel (SKELETAL.SEQV6) identified a heterozygous mutation in TGFB1: c.653G>A (p.Arg218His), which has been previously reported in CED. Reaching a diagnosis was difficult because of the prior history of limb fracture and asymmetric radiological features, in contrast with the more typical symmetry observed in CED. Molecular confirmation of CED is important for clinical management. Reduced penetrance and great clinical variability complicate genetic counseling. Interestingly, the patient had an anorectal malformation and a mitral valve cleft. These features are probably unrelated to CED as CED stigmata seem to be confined to the skeleton. The latter is remarkable considering TGFβ-1 is expressed in several tissues where it performs many cellular functions, and mutations in the TGFβ-1 receptor (TGFBR1) are responsible for Loyes-Dietz syndrome type 1A.

CC6 | Clinical Genetics

Prenatal Diagnosis in the Era of Next Generation Sequencing: Ciliopathies

Carvalho I[1,2], Freixo J[1,2], Oliveira N[2], Martins AT[2], Fernandes R[3], Amorim M[1], Cohen A[2]

[1]Unidade de Genética Médica, Hospital Dona Estefânia, CHLC [2]Centro de Diagnóstico Pré-Natal, Maternidade Dr. Alfredo da Costa, CHLC [3]Serviço de Anatomia Patológica (Fetopatologia), polo Maternidade Alfredo da Costa, CHLC

Introduction: Primary cilia are immotile microtubule-based structures present on the surface of most cells in vertebrates, act as sensors of extracellular signals and play an important role during tissue development. Therefore, defects in cilia function cause multisystem pathology with a broad spectrum of clinically and genetically overlapping phenotypes. Most syndromic forms of ciliopathies are genetically heterogeneous and for, Bardet-Biedl Syndrome (BBS) and Meckel-Gruber Syndrome (MKS) at least 19 and 11 genes have been identified to date, respectively.
Clinical Report: We report 3 cases of prenatal clinical presentation of Ciliopathies. Case 1: 37yo female patient, with 12w4d gestational age, non consanguineous couple; 1st trimester ultrasound revealed posterior encephalocele and bilateral multicystic renal dysplasia. Case 2: 36yo female patient, 5th pregnancy of a healthy non consanguineous couple that at 12w3d of gestation the fetus presented with occipital encephalocele, bilateral renal dysplasia and absent urinary bladder. Previous pregnancy was terminated due to fetal ultrasound anomalies. Case 3: 29yo female patient, healthy non consanguineous couple, previous pregnancy terminated due to fetal malformations (micrognathia, polydactyly, renal dysplasia and cardiac defect) with normal microarray. Current pregnancy with normal 1st trimester ultrasound and screening and at 18w2d of gestation the kidneys showed poor corticomedullary differentiation and polydactyly. Invasive prenatal testing was performed in the 3 cases. The molecular diagnosis was established by multigene NGS panel. The MKS gene panel performed in both case 1 and case 2 revealed the presence of a compound heterozigoty and homozigoty for TMEM67 pathogenic variants, respectively. In case 3 a Ciliopathy gene panel analysis revealed the presence of two heterozygous pathogenic variants in the BBS12 gene. In all cases termination of pregnancy was requested. Fetal anatomopathological examination confirmed the prenatal findings in the 3 clinical cases.
Discussion: Ciliopathies are rarely recognised in the fetus but can be appreciated by observing subtle imaging findings in kidneys, brain and extremities. Recognition of one characteristic finding should prompt search for corroborative abnormalities. Since ciliopathies are predominantly autosomal recessive with a recurrence risk of 25%, it is important to recognise antenatal findings in order to establish molecular diagnosis allowing proper genetic counseling to the parents.

CC7 | Clinical Genetics

Sensorial deafness, amelogenesis imperfecta, pigmentary retinopathy and nail abnormalities: a case report of Heimler syndrome with molecular confirmation

Rosmaninho-Salgado J.[1]; Ferreira de Barros J.[2]; Saraiva J.[1,3]. Sousa SB.[1,4]; Ramos F.[1]

[1]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal [2]Maxillofacial Surgery Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal [3]University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra, Portugal [4]University Clinic of Genetics, Faculty of Medicine, University of Coimbra, Portugal

Introduction: Heimler syndrome (HS; #234580) is a rare autosomal recessive disorder characterized by sensorineural hearing loss (SNHL), amelogenesis imperfecta, nail abnormalities, and often retinal dystrophy. Recently, HS was recognized as a discrete phenotype at the mildest end of the Zellweger syndrome spectrum (ZSS) subgroup of peroxisomal biogenesis disorders (PBDs), caused by hypomorphic biallelic mutations in PEX1 or PEX6 genes. The present report constitutes the 8th molecularly confirmed case.
Clinical report: We describe a six-year-old girl with consanguineous parents who presented with a progressive profound bilateral SNHL since the age of 2 years. Subsequently, a progressive decline in vision and also nyctalopia was noticed at the age of 3 years and a pigmentary retinopathy was diagnosed. Dental examination showed amelogenesis imperfecta with severe caries. Nails abnormalities were present, compatible with Beau's line of the toenails. A mild cognitive impairment is also present The diagnosis of HS was suggested and a targeted next-generation sequencing panel for 12 genes associated with PBDs was performed. A homozygous missense variant in PEX1 gene, c.2528G>A(p.Gly843Asp), was found, which was classified as pathogenic as had been previously described in a case with a mild form of ZSS.
Discussion: PEX1 encodes a protein that belongs to the peroxisomal import machinery and mutations in this gene are the most common cause of ZSS disorders. The severity of the phenotype seen in this group of disorders is related to the degree of PEX1 function impairment caused by underlying mutations. The identified missense variant c.2528G>A(p.Gly843Asp) on PEX1 gene fits well the mild phenotype observed in this patient. It had already been shown to be hypomorphic, inducing a minor defect on peroxisomal import, and interestingly not detectable by the usual screening methods for PBDs in clinical practice. The presence of amelogenesis imperfecta represents the main phenotypic feature that differentiates HS from other ZSS spectrum disorders, but the absence of abnormal brain findings or impaired liver function also supports this distinction. In clinical practice, having a molecular confirmation of the clinical and biochemical diagnosis allows a better genotype-based prediction of the prognosis, clinical course of the disease, and management, besides enabling an accurate genetic counselling and prenatal diagnosis.

CC8 | Clinical Genetics

Neurodegenerative Lysosomal Diseases Approached by Next Generation Sequencing

Encarnação M[1*], Coutinho MF[2*], Silva L[1], Ribeiro D[1], Nogueira C[1], Vilarinho L[1], and Alves S[2]
[*]Equally contributed

[1]Unidade de Rastreio Neonatal, Metabolismo e Genética, Departamento de Genética Humana—Instituto Nacional Ricardo Jorge, Porto; [2]Unidade de Investigação e Desenvolvimento, Departamento de Genética Humana—Instituto Nacional Ricardo Jorge, Porto

Introduction: Lysosomal Storage Disorders (LSD) are a heterogenous group of rare, monogenic diseases with significant phenotypic overlap and clinical variability. For this reason, the diagnosis is difficult and time consuming, with multiple tests/samples being often required before a definitive diagnosis is reached. Next Generation Sequencing (NGS) is changing this scenario by allowing the variant assessment at a large scale in a single run. The aim of this work, was to develop an NGS-based workflow for the identification of LSD-causing variants.
Methods: We designed a panel including exons and intronic flanking regions from 96 genes involved in lysosome homeostasis and function. The workflow was performed using an Agilent SureSelect QXT Target Enrichment protocol followed by sequencing in an Illumina MiSeq® platform. For alignment and variant anotation the softwares Surecall and wANNOVAR were used.
Results and Discussion: To adress sensitivity and coverage of this custom-targeted panel, 14 patients were analyzed. In 5 patients, used as positive controls, the disease-causing mutations had been previously identified by Sanger. For the remaining 9, we could reach molecular diagnosis consistent with the clinical and biochemical diagnosis in 5 patients. For the 4 undiagnosed patients (suspicion of LSDs), other NGS approaches are envisaged. From our results we would like to highlight the detection of a novel frameshift mutation in the GM2A gene, which is associated with an extremely rare AB variant of Gangliosidosis, biochemically and clinically undistinguishable from the other two (Tay Sachs and Sandhoff Diseases). Also noteworthy, we were able to detect the molecular defect of a patient with a clinical suspicion of Neuronal Ceroid Lipofuscinosis (CLN). From the 14 possible genes associated to these disorders, we could detect the molecular defect with a single analysis. We detected a novel missense variant in the MFSD8 gene reaching the diagnosis of a CLN7. Additionally, we have also found novel mutations in GLA, ARSB, GALC and NAGLU genes. This NGS panel that we have now available in our department offers a unique testing strategy for the LSD diagnosis, especially those for which biochemical testing is currently unavailable. Besides decreasing the delay in diagnosis for many patients, a precise molecular diagnosis is extremely important as new therapies are becoming available for patients who share specific types of mutations.
Acknowledgments: This work is partially supported by NORTE-46-2015-03 “DESVENDAR – DEScobrir, VENcer as Doenças rARas.

CC9 | Clinical Genetics

Disruption of WDR26 by a translocation breakpoint confirms its causal role in Skraban-Deardorff and 1q41q42 microdeletion syndromes

Freixo, JP[1]; Marques, M[2]; Fino, J[2]; Carvalho, I[1],Talkowsky, ME[3–5]; Morton, C[4–7] David, D[2]

[1]Unidade de Genética Médica, Centro Hospitalar de Lisboa Central, Lisboa; [2]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, INSA IP. Lisboa; [3]Center for Human Genetic Research, Departments of Neurology, Pathology and Psychiatry, Massachusetts General Hospital, Boston, USA; [4]Harvard Medical School, Boston; [5]Broad Institute of MIT and Harvard, Cambridge; [6]Departments of Obstetrics and Gynecology, and of Pathology, Brigham and Women's Hospital, Boston; [7]University of Manchester, Manchester Academic Health Science Center, Manchester, UK

Introduction: Microdeletions or contiguous gene syndromes (CGSs) are characterized by variable complex clinical phenotypes caused by hemizygosity of contiguous genes, defined mainly by a common deletion region, or of a major causal gene locus. Delineation of the pathogenic genes within these CGS regions is a major challenge. Identification of breakpoints at nucleotide resolution of balanced chromosomal rearrangements localized within these regions constitutes a key strategy for definition of the phenotypically important genes. The aim of this study is the identification of molecular alterations responsible for an extremely complex clinical phenotype resembling 1q41q42 microdeletion syndrome (coarse facial features, severe developmental delay, congenital heart disease and congenital microcephaly) presented by an individual with the t(1;3)(q42.11;p25.3)dn.
Methods: Translocation breakpoints were localized by large-insert whole-genome sequencing. Nucleotide-level resolution of the breakpoints was carried out by amplification of the junction fragments and Sanger sequencing.
Results: The 1q42.11 breakpoint disrupts exon 12 of WD repeat domain 26 (WDR26), reported in 2017 as the causative gene of the autosomal dominant Skraban-Deardorff syndrome (SKDEAS, OMIM #617616), with clinical features that almost completely overlap the 1q41q42 microdeletion syndrome. WDR26 is WD40 repeat-containing protein presumably involved in multiple disease-associated signalling pathways. The 3p25.3 breakpoint disrupts IVS 1 of the ATPase, Ca++ transporting, plasma membrane 2 (ATP2B2, OMIM *108733), reported as a modifier of the autosomal recessive deafness-12.
Discussion: The proband's clinical features basically confirm the phenotypical overlaps between SKDEAS and 1q41q42 microdeletion syndromes. Nevertheless, deep phenotyping showed clinical features dissimilar to both syndromes, namely, aggravated congenital heart disease, hyperactivity, enuresis and encopresis. Although genes from both breakpoint regions may well contributed to the observes additional clinical features, surprisingly, their overall contribution seems marginal. In conclusion, disruption of WDR26 by the 1q42.11 breakpoint most likely leads to its haploinsufficiency due to nonsense mediated RNA decay, resulting in an extremely severe complex clinical phenotype basically matching both SKDEAS and 1q41q42 microdeletion syndromes. Therefore, we confirm its major causative role in these phenocopic syndromes.

CC10 | Clinical Genetics

National study on cleidocranial dysplasia - clinical and molecular characterization of 14 Portuguese patients

Fernandes S[1], Machado C[2], Soares AR[3], Aza-Carmona M[4], Oliveira R[5], Louro P[1,6], Ramos L[1,6], Fortuna AM[3,7], Dupont J[2], Saraiva JM[1,8], Karen Heath[4], Sousa SB[1,9]

[1]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; [2]Serviço de Genética Médica, Departamento de Pediatria, Centro Hospitalar Lisboa Norte, Hospital de Santa Maria, EPE, Centro Académico de Medicina de Lisboa, Lisboa, Portugal; [3]Serviço de Genética Médica Dr. Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal; [4]Institute of Medical and Molecular Genetics (INGEMM), IdiPAZ and Skeletal dysplasia multidisciplinary Unit (UMDE), Hospital Universitário La Paz, Madrid, Spain and CIBERER, ISCIII, Madrid, Spain; [5]Serviço de Genética Médica, Centro Hospitalar de São João, Portugal (former worker at Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal); [6]Faculty of Health Sciences, Universidade da Beira Interior, Covilhã, Portugal; [7]UMIB—Unidade Multidisciplinar de Informação Biomédica, Portugal; [8]University Clinic of Pediatrics, Faculty of Medicine, Universidade de Coimbra, Coimbra, Portugal; [9]University Clinic of Genetics, Faculty of Medicine, Universidade de Coimbra, Coimbra, Portugal Correspondance: sfreirefernandes@chuc.min-saude.pt; bsousa@chuc.min-saude.pt

Introduction: Cleidocranial dysplasia (CCD) is an autosomal dominant condition characterized by clavicles' hypo/aplasia, delayed closure of fontanelles, complex dental abnormalities, short stature and other skeletal changes. This condition is caused by RUNX2 heterozygous loss of function mutations in 70% of CCD patients, a gene that encodes a transcription factor involved in osteoblasts differentiation and skeletal morphogenesis. Despite the extensive literature on CCD, specific population data and management guidelines are lacking. We aimed to perform a national study on CCD and here we present the first results. Methods: Clinical and molecular characterization of all cases with CCD observed at 3 Portuguese hospital centres based on retrospective analysis of patient medical records and clinical re-evaluation when indicated. Sanger sequencing of RUNX2 was or is being performed in 11/14 patients, followed by multiplex ligation-dependent probe amplification, MLPA, if negative results. Results: We describe 14 patients, from 12 unrelated families, 8 males and 6 females, with ages between 2 and 46 years at last clinical evaluation. The age of clinical diagnosis in each case ranged between 1 month and 34 years (median 10 years). Four individuals have a clinically affected parent, 2 of them were included in this study. The main reasons for referral were dysmorphic features (6/14), followed by skeletal anomalies (6/14), short stature (3/14), dental anomalies (3/14) and specific suspicion of CCD (2/14). All individuals had typical skeletal and radiographic features (14/14), dental abnormalities were present in 12/12, short stature in 7/14, hand anomalies in 7/10 and scoliosis in 5/12 patients. The main medical interventions required were: dentistry/orthodontics follow-up in all patients (12/12) and orthopaedic surgery due to coxa vara in 3/13. RUNX2 pathogenic mutations were identified in 7/8 families in which results are available (6 missense and 1 nonsense). Discussion: In general, our results are in accordance with the literature. Thus far, we observed a higher rate of complications, in particular coxa vara, and of mutation positive cases than reported. This may indicate a bias towards the diagnosis of the more severe cases. It is widely considered that this dysplasia is underdiagnosed. Detailed description of populational cohorts of CCD patients are crucial for families and health professionals, leading to a better-informed management and surveillance of possible complications.

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P1 | Cancer Genetics

Germline and Somatic ALK Alterations in Neuroblastoma Patients

Rocha A.C.[1,2]; Vieira J.[1,3]; Peixoto A.[1,3]; Pinheiro M.[1]; Santos R.[3]; Lobo J.[4]; Henrique R.[2,4]; Teixeira M.R.[1,2,3]

[1]Cancer Genetics Group, Portuguese Oncology Institute of Porto Research Center (CI-IPOP), Portuguese Oncology Institute of Porto, Porto, Portugal. [2]Department of Pathology and Molecular Immunology, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal. [3]Department of Genetics, Portuguese Oncology Institute of Porto, Porto, Portugal. [4]Department of Pathology, Portuguese Oncology Institute of Porto, Porto, Portugal.

Neuroblastoma (NB) is the most common extracranial solid tumor diagnosed during infancy, accounting for more than 7% of all childhood cancers and being responsible for around 10 to 12% of childhood cancer-related deaths. Recently, somatic and germline alterations in the anaplastic lymphoma kinase (ALK) gene have been identified in a subset of NB patients. Tumors harboring these alterations might be sensitive to ALK inhibitors, which may have therapeutic consequences. To evaluate the pattern of ALK alterations in NB patients, we searched for genomic rearrangements by fluorescence in situ hybridization (FISH) with a break-apart ALK probe in 41 cell suspension tumor samples and for point mutations by Sanger sequencing in 29 fresh-frozen tumor samples. Normal tissue was analyzed from patients presenting ALK mutations in tumor samples from patients diagnosed at IPO-Porto and tested for MYCN amplification at the Department of Genetics of that institution. We found ALK copy number changes in 19 of the 41 cases (46.3%), including one case (2.4%) with ALK amplification, 13 cases (31.7%) with ALK gain, and five cases (12.2%) with ALK loss/imbalance. Moreover, we found four different activating ALK point mutations in five tumors, the c.3824 G>A, p.(Arg1275Gln), the c.3522C>G, p.(Phe1174Leu), the c.3575G>C, p.(Arg1192Pro) and the c.3520T>A, p.(Phen1174Ile). Of these, the mutations c.3824 G>A, p.(Arg1275Gln) and c.3575G>C, p.(Arg1192Pro) were found in the germline of two patients. ALK alterations are a frequent event in NB patients and could be a predictive and prognostic biomarker, as well as a potential therapeutic target in those patients. We also show the importance of understanding which ALK mutations are more likely to predispose to NB tumors, which can help direct appropriate screening in families carrying ALK germline mutations.

P2 | Cancer Genetics

Shorter p53 isoform expression through an Internal Ribosome Entry Site (IRES) in p53 mRNA

Neves, Ana Rita[1,2]; Lacerda, Rafaela[1,2]; Marques-Ramos, Ana[1,2]; Romão, Luísa[1,2]; Matsuda, Michiyuki[3]; Candeias, Marco M.[1,4]

[1]Department of Human Genetics, National Health Institute Doutor Ricardo Jorge, Lisbon, Portugal; [2]Biosystems and Integrative Sciences Institute (BioISI), Faculty of Sciences of the University of Lisbon, Lisbon, Portugal; [3]Laboratory of Bioimaging and Cell Signalling, Graduate School of Biostudies, Kyoto University, Kyoto, Japan; [4]Molecular and RNA Cancer Unit, Department of Anatomy and Developmental Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

The tumour suppressor p53 gene is one of the most studied cancer-related genes. So far, many p53 isoforms have been identified either resulting from alternative splicing, alternative translation or alternative promoter usage. It is known that cap-dependent translation is repressed under stress conditions to preserve energy. Therefore, other translational mechanisms are required to keep the synthesis of stress-response proteins. Internal Ribosome Entry Sites (IRES) were first discovered in viruses, and then observed in eukaryotes, as secondary structures present in RNA that were capable of recruiting ribosomes to the vicinity of an initiation codon inserted in an optimal environment allowing cap-independent translation of mRNAs. Translation of Δ40p53, a p53 isoform, is one example of this non-canonical mechanism due to the presence of an IRES near an alternative initiation codon (AUG40). Here, we will present a new IRES in p53 mRNA, including details on the localization and regulation of this IRES under normal and stress conditions.

P3 | Cancer Genetics

Regulation of the Alternative Splicing of Tumor-Related RAC1b by Signal Transduction Pathways

Gonçalves V.[1,2]; Matos P.[1,2,3]; Pereira JF.[1,2]; Henriques AF.[1,2] and Jordan P.[1,2]

[1]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa; [2]BioISI—Biosystems & Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa; [3]Departmento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa

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 qRT-PCR. 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, which inhibits RAC1b overexpression. Together, our results demonstrate that oncogenic signal transduction pathways deregulate alternative splicing and this may be drug revertable.

P4 | Cancer Genetics

A germline SMAD4 pathogenic variant associated with massive gastric polyposis and young-onset colorectal cancer without colonic polyposis: an unusual presentation

Soeiro e Sá M[1], Dias P[1], Dupont J[1], Vitorino E[2] e Sousa AB[1]

[1]Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal; [2]Serviço de Anatomia Patológica, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal

Introduction: SMAD4 heterozygous loss-of-function mutations are associated with Juvenile Polyposis/Hereditary Hemorrhagic Telangiectasia (JP/HHT), an autosomal dominant condition characterized by multiple gastrointestinal (GI) juvenile polyps with mucocutaneous telangiectasias and arteriovenous malformations in several organs. Although expression is variable, the overwhelming majority of patients have polyps distributed throughout the colon and exclusive gastric involvement is exceptional. Increased cancer risk is mainly associated with colorectal cancer (CRC), with lifetime estimates up to 50% and a mean age at diagnosis of 42 years. We report a family with a pathogenic mutation in SMAD4 presenting with massive gastric polyposis and CRC, with little to none colonic polyps.

Clinical Report: The proband is a 27-year-old female diagnosed with metastatic right-sided intestinal adenocarcinoma of the colon. She underwent right hemicolectomy which revealed, besides the tumor, only three polyps, corresponding to tubular and tubulovillous adenomas with low grade dysplasia. Upper GI endoscopy did not show polyps on her stomach or duodenum. Immunohistochemical staining for DNA mismatch-repair proteins associated with Lynch Syndrome and MUTYH sequencing were normal. Her mother had undergone total gastrectomy at 45 years of age due to persistent iron deficient anemia attributed to the presence of massive gastric polyposis of the fundus and body, histologically indistinguishable between juvenile or hyperplastic polyposis; her colonoscopy was normal. The diagnosis of JP associated with SMAD4 was suspected, and a next-generation sequencing (NGS) multigene panel including several CRC-predisposing genes was performed on the mother's blood, which identified a heterozygous frameshift truncating mutation in SMAD4, previously described as pathogenic. The same variant was identified in the daughter, thus confirming the clinical diagnosis in this family. Further workup is still underway to assess the presence of manifestations of HHT on the mother, which were absent on the daughter.

Discussion: This case illustrates the phenotypic variability of JP associated with SMAD4, particularly due to the lack of colonic polyposis and overt manifestations of HHT. It also shows the importance of considering non-classic presentations on the differential diagnosis of CRC at young age, after exclusion of the most common causes. For this, the availability of NGS gene panels is a clear advantage.

P5 | Cancer Genetics

Loss of the Y chromosome in male patients with Myeloid Neoplasms

Ambrósio A.[1]; Geraldes M.C.[1]; Ventura C.[1]; Furtado J.[1]; Correia H.[1]

[1]Unidade de Citogenética, Departamento de Genética INSA, IP, Lisboa.

Loss of the Y chromosome (L0Y) is describe as a both a normal age - related event and a marker of a neoplastic clone in haematological diseases. In order to understand the relationship between L0Y chromosome and the different myeloid neoplasms, we retrospectively analysed cytogenetic results of 891 males' patients, from 1995 to 2016 with myeloid neoplasms. Sixty one patients showed L0Y. Of the 61 patients without Y chromosome 24 (2,7%) had Myelodysplastic Syndromes (MDS); 24 (2,7%) had Myeloproliferative Neoplasms (MN); 6 (0,67%) had Neoplasm Myelodysplastic Syndrome / Myeloproliferative Neoplasms (MDS/MN) and 7 (0,79%) had Acute Myeloid Leukaemia (AML). These percentages can be different if we consider only the pathology in which was found the L0Y: 7,7% of all patients with MDS (310); 6,1% of all patients with NM (391); 6,6% in the patients with MDS/MN (90) and 7,6% in patients with AML (92). There are few reports of L0Y associated with Myeloid Neoplasms, since this has been considered mainly an age-related event. There for the tendency of L0Y in our data, seems to indicate that careful consideration should be taken when evaluating male patients with L0Y.

P6 | Cancer Genetics

Hepatocellular carcinoma and Cholangiocarcinoma genomic characterization

Tavares I[1], Ribeiro IP[1,2], Martins R, Abrantes M, Botelho F, Tralhão JG, Melo JB[1,2,3], Carreira IM[1,2,3]

[1]Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [2]CIMAGO—Center of Investigation on Environment Genetics and Oncobiology - Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [3]CNC-IBILI—Group of Aging and Brain Diseases: Advanced Diagnosis and Biomarkers, Coimbra, Portugal;

Introduction: Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) are the two most common primary hepatic neoplasms. While HCC arises from hepatocytes, the CC is a rare malignant tumor originating from the epithelial cells of the biliary tree and is commonly classified as intrahepatic and extrahepatic, based on anatomical location. The HCC is the most common primary hepatic tumor, representing 85-90% of all cases. CC represents less than 2% of all human malignant neoplasms and about 3% of all gastrointestinal tumors but it is the second most common primary liver cancer representing 10-25% of all cases. The prognosis of these two types of tumors is usually poor since the diagnosis is not easy to obtain. The aim of this study was to perform a genomic characterization of HCC and intrahepatic (ICC) and extrahepatic (ECC) CC patients.
Methods: The genomic characterization was performed by Array Comparative Genomic Hybridization (aCGH) in 11 HCC, 6 ECC and 7 ICC patients. Results and discussion: The results obtained revealed some common alterations between the patients of each group. Several HCC patients revealed gain of 1q, 2q37.2, 8q, 14q32.33 and 17q21.31 and loss of 3q26.1, 6p22.2 and 12p13.31. Regarding the ICC patients, the most common alterations observed were gain of 2q37.3 and Xp and loss of 3p, 6p25.3, 11q11, 14q, 16q, Yp and Yq. The patients of ECC also revealed some common alterations namely gain of 2q37.3, 6p25.3 and 16p25.3 and loss of 3q26.1, 6p25.3-22.3, 12p13.31, 17p, 18q, Yp. Some of these alterations are also common between patients of these three different groups. These regions contain genes whose alteration may be related to the development of these tumors. The genomic characterization of these patients is important to the study of such tumors since it allows to find potential biomarkers of both diagnosis and prognosis which is essential for achieving an earlier diagnosis and improving treatments.

P7 | Cancer Genetics

MCL1 and COL9A2 as players in recurrence of head and neck tumors

Ribeiro IP[1,2], Esteves L[1], Marques F[2,3], Barroso L[4], Caramelo F[5], Melo JB[1,2], Carreira IM[1,2]

[1]Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, 3000-354 Coimbra, Portugal; [2]CIMAGO—Center of Investigation on Environment Genetics and Oncobiology - Faculty of Medicine, University of Coimbra, 3000-354 Coimbra, Portugal; [3]Department of Dentistry, Faculty of Medicine, University of Coimbra, 3000-075 Coimbra, Portugal; [4]Maxillofacial Surgery Department, Coimbra Hospital and University Centre, CHUC, EPE, 3000-075 Coimbra, Portugal; [5]Laboratory of Biostatistics and Medical Informatics, IBILI—Faculty of Medicine, University of Coimbra, 3000-354 Coimbra, Portugal

Introduction: Head and neck squamous cell carcinoma (HNSCC) presents a high incidence and mortality worldwide. There is an urgent need to develop effective therapeutic approaches to prevent and treat these tumors. The progress of whole-genome technologies has opened new opportunities to explore cancer-associated pathways with therapeutic applications. This study aimed to perform a genome-wide characterization of HNSCC patients and to identify the most common altered signaling pathways and genes related to the different anatomic subsites and stages of the primary tumor as well as with the development of metastasis/relapses.
Methods: The genomic characterization of 102 HNSCC cohort was performed using array comparative genomic hybridization technique, Agilent oligonucleotide microarray 4x180K. Copy number variation data was analyzed using Matlab, R programming language and SPSS. A pathway enrichment analysis was performed, the overrepresented pathways and the respective genes altered in our cohort were determined. The association between gene alteration and staging, location of the primary tumor and metastasis/relapses development was tested.
Results: With this whole genome approach, we detected imbalances in all chromosomes; however, it was possible to verify that the most common deletions and amplifications were observed in specific chromosomal regions. Chromosomes 3, 5, 8 and 11 were the most frequently altered in our cohort. The two most statistically significant pathways associated with the amplified and deleted genes were Cell cycle and PI3K-Akt signaling pathways, and Cytokine-cytokine receptor interaction and Ubiquitin mediated proteolysis signaling pathways, respectively. From the cell cycle and PI3K-Akt signaling pathways we verified statistically significant amplification of MYC and RBL2 genes in the different tumor anatomic subsites. From the PI3K-Akt signaling pathway we found that MCL1 and COL9A2 genes seem to be related to the development of relapse/metastasis (OR> 4,7 and 2,7 respectively).
Discussion: These highlighted pathways are commonly activated in cancer, occurring frequent interactions between the signaling pathways. The correlation between molecular and clinic-pathological data has the power to identify specific genes that could help to understand the disease progression and, consequently that could have prognostic value. Further studies are required to validate the role of these genes and signaling pathways in the tumor evolution and behavior.

P8 | Cancer Genetics

Cytogenetic Characterization of a Cell Line Derived from a Pharyngeal Tumor - Preliminary Data

Marques V[1]; Ribeiro IP[1,2]; Mascarenhas A[1]; Rodrigues JM[1];Lopes-Aguiar L[3]; Costa E[3]; Lima C[3]; Botelho MF[2,4]; Abrantes AM[2,4]; Melo JB[1,2,5]; Carreira IM[1,2,5]

[1]Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [2]CIMAGO—Center of Investigation on Environment, Genetics and Oncobiology—Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [3]Laboratory of Cancer Genetics, Faculty of Medical Sciences, University of Campinas, Campinas, São Paulo, Brazil; [4]Biophysics Unit, IBILI, Faculty of Medicine, University of Coimbra, Coimbra, Portugal;[5]CNC-IBILI—Group of Aging and Brain Diseases: Advanced Diagnosis and Biomarkers, Coimbra, Portugal

Introduction: Pharynx cancer lies within the head and neck malignancies. It tends to grow silently, being the patients usually asymptomatic. Therefore, the diagnosis is frequently made in a late stage, when the disease has a poor prognosis. In addition, pharynx cancer has one of the highest frequencies of metastases among all head and neck cancers. Pharyngeal carcinoma may cause pain, bleeding and impairment of vital functions such as swallowing, breathing and speaking. The characterization of tumor cell lines has been helpful to find genetic alterations that seem to have a major role in cancer development and progression, thus having the potential to identify biomarkers with clinical and therapeutic value. We made a cytogenetic characterization of the FaDu cell line, a human epithelial cell line derived from a squamous cell carcinoma of the hypopharynx.

Materials and Methods: The cell line was cultured in DMEM supplemented with FBS. The characterization was assessed by karyotype.

Results and Discussion: The cytogenetic results showed complex karyotypes with several structural alterations. This cell line is near-triploid, with an average number of 69 chromosomes. We identified several chromosomal rearrangements, including: (1) the formation of isochromosomes such as i(1)(q10), i(5)(p10) and i(8)(q10), which are commonly associated with the carcinogenesis process; (2) all arm translocations involving chromosomes 3, 5, 6, 8 and 13; (3) the presence of a small marker chromosome in all metaphases; and (4) a few reciprocal translocations involving other chromosomes. In addition, three copies of apparently normal chromosomes were frequently observed. These preliminary findings are useful to establish the genetic profile of hypopharynx carcinoma. The use of well-characterized cancer cell lines constitute a powerful tool to improve our understanding of the molecular mechanisms underlying pharyngeal tumors, as well as to provide a research basis for pharmacological studies.

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Hereditary Breast and Ovarian Cancer: When a BRCA2 is missing

Carvalho I[1], Freixo J[1], Amorim M[1], Kay T[1], Sousa S[2], Castedo S[2]

[1]Unidade de Genética Médica, Hospital Dona Estefânia, Centro Hospitalar Lisboa Central, EPE. [2]Unidade de Patologia e Patologia Molecular do IPATIMUP/i3S

Introduction: Hereditary breast and ovarian cancer (HBOC) due to mutations in the BRCA1 and BRCA2 genes is the most common cause of hereditary forms of both breast and ovarian cancer. The majority of BRCA1 and BRCA2 mutations (≥80%) consist of single base changes or deletions/insertions of small numbers of bases that have significant impact on protein function. A minority of mutations in these genes are large rearrangements of DNA segments that disrupt gene function, consisting primarily of deletions and duplications of 1 or more exons.
Methods: We report a 55yo female patient referred to our Medical Genetics Clinic due to personal and family history of breast cancer. At 53yo she was diagnosed with breast cancer and underwent tumorectomy of the left breast and sentinel lymph node biopsy. The histological diagnosis established an invasive carcinoma type luminal B with negative sentinel lymph node. From the family history to note: mother diagnosed with breast cancer at 57yo and maternal grandmother diagnosed with metastatic breast cancer at 67yo. Both died shortly after diagnosis.
Results: BRCA1 and BRCA2 gene panel concurrently with deletion/duplication analysis was performed and a heterozygous deletion of BRCA2 was detected and classified as a pathogenic variant. In order to clarify the extend of the BRCA2 deletion, microarray was performed and revealed a 13q13.1 microdeletion involving the BRCA2 gene. At-risk relatives await study for detection of the familial variant on BRCA2.
Discussion: Genomic rearrangements of the BRCA2 gene account for approximately 10% of the BRCA2 mutational spectrum. According to the data available in the literature this is the first case of HBOC due to deletion of the entire BRCA2 gene to be described in Portugal. The 13q13.1 deletion is not associated with other clinical features besides HBOC due to BRCA2 deletion, and the phenotype overlaps with HBOC cause by point mutations and smaller deletions/duplications. Once a BRCA2 mutation has been identified in a family, testing of at-risk relatives can identify those family members who also have the familial mutation and thus need increased surveillance/screening and primary prevention options should be suggested, including prophylactic surgery. With this in mind, it is appropriate to consider the routine inclusion of assays for the comprehensive detection of large rearrangements as part of routine testing for BRCA1 and BRCA2 for all patients at risk for HBOC.

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Cytogenomic characterization and DNA methylation patterns of Laryngeal Cancer

Ribeiro MS[1*]; Ribeiro IP[1,2]; Ferreira SI[1,2]; Migueis J[2,3]; Carreira IM[1,2,4]; Melo JB[1,2,4]

[1]Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [2]CIMAGO—Center of Investigation on Environment Genetics and Oncobiology—Faculty of Medicine University of Coimbra, Coimbra, Portugal; [3]Departments of Otolaryngology, Coimbra University Hospitals, Coimbra, Portugal; [4]CNC.IBILI Consortium
[*]margaridasaribeiro@gmail.com

Background: Laryngeal cancer is the second most common malignancy of the head and neck, accounting for approximately 20% of cases. In Portugal, the third European country with the highest incidence of laryngeal cancer, approximately 600 new cases are diagnosed per year. With a low 5 year-survival rate, mainly explained by a late diagnosis, tumour aggressiveness and rapid metastatic process, it is essential to identify biomarkers to anticipate the cancer detection in an early stage. Aim: The main goal of this study was the cytogenomic evaluation and DNA methylation patterns characterization of laryngeal cancer in order to identify putative diagnostic and prognostic biomarkers.
Methods: Tumour and non-tumour laryngeal tissue samples obtained from twenty one patients diagnosed with laryngeal cancer were used. Detection of copy number variations (CNVs) was performed using array Comparative Genomic Hybridization (aCGH) and Methylation Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA). Furthermore, methylation patterns of some target genes were assessed by MS-MLPA analysis.
Results: aCGH revealed gains of chromosomes 3q, 7p, 8, 9q, 11q, 12p, 17q and 18p while losses were frequently found in chromosome 3p, 9p, 11p and Y. Amplifications of GATA5 and CDK6 were the most common events among tumour samples. VHL, CDKN2A, ATM and CADM genes were found to be frequently deleted. Methylation of GATA5 was frequent in tumour samples suggesting an association with late stages, while WT1 was highly methylated in non-tumour samples, being an early epigenetic event in laryngeal cancer.
Conclusion: This study confirmed some cytogenomic alterations associated with laryngeal carcinoma that have already been reported. Additionally new alterations have been identified. Some cytogenetic regions and genes found altered can be furthered studied as possible biomarkers in laryngeal cancer.

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Epidemiological study of the incidence of RAS mutations in patients with colorectal cancer in the hospital center of Trás-os-Montes and Alto Douro

Coelho A.[1]; Sousa M.[1]; Teira A.[1]; Lemos S.[2]; Ferreira J.[2]; Moutinho O.[3]; Pinto-Leite R.[4]; Barbosa M.[1]

[1]Serviço de Oncologia Médica, Centro Hospitalar de Trás-os-Montes e Alto Douro [2]Universidade de Trás-os-Montes e Alto Douro [3]Departamento da Mulher e da Criança, Centro Hospitalar de Trás-os-Montes e Alto Douro [4]Laboratório de Genética, Centro Hospitalar de Trás-os-Montes e Alto Douro

Introduction: Colorectal cancer ranks third in both cancer incidence and cancer mortality in Portugal. Several studies demonstrated the impact of RAS mutations on metastatic colorectal cancer therapy as a predictive biomarker of response. The research of this mutations is mandatory in the therapeutic approach of these patients. This study evaluates the prevalence of KRAS/NRAS mutations in 236 patients diagnosed with colorectal cancer in the hospital center of Trás-os-Montes and Alto Douro (CHTMAD). Methods: The samples used in the study were obtained patients attending the Service of Medical Oncology of CHTMAD between 01/2013 to 12/2014. The research of mutations in KRAS/NRAS oncogenes was carried using Real-Time Polymerase Chain Reaction (RT-PCR), with the amplification of two gene regions with specific allelic primers (Applied BioSystem). Results and Discussion: In this study, a total of 239 samples of 236 patients were analyzed. The majority of the samples were collected during surgical procedure; in 5.4% (n=13) the source was primary tumor biopsies and in 1.3 % (n=3) was hepatic metastases biopsies. Approximately 64% (n= 154) of the cases corresponded to patients with colon cancer and about 36% (n=85) with rectal cancer. Between the patients with colon cancer, 53.9% (n=83) had a localized tumor in left colon and 44.2% (n=68) in right colon. A total of 8 patients had synchronous tumors, the majority (n=5) had both tumors in the right colon and almost 2% (n=3) presented synchronous tumors in the right and left colon. The mutation on KRAS or NRAS gene has an incidence of 40.6% (n=97), being the majority correspondent to a mutation in KRAS gene (91.8%; n=89). The most common mutation found in KRAS gene was p.Gly12Asp, with an incidence of 30.3% (n=27) and in NRAS gene was p.Gln61Arg. Statistical analysis showed significant differences in the prevalence of the mutations, dependent on the primary tumor localization, with the right colon presenting the most RAS mutations (56.2 %; p= 0.044). The Median Overall Survival (OS) of the sample was of 55 months (IC 95 % 40.6-69.4) and the median Progression Free-Survival (PFS) of the sample was of 20 months (IC 95 % 18.0-21.9). In PFS and OS there weren't any significant statistical changes in the mutational status of RAS (p=0.209 and p=0.630, respectively). The analysis of RAS mutational status and the most common types of mutations, in this region of Portugal, proved to be similar to the rest of the Western World.

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A novel ATM mutation in familial breast cancer

Cardoso J.[1], Pereira A.[2], Rego de Sousa M.J.[2], Germano de Sousa J.[2] Germano de Sousa[2]

[1]Ophiomics—Precision Medicine, Lisboa, Portugal; 2. Centro de Medicina Laboratorial Germano de Sousa, Lisboa, Portugal

A 38 year old woman diagnosed with an invasive ductal breast cancer type (HER2+,ER-,PR-) and family history of cancer (multiple cases of breast cancer, melanoma and ovarian cancer) was referred to genetic counselling. Previous investigations revealed her to be negative for BRCA1 and BRCA2 mutations. As per NCCN guidelines at the time we investigated this patient using a panel of six genes, comprising NGS and Sanger sequencing, MLPA and an additional PCR-based investigation of a specific Alu insertion in the BRCA2 gene. This revealed a mutation in heterozygosity in the gene ATM (p.L1456P; c4394T>C).This mutation had previously been identified as pathogenic in homozigosity, in the context of ataxia telangiectasia. It has never been associated, in heterozygosity to breast cancer, but instead was identified in the context of familial gastro-intestinal cancers. This same mutation was further detected in other family members affected with early onset breast cancer, further supporting its pathogenic nature. Analysis of this family highlights the importance of multi-gene panel testing in familial cancer syndromes and reveals a novel pathogenic mutation in the ATM gene in the context of familiar breast cancer.

P13 | Cancer Genetics

Evaluation of the effectiveness of cytogenetic protocols in chronic lymphatic leukemia

Valença C.[1]; Souto M.[2]; Botelho P.[2]; Costa M.[3]; Carvalho A.[3]; Guerra M.[3]; Magalhães R.[3]; Cunha M.[3]; Moutinho O[4]; Leite R.[2]

[1]Universidade de Trás-os-Montes e Alto Douro; [2]Laboratório de Genética, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real; [3]Serviço de Hematologia, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real; [4]Serviço de Genética, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real

Introduction: Chronic lymphocytic leukemia B (CLL-B) is the most prevalent form of leukemia in adult individuals in the Western World, especially affecting the elderly. The disease results from slow clonal accumulation of neoplasm B-lymphoid cells with a phenotype of mature B-lymphocytes. Cytogenetic analysis plays a very important role in the study of this pathology, since the detection of specific chromosomal alterations has prognostic and therapeutic implications. The anomalies associated to this type of leukemia are trisomy of chromosome 12 and deletions in loci located on chromosomes 6q21, 13q14, 11q22-q23 and 17p13. The stimulators currently used to obtain metaphases are 1,2-O-tetradecanoylforor (TPA) and Pokeweed, that detects chromosomal alterations in 40-50% of cases. The development of new molecular genetic techniques, namely Fluorescent in situ hybridization (FISH), has made a great contribution to the study of CLL, increasing the detection until 80% of the cases. The aim of this study was to evaluate which of the stimulators (TPA and Pokeweed) allows better results, concerning number and metaphases quality, and the FISH effectiveness in CLL-B samples analysis.

Methods: Two peripheral blood cultures, with TPA and pokeweed, were performed, for each sample, in 70 CLL-B patients. GTL banding and FISH were done according to the protocols in the laboratory. Cytogenetic analysis followed the standard cytogenetic guidelines.

Results: With the TPA stimulator, metaphases were obtained in 94% of the samples. Of these 51.5% presented chromosomal alterations that were divided into 3 groups: a) chromosomal anomalies associated with CLL-B (24%), b) associated anomalies and other alterations (7.5%), and c) other alterations (20%). Concerning to Pokeweed, cell growth was achieved in 83% of the samples, of which 16% had chromosomal alterations: 5% with anomalies associated with the pathology; 2% with associated anomalies and other alterations, and 9% with other alterations. FISH was performed on 89% of the samples and detected chromosomal anomalies associated with CLL-B in 50% of the samples.

Conclusion: TPA was the most efficient stimulator in obtaining metaphases and detecting chromosomal alterations. Although FISH detected a higher percentage of the expected chromosomal alterations associated with CLL-B, it was not able to detect the other chromosomal anomalies found by classical cytogenetics.

Keywords: CLL; TPA; Pokeweed; Classical Cytogenetics; FISH

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SYNLAB COLONPLUS panel®: Testing hereditary and familial colorectal cancer using a NGS multi-gene panel

Pinto D[1], Martins C[1], Salgueiro N[1], Nabais Sá MJ[2], Pinto R[5], Dupont J[3], Soares G[2], Dias P[3], Gonçalves-Rocha M[4], Conceição A[1], Moreira M[1], Garcia E[1], Fortuna A[2], Sousa A[3], Reis-Lima M[1]

[1]Unidade de Biologia Molecular, Synlab—GDPN, Porto; [2]Serviço de Genética, Centro Hospitalar do Porto; [3]Serviço de Genética, Centro Hospitalar de Lisboa Norte; [4]Unidade de Genética Médica, Hospital de Braga; [5]Serviço de Gastrenterologia, Centro Hospitalar do Porto—ICBAS, UP; CIDES/CINTESIS FMUP, UP

Introduction: Colorectal cancer (CRC) is the second cause of death, for both sexes, in developed countries, only exceeded by lung cancer (men) and breast cancer (women). In Europe, 53/100000 inhabitants new cases of CCR are diagnosed per year. Around 30% of CRC show a familial aggregation but is estimated that only 5-10% arise as hereditary CRC syndromes. Hereditary Colorectal cancer and polyposis (CRCP) syndromes are a group of diseases characterized by a strong individual and/or family history of colon cancer and/or polyps, especially in early ages. The most frequent inherited CRC syndromes are: familial adenomatous polyposis (FAP) due to mutations in APC gene; MUTYH-associated polyposis (MAP) due to mutations in MUTYH gene and hereditary nonpolyposis colorectal cancer (HNPCC) with mutations in MLH1, MSH2, MSH6 and PMS2 genes. The emergence of NGS panels has enabled the simultaneously analysis of multiple genes in patients with suspected CRCP syndromes.

Methods: We performed NGS analysis of 14 genes (SYNLAB COLONPLUS panel®) associated with gastric, colorectal and small bowel cancers: APC, BMPR1A, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53, in 43 patients fulfilling clinical criteria for genetic testing. MLPA was performed for APC, MLH1, MSH2, MSH6, PMS2 and EPCAM genes. Additionally Sanger sequencing was performed for POLD1 exon 12 and POLE exon 13.

Results: Our study detected 8 pathogenic mutations (18,6%) and 7 variants of uncertain significance (VUS) (16%). Pathogenic mutations were found in MUTYH, APC, PMS2 and SMAD4 genes.

Discussion: The SYNLAB COLONPLUS panel® allowed the analysis of a greater number of genes in a very short time and a reduced price, as well as mutation detection in uncomon genes. The poor prognostic despite de immediate clinical managment when a CCR is diagnosed, enhances the needs for early detection of those at risk. The efectiveness of screening and prevention proposes is likely to be optimized with prompt identification of those at risk. The increase of multigene panel use, allows the early, quick and cost effective identification of individuals at risk, enabling surveillance plans to be early established. Again, we have the example of the value of the genetic information in the context of population screening programs, along with the incorporation of non-genetic risk factor assement.

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SYNLAB BRCA+16 genes panel®: Multi-gene panel analysis for Hereditary Breast, Ovarian and Endometrial Cancer predisposition

Salgueiro N[1], Conceição A[1], Nabais Sá MJ[2], Dupont J[3], Soares G[2], Gonçalves-Rocha M[4], Moreira M[1], Machado C[3], Moldovan O[3], Gonçalves J[1], Fortuna A[2], Sousa A[3], Reis-Lima M[1]

[1]Unidade de Biologia Molecular, Synlab—GDPN, Porto; [2]Serviço de Genética, Centro Hospitalar do Porto; [3]Serviço de Genética, Centro Hospitalar de Lisboa Norte; [4]Unidade de Genética Médica, Hospital de Braga

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, given the high mortality rate often due to late diagnosis. 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 (HBCO) are BRCA1 and BRCA2. Patients who carry a mutation in BRCA1 gene have 65% and 39% risk of developing BC and OC, respectively, by the age of 70. Patients who carry a mutation in BRCA2 gene have 45% and 11% risk of developing BC and OC, respectively, by the age of 70.

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 the last few years, Next-Generation-Sequencing (NGS) has enabled the analysis of a greater number of genes in patients with suspected genetic syndromes.

Methods: We performed NGS analysis of 18 genes (SYNLAB BRCA+16 genes panel®): BRCA1, BRCA2, ATM, BRIP1, CDH1, CHEK2, NBN, PALB2, RAD51C, RAD51D, PTEN, TP53, EPCAM, STK11, MLH1, MSH2, MSH6 e PMS2 in 68 women, with the National Comprehensive Cancer Network (NCCN 2. 2017) clinical criteria for HBOC genetic testing. MLPA for BRCA1, BRCA2, EPCAM and BRCA2 c.156_157insAlu was performed in all cases.

Results: Our study revealed 11 pathogenic/likely-pathogenic variants in the 18 genes analysed (11/68=16%). Of these 11 pathogenic/likely-pathogenic variants, 6 (54,5%) were detected in BRCA1 and BRCA2 and 5 (45,5%) in the other panel genes. This study showed 26 (38%) patients with one or two variants of uncertain significance (VUS).

Discussion: Current clinical genetics tests for BC and OC familial risks have been based on the analysis of BRCA1 and BRCA2 genes. The discovery of new genes involved in susceptibility to HBCO and the advent of NGS have enabled the introduction of multigene panel testing for hereditary forms of cancers. This approach has increased the detection of pathogenic mutations in patients with suspected genetic syndromes, with the advantage of lower costs and less time consuming. We believe that our SYNLAB BRCA+16 genes panel® can improve the detection of pathogenic mutations in unsolved high-risk HBCO patients.

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Deep-intronic genetic variation in hypertrophic cardiomyopathy

Mendes de Almeida R.[1§]; Tavares J.[1§]; Martins S.[1]; Carvalho T.[1]; Enguita FJ.[1]; Brito D.[2,4]; Carmo-Fonseca M.[1]; and Lopes LR.[4,5]

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa [2]Departamento de Cardiologia, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa [3]Serviço de Cardiologia, Hospital Garcia de Orta, Almada, Portugal [4]Centro Cardiovascular da Universidade de Lisboa [5]Institute of Cardiovascular Science, University College London. [§]These authors have equally contributed to the work.

Introduction: High throughput sequencing technologies have revolutionized the identification of mutations responsible for genetic diseases such as hypertrophic cardiomyopathy (HCM). However, approximately 50% of individuals with a clinical diagnosis of HCM have no causal mutation identified. This may be due to the presence of pathogenic mutations located deep within the introns, which are not detected by conventional sequencing analysis restricted to exons and exon-intron boundaries. The aim of this study was to develop a whole-gene sequencing strategy to prioritize deep intronic variants that may play a role in HCM pathogenesis.

Methods: The full genomic DNA sequence of 26 genes previously associated with HCM was analysed in 16 unrelated patients. Family members of two selected probands were also clinically and genetically tested. Only variants with a read depth of 20 or more, were evaluated with NCBI ClinVar and classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines. Noncoding variants were prioritized with GWAVA, CADD, SPIDEX and Genomiser. We used Human Splicing Finder, RegRNA and Regulatory Genomics: Branch point analyser to determine whether a variant may disrupt splicing motifs. Forasmuch as deep intronic variants may result in altered gene expression, the disruption of transcription factor binding sites (TFBS) were also assessed with available tracks in the UCSC genome browser.

Results: We identified likely pathogenic deep intronic variants in VCL, PRKAG2 and TTN genes. These variants, which are predicted to act through disruption of either splicing or transcription factor binding sites, are 3-fold more frequent in our cohort of probands than in normal European populations. Moreover, we found a patient that is compound heterozygous for a splice site mutation in MYBPC3 and the deep intronic VCL variant. Analysis of family members revealed that carriers of the MYBPC3 mutation alone do not manifest the disease, while family members that are compound heterozygous are clinically affected.

Discussion: This study provides a framework for scrutinizing variation along the complete intronic sequence of HCM-associated genes and prioritizing candidates for mechanistic and functional analysis. Our data suggest that deep intronic variation contributes to HCM phenotype.

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Phenotypic comparison of patients with Pitt-Hopkins syndrome with two common differential diagnosis

Soares C.A.[1]; Soares A.R.[1]; Tkachenko N.[1]; Sá M.J.[1]; Fortuna AM.[1, 2]; Soares G.[1]

[1]Centro de Genética Médica Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal; [2]Unit for Multidisciplinary Research in Biomedicine, Instituto de Ciências Biomédicas Abel Salazar/Universidade do Porto, Porto, Portugal

Introduction: In Pitt-Hopkins Syndrome (PHS), pathogenic variants or deletions in TCF4 lead to loss of function of the homonym protein. PHS is characterized by facial dysmorphisms, neurological and ophthalmologic disorders. Some of PHS phenotypic characteristics are common to other developmental disorders, making the clinical differential diagnosis challenging.
Methods: The clinical records of eight patients (four males and four females, including a pair of female twins) with clinical and molecular confirmation of PHS, were reviewed retrospectively and the phenotypic characteristics were coded using the Human Phenotype Ontogeny (HPO). The PHS patient's phenotypes were compared with the HPO revised list for the Angelman (AS) and Mowat-Wilson Syndrome (MWS). The information content (IC) was calculated for the HPO terms. The Resnik's similarity score (RSS) and p value for different developmental disorders was computed with the online tool, Phenomizer.
Results: On the HPO revision, PHS shared 11 phenotypic characteristics with AS and 12 with MWS, while 20 characteristics were unique to PHS. Of those 20 characteristics, not all were present in our patients, but presenting an open mouth (HP:0000194) was almost ubiquitous in our population. All our PHS patients presented motor delay (HP:0001270), intellectual disability(HP:0010864), wide nasal bridge (HP:0000431) and hand stereotypic movements (reviewed by the authors). Intermittent hyperventilation (HP:0004879), a characteristic phenotype often associated with PHS, was described in only five patients. All patients, except the pair of twins, presented a higher RSS for PHS than for AS and MWS.
Discussion: The PHS, AS and MWS share many phenotypic characteristics with no single characteristic being pathognomonic. Still, the RSS is a useful tool to precisely distinguish these developmental syndromes in most, but not all cases.

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New recessive mutation in LTPB3 in a family with short stature and amelogenesis imperfecta

Dias P[1], Travessa A[1], Medeira A[1], Moldovan O[1], Sousa AB[1]

[1]Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portuga

Introduction: The latent TGF-β binding protein 3, codified by LTBP3 gene (MIM 602090), belongs to a family of proteins involved in the regulation of TGF-β bioavailability in the extracellular space. Recessive pathogenic variants in LTBP3 were described in brachyolmia with amelogenesis imperfecta. Recently, heterozygous variants were described in patients with acromelic dysplasia, a phenotype previously associated with other genes in the TGF-β signaling pathway. We describe two brothers with a new homozygous pathogenic variant c.3395-3397delinsA p.(Pro1132Glnfs*79) in LTBP3 gene, with acromicric phenotype, mild spondyloepimetaphyseal changes and amelogenesis imperfecta.
Case reports: The parents are first cousins. The mother has proportionate short stature and the father has normal stature; they are both healthy and have normal teeth. The index case was born with normal length. Limitations of wrist and finger mobility were noticed at 3 yrs and linear growth arrest at 4 yrs. Skeletal X-rays at 9 yrs demonstrated mild spondyloepimetaphyseal changes. He had multiple exodontias at 10 yrs for early dental decay. Observation at 17 yrs revealed proportionate short stature with height 138.5 cm, pseudomuscular build, mild prognathism, small hands with enlarged interphalangeal joints, failure to make a fist, incomplete extension of the elbows, limited supination/pronation of the forearms, oligodontia with residual yellow and black dental pieces. Other problems included learning disability, mild restrictive lung disease, hypermetropia, traumatic fractures of the left humerus at 8 yrs and the left radius at 10 yrs. His younger brother had similar characteristics with post-natal growth restriction, short stature with mild short trunk, stocky build, limited joint flexion starting at 2.5 yrs, small spaced yellow teeth, and analogous radiological abnormalities. A LTBP3 phenotype was suspected and confirmed by WES trio analysis (both brothers and mother) with the identification of a homozygous variant, c.3395-3397delinsA, p. (Pro1132Glnfs*79), in both patients and in heterozygous state in their mother.
Discussion: Our patients have characteristics associated with both phenotypes attributed to the LTBP3 gene, the acromicric phenotype (autosomal dominant) and the amelogenesis imperfecta phenotype (autosomal recessive). Brachyolmia describes a radiological phenotype with generalized platyspondyly without significant epiphyseal or metaphyseal involvement. However radiological abnormalities in our patients are better described as mild spondyloepimetaphyseal dysplasia. It was proposed that dominant mutations in LTBP3 would have a gain of function effect and recessive hypomorphic mutations would explain the amelogenesis imperfecta and spine abnormalities. Our patients expand the spectrum of LTBP3 pathologies. Additional studies to understand the effect of this particular variant on protein function are warranted.

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Desafios e Questões Éticas Associados às Novas Tecnologias de Sequenciação no Diagnóstico Genético

Garcia B[1], Ribeiro M[1], Tavares J[1] and Carmo-Fonseca M[1]

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa

Introduction: Next-generation sequencing (NGS) technologies have allowed great progress in the diagnosis of genetic diseases, facilitating further advances in therapeutic decision making and disease prediction for at-risk patients. However, this approach raises additional challenges involving clinical validation and interpretation of genetic variants.
Methods: The entire coding sequences of 58 genes associated with heart function were analyzed in 55 patients without clinical evidence of cardiac disease.
Results: We identified 10 different genetic variants that are described as pathogenic or likely pathogenic in the ClinVar database. However, only one of these variants fulfills pathogenicity criteria, based on the published literature/databases and according to American College of Medical Genetics and Genomics (ACMG) guidelines. This variant is associated with a high risk of cardiac arrhythmia.
Discussion: Determining whether genetic variants identified by NGS fulfill pathogenicity criteria is critical in order to make the best medical decisions for patients. Another important issue concerns the ethical problems related with incidental findings unrelated to the disease that prompted the genetic test.

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LDLR, APOB and PCSK9 variant interpretation in Familial Hypercholesterolaemia—application of ACMG guidelines

Chora J.R.[1,2]; Medeiros A.M.[1,2]; Alves A.C.[1,2]; Bourbon M[1,2]

[1]Unidade de I&D, Grupo de Investigação Cardiovascular, Departamento de Promoção da Saúde e Prevenção de Doenças Não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [2]BioISI—Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

Introduction: Familial hypercholesterolaemia (FH) is an autosomal disorder of lipid metabolism presenting with increased cardiovascular risk. It is caused by functional mutations in LDLR (>90%), APOB (5-10%) and PCSK9 (1-3%). Although more than 2,000 variants have been associated with FH, the great majority have not been proved to functionally affect the low-density lipoprotein receptor cycle. The American College of Medical Genetics and Genomics (ACMG) recently published a guideline for variant interpretation in clinical settings; however variants associated with FH have not been classified by this algorithm.
Methods: We aimed to classify, following ACMG guidelines, all described variants associated with FH in different databases and individual reports (last 10 years) to establish the proportion of variants that lack evidence to support their pathogenicity. A worldwide overview of FH variants has also been performed.
Results: A total of 2,104 unique variants were found associated with FH, 1894 in LDLR, 97 in APOB and 113 in PCSK9. Only 166 variants, less than 10% of total, have been proven by complete in vitro functional studies to be causative of disease. By application of ACMG guidelines, 1,097 variants were considered pathogenic or likely pathogenic, 21 as benign or likely benign, but 986 variants are still of unknown significance. The largest number of identified variants is found in Europe, (n = 1,491), followed by Asia (n = 332), America (n = 265), Oceania (n = 134), and lastly Africa (n = 77). No APOB or PSCK9 variants were identified in African countries and only seven variants were found in all five continents.
Discussion: The lack of functional evidence for about 85% of all variants found in FH patients can compromise FH diagnosis and patient prognosis. ACMG classification improves variant interpretation, but some issues were identified. Functional studies are still necessary to understand the effect of about 40% of all variants reported. A more in depth adaptation of ACMG guidelines to FH is essential to a better diagnosis.

P21 | Clinical Genetics

Unsuspicious family with a pathogenic mutation in FOXC2 gene The importance of examining family members

Freixo JP[1]; Rodrigues M[2]; Carvalho I[1]; Gonçalves R[1]; Antunes A[1]; Kay T[1]

[1]Unidade de Genética Médica, CHLC; [2]Centro Hospitalar Universitário do Algarve, E.P.E.

Introduction: Multisystemic diseases represent a challenge to interpret clinical findings and complex genomic test results. A thorough family evaluation provides valuable clues and assumes a major role in the clinical practice of a Medical Genetics Centre. Sometimes these clues are crucial to achieve a definite diagnosis in multisystemic diseases with high phenotypic variability.
Method: We report a case of a 3 years-old girl referred to our Genetics Department due to a cardiopathy, cleft palate, microretrognathism, scoliosis, and minor dysmorphic features. Her father was healthy and her mother was obese and presented with lower limb lymphedema with onset in adolescence. A more subtle evaluation of the mother allowed us to identify a double row of lashes in the upper eyelids, suggesting the diagnosis of lymphedema - distichiasis.
Results: We performed Sanger sequencing of FOXC2 gene in mother's DNA sample which revealed the presence of a heterozygous pathogenic variant c.1024del (p.Ala342Profd*28), confirming the clinical diagnosis. The proband had the same point mutation.
Discussion: Although the classical presentation of lymphedema - distichiasis is characterized by lower limb lymphedema with onset at adolescence and the presence of anomalies of the eyelids, there are rarer presentations that include congenital cardiopathy (7%), cleft palate (4%) and scoliosis. Next Generation Sequencing with its ability to sequence many genes in less time has been progressively shifting Genetics paradigm and the concept of reverse phenotype is now deep-seated in the Medical Genetic field. Despite that and although the main features of a genetic disease are of major importance to ease the diagnostic clinical workflow, basic medical genetics terminology regarding phenotypic variability shouldn't be forgotten especially when dealing with polymalformative diseases. This case illustrates the importance of a careful family and physical evaluation in the era of NGS.

P22 | Clinical Genetics

A new variant in the COL6A1 gene segregates in a family with Bethlem Myopathy

Torres F.[1], Eulálio I.[1], Gonçalves L.[1], Araújo M.[1], Negrão L.[2], Cerqueira R.[1], Pinto-Basto J.[1]

[1]CGC Genetics, Porto, Portugal; [2]Medical Genetics Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Backgroud: COL6A1 gene encodes the alpha(α)1(VI) chain of type VI collagen, an important component of the extracellular matrix which forms a microfibrillar network that is found in close association with the cell and surrounding basement membrane. Mutations in each of the following genes COL6A1, COL6A2 and COL6A3 cause two main types of muscular disorders: Ullrich congenital muscular dystrophy, a severe phenotype, and a mild to moderate phenotype Bethlem myopathy.
Case Report: In this study we report the identification of a new variant in COL6A1 gene that segregates in a family with Bethlem myopathy. The Index patient exhibited axial and distal joint contractures. Previous molecular studies did not find mutations in MYOT, Pompe disease, LMNA, CAV3, SEP1 genes. Immunohistochemistry was also negative for the presence of sarcoglycans and dysferlin. Additionally, no mutations were detected in the 31 genes analyzed in a NGS panel for congenital muscular dystrophies. Deletion/duplication analysis was performed for genes COL6A1, COL6A2 and COL6A3 and a heterozygous deletion, c.(1002+1_1003-1)_(1056+1_1057-1) was detected in COL6A1 gene, compatible with the diagnosis of Bethlem myopathy. The father, also affected was found to be heterozygous for the same deletion. This variant encompasses at least exon 14 of the COL6A1 gene and is not described in the literature nor in population databases. Although not frequent, large deletions in this gene have been described, namely in association with Bethlem myopathy [1]. With the data currently available, and as this deletion segregates with the disease, this variant should be considered likely pathogenic. This result has consequences for the genetic counseling of these patients and relatives.
1. Pepe et al. Ann Neurol. 2006, 59: 190

P23 | Clinical Genetics

Somatic mosaicism for a nonsense DMD variant detected by next-generation sequencing

Almeida L[1], Falcão de Campos C[2], Oliveira Santos M[2], Ferreira M[1], Marques S[1], Paulino M[1], Teixeira M[1], Cerqueira R[1], Pinto-Basto J[1]

[1]CGC Genetics, Porto, Portugal; [2]Centro Hospitalar Lisboa Norte, E.P.E., Portugal

Introduction: Duchene and Becker muscular dystrophies are X-linked progressive muscle wasting diseases, caused by mutations in the dystrophin (DMD) gene. We report on a male patient (28 years old), presenting with muscle pain since infancy and high creatine kinase. Skeletal muscle biopsy was performed and findings were compatible with dystrophinopathy. No deletions/duplications in the DMD gene were detected; and sequencing analysis of this gene was performed.
Methods: DMD gene was sequenced using an oligonucleotide-based target capture (QXT, Agilent Technologies) approach followed by next generation sequencing (MiSeq, Illumina). Sanger sequencing was also performed for the detected variant.
Results: Analysis of the NGS data identified a novel likely pathogenic truncating DMD variant, c.8056G>T (p.Glu2686*). This variant was detected in apparent somatic mosaicism (~80% of the mutated alleles), further confirmed by Sanger sequencing. To exclude the presence of a sexual aneuploidy, the number of X chromosomes was confirmed in the deletions/duplications MLPA analysis performed.
Discussion: Somatic mosaicism in DMD gene is a rare event, with only a few cases with nonsense mutations reported, to our knowledge. Therefore, this case expands the clinical spectrum of DMD mosaics as the reported cases have clinical characteristics confined to the heart, such as dilated cardiomyopathy. Additionally, reporting a somatic DMD variant, using a NGS approach illustrates the sensitivity of this methodology for the detection of mosaic variants. This result also has consequences for the genetic counseling of this patient and family.

P24 | Clinical Genetics

Building up a cooperative network on craniofacial anomalies

Gil-da-Silva-Lopes VL[1]

[1]Departament of Medical Genetics, Faculty of Medical Sciences, State University of Campinas, São Paulo, Brazil.

Introduction: In 2003, a cooperative network was set up for craniofacial anomalies based on clinical genetic analysis. This report describes the activities of this group, named Craniofacial Brazil Project (CFBP), to improve research and healthcare in this area.
Methods: Participation in this ongoing project is voluntary. Geneticists, researchers and post-graduate students from different health professions have developed all activities. The strategies initially proposed were related to public health, characterization and genetic investigation of specific conditions, and education in genetics.
Results and Discussion: The preliminary phase was based on regional and multicentre studies in order to recognized characteristics of healthcare for craniofacial anomalies in Brazil. It included those related to genetic evaluation and counseling, neonatal care, and recognition of educational needs of professionals engaged in the care of individuals with Oral Clefts (OC). We also developed and implemented an on line application to record and follow individuals with OC and 22q11.2 deletion syndrome though the Brazilian Database on Craniofacial Anomalies; in the future, others craniofacial anomalies will be included. Currently, this Database is used by 13 centers and follows around 1800 individuals. Professionals' continuing education has been supported by on line healthcare manuals for S. 22q11.2 Deletion Syndrome and OC and training programs focused on genetics and pre surgical feeding methods and resources for babies with OC. Recently, molecular data from different studies related to CFBP became available on line through Molecular Brazilian Database on Craniofacial Anomalies. In the long-term, all these initiatives will found research and cost-effective decision-making OC prevention, and clinical and molecular investigation in our population. This model could be replicated countries. The CFBP is available to share these strategies with interested researchers.
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P25 | Clinical Genetics

Oculofaciocardiodental Syndrome

Almeida P.[1]; Ramos F.[2]; Saraiva JM.[3]

[1]Faculty of Health Sciences, University of Beira Interior, Portugal [3]University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra, Portugal [1,2,3]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Introduction: Oculofaciocardiodental (OFCD) syndrome is a rare X-linked hereditary syndrome with an incidence estimated to be less than 1 in 1 million. It has only been described in heterozygous females. It is presumed lethal in affected males. OFCD is characterized by ocular, facial, cardiac, and dental abnormalities. Occasional reports have also mentioned other features like skeletal findings, intestinal malrotation, hearing impairment, intellectual and psychomotor deficit. Mutations in the BCOR gene, which encodes the BCL6 corepressor, have been found to cause OFCD syndrome. Clinical Report: A 10 year-old girl with a previous possible diagnosis of CHARGE Syndrome (based on iris coloboma, atrial sept defect, developmental delay, genital and uterus hypoplasia, ear abnormality, conductive hearing loss and negative molecular analysis of CHD7 gene) was re-evaluated. She also has hands with brachydactyly and dysmorphic features. The array (180K oligoarray-CGH) was normal and a broad multigene panel of genes (focused exome, Agilent) found a novel, probably pathogenic nonsense mutation [c.4455T>A (p.Tyr1485*)] in a heterozygous form, on BCOR gene. This variant was excluded in her parents. This result established the diagnosis of Oculofaciocardiodental syndrome. This patient has similar features of the cases previously described in literature. Discussion: OFCD is a rare syndrome caused by a BCOR mutation that can be clinically recognizable. The clinical revaluation and the availability of new technologies in genetic diagnosis allowed an accurate diagnosis with specific genetic counselling. This case also highlights the importance of following the patients through growth, as dysmorphic features may become more evident with growth.

P26 | Clinical Genetics

CYP21A2 gene mutations, its nature and frequency in a paediatric portuguese cohort with congenital adrenal hyperplasia

Rosmaninho-Salgado J.[1]; Serra Caetano J.[2]; Gomes S.[3]; Pereira-Caetano I.[3];Cardoso R.[2]; Dinis I.[2]; Ramos L.[1]; Ramos F.[1]; Carvalho A.L.[1,4]; Garabal A.[1]; Sá J.[1]; Maia S.[1,4]; Sousa S.B.[1,4]; Saraiva J.M.[1,5]; Gonçalves J.[3]; Mirante A.[2]

[1]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; [2]Pediatric Endocrinology Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; [3]Human Genetics Department - National Institute of Health Dr Ricardo Jorge, Lisbon, Portugal; [4]University Clinic of Genetics, Faculty of Medicine, University of Coimbra, Portugal; [5]University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra, Portugal

Introduction: The most common cause of congenital adrenal hyperplasia (CAH) is 21-hydroxylase deficiency (21-OHD) caused by alterations in CYP21A2 gene. The clinical phenotypes of this autosomal recessive disease are classified as classic (saltwasting and simple virilizing) and non-classic forms of CAH. The severity of the disease is directly related with the impairment of the 21-OH enzymatic activity. Genetic testing can confirm the disease and is crucial for familial studies and genetic counseling. Aim: The aim of this work was to perform the clinical and molecular characterization of the patients observed at the Hospital Pediátrico de Coimbra (Portugal) with the clinical suspecion of CAH.
Methods: Retrospective analysis of patient medical records of all cases observed in our hospital with suspicion of CAH and detailed literature comparison. CYP21A2 molecular analysis had been performed in 81 unrelated Portuguese patients (51 female, 30 males) with clinical and endocrine laboratorial findings suggestive of CAH, using mini-sequencing, restriction enzyme digestion, Sanger sequencing or/and multiplex ligation-dependent probe amplification (MLPA).
Results: CYP21A2 variants were identified in 74/81 (91%) of the patients. Homozygosity for CYP21A2 was found in 39.2% (29/74) of the patients while 55.4% (41/74) were compound heterozygous and, in 5.4% of the cases (4/74), only one pathogenic variant was identified. The most frequent alterations were p.Val281Leu, g.655A/C>G (splicing variant) and p.Ile172Asn, that account for more than 50% of the alleles of this patient's cohort. All variants were already described except a novel missense variant identified in a salt-wasting patient, g.1173T>C(p.Trp201Arg). The rare variant p.Gly424Ser which was detected in one patient had been previously associated with a possible founder effect in Brazil and the splicing variant g.391G>A, only described in the Portuguese population.
Conclusion: Our study provides a detailed clinical and molecular characterization of a large cohort of CAH Portuguese patients. The overall concordance between the clinical phenotype and the inferred phenotype (based on genotype) was 90%.

P27 | Clinical Genetics

Familiar 1q21.1 microdeletion syndrome: a case report

Souto M.[1], Botelho P.[1], Sousa S.[2], Martins M.[3], Moutinho O.[4], Pinto Leite R.[1]

[1]Laboratório de Genética, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real; [2]Serviço de Pediatria, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real; [3]Consulta de Genética, Centro Hospitalar de Trás-os-Montes e Alto Douro, Vila Real; [4]Serviço de Ginecologia/Obstetrícia, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real

INTRODUTION: The 1q21.1 microdeletion is a very rare syndrome, up to date there are only 46 cases described. This disorder demonstrates a wide individual variation from no symptoms to a heterogeneous spectrum of manifestations including developmental delay (speech and motor delays), mild to moderate intellectual disability, microcephaly, slight facial dysmorphic features and eye abnormalities. Less frequently described are heart defects, abnormalities of the genitalia or urinary system, bone abnormalities (particularly in the hands and feet), and hearing loss. The authors present a case of a familial microdeletion syndrome.
CLINICAL REPORT: 10 years old boy with a microdeletion on 1q21.1q21.2 chromosome region detected by array Comparative Genomic Hybridization (aCGH). The segment deleted had 2.6 Mbp and involved 23 genes. The child had development delay, particularly affecting the development of motor skills, cryptorchidism and minor dismorphias. The cerebral magnetic resonance imaging revealed left hippocampal malformation and mild cortical-subcortical atrophy. Parents' aCGH analysis revealed a similar deletion on the father, the segment deleted was smaller (1.935 Mbp) than the son and it also had a deletion of 512Kbp on the 15q11.2 region (with no clinical significant). Although the father has not yet come to the genetic consultation, it is known that he has a mild developmental delay.
DISCUSSION: The present case is a familiar deletion on 1q21.1q21.2 region, with different manifestations. The deletion was inherited from the father, which showed a slight mental retardation, while the son presented phenotypic features consistent with 1q21.1 deletion syndrome, like intellectual disability, motor developmental delay and genitourinary anomalies. In the case of an inherited syndrome, usually the parent shows a normal or a very mild phenotype compared to their child, these may due to parental imprinting, reduced penetrance, or variable expressivity. In families where the 1q21.1 microdeletion has been inherited from a parent, the possibility of having another child with the 1q21.1 microdeletion is 50 per cent in each pregnancy. However the effect of the microdeletion on the child cannot be reliably predicted. Genetic counselling is fundamental and prenatal diagnosis will also be possible. All new cases detected should be reported in order to obtain a more precise correlation between genotype/phenotype to be used in genetic counseling.

P28 | Clinical Genetics

PTCHD1 deletion: more evidence for a new disease gene

Custódio S.[1]; Rodrigues R.[1]; Moldovan O.[1]; Sousa A.[1]; Sousa AB.[1]

[1]Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal.

Introduction: PTCHD1 gene, coding for the patched domain containing protein 1, is a newly proposed disease gene for X-linked intellectual disability and autism spectrum disorder. PTCHD1 was first identified in genome wide CNV screening studies of affected patients and families. It is highly expressed in the brain, mostly in the cerebellum, and recent functional studies in male mice showed that this gene's deficiency modifies synaptic gene expression leading to cognitive and motor disfunction.
Methods and Patient: ArrayCGH analysis was carried out using CGX-HD 180K Signature Genomics, Perkin Elmer. Data was analyzed using Genoglyphix™ software v3.1 (hg 19, build 37). The patient was a 13 year-old boy referred to our Medical Genetics Department for learning disability, autism, psychiatric problems, mild scoliosis, joint laxity, and hypospadias. He had no dysmorphic features.
Results and Discussion: ArrayCGH detected a 33.21 kb deletion in PTCHD1 gene at Xp22.11. The deletion encompasses the 5' end of the gene and includes the promoter region and the first exon. There are previous reports of 5' end PTCHD1 deletions involving the first exon but, contrary to our patient's case, all others extended to PTCHD1-AS, the gene upstream of PTCHD1, and most included also DDX53 gene. To the best of our knowledge, our patient's 33 kb deletion is the smallest detected so far involving this gene. PTCHD1 deletion cases commonly show intellectual disability and autistic and abnormal behavior, which is in agreement with our patient's phenotype. Congenital anomalies, however, are an unusual finding and are probably due to other as yet unidentified causes. Should that be the case, the latter could possibly also play a role in the patient's neurodevelopmental phenotype. The mother was unavailable for segregation analysis. Our report adds to the now growing evidence that PTCHD1 loss of function is associated with non-syndromic neurodevelopmental disorder.

P29 | Clinical Genetics

Molecular Characterization of a Novel Mucopolysaccharidosis type VI-causing Mutation - Indirect Proof of Principle on its Pathogenicity

Coutinho M.F.[1*], Encarnação M.[2*], Santos J.I.[1], Alves S.[1]

[1]Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal; [2]Newborn Screening, Metabolism and Genetics Unit, Department of Human Genetics, INSA, Porto, Portugal
[*]These authors contributed equally to the work

Introduction: With its unprecedented throughput, scalability and speed, next-generation sequencing (NGS) is revolutionizing clinical research. Targeted sequencing in particular is now implemented in many labs. While being well known that this unparalleled capacity is speeding up molecular diagnostics, it is also true that whenever a novel variant is detected, its pathogenicity must be carefully assessed and every now and again, a case pops up to highlight how tricky and delicate this process can be. Here we present a case of a molecular diagnosis of a patient with a clinical suspicion of Mucopolysaccharidosis (MPS) type VI, where even though the causal mutation was easy to detect by both Sanger and NGS, only through indirect studies could we present proof of principle on its pathogenicity. Methods: Initial studies were performed in gDNA, by classical sequencing of the ARSB gene, encoding arylsulfatase B, the enzyme deficient in MPS VI. Additional analyses included segregation studies, cDNA sequencing and NGS with in a custom gene for Lysosomal Storage Diseases (LSD) that includes 86 genes implicated in lysosomal function. Results and Discussion: Sanger sequencing of all ARSB exons and their intronic flanking regions unveiled the presence of a novel c.1213+5G>T [IVS6(+5)] homozygous mutation, with several bioinformatic predictors supporting its pathogenecity. Moreover, segregation studies confirmed its presence in heretozygozity in both parents. Still, only after a proper cDNA analysis could we confirm its effect in splicing. Unfortunately, we only had access to an extremely degraded cDNA sample obtained from blood of one of his parents. Surprisingly however, the splicing pattern observed after cDNA amplification of that sample was absolutely normal.

Thus, the case was included in a set of samples subjected to a NGS-based workflow for the identification of LSD-causing variants. After variant calling, it became clear that the IVS6(+5) ARSB mutation was the most probable cause for disease. Still, its pathogenicity had yet to be proven. We then conducted a classical sequencing approach of the ARSB gDNA and cDNA on the proband's father and ended up demonstrating that, while being heterozygous for a few SNPs in the surroundings of the mutation, the same individual seemed to be wild-type homozygous for those exact same SNPs at cDNA level. This observation provided indirect proof of the mutation's effect on splicing, further suggesting that the mutant transcript is degraded by nonsense-mediated mRNA decay (NMD). Overall, this case reminds us that, whatever the technology we use, genetic testing still needs much perseverance and cunning strategies to identify the causative mutation(s).

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P30 | Clinical Genetics

KBG Syndrome: a de novo chromosomal rearrangement in prenatal diagnosis beyond conventional cytogenetics

Carvalho I[1], Freixo J[1], Cruz J[2], Oliveira N[2], Marques B[3], Correia H[3], Morton C[4,5,6,7], David D[3]

[1]Unidade de Genética Médica, Hospital Dona Estefânia, CHLC, EPE; [2]Centro de Diagnóstico Pré-Natal, Maternidade Dr. Alfredo da Costa, CHLC, EPE; [3]Departamento de Genética Humana, INSA, Lisboa; [4]Harvard Medical School, Boston, MA, USA; [5]Broad Institute of MIT and Harvard, Cambridge, MA, USA; [6]Departments of Obstetrics and Gynecology, and of Pathology, Brigham and Women's Hospital, Boston, MA, USA; [7]University of Manchester, Manchester Academic Helath Science Center, Manchester, UK

Introduction: KBG syndrome (OMIM #148050) is a rare disorder characterized by a typical facial dysmorphism, macrodontia of the upper central incisors, skeletal anomalies, short stature and developmental delay. Cutaneous syndactyly, webbed short neck, cryptorchidism, hearing loss, palatal defects, strabismus and congenital heart defects are less common findings. Although this is an autosomal dominant condition predominant maternal inheritance, mainly due to a milder clinical manifestation in females, is frequently observed. Pathogenic alterations, mainly truncating point mutations and microdeletions, leading to haploinsufficiency of ANKRD11, have been described to be the molecular basis of this syndrome.
Clinical Report: A 39 years old female with irrelevant previous medical history and ongoing 2nd pregnancy was referred to our outpatient clinic due to increased risk for aneuploidies according to 1st trimester screening. Invasive prenatal diagnosis (PND) showed a de novo balanced chromosomal aberration (dnBCA): 46,XX,t(16;17)(q24;q21.3)dn. The 20th week ultrasound revealed hypoplastic nasal bone, atrioventricular septal defect (AVSD) and ventricular septal defect (VSD). Microarray was performed and no clinical relevant CNV's were detected. Large-insert whole-genome sequencing (liWGS) for identification of dnBCA breakpoints at nucleotide resolution was performed. This approach identified the 16q24 and 17q21.3 breakpoints within IVS3 of ANKRD11 and IVS1 of WNT3, respectively. Haploinsufficiency of ANKRD11 causes dominant KBG syndrome, whereas of WNT3 results in recessive tetraamelia syndrome (OMIM #165330). Although the translocation results in fusion genes no evidence of chimeric transcripts was found. Elective C-Section was performed due to fetal distress at 35th week with no complications for the female newborn. Postnatal echocardiography confirmed the AVSD with VSD and at 20 months old she presents mild developmental delay.
Discussion: We here describe the first case of KBG syndrome due to a dnBCA identified in PND. Disruption of both genes by the translocation breakpoints results in their haploinsufficiency. While haploinsufficiency of ANKRD11 leads to the autosomal dominant KBG syndrome the one of WNT3 is benign or subclinical. Additionally, we also demonstrated the importance of whole-genome sequencing for identification of dnBCA breakpoints at nucleotide resolution allowing an improved genetic counseling to the parents. Therefore, we recommend inclusion of this approach into current PND care.

P31 | Cytogenetics & Genomics

Sotos syndrome diagnosis by SNP array in a child with multiple malformations

Gonçalves D.[1], Correia H.[1], Onofre C.[1], Ribeiro C.[1], Silva F.[1], Azevedo A. R.[1], Cruz F.[1], Santos J.[1], Correia I.[1], Cavaleiro C.[1], Luis C.[2], Barreta A.[1]

[1]Serviço de Genética Médica, Joaquim Chaves Saúde; [2]Consulta de Neurologia Pediátrica, Departamento de Pediatria do Hospital Professor Doutor Fernando Fonseca, EPE

Sotos syndrome (MIM117550) is a congenital developmental disorder characterized by overgrowth during childhood, distinctive craniofacial configuration and learning disabilities. Convulsions, scoliosis and anomalies in the renal, cardiac and central nervous systems are also associated with this syndrome. Sotos 1 syndrome is caused by haploinsufficiency, either by mutation or deletion of NSD1 gene, which is located at chromosome 5q35. Microdeletions in 5q35 region including the NSD1 gene are reported in about 10% of the Sotos syndrome cases in European and North-American populations. We report a case of an 8 month old child with moderate psychomotor development delay, epilepsy, severe strabismus, dolichocephaly and congenital cardiopathy, in which increased fetal nuchal translucency had been detected on the first trimester ultrasound. Postnatal microarray analysis revealed a copy number loss in the region 5q35.2-q35.3, compatible with a ≈2Mb deletion. This region comprises 27 OMIM referenced genes, including NSD1 (606681), which is in accordance with clinical data. This case highlights the value of SNP array in prenatal diagnosis of fetuses with ultrasound anomalies, such as increased nuchal translucency. Furthermore, it shows the usefulness of this approach in the characterization of microdeletions, establishing well-defined breakpoints and allowing a better genotype-phenotype correlation as well as a more accurate genetic counseling.

P32 | Cytogenetics & Genomics

Study of a patient cohort with suspected microdeletion/microduplication syndrome using two MLPA® Panels

Rosas M.[1,2], Candeias C.[2,3], Chaves R.[4,5], Gouveia M.[2], Mota Freitas M.[2,3], Oliva Teles N. [2,3]

[1]Universidade de Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal; [2]Centro de Genética Médica Doutor Jacinto Magalhães/Centro Hospitalar do Porto, E.P.E., Porto, Portugal; [3]Unidade Multidisciplinar de Investigação Biomédica (UMIB)/ICBAS-UP, Porto, Portugal; [4]Universidade de Trás-os-Montes e Alto Douro (UTAD), Departamento de Genética e Biotecnologia (DGB), CAG—Laboratório de Citogenómica e Genómica Animal, Vila Real, Portugal; [5]Universidade de Lisboa, Faculdade de Ciências, BioISI—Biosystems & Integrative Sciences Institute, Lisboa, Portugal

Introdution: Chromosomal microdeletions and microduplications are typically 1 to 2 Mb long and are usually associated with genetic syndromes. They have been reported as the cause of multisystemic pathologies frequently associated with intellectual disability, developmental delay, multiple congenital anomalies, autistic spectrum disorders and diverse phenotypic findings. Multiplex Ligation-dependent Probe Amplification (MLPA, MRC-Holland, The Netherlands) is a good screening method for chromosomal microdeletions and microduplications. A new MLPA microdeletion panel for laboratory use, aiming to test patients for several new microdeletion syndromes, SALSA® MLPA® probemix P297 Microdeletion syndromes-2, is recently commertially available.

Materials and methods: In this study 96 samples previously analysed for both chromosomal studies and SALSA® MLPA® Probemix P245 Microdeletion syndromes-1 with normal results, were tested using the P297 panel. Additionally, three samples of patients with a previously known diagnosis, were performed to confirm and to validate the technique.

Results: Eighty nine of the 96 samples were normal and seven revealed a copy number alteration. The abnormal results observed in the 10 cases were as follows: two revealed copy number alterations in the band 1q21.1; three showed an alteration in copy number in band 3q29; four showed an alteration in copy number in band 15q13.3; and one had an alteration in copy number in band 18q21.2. It was possible to study the origin of the alteration in five cases; four have been inherited and one was de novo.

Discussion and Conclusion: With this work the authors have demonstrated the usefulness of MLPA approach for the establishment of diagnosis in patients presenting with rare unspecific phenotypes. MLPA is a very useful and economical resource, therefore recommended for more complete investigations of regions likely to be associated with microdeletion/microduplication syndromes.

P33 | Cytogenetics & Genomics

Non-specific clinical features: karyotype, MLPA or aCGH? A PTHS case report

Gouveia M.[1], Mota Freitas M.[1,2], Rosas M.[1], Candeias C.[1,2], Soares A.R.[1], Fortuna A.M.[1,2], Oliva-Teles N.[1,2]

[1]Centro de Genética Médica Doutor Jacinto Magalhães/Centro Hospitalar do Porto, E.P.E. (CGMJM/CHP, E.P.E.), Porto, Portugal; [2]Unidade Multidisciplinar de Investigação Biomédica/Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (UMIB/ICBAS, UP), Porto, Portugal

Introduction: Pitt-Hopkins syndrome (PTHS; OMIM #610954) was first described in 1978 in two patients with similar clinical manifestations, consisting mainly of intellectual disability, wide mouth and respiratory abnormalities. This syndrome is clinically similar to other intellectual disability syndromes, such as Angelman (OMIM #105830), Rett (OMIM #312750) and Mowat-Wilson (OMIM #235730). Genetic diagnosis of the patient is crucial not only to distinguish PTHS from other syndromes, but also to understand the underlying molecular mechanisms. Currently it is known that PTHS is caused by de novo heterozygous variants involving the TCF4 gene in 18q21.2. At least 50 pathogenic variants in the TCF4 gene have been known to cause PTHS. However, the type of mutation seems not to be related to the severity of the condition, since patients with large deletions or single nucleotide variants have both been reported with similar symptoms. Case report: In this study, we report a male patient aged two years with pervasive developmental disorders, microcephaly, apnea episodes and facial dysmorphic features, referred for cytogenetic confirmation of an interstitial deletion of 5,557Mb, in 18q21.1q21.2, detected by array comparative genomic hybridization (aCGH). Conventional cytogenetics (high-resolution banded karyotype) and multiplex ligation-dependent probe amplification studies (MLPA) with SALSA® MLPA® probemix P297-C1 Microdeletion syndromes-2 (MRC-Holland, The Netherlands) were performed and both confirmed the first result. The final karyotype result of the patient is: 46,XY,del(18)(q21.1q21.2).rsa 18q21.2(TCF4)x1. Discussion: Conventional cytogenetics is still a good and an economic first option for preliminary studies of dysmorphic/syndromic facial features. MLPA technique provides a quicker and more targeted analysis of various syndromes caused by microdeletions/microduplications, thus representing a good complement to karyotyping in the study of patients affected with a syndromic phenotype. In conclusion, these results highlight the importance of considering karyotyping and MLPA screening techniques before performing aCGH as the first line approach in some cytogenetic investigations, particularly in the detection of chromosomal abnormalities in patients with apparently non-specific clinical dysmorphims.

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A case report of a patient with a complex chromosomal anomaly combining 11p15.5 duplication and 18p deletion syndrome

Oliveira F.P.[1], Candeias C.[1,2], Mota Freitas M.[1,2], Tkachenko N.[1], Soares G.[1], Oliva Teles N.[1,2]

[1]Centro de Genética Médica Doutor Jacinto Magalhães/Centro Hospitalar do Porto, E.P.E. (CGMJM/CHP, E.P.E.), Porto, Portugal; [2]Unidade Multidisciplinar de Investigação Biomédica/Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (UMIB/ICBAS, UP), Porto, Portugal

Introduction: Among the clusters of imprinted genes in humans, one of the most relevant regions involved in human growth is localized in 11p15.5. Opposite epigenetic and genomic disturbances in this chromosomal region contribute to two distinct imprinting disorders associated with disturbed growth, Silver-Russell syndrome (SRS) and Beckwith-Wiedemann syndrome (BWS). The deletion of the short arm of chromosome 18 is now a well-known chromosomal aberration. It causes a wide range of medical and developmental concerns. There is significant variation in severity. This variation is due to the variability of the deletion size and breakpoints.

Case report: We report a female aged 41 referred for cytogenetic studies, presenting with severe mental retardation and several dysmorphic features. The karyotype was obtained with high resolution GTL banding, from peripheral blood lymphocyte cultures using standard techniques. Cytogenetic analysis revealed an abnormal chromosome 18 in all the metaphases. The combination of this finding with the complementary study of subtelomeric regions of all chromosomes by Multiplex ligation-dependent probe amplification (MLPA), using Salsa® MLPA® Kit PO36-E2 and PO70-B2 (MRC-Holland, the Netherlands) clarified the abnormality, interpreted as a derivative from a t(11;18). The outcome was both a partial duplication of the short arm of a chromosome 11, apparently 11p15.4 ->ter, and a partial deletion of the short arm of the abnormal chromosome 18, in 18p11.21. Therefore this patient presents with a partial trisomy of the short arm of chromosome 11, which might be associated with one of two syndromes: BWS or SRS, and a partial monosomy of chromosome 18 compatible with 18p Deletion Syndrome. Clinical reevaluation indicated that this patient did not have clinical features compatible with neither BWS nor SRS. Parental karyotyping has been requested.

Conclusion: We present this case of a female with 11p11.4->ter duplication and an 18p deletion, in which the phenotypic features were unusual for any one of the three syndromes, which can be explained by the fact that she has a complex chromosomal anomaly, or by the presence of other unidentified genetic anomalies. Karyotyping and molecular studies of the subtelomeric regions for chromosomes 11 and 18 in the parents are important in order to establish if this alteration is de novo or inherited. Genetic counseling has been offered in order to evaluate other family members at risk.

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Derivative chromosome 7 in a newborn with hypotelorism, cleft palate, agenesis of corpus callosum and semilobar holoprosencephaly

Simão L.[1]; Serafim S.[1]; Brito F.[1]; Ventura C.[1]; Scortenschi E.[2]; Santos V.[2]; Correia H.[1]

[1]Unidade de Citogenética, Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge,I.P.,Lisboa; [2]Serviço de Medicina Intensiva Pediátrica e Neonatal, Centro Hospitalar Universitário do Algarve, Unidade de Faro.

Cytogenetically visible unbalanced chromosome rearrangements involving the euchromatic regions most often result in severe phenotypic features. Often, it is not possible at microscopic level to distinguish if a chromosomal anomaly involves one or more than one chromosome. In these cases, the parents study is fundamental and is usually the first line of study. We report a female newborn with multiple anomalies. Ultrasonography at 32+6 weeks of gestation revealed moderate ventricular dilatation, microcephaly and intrauterine growth restriction (IUGR). Delivery was at 35 weeks and microcephaly, hypotelorism, complete medium cleft palate with nasal depression, agenesis of the corpus callosum, thalamic fusion and fusion of the lateral ventricles in the frontal region suggestive of semilobar holoprosencephaly (HPE) was observed. Seizures and nistagmus were described since the eighth day. Hypotonia was present. In addition, diabetes insipidus was diagnosed. Sepsis was developed at day 14 followed by death at day 18 in consequence of seizures and respiratory insuficiency. Cytogenetic analysis revealed an abnormal chromosome 7qter as a result of an unbalanced segregation of a maternal reciprocal translocation t(7;19), with breakpoints at 7q36.1 and 19q13.42. The newborn karyotype is 46,XX,der(7)t(7;19)(q36.1;q13.42)mat. The patient presented a partial trisomy of the region 19q13.42→qter and a partial monosomy of the region 7q36.1→7qter. Partial monosomy of chromosome 7qter has been characterized by a wide phenotypic manifestations, but HPE, microcephaly, midface hypoplasia, maxillary anomalies and sacral agenesis are frequently described. However, is not often reported in newborns. Partial trisomy 19q is a rare and severe condition, and has been described associated with low birth weight, growth retardation, microcephaly, seizures, dysmorphic facial features, short neck, clynodactyly, heart malformations, anomalies of the genitor-urinary and gastrointestinal tract. To our knowledge, there is only one previous case of der(7)t(7q;19q)(q36.1;q13.43) described, in a fetus who presented severe sacral agenesis and IUGR. The case herein reported presents some of the most common features of 7q36 partial monosomy and 19q terminal trisomy, although some of them are present in both conditions. The presence of those two imbalances may complicate the final phenotype but the important matter will be the counseling of the couple and to prevent future imbalances in their offspring.

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3(p25.3) proximal interstitial de novo deletion in a female patient with severe intellectual disability, absent speech, epilepsy and craniofacial dysmorphisms

Ferreira SI[1], Pires LM[1],Val M[1], Venâncio M[2], Carreira IM[1,2,3],Melo JB[1,2,3]

[1]Laboratório de Citogenética e Genómica, Faculdade de Medicina da Universidade de Coimbra; [2]Unidade de Genética Médica, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Portugal; [3]CNC, IBILI, Group of Aging and Brain Diseases: Advanced Diagnosis and Biomarkers, Coimbra, Portugal; [4]CIMAGO—Centro de Investigação em Meio Ambiente, Genética e Oncobiologia, Coimbra, Portugal

Introduction: Deletions at the distal portion of the short arm of chromosome 3 cause a recognizable syndrome with characteristic features, most frequently arising de novo and with breakpoints at band 3(p25). Interstitial deletions involving only sub-band 3(p25.3) are less frequently reported, and within this region two deletion areas can be defined: distal and proximal deletions.

Clinical Report: We report a 24 year old female with global developmental delay (DD), severe intellectual disability (ID), absent speech, epilepsy and craniofacial dysmorphisms. Due to her severe ID, absent speech and dysmorphic features, she was initially considered an Angelman syndrome patient. However, array-CGH analysis revealed a de novo 1Mb interstitial deletion at band 3(p25.3) between positions 10,364,749 and 11,421,309 (hg19).

Discussion: The reported deletion overlaps with deletions previously reported in the most proximal area of region 3(p25.3), although there are only 5 patients reported in the literature with this imbalance. These patients present a common phenotype consisting of DD, ID, absent or poor speech and epilepsy or EEG anomalies. The commonly deleted region includes the 3 last coding exons of SLC6A11 gene, SLC6A1 gene and its antisense gene, HRH1 gene and part of ATG7 gene. Both SLC6A genes code for Gamma-aminobutyric acid (GABA) transporters, responsible from removing GABA from the synapse. SLC6A1 gene is reported in OMIM Morbid Map as heterozygous mutations are responsible for myoclonic-atonic epilepsy and is considered haploinsufficient. These data support the association of SLC6A1 gene and the phenotype of epilepsy among patients with 3(p25.3) deletion.

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Prenatal diagnosis by chorionic villus sampling: special caution needed during results interpretation

Val M.M.[1]; Marques V.[1]; Pires L.M.[1]; Lavoura N.[1]; Ferreira S.I.[1]; Mascarenhas A.[1]; Jardim A.[1]; Pinto M.C.[1]; Melo J.B.[1,2,3,4]; Carreira I.M.[1,2,3,4]

[1]Laboratório de Citogenética e Genómica, Faculdade de Medicina da Universidade de Coimbra; [2]Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal; [3]CNC, IBILI, Group of Aging and Brain Diseases: Advanced Diagnosis and Biomarkers, Coimbra, Portugal; [4]CIMAGO— Centro de Investigação em Meio Ambiente, Genética e Oncobiologia, Coimbra, Portugal

Introduction: Whole-chromosome aneuploidy is currently known as the leading cause of miscarriage and congenital birth defects in humans. Consequently, this chromosomal abnormality is the most common indication for invasive prenatal diagnosis by chorionic villus sampling (CVS) or amniocentesis, allowing the study of fetal chromosome constitution. CVS is performed earlier than amniocentesis; however it has the disadvantage that about 1-2% of CVS results may reflect confined placental mosaicism (CPM) instead of true fetal chromosomal abnormalities.

Methods: From a cohort of around 4000 samples (20% CVS and 80% amniocentesis) that were received in our laboratory for prenatal diagnosis, 23% had indication for rapid aneuploidy detection test. Highly polymorphic short tandem repeats (STRs) on chromosomes 13, 18, 21, X and Y were used to detect the most common aneuploidies by quantitative fluorescent polymerase chain reaction (QF-PCR).

Results and discussion: Of all CVS received, 40% were tested and the main indications were: increased nuchal translucency (NT), a positive biochemical screening, hygroma, omphalocele or other ultrasound abnormalities. A negative result for aneuploidy test was verified in 73% CVS, while the remaining 27% presented a positive result: 4% trisomy 13, 24% trisomy 18, 52% trisomy 21, 13% monosomy X, 6% triploidy and 1% 48,XXXX. Among the CVS with a positive result for aneuploidy testing, a rare case was detected: a trisomy 21 where only 2 different alleles were evident in 2:1/1:2 ratios meaning that 2 of the 3 alleles were exactly identical. As this result was obtained in a CVS, this could correspond to a CPM; however, the fetus presented a phenotype including increased NT (7.8 mm) and hygroma. Later, by conventional cytogenetics a trisomy 21 was observed. After pregnancy termination, STRs analysis on DNA from fetal skin biopsy confirmed the CVS result. Comparing the STRs profile of fetus and mother, it was possible to conclude that the trisomy 21 was not of maternal origin. Therefore, the trisomy 21 may be derived from a paternal meiotic II nondisjunction, an unusual condition observed in about 1% of the cases; or had origin in an early mitotic nondisjunction (≈2% of the cases). This particular result of a 2:1 ratio illustrates that caution should be made on the interpretation of rapid aneuploidy testing by QF-PCR, especially in CVS, as we tend to associate this type of results to CPM and indeed they can be due to other biological mechanisms.

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Satellited chromosome 10 (10qs) identified in a related donor after peripheral stem cell transplantation in a patient with Philadelphia-positive acute lymphoblastic leukemia

Torres L.[1,2]; Correia C.[1,2,3]; Lisboa S.[1,2]; Vieira J.[1,2]; Bizarro S.[1,2]; Cerveira N.[1,2]; Oliveira M.J.[3]; Campilho F.[4]; Campos-Júnior A.[4]; Teixeira M.R.[1,2,5]

[1]Department of Genetics, Portuguese Oncology Institute of Porto (IPO-Porto), Porto, Portugal. [2]Cancer Genetics Group, IPO-Porto Research Center (CI-IPOP), Portuguese Oncology Institute of Porto (IPO-Porto), Porto, Portugal. [3]Unidade de Citogenética, SYNLAB-GDPN, Porto, Portugal. [4]Department of Bone Marrow Transplantation, Portuguese Oncology Institute of Porto (IPO-Porto), Porto, Portugal. [5]Pathology and Molecular Immunology, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal.

Introduction: The human acrocentric chromosomes are identified by the presence of satellited short arms, which include the nucleolar organizer regions (NORs) located in the satellite stalks. NORs localization in chromosome positions other than the short arms of acrocentric chromosomes (non-acrocentric satellite chromosomes - NASC) are rare findings, and the phenotypic consequences for carriers are variable, depending namely on the underlying mechanism to the rearrangement.

Clinical Report: We present a case of a 51-year-old male with a diagnosis of Philadelphia-positive acute lymphoblastic leukemia in September 2013. In June 2014, the patient was treated with allogeneic peripheral stem cell transplantation (SCT) from a related donor (sister), from whom clinical history is not available. The bone marrow karyotype on day 52 after the SCT revealed, in all 20 metaphases analyzed, a female karyotype with a satellited chromosome 10 (10qs). The same karyotype was observed in subsequent bone marrow evaluations, until patient death 22 months after the SCT. Confronted with this unexpected finding, we proposed at the time genetic counseling to the bone marrow donor.

Discussion: Although rare, the presence of constitutional chromosomal aberrations in donor cells after SCT or bone marrow transplantation (BMT) have been reported. This case is particularly interesting since it combines two rare findings, not only the abnormal karyotype of the donor cells, but also the chromosome abnormality itself. Ectopic NORs have been reported in several chromosomal breakpoints, including 1p, 1q, 2p, 2q, 3q, 4p, 4q, 9p, 9q, 10q, 12p, 18p, Yp and Yq. Satellited chromosome 10q has been previously described in a few reports, and is associated with normal and abnormal phenotypes, which are related to the absence or presence of 10q subtelometic imbalances, respectively. This type of case presents a challenge and an alert to some ethical and practical issues, especially when the bone marrow donor is unrelated.

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aCGH still needs Karyotype to characterize derivative chromosomes

Pinto J.[1,2], Lima V.[1,2], Leão M.[3], Oliveira R.[3], Dória S.[1,2]

[1]Serviço de Genética, Departamento de Patologia, Faculdade de Medicina, Universidade do Porto; [2]i3S—Instituto de Investigação e Inovação em Saúde, Universidade do Porto; [3]Consulta de Genética—CHSJ Porto.

Introduction: Microdeletion syndromes are well-known causes of developmental delay and/or malformations. The 18q deletion syndrome are associated to a highly variable clinical phenotype, generally characterized by mild to moderate mental retardation, developmental delay, craniofacial dysmorphisms, white matter abnormalities of the brain, limb anomalies and heart defects. This study describes two patients presenting derivative 18 chromosomes identified by aCGH.

Case Reports: Patient 1: four years-old boy with cleft palate, epilepsy, cerebral abnormalities and global development delay. Patient 2: two month-old boy with complete cleft-palate, hypospadias, right cryptorchidism, craniofacial dysmorphisms, bilateral overlapping 2/3/4 toes and bilateral optic nerve dysplasia. Agilent 4x180K microarrays and cytogenomics 4.0.2.21 software, Karyotype and FISH analysis were according to standards methods. Results: In Patient 1, aCGH revealed a 17,5Mb deletion and 3,8Mb duplication in terminal regions of the chromosomes 18q and 20p, respectively. Parental karyotypes have been requested. aCGH performed in Patient 2 revealed a 11,4Mb duplication and a 21,9Mb deletion in the terminal regions of the chromosomes 6 and 18, respectively. Mean log ratios of 0,36 (dup6pter) and -0,413 (del18qter) were observed. Karyotype showed 46,XY,der(18)t(6;18)(p25.3;q21.31) in 15/30 metaphases analysed. FISH showed the der(18) in 103/200 cells analysed. Parental karyotypes were normal.

Discussion: The simultaneous presence of terminal deletions and duplications suggests the presence of derivative chromosomes resulting from reciprocal translocations in the parents. In patient 2 the mean log ratios values for the CNVs suggested mosaicism and this hypothesis was confirmed by karyotype and FISH. The parental karyotypes result indicates a de novo abnormality. These cases stress the value of the karyotype allowing the characterization of the rearrangements. A genotype-phenotype correlation could be easy to establish in these two patients, because of the recurrent 18q deletion syndrome. Although, the duplications of 20pter and 6pter chromosomes could also contribute to the phenotypes. Additionally, in patient 2, the mosaicism could also mitigate the phenotype. So, it is extremely important to correlate the type of abnormality found and the phenotype and published in the literature. This could provide a better genetic counselling in the future, particularly for prenatal cases.

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CNVs distribution in gene regions associated with typical orofacial clefts: preliminary results

Santos SP.[1]; Lustosa-Mendes E.[2]; Dos Santos AP.[2]; Torres FR.[2]; Molck MC.[2]; Barbosa FB.[1]; Araujo TK.[1]; Gil-da-Silva-Lopes VL.[2] and Simões AL.[1]

[1]Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil. [2]Department of Medical Genetics, Faculty of Medical Sciences, State University of Campinas, São Paulo, Brazil.

Introduction: Copy Number Variations (CNVs) are genomic segments usually larger than 1 kb, which vary in copy number compared to the reference genome. CNVs contribute to the variability among individuals in the risk of developing diseases, including congenital malformations as typical orofacial clefts (OFCs). OFCs include fissures in the lip or palate, which generally have isolated clinical presentation (nonsyndromic oral cleft - NSOFC) and multifactorial etiology. However, in a considerable proportion of cases, there is an association with other congenital defects (syndromic oral cleft - SOFC) and are several etiologic mechanisms. Despite efforts to understand the OFCs' etiology, molecular mechanisms underlying cleft development have not been fully characterized and little is known about the CNVs contribution. This work aims to investigate CNVs in gene regions previously associated with OFCs and to identify those that are related to the clinical picture.
Methods: We analyzed data previously obtained by CytoScan HD Array (Affymetrix®) from samples of 59 patients with OFCs (35 NSOFC and 24 SOFC) and 110 healthy Brazilians without a family history of OFC (control group).
Preliminary Results and Discussion: There were 1443 CNVs (1008 deletions and 435 duplications) in the patients and 2673 (1672 deletions and 1001 duplications) in the controls, which corresponds to an average of 24 CNVs per subject in both groups. All chromosomes presented variants and, on average, 0.076% and 0.070% of the genome was variable in patients and controls, respectively. Most CNVs were in the range of 10 to 100 kb in both groups. We identified 1010 CNVs (69.99%), in the patients, and 1867 CNVs (69.85%), in the controls, which occurred total or partially in gene regions. Of these, 19 CNVs from patients and 31 from controls involved genes associated with OFCs. In the literature review, we found 171 genes associated with NSOFC or SOFC. Our results reinforce the multifactorial character of the disease, demonstrating that genetic alterations in isolation may not generate orofacial malformations.
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A rare case of 8p inverted duplication/deletion syndrome in prenatal diagnosis

Trindade J[1], Alves C[1], Cerveira I[2], Pereira N[2], Monteiro R[1], Lopes M[1], Ventura V[1], Baltar F[1], Correia C[1], Reis Lima M[1]

[1]Unidade de Citogenética, Synlab-GDPN, Porto; [2]Unidade de Medicina Fetal-DPN, Centro Hospitalar de Tondela-Viseu

Introduction: Inverted duplication and deletion 8p syndrome or Invdupdel(8p) is a well described and very rare chromosome rearrangement, first described by Welebar et al., in 1976. Frequency is estimated in 1/10.000-1/30.000 live births. Most cases described so far were ascertained in the first months/years of life, in children with neurodevelopmental delay, facial dysmorphism, Central Nervous System (CNS) anomalies, skeletal anomalies and congenital heart defects. As ultrasound became a routine screening method for fetal anomalies, a higher number of invasive diagnostic procedures are performed when abnormalities are detected, allowing early identification of chromosome anomalies and medical intervention.

Clinical report: We present a case in which the only ecographic fetal finding detected at 21 weeks of gestation was complete corpus callosum agenesis, in an otherwise uneventful pregnancy of a 33 year-old woman. Karyotype after amniocentesis showed a structural abnormality of chromosome 8 short arm, interpreted initially as 46,XX,der(8)?del(8)(p23.1)dup(p23.1p11.2). Array-CGH analysis revealed a deletion of 8Mb involving chromosome 8p23.3p23.1 regions and a total of 162 genes, and a duplication of 31Mb of the 8p23.1p11.1 segment, encompassing 479 genes. Pregnancy was terminated. Parental karyotypes were normal.

Discussion: About 50 cases of invdupdel(8p) syndrome have been reported in the postnatal period. Only very few reports in the literature describe this chromosomal rearrangement detected prenatally. The most frequent ultrasound finding is a CNS anomaly: partial or complete corpus callosum agenesis. In the present case, the array-CGH allowed a more accurate characterization of the abnormality, showing that the rearranged chromosome consisted of a terminal deletion 8pter (segment 8p23.3-8p23.1) and inverted duplication of the segment 8p23.1-8p11.1, known as inverted duplication and deletion 8p syndrome (ORPHA96092). Although this abnormality is large enough to be detected in a conventional karyotype, array-CGH should be recommended in the presence of abnormal ultrasound scan at prenatal diagnosis. To date, all cases of this syndrome have occurred de novo. Array-CGH may help to better establish the genotype-phenotype correlation. It is a more powerful tool for the detection of genotype imbalances, empowering clinical utility and allowing early diagnosis during the gestational period and a better genetic counselling to the families.

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Potocki-Lupski Syndrome (dup 17p11.2) in a child with psychomotor developmental delay

Monteiro R[1], Trindade J[1], Lopes M[1], Guimarães MJ[2], Ventura V[1], Oliveira MJ[1], Alves C[1], Correia C[1], Reis-Lima M[1]

[1]Unidade de Citogenética, Synlab-GDPN, Porto; [2]Clínica Pediátrica, Porto

Introduction: Potocki-Lupski Syndrome (PTLS) [ORPHA1713; OMIM 610883], also known as duplication 17p11.2 syndrome, is a rare chromosomal anomaly syndrome, affecting 1 in 20.000 live births. It is characterized by infantile hypotonia, failure to thrive, cardiovascular malformations, developmental delay, intellectual disability and behavioural problems, autistic spectrum disorder, apnea and dysmorphism. The majority of the individuals with this syndrome (~67%) harbor a common recurrent 3.7Mb microduplication at 17p11.2, which is the homologous reciprocal recombination of Smith-Magenis syndrome microdeletion. The critical PTLS region was identified as a 1.3Mb segment that includes the RAI1 gene (Retinoic Acid Inducible 1), responsible for the major phenotypic features of this disorder.
Clinical Report: We present the case of a 3-year-old boy whose young and non-consanguineous parents noticed psychomotor developmental delay. The family and personal antecedents were uneventful. Karyotype analysis raised the suspicion of a small unbalanced structural anomaly in chromosome 17, interpreted as a partial duplication of the short arm of this chromosome (band 17p11.2). Array-CGH analysis confirmed the presence of a ~3.46Mb duplication on chromosome 17p11.2 band, which includes the PTLS critical region, and hence is classified as pathogenic. Cytogenetic analysis of the parents revealed normal karyotypes.
Discussion: A de novo duplication on chromosome region 17p11.2 was identified in a child with psychomotor developmental delay, that correlates with the phenotype. This duplication is characteristic of PTLS and contains the dosage sensitive RAI1 gene. Due to their generally mild phenotype, PTLS patients may go unrecognized until later in infancy or childhood. In this case, the diagnosis was established with the support of array-CGH. Although small structural abnormalities may sometimes be visible in high resolution cytogenetic analysis (average size 3-5Mb), as was the case, array-CGH analysis is recommended as a first-tier cytogenetic diagnostic test, in patients with development delay/intellectual disability.

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An unusual case of MECP2 Duplication Syndrome resulting from a t(X;13) non-reciprocal translocation

R. Silveira-Santos, S. Custódio, C. Machado, A. Sousa, A. B. Sousa.

Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal - rosario.santos@chln.min-saude.pt

MECP2 duplication syndrome (MIM#300260) is an X-linked neurodevelopmental disorder, primarily affecting males. Clinical features include severe intellectual disability, speech abnormalities, early infantile hypotonia, progressive spasticity, seizures and recurrent infections. The syndrome is caused by variable size Xq28 duplications involving the MECP2 gene, and the majority of affected males inherit the duplication from their usually asymptomatic carrier mothers. Xq28 duplications may be caused either by an intrachromosomal duplication or, rarely, by an unbalanced X/Y or X/autosome translocation. Here, we report a case of Xq28 microduplication due to an unbalanced t(X;13) translocation in a 16-year-old boy. Hypotonia was noticed at 3 months of age. At 16, the proband has severe intellectual disability, with absence of speech, no ambulation, mild facial dysmorphism, recurrent infections and seizures. Head MRI revealed periventricular and subcortical leukomalacia and. The family history is negative for neurodevelopmental conditions. ArrayCGH analysis (PerkinElmer® CGX-HD 180K, Genoglyphix v3.1) identified a 3.03 Mb terminal duplication of the long arm of chromosome X encompassing 71 OMIM genes including MECP2. Confirmatory fluorescence in situ hybridization (FISH) with BAC and subtelomeric specific probes revealed that the duplicated Xq28 region has been translocated to the tip of the long arm of chromosome 13, at 13q34. The rearrangement was not detected in the asymptomatic mother whereas the father was unavailable for FISH analysis. Since no other abnormality was detected by our arrayCGH platform, namely in chromosome 13, we assume this case results from a non-reciprocal unbalanced translocation. The molecular karyotype of the patient is thus: ish der(13)t(X;13)(Xq28;q34).arr[GRCh37] Xq28(152193146_155225290)x2. To the best of our knowledge, this is the second reported case of a cryptic unbalanced translocation between chromosomes Xq and 13q, and is the first report of such a translocation in a male leading to functional disomy of MECP2. The autosomal imbalance in der(13) is very limited or absent, and does not impact on the phenotype. In light of the molecular findings, the patient's clinical manifestations were considered a good fit for MECP2 duplication syndrome. This case highlights the essential role of FISH as a second step analysis to assess the relative position of chromosomal segments involved in microduplications.

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Cytogenetic study in infertile couples: incidence of reciprocal translocations

Arantes R.[1], Pinho P.[2], Souto M.[3], Botelho P.[3], Gomes Z.[4], Brito M.[4], Moutinho O.[4], Martins M.[5], Pinto Leite R.[3]

[1]Hospital Center of Trás-os-Montes and Alto Douro, Genetics Laboratory- Andrology Laboratory, Vila Real, Portugal [2]University of Trás-os-Montes and Alto Douro, Vila Real, Portugal [3]Hospital Center of Trás-os-Montes and Alto Douro, Genetics Laboratory, Vila Real, Portugal. [4]Hospital Center of Trás-os-Montes and Alto Douro, Maternal and Child Department, Vila Real, Portugal [5]Hospital Center of Trás-os-Montes and Alto Douro, Genetic Consultation, Vila Real, Portugal

Introduction: Infertility is a complex multifactorial condition with male and female factors involved and it affects around 15% of couples worldwide. The cytogenetic analysis is considered an important approach in this area since numerical and structural alterations can be responsible for infertility. Individuals with reciprocal translocations are phenotypically normal, but due to alterations at the meiotic segregation they have an increased risk of producing gametes with chromosomal imbalances leading to infertility, increased risk of pregnancy loss and birth of handicapped children. Some reports suggest that reciprocal translocations in men may explain the worst semen parameters, namely, sperm concentration, motility and morphology. The aim of the present study was to describe the reciprocal translocations detected in 340 couples who participated in the infertility consultation between November 2009 and December 2016 at the Hospital Center of Trás-os-Montes and Alto Douro. The relationship between the reciprocal translocations found with the semen parameters was evaluated.

Methods: Chromosome analysis was performed according to standard techniques and, at least, 20 metaphases were analyzed. Semen analysis was done according to World Health Organization (WHO, 2010) guidelines.

Results: This work presents the reciprocal translocations found in 680 cytogenetic studies. Eight cases of balanced reciprocal translocations (1.18%) were detected in different couples, 4 in women and 4 in men: 46,XX,t(12;15)(q13.3;q24.3), 46,XX,t(5;9)(p13;q34), 46,XX,t(10;15)(q11.23;q13), 46,XX,t(2;3;15)(p22;p12.1;q26.2), 46,XY,t(4;22)(p16.1;q11), 46,XY,t(6;8)(p23;q21.3), 46,XY,t(11;22)(q14.2;q13.1) and 46,XY,t(4;10)(q31.3;p15). The semen analysis in the case with a 46,XY,t(6;8) karyotype revealed normal values, in the 46,XY,t(4;22) case an oligoastenozoospermia was detected and in cases 46,XY,t(11;22) and 46,XY,t(4;10) an oligoteratozoospermia was found. Family studies are still ongoing to confirm if these cytogenetic anomalies are de novo or inherited.

Discussion: Despite the small number of cases and the low incidence of reciprocal translocations detected, the results of this study strongly point out the importance of peripheral blood karyotype analysis of infertile couples, allowing an adequate genetic counseling.

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Translocation t(17;20): an unexpected finding in a MDS case

Botelho P.[1];Mesquita B.[2], Guerra M.[2]; Souto M.[1]; Cunha M.[2]; Moutinho O.[3]; Pinto Leite R.[1]

[1]Laboratório de Genética, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real, Portugal; [2]Serviço de Hematologia, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real, Portugal; [3]Departamento da Mulher e da Criança, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real, Portugal

Introduction: The myelodysplastic syndrome(s) (MDS) represent a heterogenous group of clonal myeloid diseases characterized by cytopenia(s), dysplasia in one or more of the major myeloid cell lines, ineffective haematopoiesis and increased risk to progress to acute myeloid leukemia. Cytogenetic studies have an important role in setting up diagnosis and in evaluating the prognosis. Approximately 50% of de novo cases have cytogenetic abnormalities, like deletions, aneuploidies or unbalanced translocations. The del(5)(q22q35) is one of the most common structural rearrangements in MDS (10%), seen as an isolated abnormality (patients have a favorable prognosis) or with additional karyotypic anomalies (patients have significantly shorter survival). The authors present the results of a cytogenetic study in a 69 years old female patient diagnosed with MDS. Myelogram revealed a hypercellular marrow with a myeloid / erythroid 4.2: 1 ratio, marked myeloid dysplasia, with an increased blast number (7.6%). The other dysplastic hematopoietic lines were also increase but less pronounced. Bone marrow cytogenetic and Fluorescence in situ hybridization (FISH) analysis was required due the aggravation of anemia.
Methods: Bone marrow cell cultures and GTL banding and FISH were performed according to the protocols in the laboratory. Cytogenetic analysis followed the standard cytogenetic guidelines.
Results and Discussion: The patient karyotype was 46,XX,del(5)(q22q35)[2]/45,XX,del(5)(q22q35),der(17)t(17;20)(p10;p10)[13]/46,XX[5]: deletion on part of long arm of chromosome 5 and an unbalanced translocation involving chromosomes 17 and 20 resulting in the loss of the short arm of chromosome 17 and the long arm of chromosome 20. The 17p deletion involves the tumor suppressor geneTP53 that predicts a poorer prognosis and higher risk of transformation to AML. Breakpoint on chromosome 20 is frequently associated with other cytogenetic abnormalities as del(5q), trisomy 8, trisomy 21, deletions or translocations involving the long arm of chromosome 13. The deletion of the 20 q arm may confer a proliferative advantage to myeloid cells through deletion of one or several tumor suppressor genes, however the target genes remain unknown. The rearrangement detected may explain the aggravation of anemia with multilineage dysplasia. Cytogenetics analysis continues to be an important tool to confirm the diagnosis and offers critical information regarding to the prognosis and therapeutic strategy.

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Newborn with tetrasomy 18p following a low risk NIPT result - the importance of a good pre-test counselling

Pereira A.[1]; Sousa M.J.[1,2]; Lourenço T.[1]; Cruz G.[2]; Sousa G.[1,2]

[1]Laboratório de Genética, Centro de Medicina Laboratorial Germano de Sousa, SA; [2]Unidade de Diagnóstico Pré-Natal, Centro de Medicina Laboratorial Germano de Sousa, SA

Introduction: We report a postnatal detected de novo tetrasomy 18p in a newborn male from a 39-years-old mother who performed non invasive prenatal testing (NIPT), refusing invasive prenatal testing.
METHODS: Non invasive prenatal testing, array-CGH and cytogenetics studies.
RESULTS: Non invasive prenatal testing reported at 15th week showed low risk for aneuploidy of chromosomes 13, 18 and 21. After delivery of a male newborn, peripheral blood sample was collected for aCGH analysis due to a dysmorphic phenotype including peculiar facies, hypospadias, low set ears and member hypertonia on a male child. The microarray analysis detected a triplication of the whole short arm of chromosome 18 as a result of a supernumerary 18p isochromosome, subsequently confirmed by cytogenetic analysis.
CONCLUSIONS: The majority of tetrasomy 18p reported cases are ascertained postnatally and has a de novo occurrence strongly associated to advanced maternal age. The clinical use of non-invasive prenatal testing to screen high-risk patients for fetal aneuploidy is becoming increasingly common and is a technology with the potential to provide a variety of clinical benefits, namely miscarriages reductions. However, pregnant women who opted for NIPT need adequate counselling about the advantages but also, the limitations including that the test is not intended for detection of partial aneuploidies and that it cannot rule out all chromosomal anomalies and consequently a low risk result does not guarantee an unaffected pregnancy. The presented case highlights the importance of providing clear information about benefits and limitations of non-invasive prenatal testing.

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MIDAS Syndrome: all that glitters is not gold
A new case report with additional unsuspected CNVs

Rodrigues R.[1]; Machado C.[1]; Oliveira I.[2]; Sousa A.[1]; Sousa A.B.[1]

[1]Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal; [2]Serviço de Pediatria, Hospital de São Bernardo, Centro Hospitalar de Setúbal, Setúbal, Portugal

Introduction: Microphthalmia, dermal aplasia, and sclerocornea (MIDAS) Syndrome is a rare X-linked, male lethal, dominant disorder. The use of arrayCGH as the first laboratorial approach in the investigation of a newborn girl with a clinical diagnosis of MIDAS Syndrome, in addition to confirming the diagnosis, enabled the identification of unsuspected copy number variants (CNVs). Karyotyping was invaluable in detecting mosaicism and allowing the characterization of the cytogenetic mechanism underlying the alterations observed by arrayCGH. arrayCGH results are instrumental in this newborn's clinical follow-up, as additionally to MIDAS syndrome, there are further syndromic regions within the detected CNVs to be considered. Patient and Methods: We describe a 27-day-old newborn girl referred to our Medical Genetics Department due to dysmorphic facial features, including skin lesions of dermal aplasia arranged in a linear pattern involving eyes, cheeks and neck; right eye anophthalmia; left eye microphtalmia with corneal opacification; and right pre-auricular pit. Anterior anus was also present. A DNA sample was studied by arrayCGH (180K CGX-HD; Signature Genomics, PerkinElmer). Genomic analysis was performed with Genoglyphix v3.1 software (Signature Genomics). Karyotyping was achieved by G-banding. Results and Discussion: arrayCGH showed two de novo pathogenic CNVs in Xp: arr[GRCh37] Xp22.33p22.2(296520_12432327)x1,Xp22.2p21.1(12445083_35221042)x3dn. G-banding analysis revealed a complex mosaic karyotype, with two abnormal cell lines: mos 46,X,der(X)del(X)(p22.2)dup(X)(p21.1p22.2)[24]/45,X[6]dn. Mutations or deletions in HCSS gene at Xp22 are causative of MIDAS Syndrome. To date, there are only about 50 cases reported worldwide, some with Xp terminal deletions overlapping our case, and presenting a similar phenotype. Nevertheless, there are reports of normal carriers of such deletions in Xp22, as X inactivation pattern is key in determining the phenotype. There have been reports of hypotonia, developmental delay, intellectual disability, scoliosis, cardiovascular problems and psychiatric disorders in cases encompassed within our reported duplication. Considering that MIDAS syndrome is phenotypically manifested, additional clinical problems are predicted due to Xp22.2-p21.1 duplication.

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Translocation t(17;20): an unexpected finding in a MDS case

Botelho P.[1]; Mesquita B.[2], Guerra M[2]; Souto M[1];Cunha M[2]; Moutinho O.[3]; Pinto Leite R.[1]

[1]Laboratório de Genética, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real, Portugal; [2]Serviço de Hematologia, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real, Portugal; [3]Departamento da Mulher e da Criança, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real, Portugal

Introduction: The myelodysplastic syndrome(s) (MDS) represent a heterogenous group of clonal myeloid diseases characterized by cytopenia(s), dysplasia in one or more of the major myeloid cell lines, ineffective haematopoiesis and increased risk to progress to acute myeloid leukemia. Cytogenetic studies have an important role in setting up diagnosis and in evaluating the prognosis. Approximately 50% of de novo cases have cytogenetic abnormalities, like deletions, aneuploidies or unbalanced translocations. The del(5)(q22q35) is one of the most common structural rearrangements in MDS (10%), seen as an isolated abnormality (favorable prognosis) or with additional karyotypic anomalies (significantly shorter survival). The authors present the results of a cytogenetic study in a 69 years old female patient diagnosed with MDS. Myelogram revealed a hypercellular marrow with a myeloid / erythroid 4.2: 1 ratio, increased blast number (7.6%) and marked myeloid dysplasia, with less pronounced dysplastic features in the other hematopoietic lines. Bone marrow cytogenetic and Fluorescence in situ hybridization (FISH) analysis was required due the aggravation of anemia.

Methods: Bone marrow cell cultures and GTL banding and FISH were performed according to the protocols in the laboratory. Cytogenetic analysis followed the standard cytogenetic guidelines.

Results and Discussion: The patient karyotype was 46,XX,del(5)(q22q35)[2]/45, XX,del(5)(q22q35),der(17)t(17;20)(p10;p10)[13]/46,XX[5]: deletion on part of long arm of chromosome 5 and an unbalanced translocation involving chromosomes 17 and 20 resulting in the loss of the short arm of chromosome 17 and the long arm of chromosome 20. The 17p deletion involves the tumor suppressor geneTP53, which predicts a poorer prognosis and higher risk of transformation to AML. Breakpoint on chromosome 20 is frequently associated with other cytogenetic abnormalities as del(5q), trisomy 8, trisomy 21, deletions or translocations involving the long arm of chromosome 13. The deletion of the 20 q arm may confer a proliferative advantage to myeloid cells through deletion of tumor suppressor gene. The rearrangement detected may explain the aggravation of anemia with multilineage dysplasia. Cytogenetics analysis continues to be an important tool to confirm the diagnosis and offers critical information on the prognosis and therapeutic strategy.

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Double hit: simultaneous 17p13.3-p13.1 duplication and 17p13.1 deletion

Rolo E.A.[1*]; Rodrigues R.[1*]; Dias P.[1]; Custódio S.[1]; Sousa A.[1]; Sousa A.B.[1]

[1]Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal
[*]These authors contributed equally to this work

Copy Number Variations (CNVs) in 17p13 are associated with several conditions, including 17p13.3 Duplication Syndrome and 17p13.1 Deletion Syndrome. The latter both cause intellectual disability, hypotonia and dysmorphic features; and 17p13.3 duplication is also associated with brain defects. Interestingly, 17p13.1 encompasses the TP53 gene, which is responsible for Li-Fraumeni Syndrome, an inherited cancer syndrome predisposing to a wide spectrum of tumors from an early age. A 5-year-old girl was evaluated for severe psychomotor developmental delay, hypotonia, microcephaly with Dandy-Walker malformation and growth retardation. The patient had feeding difficulties from birth and presented with severe malnutrition, aggravated by recurrent vomits resulting from proximal esophagus stenosis. At observation, she had a long face with a high forehead, hand and feet abnormalities with hyperconvex fingernails, and scoliosis. A DNA sample was studied by array-CGH (180K CGX-HD, PerkinElmer®). Genomic analysis was performed with Genoglyphix v3.1 software (PerkinElmer®). Complementary chromosomal analysis was performed by conventional G-banding karyotyping. Array-CGH identified two contiguous pathogenic CNVs in 17p13: (i) a terminal duplication on 17p13.3-p13.1 [arr 17p13.3p13.1 (48858_7556923)x3], including the critical region for 17p13.3 Duplication Syndrome; (ii) a deletion in 17p13.1 [arr 17p13.1 (7572619_7940756)x1], encompassing the critical region for 17p13.1 Deletion Syndrome and TP53, the causative gene for Li-Fraumeni Syndrome. Conventional cytogenetic analysis confirmed a tandem 17p13.3-p13.1 duplication profile. As expected, the 17p13.1 deletion was not apparent in the patient's karyotype. The patient's mother had a normal karyotype. The father was unavailable for karyotyping. Feeding difficulties and gastroesophageal reflux are a commonly described complication in 17p13.3-p13.1 Duplication Syndrome. In this case, the contributory proximal esophageal stenosis might be related to an as yet uncharacterized congenital malformation, a feature not previously described in this chromosomal abnormality. Fortunately, although TP53 is included in the deleted 17p13.1 region, complete deletion of this gene does not seem to cause an increased oncological risk. To our knowledge, this is the first report of a patient with simultaneous and contiguous 17p13.3-p13.1 duplication and 17p13.1 deletion, derived from a complex chromosomal rearrangement.

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Nucleotide-level resolution of a complex chromosomal rearrangement associated with cognitive disabilities reveals chromothripsis

Cardoso MP[1], Oliva-Teles N[2,3], Tkachenko N[2], Talkowski ME[4–7], Morton CC[5–8], Fortuna AM[2,3], David D[1]

[1]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, INSA IP, Lisboa, Portugal; [2]Centro de Genética Médica Doutor Jacinto Magalhães/Centro Hospitalar do Porto, EPE, Porto, Portugal; [3]Unidade Multidisciplinar de Investigação Biomédica/Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; [4]Center for Human Genetic Research, Departments of Neurology, Pathology and Psychiatry, Massachusetts General Hospital, Boston, USA; [5]Harvard Medical School, Boston, USA; [6]Broad Institute of MIT and Harvard, Cambridge, USA; [7]Departments of Obstetrics and Gynecology, and of Pathology, Brigham and Women's Hospital, Boston, USA; [8]University of Manchester, Manchester Academic Health Science Center, Manchester, UK

Chromothripsis is an extreme form of complex chromosomal rearrangement (CCR), characterized by a localized shattering and random reassembly of genomic fragments. The aim of this study is the characterization at sequence-level resolution of a cytogenetically identified CCR 46,XY,t(7;14)(q21.13;q31),inv(15)(q21.2q26.1) associated with cognitive disabilities, and intrafamilial phenotype-genotype correlation analysis. Chromosomal alterations were mapped by large-insert whole genome sequencing (liWGS). Nucleotide-level resolution of breakpoints and intrafamilial segregation analysis were carried out by junction fragments amplification and Sanger sequencing, and high-resolution array analysis. The 7q21.13 breakpoint is localized 324 bp from the 3' end of CFAP69 transcript. Although in an intergenic region, the 14q31.1 breakpoint disrupts a lncRNA. Instead of the cytogenetically reported inv(15)(q21.2q26.1), liWGS analysis identified cryptic alterations resembling chromothripsis. It involves 9 breakpoints across 60 Mb, 7 of which within an 8 Mb region, disrupting PLCB2 (OMIM *604114). Of the 8 resulting fragments, sized 4.5 kb to 52 Mb, 6 were reshuffled within this region, whereas a 489 kb fragment, encompassing C15orf53, was deleted, and a 645 kb fragment was inserted into 3p14.1, disrupting FRMD4B (OMIM *617467). Additionally, both liWGS and array analysis identified a novel 5.3 Mb deletion on 3p12.1-3p12.3 encompassing the neuronal axon guidance receptor ROBO1 (OMIM *602430), associated with dyslexia, and GBE1, causing the autosomal recessive glycogen storage disease (OMIM #232500). This deletion is unreported in the Database of Genomic Variants. A 3.1 Mb deletion affecting both genes has been reported with unknown pathogenicity in the DECIPHER database. Only the proband's son with reshuffled 15q14q21.1 region is phenotypically normal. Phenotype-genotype analysis of the remaining family members shows that they share overall similar cognitive disabilities independently from their genotypes. In conclusion, the proband's revised karyotype is 46,XY,t(7;14)(q21.13;q31.1),15q14q26.2cth, ins(3;15)(p14.1;q14),del(3)(p12.1p12.3). Despite the dramatic effect of chromothripsis on the genomic architecture at the 15q14q21.1 genomic region, comprising 6 dominant disease genes, it is most likely benign or subclinical. Presently, the major candidate genes for the cognitive disabilities are those affected by the 5.3 Mb deletion on 3p12.1-3p12.3.

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G418 as a suppression therapy for β-thalassemia disease

Martins-Dias, Patrícia[1,2] Gomes-Duarte, Andreia[1,2] Romão, Luísa[1,2]

[1]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [2]Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

Premature translation-termination codons (PTCs or nonsense codons) can arise from mutations in germ or somatic cells. The introduction of a PTC into an mRNA can trigger nonsense-mediated decay (NMD), an important mRNA surveillance mechanism that typically recognizes and degrades mRNAs containing PTCs to prevent the synthesis of C-terminally truncated proteins potentially toxic for the cell. The physiological relevance of NMD is manifested by the fact that about one third of genetic disease-associated mutations generate PTCs, including β-thalassemia. In recent years, a novel therapeutic approach entitled suppression therapy has been developed based on low molecular weight compounds to induce the translation machinery to recode a PTC into a sense codon, the so called “readthrough” (or suppression). Here, by using a model of constructs containing the 5’ part of the normal, or nonsense-mutated, human β-globin gene fused to the firefly luciferase gene as a reporter, we intend to prove the principle that the suppression therapy can restore enough β-globin protein to outweight the manifestations of β-thalassemia. Our results from bioluminescence assays and Western blot analyses have shown that the aminoglycoside G418 is able to suppress a nonsense mutation at codon 15 or 39 of the human β-globin mRNA, in cultured HEK293 cells. We are now interested in stablishing how NMD inhibition can increase the efficiency of suppression therapy. A deeper study on the suppression therapy is crucial, as it offers a major approach to treat a wide range of inherited pathologies.

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Discriminating the power of computational tools for genetic diagnosis of hypertrophic cardiomyopathy

Ribeiro M.[1], Tavares J.[1], Garcia B.[1] and Carmo-Fonseca M.[1*]

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa

Introduction: Exome sequencing is an exceptional approach to identify disease-causing mutations responsible for genetic diseases such as Hypertrophic Cardiomyopathy (HCM). Nonetheless, identifying a single, likely causative, disease mutation is a challenging and time-consuming process. Efforts are being made in the review and classification of genetic variants, allowing for a better development of prediction computational tools that aid to prioritize candidate variants. However, there is still an unmet need for a good characterization of these tools and refinement of their prioritization thresholds. In this study we aimed to assess the ability of available prediction tools to retrieve accurate results by using a manually curated set of variants, and adjust their thresholds for better performance in HCM analysis.
Methods: We selected two sets of missense and splice site variants associated with HCM from the ClinVar database. They were classified either as pathogenic or benign by multiple submitters or an expert panel with no conflicting interpretations. Variants classification was then confirmed by manual curation of available data. The performance of 14 computational prediction tools was assessed by calculating the rate of false positive and false negative results. Their thresholds were then adjusted for highest accuracy. Exome sequencing of a HCM gene panel was analysed in 53 unrelated patients, comparing the previously described thresholds with the adjusted thresholds.
Results : We observed a high rate of false positive and false negative results when using the recommended thresholds for the tools tested in both variants sets. Within the group of tools that analyse both coding and noncoding variants, Genomiser and GWAVA proved to perform poorly with the proposed 0.6 and 0.4 thresholds, respectively. Globally, tools that analyse noncoding variants by evaluating their effect on splicing, functional impact or conservation, had high accuracy. By calibrating the thresholds of tools with numeric scores we got a better sensitivity. When analysing HCM patients without causal mutations, these adjustments allowed for more conclusive results by reducing the number of prioritized variants with unknown significance.
Conclusion: This study highlights the need for caution in the interpretation of likely causative disease mutation prioritized by computational tools. Our data also suggests that many of the proposed thresholds should be adjusted in order to have more reliable results.

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Induced Pluripotent Stem Cells: from fibroblast to disease models

Ana Joana Duarte[*,**], Luciana Moreira[***], Maria Francisca Coutinho[*], José Bragança[****], Olga Amaral[*]

[*]Department of Human Genetics | Unit of Research & Development—National Health Institute Dr. Ricardo Jorge (INSA-RJ) | Porto and Centro de Estudos de Ciência Animal | Universidade do Porto | Portugal; [**]Instituto de Ciências Biomédicas Abel Salazar (ICBAS) | University do Porto | Portugal; [***]Department of Genetics and Genomic Medicine | Ichan School of Medicine at Mount Sinai | New York, NY | USA; [****]Department of Biomedical Sciences and Medicine & Centre for Biomedical Reseach | University of Algarve | Faro | Portugal

Introduction: Lysosomal storage disorders (LSDs) are a group of genetic diseases characterized by lysosomal dysfunction. In these diseases, the lack of good models limits the understanding of the human pathophysiologic mechanisms and the development of new therapies. Some of the commonest LSDs are currently treated by enzyme replacement therapy (ERT), which only ameliorates the symptoms, and particularly in cases of advanced disease or late onset, results are discouraging. In 2006, Yamanaka's group expressed four transcription factors (Oct4, Sox2, Klf4, and c-Myc) producing induced pluripotent stem cells (iPSCs).The iPSCs generated from somatic cells from patients are a desirable source for patient-specific studies since they maintain the patients' genetic background, which makes them an ideal choice for creating precise disease models.

Materials and Methods: The biological material being used consists of commercially obtained human control dermal fibroblasts and patients' fibroblasts (Gaucher and Fabry disease), obtained from the 'Istituto Giannina Gaslini'. Different methods of delivery were first tested and since our cells proved to be amenable to transduction by SeV, the method of choice for the delivery of the transcription factors was by SeV transduction.

Results and Discussion: In this approach, we used a non-integrative Sendai virus (SeV) method to deliver the pluripotency transcription factors. Pluripotent cells were obtained successfully with all the cell lines used. Presently we are working on the validation of current results in order to confirm if the aim of generating iPSCs was achieved without loss of the cells' integrity. So far we obtained encouraging results, although not all validation tests have been performed. We ultimately aim at developing iPSCs from LSDs patients' fibroblasts and normal controls to produce disease models. Our final goal, with the current work, is to obtain a good cellular model for LSDs allowing the development of new strategies for pathogenesis modeling and drug testing.

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P54 | Molecular Genetics

Founder Effect of a PKD2 variation in the Northern Portuguese population

Rocha L.[1,2]; Fernandes S.[1]; Oliveira J.P.[1,2]

[1]Genetics, Department of Pathology, Faculty of Medicine of Porto, Instituto de Investigação e Inovação em Saúde, Universidade do Porto. [2]Medical Genetics - Hospital Center of São João.

Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is a multi-organic hereditary disorder, responsible for 7-10% of cases of end stage renal failure. ADPKD is the most common inherited kidney disease. Variants in PKD1 (OMIM #601313) and in PKD2 (OMIM #173910) genes account respectively for 85% and 15% of ADPKD cases. Both forms of ADPKD have similar pathogenesis and clinical features, but patients with PKD2 variants usually present later clinical manifestations and progression toward terminal nephropathy occurs 10 to 15 years later than in patients with PKD1 mutations. Although there is a high level of allelic heterogeneity in both PKD1 and PKD2 genes, herein we report a recurrently identified variant in PKD2 gene, suggesting the existence of a founder effect in a well-defined geographic region in North of Portugal.

Methods: Genomic DNA was extracted from 40 unrelated PKD patients´ peripheral blood samples using a standard method. PKD1 and PKD2 genes were analyzed by NGS sequencing using the PGM Ion Torrent (ThermoFisher). The PKD2 variant in exon 1 was confirmed by Sanger sequencing (ABI Prism 3500 genetic analyzer). The PKD2 reference sequence used is NM_000297.3.

Results: An heterozygous single-nucleotide c.181C>T substitution in exon 1, predicting a nonsense variation p.(Gln61Ter) was identified in 10 PKD patients (25%).

Discussion: The nonsense variation c.181C>T, p.(Gln61Ter), leads to an early termination of the translation, which is expected to affect the protein function. This variant was neither described on the ExAC database (http://exac.broadinstitute.org) nor on the 1000 Genomes Project, only being reported in a family on the ADPKD Mutation Database (http://pkdb.mayo.edu/index.html). According to this database, this variation is considered definitely pathogenic, which is consistent with the clinical diagnosis of the patients studied. As most of these families are from a limited region in the Douro valley, we hypothesized that this might be a 'founder' mutation. Based on these data, we changed our phenotyping approach to ADPKD families from that region testing, as first tier, for this PKD2 variant.

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No evidence for association of the FTO rs9939609 obesity risk allele with Binge Eating Disorder (BED) susceptibility

Palmeira L[1], Cunha M[1], Padez C[2], Pinto-Gouveia J[1], Manco L[2]

[1]CINEICC, Faculty of Psychology and Social Education, University of Coimbra; [2]Research Centre for Anthropology and Health (CIAS), Department of Life Sciences, University of Coimbra

Objectives: The common single nucleotide polymorphism (SNP) rs9939609 in the fat mass and obesity-associated gene (FTO) is the most frequently described variant associated with risk of obesity. The rs9939609 obesity risk A-allele was also associated with an increased vulnerability to Eating Disorders including bulimia nervosa and anorexia nervosa. As data addressing the association between FTO SNPs and Binge Eating Disorder (BED) are totally lacking, we evaluated the genotype distribution of rs9939609 in a series of BED patients.
Methods: SNP rs9939609 T/A was genotyped by TaqMan assay in 31 overweight/obese females (25≤BMI<50 kg/m2) (20-57 years old; mean age 30.09) diagnosed with BED and in a sex matched group of 60 overweight/obese females (26<BMI<45.2 kg/m2) (22-58 years old; mean age 42.85) without signs of BED psychopathology. A normal weight group of 105 females (15<BMI<25 kg/m2) (21-36 years old; mean age 23) was also enrolled in the study. Clinical data were collected through a face-to-face structured clinical interview and self-reported questionnaires assessing eating psychopathological symptoms. BED diagnosis was establish using the DSM-5 criteria. To determine the existence of a BED, the Eating Disorders Examination Interview (EDE) and the Binge Eating Scale (BES) total score were used. The overall association between the FTO genotypes (AA, AT, TT) and BED was tested by logistic regression in the additive model.
Results and discussion: Frequency of obesity risk A-allele was 0.242 in overweight/obese females classified as BED (G1), 0.40 in overweight/obese subjects with no BED symptomatology (G2) and 0.329 in the normal weight group (G3). Genotype distributions are according HWE in G1 (p=1), G2 (p=0.07) and G3 (p=0.67). Genetic comparison between BED group G1 vs. G2, showed a marginal significant association between T-allele and BED (OR=0.534; p=0.056), in concordance with a lower A-allele frequency in G1 (0.24 vs. 0.4). No significant differences were found between the normal-weight group G3 and G1 (OR=0.641; p=0.19). Comparing G3 and G2, in the recessive model, a significant association was observed between the A-allele and obesity risk (OR=2.628; p=0.035), in concordance with general studies. In conclusion, the marginally significant lower frequency of the rs9939609 A-allele in individuals with BED when comparing with overweight/obese subjects with no BED psychopathology suggest that the FTO obesity risk A-allele has no potential role in BED.

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Fetal Hemoglobin Level and Stroke Risk in Children With Sickle Cell Anemia

Nicolau M.[1]; Vargas S.[1]; Coelho A.[1]; Silva M.[1]; Mendonça J.[1]; Vieira L.[1,2]; Kjöllerström P.[3]; Maia R.[3]; Silva R.[4]; Dias A.[5]; Ferreira T.[5]; Morais A.[6]; Mota Soares I.[7]; Lavinha J.[1,8]; Faustino P.[1,9]

[1]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa; [2]ToxOmics, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa; [3]Unidade de Hematologia, Hospital de Dona Estefânia, CHLC, Lisboa; [4]Unidade de Neuropediatria, Hospital de Dona Estefânia, CHLC, Lisboa; [5]Departamento de Pediatria, Núcleo de Hematologia, Hospital Prof. Doutor Fernando da Fonseca, Amadora; [6]Departamento de Pediatria, Hospital de Santa Maria, CHLN, Lisboa; [7]Departamento de Pediatria, Hospital Garcia de Orta, Almada; [8]BioISI, Faculdade de Ciências, Universidade de Lisboa, Lisboa; [9]ISAMB, Faculdade de Medicina, Universidade de Lisboa, Lisboa; Portugal.

Sickle Cell Anemia (SCA) is a hereditary anemia caused by a missense mutation in the HBB gene and it is characterized by chronic hemolysis, recurrent episodes of vaso-occlusion and infection. Cerebral vasculopathy is one of the most devastating complications of the disease and even young children with SCA have a high risk of stroke. It is known that both environmental and genetic determinants are able to modulate the onset, course and outcome of the disease. Among those, the level of fetal hemoglobin (HbF) has been proposed as the most significant disease modulator. Thus, in this work, we aimed to investigate if the level of HbF in SCA children is related with the risk of stroke and if it is modulated by variants in genes, such as HBG2, BCL11A, HBS1L-MYB, and KLF1.

Sixty-seven children (more than 3 years of age) with SCA were enrolled in this study. Hematological and imaging data were retrospectively obtained from patients' medical records at Greater Lisbon area hospitals. Patients were grouped according to their degree of cerebral vasculopathy evaluated by transcranial Doppler velocities and magnetic resonance imaging. Molecular analyses were performed using Next-Generation Sequencing, Sanger sequencing and PCR-RFLP. In silico studies and statistical analyses were done using the PolyPhen-2 and SPSS softwares, respectively.

The association studies revealed that low HbF levels were associated with stroke events in SCA children ($p=0.005$). At the molecular level, it was observed that patients with the rarest genotypes in HBG2 (rs7482144_TT+TC) presented higher levels of HbF ($p=0.031$). Additionally, the rs11886868_C and the rs4671393_A alleles in BCL11A also seemed to predispose to higher HbF levels. Moreover, eleven distinct variants in KLF1 were detected (one of them novel, the p.Q342H) with 83% of the patients having at least one variant in this gene. The group of patients who have co-inherited the above mentioned variants in HBG2 and BCL11A together with at least one KLF1 variant presented the highest HbF levels ($p=0.021$). Our results corroborate previous studies suggesting that a low level of HbF in SCA patients is a risk factor for stroke. Furthermore, we report for the first time the importance of KLF1 variants in combination with other genetic modifiers to the final phenotypic expression of HbF in SCA children with different degrees of cerebral vasculopathy. Consequently, this study allowed the delineation of a genetic pattern with prognostic value for SCA.

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Genetically Modulated Substrate Reduction Therapy for Sanfilippo syndrome - proof of principle

Santos JI.[1*], Coutinho MF.[1*], Gaspar P.[2], Alves S.[1]

[1]Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal; [2]Newborn Screening, Metabolism and Genetics Unit, Department of Human Genetics, INSA, Porto, Portugal [*]These authors contributed equally to the work

Introduction: Sanfilippo syndrome, or Mucopolysaccharidosis (MPS) type III refers to a group of five autosomal recessive neurodegenerative lysosomal storage disorders caused by the incomplete lysosomal degradation of the glycosaminoglycan (GAG) heparan sulphate (HS) that accumulates in patient cells and triggers disease. The main characteristic of MPS III is the degeneration of the central nervous system, resulting in mental retardation and hyperactivity, with a typical early onset. Currently, there is no effective therapy available, with treatment limited to clinical management of neurological symptoms. In order to address this issue, we have designed an RNA-based therapeutic strategy based upon the selective downregulation of genes involved in HS biosynthesis.

Methods: Taking advantage of the RNA interference (RNAi) technology potential, we have designed and assayed a specific siRNA targeting an early stage of the HS biosynthetic cascade (XYLT1). Our goal is to promote an effective reduction of the accumulating substrate, ultimately decreasing or delaying the symptoms. Fibroblasts from MPS III patients' were transfected with the designed siRNA. Total RNA was extracted and target mRNA levels evaluated through real-time PCR. In order to evaluate the effect of this approach, the GAGs accumulation was quantified over time using a modified 1,9-dimethylmethylene blue assay.

Results and Discussion: Proof of principle on the effect of an siRNA targeting XYLT1 was achieved for two independent control cell lines, with 8-12 fold decreases on the target mRNA levels, after 24h of incubation with concentrations of 20nM of each siRNA. Subsequent analysis on the effect of the same siRNA on patients' cell lines resulted in significant lower expression of XYLT1 in MPS IIIA, IIIC and IIID fibroblasts. Initial studies evaluated mRNA levels after 24-48h incubation. Studies on MPS IIIB are also ongoing. For MPS IIIC, we have already assessed the treatment effect on storage and observed a significant reduction (50%) on the total GAGs levels. We are currently addressing GAGs' storage in the remaining MPS III cell lines. Here we present an overview of the preliminary results of this project and unveil its next steps towards a full characterization/evaluation of its potential therapeutic effect.

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Genetic Modifiers of the Intermediate Phenotypes in Sickle Cell Anemia

Aguiar L.[1,2,3]; Matos A.[1,2,3]; Gil A.[1,2,3]; Ferreira J.[1,2,3]; Alonso I.[1,2,3]; Rebelo I.[4,5]; Braga L.[6], Almeida S.[7]; Kjollerstrom P.[6]; Faustino P[3,8]; Bicho M.[1,2,3]; Inácio A.[1,2,3]

[1]Instituto De Investigação Científica Bento Da Rocha Cabral, Lisboa; [2]Laboratório de Genética, Faculdade de Medicina da Universidade de Lisboa, Lisboa; [3]Instituto de Saúde Ambiental, Faculdade de Medicina da Universidade de Lisboa, Lisboa; [4]Departamento de Bioquímica, Faculdade de Farmácia da Universidade do Porto, Porto; [5]Instituto de Biologia Molecular e Celular, Porto; [6]Unidade de Hematologia, Hospital de Dona Estefânia, Lisboa; [7]Serviço de Genética Médica, Hospital de Dona Estefânia, Lisboa; [8]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa

Sickle cell anemia (SCA) is an inherited blood disorder characterized by the presence of hemoglobin S (HbS). This disease is caused by a single point mutation in the beta-globin gene with a corresponding amino acid substitution at the sixth position of the beta-globin chain. Vaso-occlusion and hemolytic anemia are the major features of this disease, however, SCA patients present clinical and hematologic variability that cannot be only explained by the single mutation. Others genetic modifiers and environmental factors are important for the clinical phenotype. We studied the association between several hematological and biochemical parameters and a set of genetic variants in 26 pediatric SCA patients. Myeloperoxidase (MPO) and placental growth factor (PlGF) were determined by ELISA (R&D Systems Inc.). Amplification of DNA samples for the rs1050829 characterization, in the glucose-6-phosphate dehydrogenase (G6PD) gene, was performed by PCR followed by restriction fragment length analysis. A multiplex PCR assay was used for simultaneous amplification of glutathione S-transferases mu (GSTM1) and theta (GSTT1). All statistical tests were performed with SPSS 24.0 software. Our results show higher levels of MPO ($p<0.001$) and PlGF ($p=0.048$) in SCA patients, compared with healthy adult controls. Moreover, in these patients we found associations between: 1) lower levels of total hemoglobin and the GSTM1 null genotype ($p=0.044$); 2) higher levels of HbS with the rs1050829_G genotype (hemizygous males) in the G6PD gene ($p=0.026$). We suggest that the mentioned polymorphisms in GSTM1 and G6PD genes may act as genetic modifiers in SCA, which could be useful for the prediction of increased susceptibility to complications. Furthermore, our results reinforce the importance to study biochemical parameters for a better understanding of the clinical outcome of this disease.

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Molecular characterization of Portuguese patients with X-linked icthyosis

Seabra A[1, 2, 3], Laranjeira F[1, 4], Ribeiro I[1, 4], Caseiro C[1], Quelhas D[1, 4], Lacerda L[1, 4]

[1]Unidade de Bioquímica Genética, Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar do Porto (CGMJM/CHP); [2]Faculdade de Ciências, Universidade do Porto (FC/UP); [3]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (ICBAS/UP); [4]Unidade Multidisciplinar de Investigação Biomédica ICBAS/UP - CHP (UMIB)

INTRODUCTION: X-linked ichthyosis (XLI) is caused by deficient steroid sulfatase enzyme activity, resulting from mutations on the STS gene (located in Xp22.31). XLI follows the X-linked recessive inheritance pattern, leading males to present the typical phenotype found in this disease - scally, dry skin, with a polygonal, dark look - while females only show the phenotype if they have both gene alleles mutated, since the STS gene escapes the X chromosome inactivation. Most mutations found in this gene are large deletions, usually spanning the entire gene, making carrier screening impossible by routine PCR-based methods. Recent development of MLPA probes for STS gene has overcome that limitation. PATIENTS AND METHODS: Primers for amplification of all (10) exons of STS gene were designed using Primer-BLAST. Twenty six male patients, belonging to 26 families, with prior diagnosis confirmation by steroid sulfatase enzyme assay were enrolled in this study. gDNA was extracted from cultured fibroblasts, obtained from skin biopsies, and all STS gene exons were PCR amplified and visualised in agarose gel following electrophoreses, and sequenced, if applicable. MLPA analysis was performed in two patients and their mothers. RESULTS: STS gene was absent in all patients as none of the exons amplified, in contrast with healthy controls. Interestingly, all patients presented a suitable PCR product when exon 6 pair of primers were used, that turned out to be due to the Y chromosome pseudogene amplification, which have not been found in healthy controls. Both patient's mothers analysed by MLPA carried the deletion, allowing us to conclude the gene deletion was inherited. DISCUSSION: Primer-BLAST failed to select primers avoiding highly similar sequences and failed also on mentioning possible amplification of unintended targets. The data obtained on Portuguese XLI patients follows the patterns uncovered in molecular characterization studies of patients from other populations. However, it is distinctive from the Spanish study where only 75% of patients presented with the whole gene deletion and 25% with only partial deletion. MLPA analysis demonstrated to be reliable for carrier screening and, therefore, family study and genetic counselling will be offered to families affected by XLI.

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Functional characterization of 2 news variants in the APOB gene

Alves A.C.[1,2], Etxebarria A.[3], Medeiros A.M.[1,2], Benito-Vicente A.[3], Martin C.[3] and Bourbon M.[1,2]

[1]Unidade de I&D, Grupo de Investigação Cardiovascular, Departamento de Promoção da Saúde e Prevenção de Doenças Não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; [2]BioISI—Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal. [3]Unidad de Biofísica (CSIC, UPV/EHU) and Departamento de Bioquímica y Biología Molecular, Universidad del País Vasco, Apdo. 644, 48080 Bilbao, Spain.

Introduction: APOB mutations are a rare cause of Familial Hypercholesterolaemia (FH) and until recently time routine genetic diagnosis only included the study of two small APOB fragments (in exon 26 and 29). In the past years functional mutations have been described in APOB fragments not routinely studied and our group characterized 2/5 as causing FH. The main aim of this work was to identify and characterize novel alterations in APOB in order to identify the genetic cause of the hypercholesterolemia in the patients with clinical diagnosis of FH.
Methods: We performed next generation sequencing of 50 Portuguese clinical FH patients apparently mutation negative. All results found in APOB were analysed. For functional studies LDL from index patients and relatives was separated and marked with FITC-LDL for studies by flow cytometry in lymphocyte and U937 growth assays.
Results: A total of 29 APOB variants have been identified in this study, however only 11 variants are putative pathogenic. It was only possible to performed functional studies of 2 variants p.(Pro994Leu) and p.(Thr3826Met) found in 4 patients. In vitro analysis of the variant p.(Thr3826Met) showed a decrease in binding and internalization of LDL and in deficient growth in U937 assays, showing a similar effect as APO3527. Variant p.(Pro994Leu) had a neutral effect.
Discussion: The spectrum of functional alterations in APOB outside the fragments routinely screened is growing. Screening of all 29 exons of APOB should be performed in routine diagnosis, now possible by NGS. It is expected that a further 10% of clinical FH patients can have FH due to a novel APOB mutation.

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Pilot study of genetic risk variants in Retinal Angiomatous Proliferation using MLPA—an update

Tomás M[1], Pires LM[1,2], Val M[1,2], Marques JP[3,4], Raimundo M[5], Marques M[3], Laíns I[2,5,6], Silva R[2,3,5], Carreira IM[1,2,7,8]

[1]Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [2]Faculty of Medicine, University of Coimbra. Portugal (FMUC); [3]Association for Innovation and Biomedical Research on Light and Image (AIBILI). Coimbra. Portugal; [4]Centre of Responsibility in OphthalmologyCoimbra (CRIO-CHUC), Coimbra, Portugal; [5]Ophthalmology Department. Centro Hospitalar e Universitário de Coimbra (CHUC). Portugal;[6]Department of Ophthalmology, Massachusetts Eye and Ear, Harvard Medical School, Boston, United State; [7]Center of Investigation on Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra; [8]CNC-IBILI—Group of Aging and Brain Diseases: Advanced Diagnosis and Biomarkers, Coimbra, Portugal

Introduction: Age-related Macular Degeneration (AMD) is a late-onset multifactorial disease characterized by a progressive destruction of the macula. Although the early stages are usually asymptomatic, the late forms of the disease - geographic atrophy and neovascular/exudative AMD (eAMD) - can lead to irreversible vision loss. Retinal angiomatous proliferation (RAP) is a particularly aggressive neovascular phenotype, estimated to represent up to 20% of all eAMD cases. The phenotypic differences between RAP and typical eAMD are thought to have a genetic basis. In fact, AMD is a multifactorial disease characterized by complex interactions between environmental (age, smoking, ethnicity, etc) and genetic factors, with the latter accounting for up to 70% of the disease burden. Most genetic alterations associated with the incidence and progression of AMD are single nucleotide polymorphisms (SNPs) in CFH and ARMS2/HTRA1 genes. Moreover, copy number variations (CNVs) involving CFHR3 and CFHR1 genes have also been extensively reported.
Materials and Methods: This is a cross-sectional study, aiming to evaluate SNPs and CNVs within the genes most commonly associated with AMD in patients with RAP. Taking advantage of multiplex ligation probe amplification (MLPA) technique, a SALSA probemix (MRC-Holland) was used to evaluate 172 samples: 39 RAP and 34 eAMD patients and 99 controls.
Results and Discussion: Patients (eAMD+RAP) and controls have statistically significant differences concerning CFH intronic SNP (rs1410996; p=0.010), ARMS2 SNP (rs10490924; p<0.001), and as well as CNVs concerning CFHR3 and CFHR1 (p=0.023). The combination of these 3 genetic variants significantly increased the odds ratio of neovascularization (p<0.001). On the other hand, only CFH intronic SNP (rs1410996) was able to significantly differentiate the 2 groups of patients by itself (p=0.046). We also verified that the simultaneous presence of CFH SNP Y402H (rs1061170), CFH intronic SNP (rs1410996) and CFHR3 and CFHR1 CNVs appears to confer an increased risk of developing eAMD over RAP (p=0.031, OR=2.86, 95% CI [1.10 - 7.42]). Conclusion: The cohort's increase, as well as the addition of a new group of patients allowed a better understanding of RAP's genetic background, with promising results for the genetic distinction between this neovascular phenotype and typical eAMD. This represents one further step towards a new diagnostic approach of AMD and ultimately, the prediction of its development.

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Caracterização de regiões polimórficas em genes ligados à hipertensão—NOS3, G6PD e HBA numa população Moçambicana e numa população Portuguesa

Semente I.[1,2]; Matos A.[1,3]; Faustino P.[2,4]; Bicho M.[1,3,4]; Inácio A.[1,3,4]

[1]Laboratório de Genética, Faculdade de Medicina da Universidade de Lisboa, Lisboa; [2]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa; [3]Instituto De Investigação Científica Bento Da Rocha Cabral, Lisboa; [4]Instituto de Saúde Ambiental, Faculdade de Medicina da Universidade de Lisboa, Lisboa; Portugal

Introdução: A hipertensão arterial é uma doença multifatorial, de elevada prevalência em Moçambique e Portugal (33,1%[1] e 42,2%[2] respetivamente). Uma vez que a sintase do óxido nítrico endotelial (NOS3), a glucose-6-fosfato-desidrogenase (G6PD) e a alfa-globina (HBA) têm sido propostas como potenciais moduladoras da hipertensão arterial, pretendem-se neste estudo caraterizar as variantes genéticas mais comuns em duas populações, uma Moçambicana e outra Portuguesa.
Material e Métodos: Foram analisadas 22 amostras de DNA provenientes do Hospital Central de Maputo e 87 provenientes do Hospital de Santa Maria. Para a pesquisa do número de repetições em tandem (VNTR) no intrão 4 do gene NOS3 foi usada a PCR, para a pesquisa do SNP rs1050829 no gene G6PD foi usado a PCR-RFLP e para a pesquisa da deleção alfa-talassémica de -3,7kb no agrupamento génico da alfa-globina foi usada uma metodologia de Gap-PCR. As frequências alélicas e genotípicas foram calculadas e analisadas com recurso ao programa estatístico SPSS 22.0
Resultados: Os resultados mostram que em relação ao gene HBA, a população Moçambicana analisada apresenta a frequência de 59% para o alelo mutado e de 41% para o alelo normal, em contraste com o observado para a população Portuguesa onde foi detetada a frequência de 1% para o alelo mutado e 99% para o alelo normal. No gene G6PD, observou-se na população Moçambicana a frequência de 76% para o alelo mutado e 24% para o alelo normal e para a população Portuguesa analisada observou-se que o alelo mutado apresenta a frequência de 1% e o alelo normal a frequência de 99%. Para o VNTR em NOS3, na população Moçambicana os alelos 4a e 4b apresentam respetivamente a frequência de 32% e 68%, enquanto na população Portuguesa os mesmos alelos apresentam respetivamente a frequência de 12% e 88%.
Discussão: Estes resultados preliminares mostram a caracterização da frequência de regiões polimórficas em três genes potencialmente influentes no desenvolvimento da hipertensão em duas populações distintas. Encontra-se em estudo um outro conjunto de indivíduos controlos, não hipertensos, que permitirão através de estudos de associação avaliar a contribuição dos referidos polimorfismos para esta patologia.
[1] Damasceno A et al. Hypertension 2009; 54:77. [2] Polonia J et al. Journal of Hypertension 2014; 32:1211.

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Genotype-phenotype correlations in patients with phenylketonuria from Rio de Janeiro, Southeast Brazil

Vieira Neto E.[1,2]; Laranjeira F.[3]; Quelhas D.[3]; Seabra A.[3]; Mineiro N.[3]; Carvalho L.M.[4]; Lacerda L.[3]; Ribeiro M.G.[2]

[1]Agência Nacional de Saúde Suplementar, Brasil; [2]Instituto de Puericultura e Pediatria Martagão Gesteira, Universidade Federal do Rio de Janeiro, Brasil; [3]Centro de Genética Médica Jacinto de Magalhães, Centro Hospitalar do Porto, Portugal; [4]Instituto de Diabetes e Endocrinologia Luiz Capriglione, Rio de Janeiro, Brasil

Introduction: The clinical phenotypes of phenylketonuria (PKU) are highly variable. This has been attributed to genetic heterogeneity and frequent compound heterozygosis. The correlations between phenotypic characteristics and the causative mutations found in PKU patients from Rio de Janeiro, Brazil, were evaluated.

Methods: Pre-treatment phenylalanine (Phe) levels were obtained for a total of 95 completely genotyped patients. They were assigned to one of the following phenotypes: classic, moderate and mild PKU, and mild hyperphenylalaninemia (MHP). The predicted phenylalanine hydroxylase (PAH) residual activity was deduced for each genotypic combination. The relationship between the predicted PAH residual activities and the inverse of pre-treatment Phe levels were evaluated. A phenotype prediction system based on arbitrary assigned values was employed to compare expected and observed phenotypes. The phenotypes of functionally hemizygous patients were also analyzed.

Results: A strong relationship between mutation severity, according to the level of predicted PAH residual activity, and the inverse of pre-treatment Phe levels was observed ($t=4.76$, $P<0.0001$). Means (±SD) of predicted PAH activity associated to classic, moderate, and mild PKU were significantly different: 13.8% (12.7), 20.9% (11.6), and 30.0% (13.3), respectively ($F=9.99$, $P=0.0001$). The observed phenotype of patients that were homozygous or compound heterozygous for mutations of assigned severity matched the predicted phenotype in 48% of the cases. The majority (85%) of patients presenting two null mutations had a classic phenotype. Functionally hemizygous patients carrying mutations p.R261Q and p.V388M showed variable outcomes, while most of those (80%) carrying the mild mutations p.L48S, p.R68S, and p.L249F presented a classic phenotype.

Discussion: A high degree of agreement (85%) was found between null/null genotypes and the classic phenotype. Inconsistencies observed in other mutation combinations may be due to differences in methods used for determining PAH activities of non-null genotypes as well as to interallelic complementation. The almost even distribution of p.R261Q and p.V388M compound heterozygotes with null mutations along the phenotypic spectrum may result from epigenetic factors. Mild mutations were associated with classic PKU. Despite these discrepancies, genotype continues to be the main determinant of metabolic outcome in most patients with PKU, anticipating their dietary needs.

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NGS experience in the molecular diagnosis of Alport syndrome and related collagen type IV glomerulopathies

Ferreira S[1], Rocha L[1], Alves S[1], Fernandes S[1], Oliveira JP[1,2]

[1]Department of Medical Genetics, Faculty of Medicine, University of Porto, Porto, Portugal; [2]São João Hospital Centre, Porto, Portugal

Introduction: The mature human glomerular basement membrane (GBM) collagen IV network is assembled by interactions between triple-helical α3α4α5(IV) protomers, being each monomer respectively encoded by the autosomal genes COL4A3 and COL4A4, and the X-linked gene COL4A5. Mutations in any of these genes are in the origin a pathogenically related but genetically and clinically heterogeneous group of familial haematuric diseases that include Alport syndrome (AS), benign familial haematuria (BFH) or thin basement membrane disease (TBMD). Next-Generation Sequencing (NGS) is the appropriate first-tier molecular approach to the diagnosis of genetically heterogeneous disorders like these collagen IV glomerulopathies. However, the screening of exonic deletions/duplications requires additional multiplex ligation-dependent probe amplification (MLPA) as a second-tier genetic diagnosis in patients were no pathogenic variant is found or in the ones were only one variant is found in the autosomal located genes.
Methods: One hundred Portuguese index cases, presenting with one or more of the following criteria: family history of macro/microscopic haematuria and/or of progressive chronic kidney disease (CKD) or end stage renal failure (ESRF); (ii) Electron microscopy evidence of AS on renal biopsy; (iii) high-tone sensorineural hearing loss (SNHL); (iv) characteristic ophthalmological signs, were studied using a NGS panel that included the COL4A3, the COL4A4 and the COL4A5 genes. MLPA was used as a second-tier approach in all justifiable cases.
Results: Confirmed pathogenic variants were found in 62 patients of which, 21 were heterozygous for COL4A3, 11 were heterozygous for COL4A4, 21 were hemizygous or heterozygous for COL4A5, 4 were homozygous for COL4A3, 1 was homozygous for COL4A4, 8 were compound heterozygous for COL4A3 and one was a triple heterozygous for COL4A3/COL4A4 and COL4A5.
Discussion: Unlike reported for other populations the prevalence of pathogenic variants in the Portuguese population is not particularly higher for any of the collagen IV coding genes. It is, therefore, truly important to use a wide genetic approach like NGS to do the first-tier screening of collagen IV glomerulopathies and to extend the genetic diagnosis to the use of methods that allow to overcame the limitations of the NGS methodology whenever it is justified by the clinical diagnosis.

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Functional and disease modeling study of DAND5 variant in patients with Congenital Heart Disease and laterality defects

Fernando Cristo[1], José M. Inácio[1], Graça Rosas[1], Isabel Marques Carreira[2], Joana Barbosa Melo[2], Patrícia Mendes[3], Duarte Saraiva Martins[4], José Maio[3], Rui Anjos[4], Luís Pereira de Almeida[5] and José A. Belo[1]

[1]Stem Cells and Development Laboratory, CEDOC, NOVA Medical School / Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal; [2]Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal. CNC.IBILI Consortium, University of Coimbra, Coimbra, Portugal. CIMAGO—Center of Investigation on Environment Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Portugal; [3]Departamento Materno-Infantil, Centro Hospital do Algarve, EPE, Faro, Portugal; [4]Hospital de Santa Cruz, Centro Hospitalar Lisboa Ocidental, Lisboa, Portugal; [5]CNC—Center for Neurosciences & Cell Biology, University of Coimbra, Coimbra, Portugal.

Perturbations on Left-Right axis establishment lead to laterality defects (LD), with frequently associated Congenital Heart Defects (CHDs). The etiology of isolated cases of CHDs or cases of LD with associated CHDs is linked with variants of genes involved in the Nodal signaling pathway. We identified mouse cerberus-like2 (Cerl2) and its human counterpart DAND5 as an essential gene in the correct establishment of the laterality of visceral organs, including the heart. Cerl2/DAND5 functions as an inhibitor and master regular of Nodal signalling in a temporal and spatial precise way. Cerl2/DAND5 knockout mice display a vast array of congenital cardiac malformations associated or not with extracardiac anomalies. Importantly, these KO mice present thickening of the left ventricle and of the IVS due to hyperproliferation of cardiomyocytes, independent of L/R defects.

With this in mind, we analyzed a cohort of patients with CHD that can arise from perturbations in the formation of the Left-Right axis. Here, we report two patients with a DAND5 heterozygous variant (c.455G > A) in the functional domain of the DAND5 protein (p.R152H). A functional analysis assay showed a significant decrease in the activity of this variant protein when compared to its wild-type counterpart, supporting a model in which the imbalance in dosage-sensitive Nodal signaling is a final common way for laterality defects and associated CHDs and suggesting a possible role of this variant in the risk of disease.

To shed light into the mechanism of disease displayed by patients carrying the DAND5 variant, we generated patient-derived iPS cells. For this purpose, exfoliated renal epithelial cells isolated from a urine sample were obtained to serve as template cell for reprogramming. After reprogramming, iPSC colonies from were picked, expanded and ultimately characterized. The results from the characterization, analyzing the morphology, pluripotency state, karyotype, STR profile and differentiation potential towards three germ layers, indicate that the cell lines were successfully generated. These iPSCs are now being used to understand the cellular and molecular mechanisms of disease behind a simple nucleotide variant.

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Exploring the impact of noncoding genetic variation on lysosomal function

Ramos CG[1], Klein AD[2], Futerman AH[2] & Carmo-Fonseca M[1]

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal; [2]Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot 76100, Israel

Lysosomes play a critical role in autophagy, a process responsible for clearance of damaged, misfolded or aggregated proteins in cells. Lysosome disfunction causes Lysosomal Storage Diseases (LSDs) and is associated with neurodegenerative disorders related to the accumulation of abnormal proteins, such as Alzheimer's, Parkinson's and Huntington's disease. Homozygous mutations in the GBA1 gene, which encodes for the lysosomal enzyme beta-glucocerebrosidase, results in Gaucher disease (the most common LSD), and heterozygous GBA1 mutations are strong risk factors for Parkinson. Gaucher disease displays considerable phenotypic variability and previous work from the Futerman lab identified putative single nucleotide polymorphisms (SNPs) that may be involved in determining disease severity (Klein at al. 2016, Cell Reports 16, 2546-53). Remarkably, the majority of these SNPs are located in noncoding regions of the genome, namely in introns. We are currently exploring whether these SNPs affect gene expression. In particular, we are testing whether the modifier SNPs interfere with splicing or transcriptional activity. Funding: CGR is supported by an FCT fellowship (SFRH/BPD/75718/2011). This project is funded by EU H2020 RISE grant 734825, and LISBOA-01-0145-FEDER-007391 (project cofunded by FEDER, through POR Lisboa 2020—Programa Operacional Regional de Lisboa, PORTUGAL 2020, and Fundação para a Ciência e a Tecnologia).

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Serum lipid alterations in GBA-associated Parkinson's disease

Miltenberger-Miltenyi G[1], Guedes LC[1, 2], Chan RB[3], Gomes MA[1], Conceição VA[1], Machado RB[1], Soares T[1], Xu Y[3], Gaspar P[4], Carriço JA[1, 5], Alcalay RN[6], Ferreira JJ[1, 7], Outeiro TF8

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; [2]Department of Neurosciences and Mental Health, Neurology, Hospital de Santa Maria- CHLN, Lisbon, Portugal; [3]Columbia University Medical Center, Department of Pathology and Cell Biology, Taub Institute for Research on Alzheimer's Disease and the Aging Brain, New York, NY, USA; [4]Lysosome and Peroxisome Biology Unit (UniLiPe), Institute of Molecular and Cell Biology (IBMC), University of Oporto, Oporto, Portugal; [5]Instituto de Microbiologia, Faculty of Medicine, University of Lisbon, Portugal; [6]Columbia University Medical Center, Department of Neurology, New York, NY, USA; [7]Laboratory of Clinical Pharmacology and Therapeutics, Faculty of Medicine, University of Lisbon, Portugal; [8]Department of Experimental Neurodegeneration, Center for Nanoscale Microscopy and Molecular Physiology of the Brain, Center for Biostructural Imaging of Neurodegeneration, University Medical Center Göttingen, Göttingen, Germany.

INTRODUCTION: Mutations in the GBA gene, encoding for the lysosomal enzyme glucocerebrosidase, are associated with Gaucher disease. Alterations in plasma sphingolipids have been reported in Gaucher, and similarly in brain extracts in Lewy body disease. As GBA mutations are prevalent risk factors for Parkinson's disease and overlap of molecular pathways are presumable, here we assessed the lipid profiles in Parkinson's patients with and without GBA mutations.
METHODS: We sequenced all GBA exons in 415 Parkinson's patients, previously genotyped for LRRK2. 64 patients (29 GBA positive vs. 35 non-GBA-carriers including 18 LRRK2 positive and 17 non-mutated) were analyzed for chitotriosidase activity and for the concentration of 40 lipid classes using HPLC-MS.
RESULTS: 29/415 patients (6.9%) carried 8 different GBA mutations associated with Gaucher or Parkinson's, including one novel mutation. Chitotriosidase activity was similar across the genetic groups, while the levels of key lipids were altered in GBA mutation carriers: Monohexosylceramide, Ceramide and Sphingomyelin were elevated; while Phosphatidic acid (PA), Phosphatidylethanolamine (PE), Plasmalogen phosphatidylethanolamine (PEp) and Acyl Phosphatidylglycerol (AcylPG) were decreased.
CONCLUSION: The results suggest an important role for these lipids in GBA mediated Parkinson's disease and assist in the identification of common pathways between Gaucher and Parkinson's. Ultimately, our findings may lead to the identification of novel biomarkers for individuals at increased risk of developing Parkinson's disease.

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Spinal Muscular Atrophy: is LARP4 a positive modifier of SMN expression?

Sá J.[1], Oliveira M.[1], Gomes AL.[1], Matos AM.[1], Peixeiro I.[1], Costa G.[2], Cordeiro C.[2], Voigt A.[3,4], Gama-Carvalho M.[1]

[1]University of Lisbon, Faculty of Sciences, BioISI—Biosystems & Integrative Sciences Institute, Lisbon, Portugal; [2]University of Lisbon, Faculty of Sciences, Chemistry and Biochemistry Department, Lisbon, Portugal; [3]Department of Neurology, University Hospital, RWTH Aachen University, Aachen, Germany; [4]JARA—Institute Molecular Neuroscience and Neuroimaging, Forschungszentrum Jülich GmbH and RWTH Aachen University, Aachen, Germany

Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by deletion or mutation of the SMN1 gene, resulting in decreased production of the SMN protein, leading to motor neuron death. In the human genome, the SMN1 gene is found on chromosome 5, which has a duplicated genomic region containing an almost identical copy, the SMN2 gene. The main difference between these two genes is a silent transition that results in the inefficient splicing of exon 7 of SMN2, leading to the predominant production of an unstable protein (90%). The remaining 10% of full length transcripts support the production of a functional protein, thus being of great interest as a therapeutic target. In fact, the FDA has recently approved a new drug for SMA that can effectively modulate SMN2 splicing. In addition to splicing modulation, the study of mechanisms regulating mRNA stability and translation can provide additional means to enhance SMN protein expression. We have analyzed the role of the SMN1/2 5´UTR and 3´UTR regions in the regulations of mRNA expression using a luciferase reporter system and show that the 3'UTR region has a positive effect on protein expression. In contrast, the 5'UTR region has a negative effect, possibly due to the presence of a secondary structure element. We have further tried to identify RNA binding proteins that bind to the 3´UTR of SMN2 and thus may be involved in mRNA stability control. Through RNA-protein pull downs coupled to mass spectrometry, we identified LARP4 as a SMN1/2 mRNA binding protein and have shown a positive effect of this protein on the translation of the SMN-3'UTR luciferase reporter. We further show that LARP4 levels impact both the stability and translation of the endogenous SMN mRNA, acting as a positive regulator of its expression. Interestingly, in Drosophila melanogaster, the LARP4 homolog, CG11505, was identified as a putative regulator of dendritic spine growth (Laviolette et al 2005) and a positive modifier of the SMA phenotype (Sen et al 2014) in the context of large scale genetic screens. To confirm the role of the LARP4 homolog in the nervous system and in SMN regulation, we have generated fly lines that overexpress this protein. Preliminary analysis of the CG11505 transgene flies suggests that there is a degeneration in photoreceptor cells with decreased eye size. In summary, our results point to LARP4 as a novel regulator of SMN mRNA expression that may play a role in central nervous system function.

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Association between TOMM40 Poly-T Repeat Variants and risk of Mild Cognitive Impairment convertion to Alzheimer's Disease

Cardoso R.[1,2]; Almeida MR.[2]; Baldeiras IE.[3]; Pereira CM.[2,4]; Oliveiros B.[5]; Santana I.[6]; Oliveira CR.[2,4]

[1]NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa; [2]Center for Neuroscience and Cell Biology, University of Coimbra; [3]Laboratory of Neurochemistry, Coimbra University Hospital Center; [4]Faculty of Medicine, University of Coimbra; [5]Laboratory of Biostatistics and Medical Informatics, Faculty of Medicine, University of Coimbra; [6]Department of Neurology, Coimbra University Hospital Center

Introduction: Mild Cognitive Impairment (MCI) has a rate of conversion to Alzheimer's Disease (AD) estimated to reach 10-15% per year. When considering MCI as precursor of AD is of major importance to study the risk factors that predict which MCI patients will convert to AD, namely risk genes. One Poly-T Repeat polymorphism on TOMM40 gene (TOMM'523) has been associated with increased risk of AD, however very few studies addressed its relation with MCI to AD conversion. The aim of this study was to correlate the different poli-T repeats variants of this polymorphism with risk and time of conversion from MCI to AD.

Methods: The MCI patients were recruited and diagnosed at the Dementia Clinic, Neurology Department of Coimbra University Hospital Center (CHUC). The group consisted in 62 patients that converted to AD and 44 patients that remained stable. Poli-T lengths were accessed using fragment analysis and classified as short (S, ≤ 19), long (L, 20-29) or very long (VL, ≥ 30). We performed logistic analysis in order to access the risk of conversion from MCI to AD, Kaplan-Meier curves to access the time of conversion and Krusk-Wallis test to compare the levels of biomarkers among TOMM'523 genotypes.

Results: In this study we observed that the poli-T distribution was different when comparing MCI patients that remained stable to the ones that converted to AD. We then showed that the presence of at least one L allele (L+) is a risk factor for MCI conversion, whereas S/S, S/VL and VL/VL are protective. Within these protective alleles VL/VL patients convert significantly slower when comparing with L+ patients. Using a pairwise comparison we showed that VL/VL genotype is significantly associated with non-compatible AD biomarkers.

Discussion: TOMM40 is in linkage disequilibrium with APOE and due to this fact we observed that the APOE ε3 allele is associated with S and VL genotypes and ε4 allele is almost exclusively associated with L TOMM40 allele. On the other hand, we found that VL/VL patients convert significantly slower and are associated with normal AD-core biomarkers when compared with S/VL and S/S genotypes. In conclusion, VL/VL genotype seems to be protective, when comparing to S/VL and S/S genotypes, giving us extra information within the e3/e3 allele that is considered neutral regarding AD risk.

P70 | Quality Control & Public Services

A molecular genetics laboratory accredited for the diagnosis of familial dementias by NGS

Sousa S[1]; Silva P[1]; Lopes A[1]; Brandão A[1]; Seada P[1]; Arinto P[1]; Bastos-Ferreira R[1]; Alonso I[1,2]; Sequeiros J[1,2,3]

[1]CGPP—Centro de Genética Preditiva e Preventiva, IBMC—Instituto de Biologia Molecular e Celular, i3S—Instituto de Investigação e Inovação em Saúde; [2]UnIGENe, IBMC—Instituto de Biologia Molecular e Celular, i3S—Instituto de Investigação e Inovação em Saúde; e [3]ICBAS—Instituto de Ciências Biomédicas Abel Balazar; Universidade do Porto, Portugal

Requirements are particularly high for quality of genetic testing, as tests are typically performed once in a lifetime and possible errors having tremendous implications for the tested persons and their relatives. The OECD Guidelines for Quality Assurance in Molecular Genetic Testing aimed to improve quality, recommending that laboratories reporting molecular genetic testing results for clinical care purposes should be accredited. Next-generation sequencing (NGS) is becoming a standard in clinical genetics laboratories and is particularly helpful for genetically heterogeneous diseases. We worked towards accreditation of NGS, in accordance with ISO15189. For that we documented standard operation procedures, reference materials, equipment's maintenance, personal competence (training), EQA, IQC and validation procedures. The validation process of NGS protocols comprised several challenging steps and few guidelines have been established to harmonize validation of this methodology. Using the Ion Torrent PGM, our first validation approach was to select and sequence amplicons harbouring disease-causing variants previously identified by Sanger sequencing, representing the most common diseases at our lab. A set of 71 unique disease-causing variants, in 40 genes, was screened. In our second approach, we sequenced the Genome in a Bottle (GiaB) NA12878 DNA from Coriell, and compared the variants found using our methodologies with the set of high-confidence variants made available by the GiaB consortium. Our analysis pipeline resulted in a sensitivity of 100% [TP/(TP+FN)] for this dataset, successfully identifying all 71 variants screened. For the dementia panel, analysis of variants in NA12878 identified 16 variants, but failed to detect 4 (80% sensitivity). The undetected variants were located in low or non-covered regions; Sanger sequencing of these regions is routinely performed at our lab. We present here an established, tested, optimised, validated and accredited NGS workflow with high sensitivity, currently in use in our diagnostic routine, allowing physicians to focus on the clinical presentation of their patients and correlate this with the genetic findings. CGPP is, so far, the only clinical genetics laboratory in Portugal accredited by ISO15189, for molecular genetic testing of neurological diseases and for NGS in Alzheimer and other familial dementias.

P71 | Quality Control & Public Services

Science communication: a matter of shared experiences

Olga Amaral*, Ana Joana Duarte*, J. Inês Santos*, Liliana Matos*, M. Francisca Coutinho*, Pedro Lacerda*, Sandra Alves*

*DEPARTMENT OF HUMAN GENETICS | UNIT OF RESEARCH & DEVELOPMENT NATIONAL HEALTH INSTITUTE RICARDO JORGE (INSA) PORTO | PORTUGAL

Introduction: During the last decades, raising public awareness of science and technology has been a major focus of interest for Science Societies as well as Public and Private Institutions. In Portugal, the 'Ciência Viva' programme is the most well-known example of an action for science communication. Promoting public awareness since 1997, it began with summer activities and the 'Semana da Ciência e da Tecnologia'.
Methods: Throughout the years, our participation in science awareness events such as 'Ciência Viva', 'Semana Aberta do INSA', 'European Researchers Night', 'Brain Awareness Week' and presentations in schools have been possible through inter-departmental collaborations, shared infrastructures, support of informatics platforms, international scope of events and the motivation of professors, teachers and researchers.
Results: In our experience, the involvement in science communication events has always been rewarding. Along the years, the impact of these activities could be observed by the increasing number of participants. Effective communication was achieved, mostly, by collaborating with others with an open mind, sharing experiences and trying to breach the scientific terminology gap. By using analogies adapted to the target audience and keeping the message simple the public becomes more attentive and responsive (https://www.youtube.com/watch?v=TlOA_LSvaJ4).
Discussion/conclusion: Even complex subjects can be explained in simple terms. To get the message across, you need to invest the time and effort to grasp the opportunity of turning a privileged experience into a memorable and enjoyable one. A successful approach requires sharing experiences and establishing a two-way learning opportunity rather than teaching. Moreover, greater fidelization of the public is achieved through the scheduling of a constant timing for science communication initiatives. Events with a predetermined time frame tend to show greater public participation, and better organization, than those that have fluctuating dates.
Acknowledgements: We thank all students, public in general and institutional co-workers who, over the past 20 years, collaborated at both sides of these events and helped to establish a better two-way channel of communication. We also thank all the sponsors of 'Semana aberta do INSA', Marie Curie action 'European Researcher's night', Dana Foundation, University of Porto, 'Programa Ciência Viva' and Fundação para a Ciência e a Tecnologia (FCT). FCT funding of OA: PTDC/BIM-MEC/4762/2014 (2016-); SA: PTDC/BBB-BD/6301/2014; AJD: SFRH/BD/118009/2016; MFC: SFRH/BPD/101965/2014 and IS: SFRH/BD/124372/2016.

P72 | Other: Clinical Exome Sequencing

Concordance between variants detected by clinical exome, gene panel and Sanger sequencing

Mendonça J[1], Silva C[1], Theisen P[2], Gonçalves J[2], Vieira L[1]

[1]Unidade de Tecnologia e Inovação, Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa; [2]Unidade de Genética Molecular, Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa; [3]ToxOmics, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa

Introduction: Exome sequencing (ES) is becoming a preferred methodology for detecting DNA changes in genetic diseases with no known molecular cause or no definitive diagnosis. This results from the fact that next-generation sequencing technology allows a greater number of bases to be sequenced at an increasingly lower cost. However, sequencing a high number of genes requires an evaluation of the analytical performance of ES before it is used in the clinical setting.

Methods: Fifteen genomic DNA samples were used to prepare sequencing libraries with the TruSight One Sequencing Panel (Illumina) consisting of 4813 disease-associated genes ('clinical exome'), according to the manufacturer's procedures. Libraries were sequenced on the MiSeq (Illumina) and the results were analyzed using the MiSeq Reporter and IGV. Variants identified in ES were compared with those validated previously in a subset of genes using the TruSight Cancer gene panel (Illumina) and Sanger sequencing. This study was conducted in 2 phases. In the first, the clinical exome of 9 samples was sequenced and the variants obtained were compared with known variants in 8 genes. In the second phase, 6 samples were sequenced and the variants in 8 genes were analyzed without prior knowledge of the results obtained in the other methods. Furthermore, it was not known that one of these samples had been sequenced in the first phase of the study.

Results: In the first phase, ES identified all the exonic (n=41) and intronic flanking (n=15) variants validated in the MSH2, MLH1, APC, MUTYH, BRCA1, BRCA2, STK11 and TP53 genes, while no additional changes have been detected. In the second phase, ES detected a total of 50 variants in MSH2, MLH1, APC, BRCA1, BRCA2, TP53, CDH1 and ATM genes which were found to include each of the 46 variants previously validated and 4 additional changes located outside the genomic regions defined in the gene panel. The same 15 exonic variants were identified in the sample independently processed and sequenced in both phases. Taken together, 87 variants were independently identified using different sequencing approaches.

Discussion: The results of this work showed a complete agreement between variants identified by clinical exome, gene panel and Sanger sequencing. Moreover, these results support the notion that the clinical exome panel can also be used as a set of sub-panels of genes applicable to different genetic diseases.

P73 | Other: Genetic Disease Diagnosis

In2Genome Project—A multidisciplinary approach to accelerate the diagnosis of genetic diseases.

Sérgio B. Sousa[1], Margarida Venâncio[1], Joaquim Sá[1], Belinda Campos-Xavier[1], Maria José Simões[2], Cristina Barroso[2,3], Hugo Froufe[2], Catarina Correia[4], Sofia Pinto[4], Jorge M. Saraiva[1], Conceição Egas[2,3]

[1]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra; [2]Biocant, Transfer Technology Association, Cantanhede; [3]Center for Neuroscience and Cell Biology, University of Coimbra; [4]Coimbra Genomics, Biocant Park, Cantanhede

There is an unprecedented number of cases in which a genetic diagnosis can result in determinant information for clinical practice. However, the average success rate of genetic diagnosis in patients referred for genetic counseling is below 50%. A genetic diagnosis is still often achieved by conventional approaches (e.g., cascade of gene-targeted testing), which is time and cost consuming. The increasing use of untargeted approaches based on next-generation sequencing has shown to be cost-effective in the diagnosis of a wide range of conditions. When solving complex clinical cases with significant genetic heterogeneity and/or unspecific phenotype, whole-exome sequencing trio analysis improved both the level of precision and detection of genetic variations. In Portugal and many other places, the main difficulty is the lack of experienced multidisciplinary teams that can accurately interpret the many identified variants. The goal of the In2Genome Project, which started in July 2017, is to integrate whole-exome sequencing in routine clinical practice by a team that can adequately address its multiple challenges and prepare the close future: whole-genome sequencing as first-tier test. Financed by CENTRO 2020, In2Genome gathers a team of sequencing, clinical genetics and personalized medicine experts whose complementary skills will make possible the development of a new approach to the diagnosis of genetic diseases. The Medical Genetics Unit of CHUC, besides the patient evaluation, selection and counseling, will be directly involved in the variant interpretation and issuing of the diagnostic report. Genoinseq will sequence the whole-exomes of patients and Coimbra Genomics will participate through its personalized medicine platform ELSIE. This project will focus on neurodevelopmental disorders and will analyze a group of patients with intellectual disability/multiple congenital anomalies syndromes. An initial group of patients with previous molecular diagnosis will be resequenced, analyzed and compared to previous results to verify and optimize the process. The analysis of a large group of undiagnosed patients will constitute the following and main phase. In parallel, a database of Portuguese genetic variants will be developed.

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P74 | Other: Forensic Sciences

Bisulfite PCR-sequencing as a method to evaluate the DNA methylation patterns of the age predictor gene ELOVL2

Dias H[1,2,3]; Cunha E[2]; Corte Real F[3]; Manco L[1]

[1]Research Centre for Anthropology and Health (CIAS), Department of Life Sciences, University of Coimbra, Portugal; [2]Centre for Functional Ecology (CEF), Laboratory of Forensic Anthropology, Department of Life Sciences, University of Coimbra, Portugal; [3]National Institute of Legal Medicine and Forensic Sciences, Portugal

Introduction: In forensic investigations, age estimation has a crucial role, complementing the prediction of externally visible characteristics. Recently, DNA methylation of some genes emerges as a powerful tool of Forensic Genetics in many contexts, including age-at-death estimation. Some studies shown a strong correlation between DNA methylation status of the ELOVL2 (fatty acid elongase 2) gene (6p24.2) and chronological age of individuals. In this study, we have investigated the correlation between DNA methylation patterns of nine CpGs from ELOVL2 and chronological age based on the bisulfite PCR-sequencing method, which has not been used until now for human age estimations.
Methods: Blood samples of 48 Portuguese healthy subjects (32 females, 16 males; aged 1-95 years old), were collected after informed consent and according institutional and ethical guidelines. Genomic DNA was extracted using a commercial kit and subjected to bisulfite conversion using the EZ DNA Methylation-GoldTM Kit (Zymo Research, CA, USA). PCR amplification of ELOVL2 gene fragment was performed using primers previously designed, followed by Sanger sequencing. The methylation status of cytosines in each CpG dinucleotide was estimated by measuring the ratio of the cytosine peak height to the sum of cytosine and thymine peak heights (C/C+T). Simple linear regressions were used to analyze relationships between each CpG methylation level and chronological age. Statistical analysis was performed using SPSS software, version 24.0.
Results and discussion: A positive correlation between ELOVL2 DNA methylation and chronological age was observed. Simple linear regression testing the association between DNA methylation levels and age revealed strong correlations for all CpGs (R>0.80). The strongest correlation was observed for C6 (R=0.943; p=1.5×10-23), explaining 88.6% of variation in age (adjusted R2=0.886), followed by C5 (R=0.937; p=1.24×10-22) (adjusted R2=0.875) and C7 (R=0.932; p=6.10×10-22) (adjusted R2=0.866). In conclusion, the bisulfite PCR-sequencing method showed to be an efficient and economic method for quantification of DNA methylation patterns in ELOVL2, a powerful age predictor, in concordance with previous studies. This method, consisting of bisulfite conversion followed by Sanger sequencing, can be considered as a basis for future age estimation models.

P75 | Other: Genetic Counselling/Community Genetics

Opposing pre-symptomatic testing for late onset neurological disorders: accounts of non-engagement in medical genetics

Mendes Á[1], Paneque M[1], Sequeiros J[1,2]

[1]UnIGENe and CGPP—Centre for Predictive and Preventive Genetics, IBMC - Institute for Molecular and Cell Biology, i3S—Instituto de Investigação e Inovação em Saúde, Universidade do Porto; [2]ICBAS—Instituto de Ciências Biomédicas Abel Salazar; Universidade do Porto

Engagement with genetic knowledge and services is commonly described, among other motivations, as the morally sound way to conduct life with severe hereditary diseases. There are few studies, however, that focus on those who decide not to undertake genetic testing in the presence of genetic risks. We present the accounts from people at-risk or affected by late onset neurological disorders, and their family members, who have avoided contacting with genetic services, thus remaining uninformed about their genetic status and not undertaking pre-symptomatic testing (PST) for Huntington disease, familial amyloid polyneuropathy TTR Val30Met and Machado-Joseph disease. Through a qualitative study we undertook a discourse analysis of 6 semi-structured interviews conducted with persons at-risk or affected by those disorders, and of 4 family interviews, involving a total of 21 participants. Recruitment occurred through patients' associations. Preliminary findings indicate that some people frame access to predictive genetic information as being worthless as, although removing uncertainty, no effective treatment is yet available for their family´s disease. Therefore, those participants seek to avoid potentially burdensome knowledge, although they relied on experiential ways to gather knowledge about the disease (by relating with other at-risk and affected family members or close friends). By choosing 'not to know', participants prioritize to focus their lives on everyday pressing concerns (such as parenting their children, or assuming the caregiving of affected relatives), without the possible burden of knowledge of an impending disease. Other participants described their eagerness to undertake PST when making decisions about reproduction, or when their children decide they want to know their genetic status or to have children themselves. There were also accounts where non-engagement was deemed as inadequate or even irresponsible. Findings will hopefully clarify the reasoning of those who opt for non-engagement with medical genetic services and PST. It also points out to (1) a range of views about the value of genetic information, especially when no effective treatment or cure is available; and that (2) people at risk have different tolerance thresholds for predictive information. These results may be relevant for the genetic counselling practice, by bringing further insights into the decision-making process and to the professionals approach and support.

P76 | Other: Genetic Epidemiology

Population genetics of IFITM3 in Portugal and Central Africa reveals a potential modifier of influenza severity

David S.[1,2]; Correia V.[3]; Antunes L.[4]; Faria R.[1]; Ferrão J.[1]; Faustino P.[1,5]; Nunes B.[4]; Maltez F.[6]; Lavinha J.[1,7]; Rebelo de Andrade H.[3,8]

[1]Dept. Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Lisboa; [2]Centro de Estudos de Ciência Animal, Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares, Universidade do Porto, Porto; [3]Dept. Doenças Infeciosas, INSA, Lisboa; [4]Dept. Epidemiologia, INSA, Lisboa; [5]ISAMB, Faculdade de Medicina, Universidade de Lisboa, Lisboa; [6]Serviço de Doenças Infeciosas, Hosp Curry Cabral, Centro Hospitalar de Lisboa Central, Lisboa; [7]BioISI, Faculdade de Ciências, Universidade de Lisboa, Lisboa; [8]Centro de Patogénese Molecular—Retrovirus e Infeções Associadas (CPM-URIA), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Introduction: Influenza epidemics are a serious global public health and economic problem. The IFITM3 allele (rs12252-C) was suggested as a strong population-based genetic risk factor for severe influenza virus infection by A(H1N1)pdm09. We analyzed the population genetics of IFITM3 variants in the Portuguese general population (n=200) and Central Africans (largely Angolan) (n=148) as well as its association to influenza severity in Portuguese patients (n=41).
Methods: For genotyping, we used PCR-sequencing of the 352 bp fragment in the first exon of IFITM3 around rs12252. The Fisher Exact Probability and Chi-square tests were used for testing association to mild vs. severe influenza. The latter was also used for analyzing the frequencies of the SNPs and haplotypes between each patients' group and the general population.
Results: Seven SNPs were identified. SNP distributions in the Portuguese appeared at an intermediate level between the Africans and other Europeans. According to HapMap rs34481144 belongs to the same linkage disequilibrium (LD) block as rs12252, and is in strong LD with rs6421983. A negative association with severe relative to mild disease was observed for allele rs34481144-A, indicating a protective effect under the dominant model. Moreover, haplotype Hap4 with rs34481144-A, not including rs12252-C, was significantly associated to mild influenza. Conversely, although with borderline significance, haplotype Hap1 with rs34481144-G, not including rs12252-C, was associated to severe disease. Moreover, in comparison to the general Portuguese population, statistical significant differences in the frequencies of the protective allele rs34481144-A in the severe disease group, the deleterious Hap1 in the mild disease group and the protective Hap4 in the severe disease group, were observed. The population attributable risk (PAR) for the targeted rs34481144 allele or genotype was of 55.91% and 64.44% in the general population and the mildly infected individuals, respectively.
Discussion: These findings point to important differences in the risk of developing severe disease due to A(H1N1) influenza virus infection in populations of different genetic background. Confirmation in larger and different population sets and further studies to determine possible functional alterations are needed.

P77 | Other: Genetic Epidemiology

Variants in the non-coding region of the TLR2 gene associated with infectious subphenotypes in pediatric sickle cell anemia

David S.[1,2]; Aguiar P.[3]; Antunes L.[4]; Dias A.[5]; Morais A.[6]; Sakuntabhai A.[7,8]; Lavinha J.[1,9]

[1]Dept. Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Lisboa, Portugal; [2]Centro de Estudos de Ciência Animal, Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares, Universidade do Porto, Porto, Portugal; [3]Centro de Investigação em Saúde Pública, Escola Nacional de Saúde Publica, Universidade Nova de Lisboa, Lisboa; [4]Dept. Epidemiologia, INSA, Lisboa; [5]Dept. Pediatria, Hosp. Prof Doutor Fernando Fonseca, Amadora, Portugal; [6]Dept. Pediatria, Hosp. Santa Maria, Lisboa; [7]Unité de la Génétique Fonctionnelle des Maladies Infectieuses, Institut, Pasteur, Paris; [8]Centre National de la Recherche Scientifique, URA3012 Paris, France; [9]BioISI, Faculdade de Ciências, Universidade de Lisboa, Lisboa

Introduction: Sickle cell anemia (SCA) is characterized by chronic hemolysis, severe vasoocclusive crises (VOC) and recurrent often severe infections. Ou purpose was to investigate the association of genetic variation in three noncoding polymorphic regions of the innate immunity-associated TLR2 gene, the -196 to -174 indel, SNP rs4696480 and a (GT)n short tandem repeat, on the infection phenotype in a cohort of 95 SCA pediatric patients. Methods: A stepwise approach in the statistical analysis of genotype-to-phenotype association was used. The PLINK program was used in exploratory association testing for the identification of infectious disease subphenotypes. This was followed by descriptive statistics for the comparison of rates or the chi-square test for dichotomous traits. Lastly, a quasipoisson regression model was used in the association studies between the TLR2 polymorphic loci and the selected disease subphenotypes and correlated dependent variables, including a derived hemolytic component. Statistical significance was considered at $p \leq 0.05$. Results: The infectious subphenotypes included: (A) recurrent respiratory infections, and (B) severe bacterial infection at least once during the patient's follow-up. The absence of the haplotype [Del]-T-[n≥17](Hap7) in homozygocity protected against subphenotype (B), in a statistically significant association, resisting correction for multiple testing. For the individual loci, the same association tendencies were observed as in the haplotype, including a deleterious association between the SNP rs4696480 T allele and subphenotype (A),whereas the A/A genotype was protective, and a deleterious effect of the A/T genotype with subphenotype (B), as well as including the protective effect of -196 to -174 insert (Ins) and deleterious effect of the deletion (Del) in homozygocity, against subphenotype (B). Moreover, a reduction in the incidence rate of severe bacterial infection was associated to a rise in the hemolytic score, fetal hemoglobin levels (prior to hydroxyureia treatment) and 3.7-kb alpha-thalassemia. Interestingly, differences between the effects of the two latter covariables favoring a reduction in the incidence rate of subphenotype (B) contrast with a resulting increase in relation to subphenotype (A). Discussion: The development and validation of these early predictors of disease severity could have practical implications in health care strategies to lower the morbidity and mortality of SCA patients.

P78 | Other: Population Genetics

Highest number of AMY1 copy number suggest to protect against obesity risk in Portuguese young adults: a preliminary study

Pinho S[1]; Padez C[1]; Manco L[1]

[1]Research Centre for Anthropology and Health (CIAS), Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Introduction: Genome-wide association studies have identified many single nucleotide polymorphisms associated with Body Mass Index (BMI). Additional genetic variants, such as copy number variations (CNV), have also been investigated in relation to BMI. Recently, the highly polymorphic CNV in the salivary amylase gene (AMY1A) has been associated with obesity in adults and children from different populations, supporting for a potential role of a higher copy number of AMY1A gene in protecting from excess of weight gain. However, other studies failed to reproduce these findings. In the present study, we investigate the association between AMY1A CNV and obesity in young adults of Portuguese origin.
Methods. We evaluated the number of AMY1 copies in 180 Portuguese young adults (109 females, 71 males; age 17 to 35 years old, mean 21.3): 22 obese (30.13≤BMI≤44 kg/m2), 58 overweight (25.02≤BMI≤29.81 kg/m2) and 100 normal weight controls (18.66≤BMI≤23.75 kg/m2). A TaqMan assay targeting AMY1A gene (Hs07226362_cn; Applied Biosystems) and the reference RNase P Assay (#4403326; Applied Biosystems) were used for genotyping in the QX100 droplet digital PCR (ddPCR) system (Bio-Rad Laboratories, Hercules, CA, USA), following the manufacturer's recommendations. Previous gDNA enzymatic digestion was done with HindIII. Statistical analyses were performed with SPSS (vs 24).
Results and Discussion: Median AMY1A copy number within groups were: obese 7 (range 3-10); overweight 6.5 (range 3-12); normal weight 7 (range 2-14). Although the lowest AMY1A copy number was observed in the obese group (≤10), relative copy number distributions and medians did not differ significantly between normal, overweight and obese subgroups (Kruskall-Wallis test p=0.996 and Independent Samples Median test p=0.848). Moreover, defining a case group with obese and overweight individuals, logistic regression did not show a significant association between AMY1A copy number and obesity risk in the whole population (p=0.984), as well as in the lower (<7) or upper half (≥7) of AMY1A copy number distribution (p=0.981 and p=0.835, respectively). However, testing both groups in the subset samples above the third quartile of the AMY1A copy number distribution (>9) a marginal significant association was found between lower copy number and obesity risk (OR=0.45; p=0.056). In conclusion, our results suggest that the highest AMY1A copy number protect against obesity in the study sample of Portuguese young adults.

P79 | Other: Translation Regulation

Integrative network approach to identify new players involved in NMD or its regulation

Nogueira G.[1, 2]; R Pinto F.[1]; Romão L.[1, 2]

[1]Biosystems and Integrative Sciences Institute, Faculdade de Ciências - Universidade de Lisboa; [2]Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge

Nonsense-mediated mRNA decay (NMD) is a surveillance pathway that recognizes and selectively degrades mRNAs carrying premature translation-termination codons (PTCs). The physiological importance of NMD is manifested by the fact that about one third of all genetic diseases and some forms of cancer are caused by nonsense or frameshift mutations that introduce PTCs, and NMD can modulate the clinical phenotype of these diseases. Noteworthy, in total, genetic diseases attributable to PTCs affect millions of patients worldwide. Recent studies have shown that NMD also targets mRNAs transcribed from a large subset of wild-type genes, shaping their levels. NMD is a complex process where several proteins interact with each other and cooperate to induce degradation of a given transcript. Although this pathway has been extensively studied, the interactions and connectivity among these components is only partly elucidated. Aiming to expand the knowledge about the NMD pathway, we are combining bioinformatics, network analysis and experimental work to identify new proteins involved in NMD or its regulation. Our work, begins with a network analysis approach that integrates publicly available data regarding different types of interactions: 1) protein-protein, 2) kinase-target, 3) phosphatase-target, 4) miRNA-target, 5) transcription factor-target, 6) gene co-expression and 7) ubiquitination-target. Additionally, our network include data regarding known NMD-targets and NMD-triggering features. The generated network will be used to find novel NMD-associated proteins, prioritizing candidates with simultaneous interactions with different mRNA processing pathways (mRNA splicing, mRNA transport, mRNA translation and mRNA decay). Following data integration, we will develop a scoring algorithm to select the most central proteins in the generated network, which can be essential to further understand NMD and its regulation. The predicted candidates will be experimentally validated and their role in NMD will be tested. Due to the diversity of regulatory links integrated in this workflow, we propose it can be applied to find molecular bridges between related biological processes and generate novel hypotheses about the molecular mechanisms co-regulating these phenomena.

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“City tour by night”

The bus will leave the hotel at 19:00 (7p.m.) on November 17th, go by the main monuments of the city and will end up in the conference dinner restaurant.

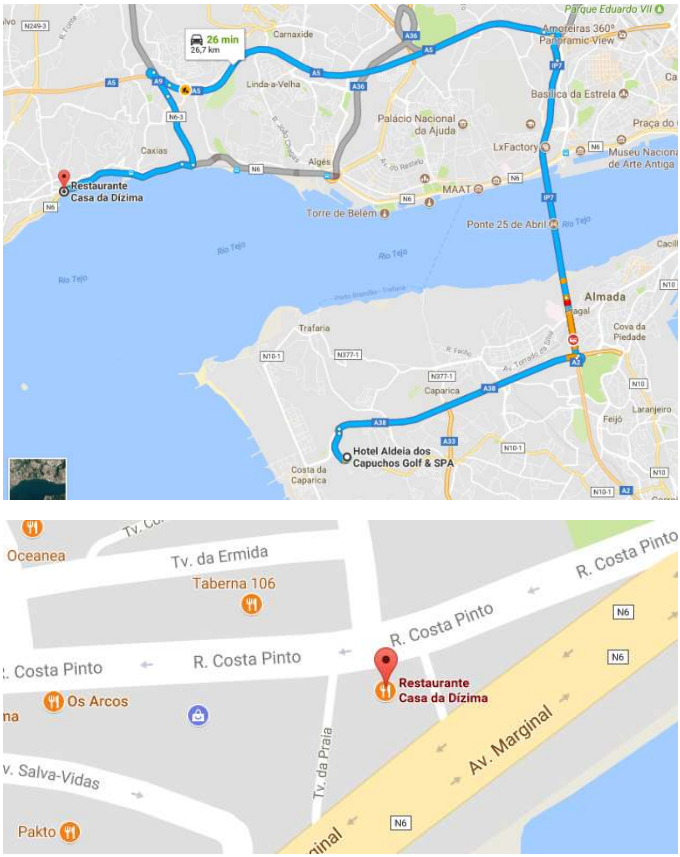
Price: 20€ (transportation back to the hotel after the dinner included)

Conference dinner – 21:00h

Casa da Dízima (http://casadadizima.com/en/)
Rua Costa Pinto, 17
2770-046 Paço de Arcos

Price: 35 €*

*SPGH subsidizes part of the conference dinner price



Casa da Dízima localization

Useful phone numbers:

Police (GNR-Caparica): 212 909 340

Fire department (Caparica): 212 900 030

Ambulance (emergencies): 112

Taxis (Caparica): 917 253 636

Taxis (Lisbon): 217 932 756 / 962 754 085

International code for Portugal: 00 351

Luísa Romão, President of SPGH 2017: +351 929 190 063 (from an international call)

Staff member Rafaela Lacerda: +351 919 232 080 (from an international call)

Staff member Paulo Costa: +351 928 026 851 (from an international call)

Notas

Notes

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