20ª Reunião Anual

Sociedade Portuguesa de Genética Humana

10-12 Novembro 2016

Coimbra

20ª Reunião Anual

Sociedade Portuguesa de Genética Humana

SPGH

10 a 12 de Novembro de 2016
Fundação Bissaya Barreto
Bencanta – Coimbra

Caros Colegas

É com muito gosto e orgulho que que vos damos as boas-vindas a Coimbra e à 20ª Reunião Anual da Sociedade Portuguesa de Genética Humana (SPGH)! Nesta Reunião festejamos o 20º aniversário da nossa Sociedade Científica. De facto, foi já há duas dezenas de anos, no dia 6 de Dezembro de 1996, que no Auditório da Ordem dos Médicos em Lisboa se realizou a sessão constituinte da SPGH.

Como vem sendo hábito nas nossas Reuniões Anuais, este ano quisemos trazer à discussão vários temas emergentes, apresentando diferentes exemplos do que tão bem se faz dentro e fora do nosso país! A excelente investigação científica produzida nesta área tem permitido não só clarificar mecanismos fundamentais, mas também levar a uma translação do conhecimento científico ao diagnóstico e à clínica, a um ritmo vertiginoso. No programa deste ano teremos sessões dedicadas à oncogenética e à neurogenética e aos novos avanços diagnósticos e terapêuticos. Discutiremos o papel do mosaicismo e da descrição de um número crescente de patologias associadas a mutações pós-zigóticas, os mecanismos de doença associados a variações da estrutura e da arquitectura do genoma codificante e não-codificante, bem como os avanços no âmbito do diagnóstico pré-natal. Organizámos ainda, como um dos temas centrais da nossa reunião, uma sessão abrangente e transversal para debater os vários desafios atuais na interpretação de variações de sequência, tarefa árdua tanto para investigadores como para geneticistas clínicos e laboratoriais aquando do diagnóstico de doenças genéticas por técnicas de sequenciação massiva em paralelo. Teremos uma mesa de Políticas Públicas sobre a estratégia para os Centros de Referência em doenças Genéticas e uma mesa de Bioética onde se pretende debater diferentes aspectos do *screening* genético alargado de portadores. Para além das duas sessões de comunicações orais seleccionadas de entre os trabalhos submetidos, introduzimos este ano uma novidade, com a organização de duas sessões mais curtas de apresentações de casos clínicos.

Agradecemos à nossa Comissão Científica o inexcedível contributo e continuado trabalho na selecção dos resumos submetidos e na escolha dos trabalhos premiados. A organização quis elaborar um programa científico apelativo e actual, contando com a vossa animada participação para que esta reunião seja cientificamente rica e proveitosa.

Preparámos ainda um programa social local para que possamos juntar-nos num ambiente relaxado, propício ao convívio, ao *networking* e sobretudo à consolidação da nossa comunidade, ao mesmo tempo que disfrutamos da nossa Cidade, Património Mundial da Unesco. Coimbra tem muitos encantos a (re)descobrir e a conhecer!

A Comissão Organizadora,
Joana Barbosa de Melo Sérgio Bernardo de Sousa Cecília Correia

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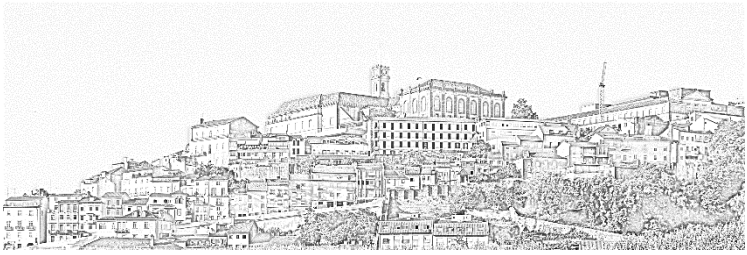

PROGRAMA CIENTÍFICO | SCIENTIFIC PROGRAMME

Day 1 Thursday, November 10th

09:30 – REGISTRATION OPENING

10:00 – CLUBS *(Parallel Sessions)*
Cytogenetic and Molecular Genetics Club
Medical Genetics and Clinical Dysmorphology Club

14:15 – OPENING & WELCOME
Joana Barbosa de Melo, SPGH President
Sérgio Bernardo Sousa, SPGH Secretary
Cecília Correia, SPGH Treasurer

14:30 – NEUROGENETICS
Chairs: Catarina Resende de Oliveira; Lina Ramos

Molecular approaches for Machado-Joseph disease
Luis Almeida, CNC, Faculty of Pharmacy, Coimbra, Portugal
Genomics in neurodegenerative diseases
José Miguel Brás, London, UK

15:30 – SELECTED CLINICAL CASES I
Chairs: Ana Berta Sousa; Fabiana Ramos

16:00 – Coffee Break / Poster Viewing

16:25 – Corporate Symposium (Illumina)
Furthering clinical testing with genomics solutions from Illumina
Szabolcs Kokeny, PhD., Sr. Sales Product Specialist EMEA, Channel Partners

16:45 – Genome structure and phenotype: 16p11.2 Rearrangements as an example
Chair: Joana Barbosa de Melo
Alexandre Reymond, Un. Lausanne, Switzerland

17:15 – SELECTED ORAL COMMUNICATIONS I
Chairs: Filipa Carvalho, Ana Cristina Sousa

18:45 – SPGH ASSEMBLY

Day 2 Friday, November 11th

08:45 – SELECTED ORAL COMMUNICATIONS II
Chairs: Sofia Dória; Rosário Pinto-Leite

10:30 – Copy Number Variations (Cnvs) can cause disease by changing the 3D-Structure of the genome
Chair: Isabel Marques Carreira
Stefan Mundlos, Max Planck Institute, Berlin, Germany

11:15 – COFFEE BREAK / POSTER VIEWING AND DISCUSSION

12:00 – ONCOGENETICS
Chairs: Carla Oliveira; José Manuel Nascimento Costa

From Li-Fraumeni syndrome to p53-related cancers
Thierry Frebourg, Rouen University Hospital, Rouen, France
Thyroid cancer: from transformation to (quasi) immortalization
Paula Soares, Ipatimup, Porto, Portugal

13:00 – Lunch

14:15 – CHALLENGES IN THE INTERPRETATION OF SEQUENCE VARIANTS
Chairs: Sérgio B. Sousa; Susana Fernandes

Implementation of the ACMG Guidelines for Variant Interpretation
Steven Harrison, Harvard University, USA
Towards a quantitative Bayesian pathogenicity and diagnosis framework
Leslie Biesecker, National Human Genome Research Institute, USA
Genetic variants of uncertain clinical significance in hereditary breast cancer: challenges for clinical management
Encarna Gomez, Maastricht, The Netherlands
Classification of genetic variants: lessons and challenges from cardiogenetics
José Carlos Machado, IPATIMUP, Portugal

16:15 – Coffee Break / Poster Viewing

16:40 – Corporate Symposium (Sophia Genetics)
Leveraging the collective knowledge of the largest clinical genomics community to democratize Data-Driven Medicine
Jean-François Vanbellinghen, Subject Matter Expert

17:00 – PRENATAL DIAGNOSIS
Chairs: Jorge Saraiva; Maria do Céu Almeida

Prenatal: where are we going?
Isabel Marques Carreira, FMUC, Coimbra, Portugal
Fabiana Ramos, HP-CHUC, Coimbra, Portugal

17:45 – PUBLIC POLICY SESSION "CENTROS DE REFERÊNCIA EM PORTUGAL – ESTRATÉGIA PARA AS DOENÇAS GENÉTICAS"
Chairs: Jorge Sequeiros; Luísa Romão

Research on Rare Diseases, Reference Centres in Portugal and European Reference Networks: are we following the right strategy to meet our national needs?
Jorge Sequeiros, IBMC, i3S, Porto, Portugal
Reference Centres for rare diseases in Portugal: Will we meet the expectations?
João Lavinha, INSA, Lisboa, Portugal

20:00 – Conference Dinner

Day 3 Saturday, November 12th

08:45 – SELECTED CLINICAL CASES II
Chairs: Ana Berta Sousa; Jorge Saraiva

09:30 – BIOETHICS DEBATE: EXPANDED CARRIER SCREENING – A NEW TOOL IN PRIMARY GENETIC PREVENTION
Chairs: Heloísa Santos; Célia Ventura; Francisco Corte Real

Introduction
Heloísa Santos, SPGH, Lisboa, Portugal
Classic carrier screening in Portugal - Haemoglobinopathies
João Lavinha, INSA, Lisboa, Portugal
Responsible implementation of expanded carrier screening
Lidewij Henneman, VU University Medical Center, Amsterdam, The Netherlands

10:30 – SESSÃO COMEMORATIVA DO 20º ANIVERSÁRIO DA SPGH

10:45 – Coffee Break / Poster Viewing

11:15 – Mosaicism And The Molecular Taxonomy Of Human Disease
Leslie Biesecker, National Human Genome Research Institute, USA
Chair: Margarida Reis Lima

12:15 – SPGH Award Conference

12:45 – Basic and Clinical Research Awards Ceremony

12:55 – Closing Session

13:30 – "Brunch com Ciência"

15:00 – "Coimbra Património Mundial"

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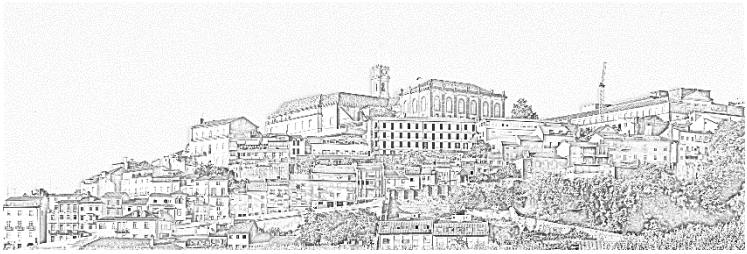

LUÍS ALMEIDA

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Luís Pereira de Almeida research activity is developed at the Center for Neuroscience and Cell Biology of the University of Coimbra (CNC), Portugal where he is Principal Investigator and Vice-President. Luis is a tenured assistant professor at the Faculty of Pharmacy, University of Coimbra since 2003. He did his PhD in the Gene Therapy Center of Lausanne, CHUV, Switzerland and spent short sabbatical leaves at CEA, Saclay in France (2005), at the Massachussetts Institute of Technology (2010) and has been vice-president of the Portuguese Society for Stem Cells and Cell Therapy (2013-2015).

The research of his group is focused on molecular/gene therapy approaches for brain disorders with a focus on Machado-Joseph disease/spinocerebellar ataxia type 3, including strategies that span from disease-modifying and gene silencing approaches, to autophagy activation, proteolysis inhibition and stem cell transplantation, works published in over 60 manuscripts, cited over a thousand times, and awarded with prizes by the Portuguese Society for Neurosciences (2009, 2011-2014), the Portuguese Society of Human Genetics (2009), and Fundação Pulido Valente.

Luis has been responsible for over 20 research projects funded by the National Ataxia Foundation (USA), Association Française de Myopathies, The Portuguese Foundation for Science and Technology (FCT) and private funds. His group integrated a Marie Curie Initial Training Network "TreatPolyQ" within the 7th Framework Program of the European Union, and presently an E-rare Eranet. He coordinates the transnational projects SynSpread and ModelPolyQ within the European Joint Programme for Neurodegenerative Diseases (JPND).

JOSÉ MIGUEL BRÁS

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José Brás is a human geneticist whose research interests focus on the genetics of neurological disease and how genetic variability impacts on phenotype. José obtained his PhD from the University of Coimbra for work conducted largely at the Laboratory of Neurogenetics, NIA, NIH. He then moved to UCL in London where he performed postdoctoral training under the supervision of Professor John Hardy. After this period, José started his independent group within the Department of Molecular Neuroscience. His lab investigates the genetic and molecular mechanisms underlying simple Mendelian and complex neurological diseases. José is currently a Proleptic Lecturer and Alzheimer's Society Research Fellow.

ALEXANDRE REYMOND

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Alexandre Reymond carried out his thesis in the laboratory of Dr. Viesturs Simanis at the Swiss Institute for Experimental Cancer Research (ISREC) and received his Ph.D. from the University of Lausanne in 1993. After completion of his postdoctoral training with Dr Roger Brent in the Department of Molecular Biology, Massachusetts General Hospital and in the Department of Genetics, Harvard Medical School in Boston, he moved to the Telethon Institute of Genetics and Medicine (TIGEM) in Milan in 1998 to lead a research group. He joined in 2000 the Department of Genetic Medicine and Development, University of Geneva Medical School. He moved to the Center for Integrative Genomics, University of Lausanne in October 2004 and became its Director in 2015.

STEFAN MUNDLOS

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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.

Stefan Mundlos is working on fundamental aspects of genomic diseases. 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 and 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.

He has been honored with ESHG Award 2016 for his fundamental work on the identification and characterization of disease genes and disease-causing mechanisms of gene regulation.

THIERRY FREBOURG

Department of Genetics, Rouen University Hospital
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Thierry Frebourg is Professor of Genetics, Head of the Department of Genetics, Rouen University Hospital, Director of the Inserm U1079 and of the Normandy Centre for Medical Genomics and Personalized Medicine in France. He obtained his M.D at Rouen University in 1986, his Ph.D in Molecular Biology at the University of Paris VII in 1990 and did his post-doc at the Massachusetts General Hospital, Harvard Medical School in Boston. He is a clinical and molecular geneticist whose research is focused on inherited forms of cancer, especially Li-Fraumeni syndrome and inherited forms of colorectal and breast cancers, medical applications of NGS and interpretation of genomic variations. His group combines expertise in clinics, genomic technologies, bioinformatics, statistical analyses and functional analyses of genetic variants in cellular assays. He has co-authored 382 publications in medical genetics and molecular biology (H-index 56).

PAULA SOARES
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Paula Soares, BSc, MSc, PhD, is Assistant Professor of Biopathology at the Medical Faculty of the University of Porto and coordinates the Group of Cancer Biology at the Institute of Pathology and Immunology of the University of Porto (Ipatimup) – Instituto de Investigação e Inovação em Sáude (i3S), Portugal. Her main interests include oncobiology of thyroid and neuroendocrine tumours mainly addressing thyroid cancer genetic alterations and signal transduction molecules involved in the MAPK and mTOR pathways. Paula Soares has a particular interest in the clinical translation of the pathologic meaning of genetic alterations. The metabolic deregulation in cancer is also an interest in her research. She has over 200 papers (including book chapters) in peer-reviewed journals and an h-index of 37. Paula Soares is also a member of numerous scientific committees and evaluation boards and serves on grant review committees and in journal editorial and review boards.

STEVEN HARRISON

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Steven Harrison is a Clinical Molecular Genetics Fellow at Harvard Medical School and the Laboratory for Molecular Medicine (Partners HealthCare Personalized Medicine). His work focuses on variant interpretation approaches and standardization at both a CLIA-certified molecular diagnostic laboratory and as part of the NIH-funded Clinical Genome Resource (ClinGen) program. As a member of multiple ClinGen working groups, Steven works to facilitate the centralized sharing of genetic data with ClinVar, develop a variant curation interface utilizing the American College of Medical Genetics and Genomics (ACMG) guidelines for variant interpretation, and co-chairs the Sequence Variant Inter-Laboratory Discrepancy Resolution task team which aims to resolve variants with interpretation differences between clinical laboratories. He completed his PhD in Genetics & Development in 2014 at the University of Texas Southwestern Medical Center under the mentorship of Dr. Linda A. Baker.

LESLIE BIESECKER

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Dr. Biesecker is a clinical and molecular geneticist and Chief of the Medical Genomics and Metabolic Branch and Director of the Physician-Scientist Development Program at the National Human Genome Research Institute of the National Institutes of Health, which he joined in 1993. He uses genetic and genomic technologies to study the etiology of genetic disorders and has published nearly 300 primary research articles, reviews, and chapters. He received his medical training at the Univ. of Illinois, Pediatrics at the Univ. of Wisconsin, and Clinical and Molecular Genetics at the Univ. of Michigan. His laboratory has elucidated the etiology and natural history of numerous diseases. In addition, he developed the ClinSeq® program, which began clinical genomics research in 2006, before the widespread availability of next generation sequencing. He co-directs a CLIA-certified molecular diagnostic laboratory within NHGRI.

Dr. Biesecker serves as an editor or board member for four biomedical journals, was a member of the board of directors for the American Society of Human Genetics, is an advisor to the Illumina Corporation, and served on the advisory panels for the World Trade Center and Hurricane Katrina victim identification efforts.

ENCARNA GOMEZ

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Current appointments: Staff member dept. Clinical Genetics and Head Hereditary tumors at the Oncology Centre of Maastricht University Medical Centre. Associate professor (UHD) dept. of Genetics and Cell Biology, Maastricht University, The Netherlands. **Education:** 1979-85: M.D. Degree. University of Salamanca, Spain; 1985-89: Ph.D. Degree. University of Salamanca, Spain. Thesis title: "Acute lymphoblastic leukemias: immunological and biological features and prognostic factors". *Cum laude*. 1987-91: Internship Hematology. Salamanca, Spain. 1997-98: Internship Internal Medicine. Rotterdam, The Netherlands. 2015-present: Internship Clinical Genetics. Maastricht, The Netherlands. **Previous appointments:** 1991-1994: Staff member Dept. Hematology, Academic Hospital Salamanca, Spain. 1992-1993: Post-Doctoral fellowship: Thrombosis and Hemostasis Research Center, LUMC, Leiden. 1994-96: Post-Doctoral fellowship: Center for Thrombosis and Hemostasis, Univ. North Carolina, Chapel Hill, U.S.A. 1996-2002: Staff member Dept. Hematology, Erasmus MC, Rotterdam. **Current Committee and Board Memberships:** 2002- National Workgroup for Clinical Oncogenetics (WKO), The Netherlands. 2003- Hereditary Breast/Ovarian cancer research Nederland (HEBON) workgroup. Steering committee member. 2009- Evidence–Based Network for the Interpretation of Germline Mutant Alleles (ENIGMA). Steering committee member and Chair of the Clinical Working Group.

My research interests in the genetic counselling revolve around the clinical translation of knowledge about the genetic predisposition to familial cancers, and in particular towards the classification and clinical management of patients with a genetic Variant of Uncertain Significance (VUS) in breast cancer predisposition genes, within an International framework.

In the same line of research, I also coordinate projects to determine the influence of modifier genes on the phenotypes of hereditary cancer syndromes, and conduct research aimed at understanding the biology underlying developmental abnormalities associated with hereditary cancer syndromes.

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José C. Machado (JCM) is vice-president of the Institute of Molecular Pathology and Immunology of the University of Porto (Ipatimup), where he is also group coordinator. JCM is Associate Professor in the Faculty of Medicine of Porto. His research interest stands in the area of molecular genetics of cancer, and genetics of complex diseases. He is internationally best known for his research in the area of Helicobacter pylori-related gastric carcinoma. The scientific question that drives JCM's research group is how is genetic information transferred between cancer cells, and how does that transference impact on the heterogeneity, diversity and plasticity of cancer cells. JCM has been awarded research projects by the Portuguese Foundation for Science and Technology, by the Portuguese Agency for Innovation, by the Portuguese Ministry of Health and by the European Union. JCM is also the director of the genetic diagnosis laboratory of IPATIMUP. This laboratory is accredited by the College of American Pathologists (CAP) and ISO9001:2008 certified. This is one of the reference laboratories in Portugal for the detection of KRAS mutations in colorectal cancer, EGFR mutations in Lung cancer, HER2 testing in breast and gastric cancer, among other tests. It is also one of the reference labs for the genetic diagnosis of hereditary cancer syndromes and hereditary cardiovascular diseases. As of October, 2016, JCM (Scopus Author ID 7102792651) co-authors 132 peer-reviewed publications in international journals, with 5897 total citations, and with an h-index of 36.

ISABEL MARQUES CARREIRA

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Isabel Marques Carreira (IMC) is the Head of the Cytogenetics and Genomics Laboratory of the Faculty of Medicine of the University of Coimbra (LCG-FMUC) since 1992. She is an Associated Professor with "Agregação" in FMUC. LCG-FMUC is a ISO9001:2008 certified laboratory performing cytogenetics, molecular cytogenetics, molecular genetics and genomics in prenatal and postnatal diagnosis. IMC is the Co-chair of the Clinical Laboratory Geneticists speciality of the European Board of Medical Genetics; Coordinator of the Commission for the speciality of Clinical Laboratory Geneticists of the Portuguese Society of Human Genetics; Co-coordinator of the "marker chromosomes" Permanent Working Group of the European Cytogenetics Association; Coordinator of CIMAGO (Centro de Investigação em Meio Ambiente, Genética e Oncologia); responsible for the quality system of the Division of Laboratories of the University of Coimbra. IMC participated as PI or collaborator in more than 40 research projects. Her main research interest stands in the areas of Aging and Brain diseases: in search of biomarkers; and omic signatures in Head and Neck cancer. IMC co-authors more than 150 peer review publications in international journals and has more than 650 participations in scientific events.

FABIANA RAMOS

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Fabiana Ramos (born in Monte Carmelo, Brasil) obtained her MD from Faculdade de Medicina of Universidade Federal de Minas Gerais (Brasil) in 2000. She completed her residence training in Gynecology and Obstetrician in Hospital das Clínicas da Universidade Federal de Minas Gerais in 2004. She moved to Portugal where She undertook a position of one year, as a fellow in Fetal Medicine in Gynecology and Obstetrician Department of São João Hospital (Porto) before becoming a Medical Genetics Specialist in 2009.

Until last year, Fabiana Ramos was also teaching assistant of Medical Genetics in Integrated Master of Medicine in Faculty of Medicine of the University of Coimbra.

Currently, she is a Medical Genetics consultant in Medical Genetics Department of Centro Hospitalar e Universitário de Coimbra and Coordinator of the Prenatal Sector of the Medical Genetics Department in Bissaya Barreto Maternity Hospital (Coimbra, Portugal).

JORGE SEQUEIROS

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Obtained his MD from Fac. Medicina, Univ. Porto (1975), and a PhD in Genetics, ICBAS, Univ. Porto (1990). Fellow in Medical Genetics (1982-85) at Johns Hopkins Hosp., Baltimore, MD, USA; specialist in Internal Medicine and in Medical Genetics (1987). Currently Full Professor of Medical Genetics and Director of the MSc on Genetic Counselling, ICBAS, Univ. Porto (accredited by EBMG). Research group leader of UnIGENe and Director of CGPP (Centre for Predictive and Preventive Genetics), for clinical and laboratory genetic services at IBMC, i3S, Univ. Porto. He supervised or co-supervised 28 PhD students theses successfully completed. Past president of the Ethics Commission, Univ. Porto; past member of the National Bioethics Council (CNECV); and consultant (Ethics), at the Directorate-General of Health (DGS). Past Member of the EBMG. National Coordinator of ORPHANET-PT (2009-15) and past president of the National Human Genetics Commission (DGS). Founder and first President (1999-2009) of the College of Medical Genetics, at Ordem dos Médicos. Founder and first Board of the SPGH (1996-99). Secretary-general (and interim president), Ataxia Research Group, World Federation of Neurology (1993-97). ataxias (2004-14). Member of several working groups at OECD, namely on human research biobanks and databases, on pharmacogenetics and the steering group on Quality Assurance and Proficiency Testing Schemes for Molecular Genetic Testing in OECD countries (2001-2010); and of an ad-hoc expert group on genetic testing at the European Commission. Working group on "Direct-to-Consumer Genetic Testing", of EASAC (European Academies Science Advisory Council) and FEAM (Federation of European Academies of Medicine) (2011-12). Participant, unit leader and member of steering group of EuroGentest – harmonizing genetic testing in Europe (NoE, FP6) and participant in EuroGentest2. Member of several research networks on Rare Diseases, including, Euro-HD (EHDN), HD-MAPS, SPATAX, RIBERMOV, Safe, Euro-Wilson. He is member of the scientific committees of several lay associations, including the Joseph Diseases' Foundation (USA, 1982 on), EUROATAXIA (2002-06), MJD Foundation (Australia, 2009-); also honorary member and/or scientific committee member of the Portuguese associations for familial amyloid neuropathy (2001-), hereditary ataxias (2008-) and Huntington disease (2001-). He is the author or co-author of more than 200 full scientific papers, including over 160 original articles in refereed international journals. Member of the editorial boards of Clinical Genetics and Journal of Community Genetics.

JOÃO LAVINHA

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João Lavinha (born in Sintra, 1949) is Head of the Research & Development Unit, Human Genetics Department, National Institute of Health, INSA, Lisbon. He has held other positions at INSA over the years, e.g., Head, Centre for Human Genetics (2005-2008); Director-general (2000-2004); and Head, Molecular Biology Laboratory, Human Genetics Department (1993-2000). He received his *Provas para Investigador Auxiliar* (equivalent to PhD) from INSA (1994), and his MSc (Medical Science) from the University of Glasgow (1993). He is (co)-author of 95 papers, 69 of which in international peer-reviewed journals. His current research interests include molecular etio-pathogenesis and epidemiology of genetic disease, genetics of disease susceptibility and public health genomics. Between 2005 and 2012 he served in the Portuguese National Council for Environment and Sustainable Development. In 2012 he was appointed to the Portuguese National Council for Science & Technology. He has been elected to the board of the European Society of Human Genetics (1997-2002) and of the Sociedade Portuguesa de Genética Humana (2004-2006) and is currently a board member of APF, the Portuguese Planned Parenthood association.

HELOÍSA G. SANTOS

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- Geneticista Médica e Pediatra
- Directora do Serviço de Genética Médica do Hospital S. Maria, Lisboa (1999-2004)
- Doutoramento em Genética em 1991 (Classificação: Muito Bom com Distinção e Louvor)
- Professora Auxiliar Convidada da disciplina de Genética da Faculdade de Medicina de Lisboa
- Prémio Nacional de Genética em 1991
- Consultora Permanente de Genética da Direcção Geral da Saúde. Actualmente é também membro da Comissão de Genética
- Sócia fundadora e primeira Presidente da SPGH. Presidente da Comissão de Bioética da SPGH. Eleita em 2011 Sócia Honorária desta Sociedade
- Membro do International Bioethics Committee da UNESCO (IBC) (2002-2006)
- Além de Presidente da Comissão de Bioética da Sociedade Portuguesa de Genética Humana, é igualmente Presidente da Comissão de Bioética da Sociedade Portuguesa de Pediatria (SPP) e da CES do INSA. É membro do Conselho de Ética e Deontologia Médica da Ordem dos Médicos
- Cento e dezanove trabalhos publicados, 51 em revistas internacionais
- Cerca de 400 participações activas em reuniões portuguesas e internacionais
- Participou em múltiplas acções de formação Pós-Graduada dirigidas a médicos e outros técnicos de saúde (enfermagem, áreas laboratoriais, etc)
- Investigadora do Projecto "Estratégias para Implementação de Programas de Prevenção de Doenças Genéticas e Malformativas: um Estudo Conjunto Brasil/Portugal" (JNICT)
- Investigadora Principal do Projecto "Estudo Clínico e Genético das Osteocondrodisplasias. Registo Português" (FLAD)
- Investigadora Principal do Projecto "Características clínicas, citogenéticas e psicológicas do Síndrome de Turner", que foi realizado no Serviço de Genética do HSM (Ministério da Saúde)
- Investigadora Principal do Projecto "Estudo molecular das condrodisplasias associadas a mutações do gene FGFR3 na população portuguesa" (FLAD)

LIDEWIJ HENNEMAN

Department of Clinical Genetics, Section Community Genetics and EMGO Institute for Health and Care Research, VU University Medical Center
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Dr. **Lidewij Henneman** is Associate Professor at the Department of Clinical Genetics, VU University Medical Center Amsterdam. She is a certified teacher at the faculties of Health Sciences and Medicine.

Her research focuses on the implementation and impact of (new) genetic applications, including the views, prior knowledge and expectations of potential users such as health care professionals, patients and wider society. She published over 90 international articles.

Specific projects are in the field of community genetics and public health genomics such as the implementation of population-based carrier screening, non-invasive prenatal testing (NIPT, cfDNA screening), genetic risk perception and (reproductive) decision making. Her research group is involved in the evaluation of several carrier screening initiatives, including screening for founder populations, ancestry-based screening and an expanded carrier test for 50 severe recessive disorders.

She won the EMGO+ Societal Impact Award 2016 for her role in the implementation of NIPT in the Netherlands, and received the Dutch Public Health Award for her PhD thesis on preconceptional cystic fibrosis carrier screening.

She is chair of the scientific Netherlands Association of Community Genetics and Public Health Genomics (NACGG) and board member of the Dutch Society for Human Genetics (NVHG).

Research group: www.vumc.com/researchcommunitygenetics
Personal Page: www.emgo.nl/team/196/lidewijhenneman/personal-information/

PALESTRAS
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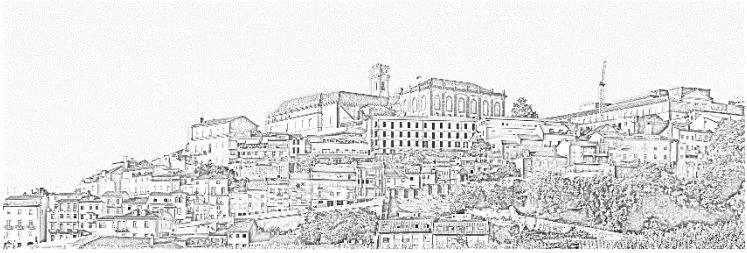

MOLECULAR APPROACHES FOR MACHADO-JOSEPH DISEASE

***Luís Almeida**, CNC, Faculty of Pharmacy, Coimbra Portugal*

Thursday, 10th November - 14:30 h

Machado-Joseph disease (MJD) is caused by the overrepetition of a CAG trinucleotide in the ATXN3/MJD1 gene, which translates into an expanded polyglutamine tract within the protein ataxin-3. The mutant ataxin-3 protein becomes prone to misfolding, accumulating as nuclear and intracellular inclusions, and acquires toxic properties which lead to neuronal dysfunction and cell death. In this talk

I will present some promising strategies to alleviate MJD with a particular focus on gene silencing, the microRNA pathway and caloric restriction.

Supported by National Ataxia Foundation, the Richard Chin and Lily Lock Machado-Joseph disease research fund, the Portuguese Foundation for Science and Technology (FCT), the EU Joint Programme – Neurodegenerative Disease Research (JPND Projects SynSpread, ESMI, and ModelPolyQ) and Association Française de Myopathies.

GENOMICS IN NEURODEGENERATIVE DISEASES

José Miguel Brás, *Department of Molecular Neuroscience, UCL, Institute of Neurology, London, UK*

Thursday, 10th November - 14:30 h

Advances in genomic technology in the past decade have revolutionized our understanding of the genetic basis of many diseases. Neurodegenerative diseases have been no exception and here we have moved from a handful of genes we knew were involved in causing these diseases, to a much larger number of genes we now know are involved in modulating risk.

Here, I will review and discuss some of these findings and how they are starting to show us shared biological pathways that are involved in otherwise apparently disparate diseases. Knowledge of these pathways will allow us to compare and contrast molecular phenotypes with much greater accuracy and will, in all likelihood, pave the way for truly personalized medicine.

CORPORATE SYMPOSIUM Illumina

FURTHERING CLINICAL TESTING WITH GENOMICS SOLUTIONS FROM ILLUMINA

***Szabolcs Kokeny**, PhD., Sr. Sales Product Specialist EMEA, Channel Partners*

Thursday, 10th November - 16:25 h

Nowadays, the personalized approach to health care and cancer care in particular is becoming more and more popular and is taking an important place in the translational medicine paradigm. Wider panels of genetic markers are also on the market which cover a greater number of possible oncogenes including those with lower reliability of resulting medical conclusions. In light of the large availability of high-throughput technologies, it is very tempting to use complete patient-specific New Generation Sequencing (NGS) or other "omics" data for cancer treatment guidance. However, there are still no gold standard methods and protocols to evaluate them.

In some cases, detection of the patient-specific individual mutations that point to a targeted therapy has already become a routine practice for clinicians and oncologists. Here we will discuss the clinical utility of each of the data types and describe a systems biology approach adapted for single patient measurements.

The aim of this presentation is to describe shortly the NGS platforms and solutions for DNA/RNA sequencing and in more detail key achievements and unresolved hurdles. We will try to summarize the current state of the field focusing on the clinically relevant case-studies and practical aspects of data processing. A special focus will be given on potential clinical applications and future perspectives of this innovative technique in the field of reproductive genetic health and oncology.

GENOME STRUCTURE AND PHENOTYPE: 16p11.2 REARRANGEMENTS AS AN EXAMPLE

***Alexandre Reymond**, Un. Lausanne, Switzerland*

Thursday, 10th November - 16:45 h

Phenotyping of more than 650 deletion and reciprocal duplication carriers of the proximal 16p11.2 600kb BP4-BP5 interval showed that these rearrangements are associated with autism spectrum disorders and mirror phenotypes of obesity/underweight and macro-/microcephaly. The considerable variance of the resulting phenotypes suggests that yet unidentified modifying factors may contribute. To potentially identify modifier genes of these phenotypes we sequenced the exomes and the transcriptomes of 250 individuals and identified slightly deleterious variants that potentially contribute to some of the traits.

The above-mentioned recurrent pathogenic deletions and duplications at chromosome 16p11.2 are mediated by a complex set of highly identical and directly oriented segmental duplications. This disease-predisposing architecture results from recent, Homo sapiens-specific duplications (i.e. absent in Neandertal and Denisova) of a segment including the BOLA2 gene, the latest among a series of genomic changes that dramatically restructured the region during hominid evolution. All humans (n = 2,359) examined carry one or more copies of the duplication, which was fixed early in the human lineage—a pattern unlikely to have arisen so rapidly in the absence of selection ($p < 0.0097$) and suggestive of a potential adaptive role of human BOLA2 duplication.

COPY NUMBER VARIATIONS (CNVS) CAN CAUSE DISEASE BY CHANGING THE 3D-STRUCTURE OF THE GENOME

Stefan Mundlos, *Institute for Medical and Human Genetics, Charité, Berlin and RG Development & Disease, Max Planck Institute for Molecular Genetics, Berlin, Germany*

Friday, 11th November - 10:30 h

Approximately 5% of the human genome is structurally variable in the normal population, which includes deletions and duplications (collectively referred to as copy number variants, CNVs), as well as inversions, and translocations. CNVs have received considerable attention as a major cause for genetic disease, promoting the search for CNVs as a standard diagnostic procedure in conditions such as intellectual disability and congenital malformations. The pathogenicity of CNVs is generally explained by their effect on gene dosage. However, such variants have the potential to disrupt the integrity of the genome, causing changes in the regulatory architecture that lead to pathogenic alterations of gene expression levels and patterns.

Technology-based approaches for the quantification of chromatin contacts including chromosome conformation capture and FISH have shown that the genome is folded in a highly controlled manner and that the resulting 3D-configuration directly influences gene regulation. In particular, one of its genome-wide variants of chromosome conformation capture (Hi-C) revealed that mammalian genomes are organized in topologically associating domains (TADs), large genomic regions that display a high degree of interaction compared to the rest of the genome thereby restricting the contacts that enhancers establish with their target genes. TADs have been identified as a fundamental organisational unit that is critical for accurate spatio-temporal gene expression.

We demonstrated that CNVs can disrupt the 3D architecture of the genome thereby disturbing the intricate regulation of gene expression. Disruption of TADs can rewire long-range regulatory architecture and result in pathogenic phenotypes.

Using CRISPR/Cas genome editing, we generated mice with corresponding rearrangements. Both in mouse limb tissue and patient-derived fibroblasts, disease-relevant structural changes cause ectopic interactions between promoters and non-coding DNA. Using chromosome conformation capture assays (4C and Hi-C), we show that duplications can result in specific effects depending on their size an position relative to TADs and their boundaries. Duplications that span a TAD boundary result in the formation of a novel chromatin domain, or neo-TAD. This formation of neo-TADs explains the divergent effects 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.

Our results demonstrate the functional importance of TADs for orchestrating gene expression via genome architecture and indicate criteria for predicting the pathogenicity of human structural variants, particularly in non-coding regions of the human genome.

Literature:

Franke M, et al. Formation of new chromatin domains determines pathogenicity of genomic duplications. Nature. 2016 Oct 5;538(7624):265-269;

Lupiáñez DG, Spielmann M, Mundlos S. Breaking TADs: How Alterations of Chromatin Domains Result in Disease. Trends Genet. 2016 Apr;32(4):225-37.

FROM LI-FRAUMENI SYNDROME TO P53-RELATED CANCERS

Thierry Frebourg, *Department of Genetics, Rouen University Hospital and Inserm U1079, IRIB, Normandy Centre for Genomic and Personalized Medicine, France*

Friday, 11th November - 12:00 h

Li-Fraumeni syndrome (LFS; MIM#151623) is a remarkable inherited cancer susceptibility disorder characterized by a wide tumor spectrum, which complicates its clinical recognition and medical management. LFS results from germline mutations of the *TP53* tumor suppressor gene, which plays a key role in response to DNA damage. The "Chompret criteria" for LFS criteria have sequentially been updated to facilitate the clinical recognition of the syndrome and to take into account the four clinical situations suggestive of LFS:

(1) familial presentation [a proband with a LFS tumor (breast cancer, soft-tissue sarcoma (STS), osteosarcoma, CNS tumor, adrenocortical carcinoma (ACC) under 46 years and one first- or second-degree relative with a LFS tumor under 56 years or with multiple tumors],

(2) multiple primary tumors [two of which belonging to the narrow LFS spectrum, the first being developed before 46 years] or

(3) rare cancers in childhood [ACC or choroid plexus carcinoma (CPC) or rhabdomyosarcoma of embryonal anaplastic subtype irrespective of the family history], or

(4) early-onset breast cancer before 31 years of age. ~

In *TP53* mutation carriers, the mean age of first tumor onset has been estimated to 25 years, 41% having developed a tumor by age 18. In childhood, the LFS tumor spectrum is characterized by osteosarcomas, ACC, CNS tumors, and STS. In adults, the tumor distribution is characterized by the predominance of female breast carcinomas and STS. Germline *TP53* mutation carriers have an exceptionally high risk of developing multiple primary tumors, estimated at least to 40%. Several lines of evidence suggest, in agreement with the key role of p53 in response to DNA damage, that radiotherapy and chemotherapy contribute to the development of secondary tumors in LFS. Therefore, in germline *TP53* mutations affected carriers, radiotherapy should be avoided, if possible. Because *TP53* is now included in cancer gene panels, the number of *TP53* tests has increased exponentially over the last two years and germline *TP53* mutations are now more frequently identified in patients who have developed only adult cancers.

Although modifier genes probably contribute to the age of onset and tumor type variability, a genotype-phenotype correlation has emerged among *TP53* mutation carriers. The most severe mutations are the dominant-negative missense mutations: they are significantly associated with earlier tumor onset and they represent the predominant germline alterations in carriers who develop childhood cancers except ACC. The less severe alterations correspond to loss of function mutations, such as nonsense mutations, frameshift mutations or genomic rearrangements: these alterations are associated with later tumor onset. These inactivating mutations are predominantly detected in pedigrees characterized by cancers occurring in adults.

The non-dominant-negative missense mutations define an intermediate class, in terms of clinical severity. Among them, the p.(Arg337His) associated to a founder effect in Southern Brazil and the (Arg158His) mutation are associated with ACC. The

predominance and clinical severity of the germline dominant-negative missense mutations can be explained by their drastic impact on p53-mediated transcriptional response to DNA damage. Therefore, it would be probably appropriate to stratify the screening protocols of *TP53* mutation carriers according to the type of mutation, considering the clinical gradient of germline *TP53* mutations, and to the familial history of cancer: In families harbouring *TP53* dominant-negative missense mutations and/or presenting with childhood tumors, the severity of the mutations and/or the familial history constitute arguments for performing pre-symptomatic testing in children and annual screening protocols, including total body-MRI that is under evaluation in different countries. In contrast, in families harbouring other classes of mutations, such as other non dominant-negative missense mutations and null mutations, and with only adult tumors, it seems appropriate to restrict pre-symptomatic testing to adults, with the aim, in particular, to offer to female mutation carriers annual breast MRI from 20 years of age.

Therefore, our perception of LFS has evolved from a very rare condition characterized by an aggregation of childhood and early-onset breast cancers to a wide range of clinical presentations sometimes limited to adult tumours. For these reasons, the term "Li-Fraumeni syndrome" is probably too restrictive since it corresponds to highly penetrant mutations associated with the classical form of the disease characterized by childhood tumors. Considering the progressive extension of the criteria since the original description of the syndrome, it would be appropriate, according to the paradigm of *CFTR* mutations involved not only in cystic fibrosis but also in CFTR-related disorders, to include Li-Fraumeni syndrome within an expanded category designated *TP53*-related cancers characterized by broader tumor spectrum and age of tumor onset.

THYROID CANCER: FROM TRANSFORMATION TO (QUASI) IMMORTALIZATION

***Paula Soares**, Cancer Signaling and Metabolism Group, IPATIMUP / I3S, Porto, Portugal*

Friday, 11th November - 12:00 h

Thyroid cancer is the most common endocrine neoplasia, presenting an increasing incidence worldwide over the last few decades. Thyroid tumours comprise a large spectrum in terms of biological behaviour; the well-differentiated (papillary and follicular) thyroid carcinomas that usually carry an excellent prognosis and, in the other side of the spectrum, the highly aggressive and lethal undifferentiated carcinoma.

The overall good prognosis of well-differentiated thyroid carcinomas is due, in part, to the effective treatment that comprises surgery followed by 131I radioactive iodine (RAI) ablation. Yet, a minority follow a more aggressive clinical course. Indeed, 5-10% of WDTC develop regional recurrences or distant metastases, and 26-60% of those recurrences or metastases become refractory to RAI therapy which may lead to a fatal outcome.

The genetic alterations most frequently detected in PTC belong to the MAPK and PI3KCA/AKT pathways and include BRAF (V600E), RAS mutations (all the three members of the RAS family) and also RET/PTC and PAX8-PPARG rearrangements. Recently, telomerase promoter mutations were reported as a newly discovered genetic alteration with prognostic significance in thyroid cancer.

The challenges in thyroid cancer research are, in one hand, the identification of molecular biomarkers that can identify those patients that will have a dismal prognosis. In the other hand, identify new molecules that can be efficiently used to treat patients with thyroid carcinomas not responding to radioactive iodine and, in these settings, the focus will rest on the identification of specific molecular targets and predictive biomarkers.

IMPLEMENTATION OF THE ACMG GUIDELINES FOR VARIANT INTERPRETATION

***Steven Harrison**, Harvard University, USA*

Friday, 11th November - 14:15 h

The American College of Medical Genetics and Genomics (ACMG), in collaboration with the Association for Molecular Pathology (AMP), has published a guideline to enable a more systematic assessment of evidence for and against pathogenicity of DNA variants for Mendelian disease. Variants are classified based on combinations of criteria of varying strength (e.g. supporting, moderate, strong, very strong) and type (e.g. population data, computational data, functional data, segregation data, etc.). Clinical laboratories and expert working groups operating within the NIH funded Clinical Genome Resource Program (ClinGen) are applying the ACMG/AMP guidelines to resolve differences in variant interpretation identified in NCBI's ClinVar database and build more granularity into the criteria using gene and disease specific parameters to enable expert review of variants in ClinVar.

As of Sept 2016, 20% (29,885) of all variants in ClinVar have interpretations from two or more submitters, of which 15% (4,498) have different submitted interpretations. Through pilot efforts the majority of these differences in variant interpretation appear to be resolvable and efforts are underway to resolve these differences using the ACMG/AMP guidelines.

TOWARDS A QUANTITATIVE BAYESIAN PATHOGENICITY AND DIAGNOSIS FRAMEWORK

***Leslie Biesecker**, National Human Genome Research Institute, USA*

Friday, 11th November - 14:15 h

The ACMG Richards et al pathogenicity criteria represent a landmark effort to organize and systematize laboratory practice and judgment regarding the determination of the pathogenicity of variants from genetic or genomic testing. These criteria define a categorical, semi-quantitative, informal Bayesian approach to pathogenicity that incorporates a number of variant and patient attributes to arrive at a pathogenicity assertion. The ClinGen Sequence Variant Interpretation working group has been charged to refine and operationalize the Richards et al criteria in the short run, but in the long run, is setting out to develop a system that will transform the criteria.

The long-term objective is to create a system that is transparent, modifiable, fully quantitative, formally Bayesian, and distinguishes variant data from case data. In this lecture I will describe the skeleton of this new approach and demonstrate how it fits into the bigger picture of genomic diagnosis.

GENETIC VARIANTS OF UNCERTAIN CLINICAL SIGNIFICANCE IN HEREDITARY BREAST CANCER: CHALLENGES FOR CLINICAL MANAGEMENT

***Encarna Gomez**, Maastricht, The Netherlands*

Friday, 11th November - 14:15 h

Germline pathogenic variants (also known as mutations or deleterious variants) in the tumor suppressor genes BRCA1 and BRCA2 confer high life time risks of breast cancer, ovarian cancer and less frequently also other cancers.

A considerable number of gene tests will identify rare variants where clinical significance cannot be inferred from sequence information alone, known as Variants of Uncertain clinical Significance (VUS). The International Agency for Research on Cancer (IARC) has proposed a standardized 5-tier classification system applicable to sequence-based results in highly penetrant cancer predisposition genes and linked the likelihood of pathogenicity to clinical actions. The multifactorial likelihood model, developed by members of the ENIGMA consortium (Evidence-based Network for the Interpretation of Germline Mutant Alleles) is used in this classification to calculate the probability of pathogenicity that a given variant has risk equivalent to known high risk pathogenic BRCA1 and BRCA2 variants, and so categorizes each variant into a specific class. The ENIGMA consortium, an International consortium of researchers and clinicians recognised by ClinGen as expert panel for BRCA1/2 classification, conduct multidisciplinary research to develop and apply methods to assess the clinical significance of sequence variants in breast cancer predisposition genes.

The proportion of VUS is likely to grow with increasing use of BRCA1/2 testing for tailoring cancer treatment and extension of testing to tumours for somatic mutations. Most VUS will not be associated with a high risk of cancer, but a misinterpreted VUS has the potential to lead to mismanagement of both the patient and their relatives. I will focus on the new challenges for clinical management represented by moderate risk variants, (i.e. variants with reduced penetrance), such as the BRCA1 c.5096G>A p.Arg1699Gln, and moderate risk candidate breast cancer genes, tested using massive parallel sequencing technologies.

CLASSIFICATION OF GENETIC VARIANTS: LESSONS AND CHALLENGES FROM CARDIOGENETICS

***José Carlos Machado**, IPATIMUP, Portugal*

Friday, 11th November - 14:15 h

The advent of Next Generation Sequencing (NGS) technologies provided the genetic diagnostics laboratory with unprecedented capacity to approach genetic diagnosis in a multigene fashion, and to produce large amounts of data. Genetic diseases which were diagnostically addressed only in specific laboratories, and genes which were hardly included in genetic analyses, are now commonly screened in routine laboratories using multigene panel and whole exome sequencing approaches. The establishment of such approaches led to a situation where an increasing number of genetic variants are detected and reported.

The latest guidelines for the classification of genetic variants put a lot of emphasis on the need to proceed with caution and on the use of stringent criteria in order to maximize clinical utility. Genetically heterogeneous diseases with incomplete penetrance, such as those included in the group of cardiomyopathies, make a perfect case to discuss the problem of genetic variant classification and its clinical relevance. In this presentation, we will discuss specific examples of genetic variant classification, using cardiomyopathies as a model, and following the recent guidelines published by ACMG.

CORPORATE SYMPOSIUM Sophia Genetics

LEVERAGING THE COLLECTIVE KNOWLEDGE OF THE LARGEST CLINICAL GENOMICS COMMUNITY TO DEMOCRATIZE DATA-DRIVEN MEDICINE

Jean-François Vanbellinghen, *Subject Matter Expert*

Friday, 11th November - 16:40 h

Today Sophia Genetics is the global leader in Data-Driven Medicine (DDM). The Sophia DDM® platform facilitates and accelerates patients' diagnosis. Powered by SOPHiA, the collective artificial intelligence, our core technologies PEPPER™, MUSKAT™ and MOKA™ process and analyse raw genomic data to spot pathogenic variants responsible for diseases. Our intuitive user interface allows you to directly interpret results and generate detailed variant reports.

From DNA extraction to data analysis, we understand your requirements and help you validate your NGS tests in the lab. We are both ISO 13485 (Medical Devices Quality Management) and ISO 27001 (Information Security Management) certified. Since inception, Privacy and Security have always been part of our corporate DNA. Over 180 healthcare institutions in more than 30 countries trust Sophia Genetics, performing thousands of genome analyses every month… Come and benefit from the world's largest clinical genomics community!

PRENATAL: WHERE ARE WE GOING?

Isabel Marques Carreira. Cytogenetics and Genomics Laboratory, Faculty of Medicine of the University of Coimbra

Fabiana Ramos. Medical Genetics Department, Paediatric Hospital of Coimbra, CHUC

Friday, 11th November - 17:00 h

For the past 40 years obstetricians, medical geneticists and clinical laboratory professionals have worked hand in hand to provide reliable, safe and cost effective diagnosis in prenatal area in order to allow a clinical intervention as soon as possible in a at risk gestations.

A small but "significant" risk of miscarriage has always been associated with the reliability of the prenatal diagnosis (PND) testing at our disposal (chorionic villus sampling, amniocentesis and cordocentesis). Increasing accuracy in ultrasound and biochemical tests have been used to "refine" the risks of screening tests for aneuploidy or for other genetic conditions and to reduce the invasive procedures. The karyotype, a gold standard test in PND, made the difference in the last decades to the diagnosis of chromosome aberrations. Now array chromosome analysis is becoming in the first line of investigation, particularly in foetuses with multiple anomalies.

Beside the experience in invasive procedure and reduced rate of complications, for more than 20 years the big goal in prenatal screening and diagnosis has been to develop a reliable non-invasive PND test (NIPT). A non-invasive foetal sex determination and foetal Rhesus D genotyping in maternal blood become common practice in PND and a molecular screening of trisomy 21 is becoming real for all pregnant.

In these past decade, tremendous laboratory technological advances have allowed an enormous step forward! Since 2011 it has been possible to analyse the genome of a foetus by sequencing foetal DNA fragments in the maternal circulation. In the last 4 years this screening test has revolutionized not only prenatal care but has also open up new prospects for personalized medicine for the foetus and the mother.

NIPT is disrupting the 40 year old prenatal screening paradigm! Its impact is yet to be truly comprehended. There are extensive implications on the enormous scope of human genetic variation that can be detected before birth. Pregnant women also face the challenge of having to deal with the unexpected incidental findings about their own health. A large accumulation datasets of the genomic information from the pregnant woman and from the foetus is indeed a reality, so it is of the outmost urgency and importance to discuss ethical issues regarding future data mining and intellectual property as more technological advances occur.

Industry, rather than academic laboratories, has driven innovation in NIPT. No doubt that they have propelled the field forward allowing an increasing number of women to be tested. However there are many gaps in the follow ups, as well as on the understanding of many of the biological mechanisms. There are contradictory results which are causing a lot of anxiety in thousands of women worldwide. There is the need to handle these issues

properly. If genetic counselling and informed consent has always been an important issue in PND now it is even more crucial because discrepancies on results have to be envisaged and explained as well as incidental findings that can have major implications for example in mothers' healthcare.

RESEARCH ON RARE DISEASES, REFERENCE CENTRES IN PORTUGAL AND EUROPEAN REFERENCE NETWORKS: ARE WE FOLLOWING THE RIGHT STRATEGY TO MEET OUR NATIONAL NEEDS?

***Jorge Sequeiros**, ICBAS and IBMC, i3S, Univ. Porto, Porto, Portugal*

Friday, 11th November - 17:45 h

A National Plan for Rare Diseases (RD) and a document on Reference Centres (RC) for RD were published in 2008. Portugal was one the first European countries to adopt such a plan, but these did not receive any funds and were never implemented. The "Rare Disease Card", the only official action in the field, has been having a very limited reach. EUCERD had a national expert participating only 2012-2015. Orphanet was initiated in Portugal in 2003, at IGM (where it stayed until this was absorbed by INSA, in 2006/7, but was not managed thereafter). Rebuilding and recovering the portal in Portugal and in Portuguese was led by a team at IBMC, from 2009 until 2015, when DGS appropriated it. As in most other European countries, there is no funding program for research, nor infrastructures dedicated to RD in Portugal. Portugal did not enter IRDiRC, the programme coordinating research on RD at the global level, though FCT has been a partner through the ERA-NET E-Rare3 consortium.

Portugal has two national federations of national patient associations (Fedra and Aliança), but an effort is ongoing to try to join them in a single umbrella organization. On February 2015, the national meeting of the EUROPLAN project took place at Assembleia da República, joining the various stakeholders, and a detailed report of the existing resources and needs on RD was published (www.eurordis.org/content/reports-europlan-national-conferences-2012-2015). As this meeting was taking place, the new Integrated Strategy on RD was published, revoking the NPRD and the document on RC.

A few RC have been established in the last two years (initially as Centres of Excellence, following the same approaches as those for common diseases). Those approved are limited to metabolic diseases, rare tumours and familial amyloid neuropathy, i.e., all diseases where some treatment is available. Due to the delay in approving official RC, de facto reference and research centres, dispersed within Hospitals of the NHS, Universities and Research Institutes will have a very tough job competing for European funds and joining the European Reference Networks (24 ERN were proposed, most expected to be approved and operational by 2017).

The EC is setting now a new Horizon 2020 EJP Cofund, "to support coordinated national research and innovation programmes", "in a strategy from bench to bedside". This tool will rest on three pillars: one similar to E-Rare, based on national funding agencies; another based on the ERNs; a 3rd for disseminating results, training activities and BP guidelines; and an overarching structure for coordination and overall strategy. But, will we be prepared and on time to be up to these challenges and meet its requirements?

REFERENCE CENTRES FOR RARE DISEASES IN PORTUGAL: WILL WE MEET THE EXPECTATIONS?

***João Lavinha**, Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal*

Friday, 11th November - 17:45 h

Due to their high rarity, diversity and severity rare diseases (RD) as a whole are sometimes under-recognised and not adequately dealt with. Hence the need to create national centres of expertise and establish international reference networks devoted to RD control. The main expected benefits for patients and relatives would be to allow them identify the appropriate health/education/welfare care resource for their case.

The Portuguese "Estratégia Integrada para as Doenças Raras" (established by Despacho 2129-B/2015) aims at recognising national reference centres for RD and to promote their participation in the corresponding EU reference networks, in order to:

1) Overcome the limited experience of (health, education and welfare) professionals confronted with very rare conditions.

2) Improve access for EU citizens to treatment requiring a particular concentration/pooling of resources (infrastructure and knowledge) or expertise.

3) Offer patients the highest possible chance of success through sharing of expertise and resources.

4) Maximise cost-effective use of resources by concentrating them where appropriate.

5) Help to share knowledge and provide training for health professionals.

6) Act as benchmarks to help develop and spread best practice throughout Europe and help small countries with insufficient resources from their health/education/welfare sectors to provide a full range of highly specialised services of the highest quality.

All stakeholders (namely, patients, health/education/welfare professionals and managers) pressure for change in this direction. However, there are difficulties in establishing and funding such multisector cooperation. The latter will be illustrated with empirical data derived from the first one and a half year of the strategy implementation. In any case, we should always distinguish between structures, processes and outcomes, i.e. the ultimate goal of our endeavour (cf Edmund Jessop, National Commissioning Group, UK).

BIOETHICS DEBATE: EXPANDED CARRIER SCREENING – A NEW TOOL IN PRIMARY GENETIC PREVENTION

INTRODUCTION

***Heloísa Santos**, Comissão de Bioética SPGH, Serviço Genética Médica HSM, Faculdade Medicina Universidade Lisboa*

Saturday, 12th November - 09:30 h

Classic carrier screening is a well-known activity in primary genetics prevention and, after many years of experience, we know the main advantages and risks for the couples and the children.

We have now strategic screening programs and usually excellent results in recessive disorders with high morbidity, some of them with higher prevalence in a specific population.

Expanded carrier screening and other new strategies based in panels with multiple genes implicated in many conditions including some diseases of low clinical significance, needs new criteria and new recommendations. The low price and, in consequence, the offering to the public by commercial agents without ethical rules is another aspect to be emphasized.

ESHG published in 2016 a very important document about this expanded screenings (Eur J Hum Genet 2016; 24: 781-783). The Bioethics Committee believe that the members of SPGH need to be more informed and further discuss the characteristics and ethical problems linked with this new growing tool.

CLASSIC CARRIER SCREENING IN PORTUGAL - HAEMOGLOBINOPATHIES

***João Lavinha**, Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal*

Saturday, 12th November - 09:30 h

Haemoglobinopathies are among the most frequent genetic conditions worldwide, with a markedly heterogeneous geographic distribution. In Portugal, thalassaemias and sickle cell disease are the most abundant haemoglobinopathies both in the autochthonous and migrant populations from sub-Saharan, Latin American or Far Eastern origin. In the absence of a definitive, safe and affordable cure, the best control strategy still relies on prevention through (i) population-based carrier screening, (ii) genetic counselling and (iii) prenatal (or pre-implantation) diagnosis. This approach has been successfully tested on both sides of the Atlantic (Mediterranean, USA, Cuba) resulting in a very significant reduction in the at-birth prevalence.

In Portugal, there is a clinical guideline (Circular Normativa da Direção Geral da Saúde 18/DSMIA de 07.09.2004) aiming at the prevention of the severest forms of haemoglobinopathy by offering universal carrier screening in the preconceptional or prenatal period. In this presentation empirical data will be presented summarizing 25 years of service provision in one of the largest human genetics centres in Portugal. During this long experience, no moral distress has been detected in association with haemoglobinopathy control.

RESPONSIBLE IMPLEMENTATION OF EXPANDED CARRIER SCREENING

***Lidewij Henneman**, Department of Clinical Genetics, Section Community Genetics and EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, the Netherlands*

Saturday, 12th November - 09:30 h

Carrier screening is the detection of carrier status of recessive diseases in persons who do not have an a priori increased risk of being a carrier based on their personal or family history. Carrier screening aims to facilitate informed reproductive decision-making among identified carrier couples. Expanded carrier screening offers screening for multiple recessive disorders, facilitated by new genetic testing technologies. The screening naturally expands as the range of disorders included in the screening expands; the pick up of "carrier couples" is much greater as more conditions, genes and sequence variants can be screened for simultaneously than hitherto possible. Expanded carrier screening panels that have been introduced to date have been advertised and offered to health care professionals and the public on a commercial basis. In 2016, recommendations for expanded carrier screening for health care professionals, laboratory experts and health authorities were developed by the Public and Professional Policy Committee (PPPC) of the European Society of Human Genetics (ESHG). The recommendations address the challenges that expanded carrier screening might pose in the context of the lessons learnt from decades of population-based carrier screening.

Responsible implementation of expanded genetic carrier screening raises many ethical, legal, social and technical questions. In my presentation I will address some of these questions, including, among others: Which diseases and sequence variants should be included in the panels and on what basis will these decisions be made? What are public and professional attitudes and preferences towards expanded carrier screening panels? How can pre- and post-test education and counselling be optimised to facilitate informed decision making? I will show that while having the potential to overcome some moral limits inherent in traditional carrier screening, expanded universal carrier screening comes with moral challenges of its own.

MOSAICISM AND THE MOLECULAR TAXONOMY OF HUMAN DISEASE

***Leslie Biesecker**, National Human Genome Research Institute, USA*

Saturday, 12th November - 11:15 h

Mosaic disorders provide a window into the biology of human disease that is otherwise unavailable to us. Mosaic disorders allow the manifestation of mutations that are lethal in the non-mosaic state and would be otherwise lost in gametes or early embryos. The early human recognized human disorders attributable to mosaicism were those that were visible to the human eye and caused overt dermatologic phenotypes. A wide range of such disorders have now been molecularly characterized including Proteus syndrome, PIK3CA-related overgrowth spectrum, Sturge-Weber, and others. Surprisingly, several of these disorders have proven to be caused by mutations that are well-recognized as cancer mutational driver mutations. Conceptually these disorders can be considered to be single gene reductionist model systems for cancer. Additionally, these disorders strain our definitions of clinical diagnosis and propel us toward considering a transition to a molecular taxonomy of human disease - classifying diseases by their molecular signatures instead of the manifest phenotype.

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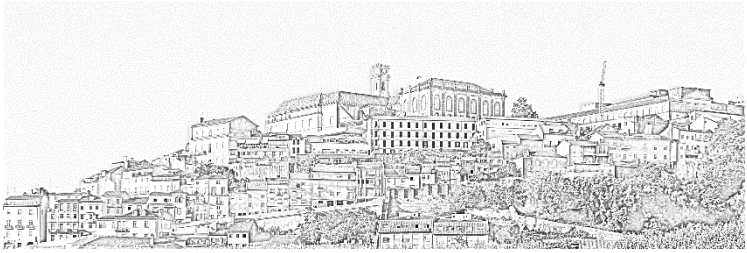

Session	Oral Communications
Session I – Thursday, 10th November, 17:15 h	OC1 – OC6
Session II – Friday, 11th November, 08:45 h	OC7 – OC13

OC1 | Neurogenetics

Comparison of CRISPR-based methods for modeling loss-of-function in iPS cells

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Introduction: Given the rapid pace of discoveries that de novo loss-of-function (LoF) mutations in highly conserved genes represent penetrant sources of genetic risk in autism spectrum disorder (ASD), it is imperative to generate robust models that recreate the human cellular landscape, particularly for neurological disorders where brain tissue is not readily available, and to eliminate the confound of different genetic backgrounds. **Methods**: We used an induced pluripotent stem cell (iPSC) line from a healthy male subject to perform dual-guide CRISPR/Cas9 gene editing of 9 independent genes for which LoF mutations represent strong risk factors for ASD. The efficiency of 30 dual guide-RNA combinations to generate deletions was then compared, using either FACS sorting or puromycin selection followed by serial dilutions to obtain single-cell derived colonies.
Results: Dual guide CRISPR successfully generated deletions in all genes, however the efficiency varied widely by guide-RNA and cellular protocol. The overall efficiency of the FACS method was 3.4% to generate predicted ablations, out of 1002 colonies screened (range = 0% - 10.6%). By contrast, the puromycin method had an average efficiency of 15.1% from 4437 colonies (range =0% - 32%).
Conclusions: This systematic survey of genome editing approaches suggests that dual-guide deletion generation varies widely by guide-pair. We also find that the increased certainty of deriving a single cell from FACS sorting comes at a significant cost in terms of efficiency and cell viability compared to serial dilutions.

Support: Portuguese Foundation for Science and Technology (SFRH/BD/52049/2012), Simons Foundation Autism Research Initiative, Autism Speaks

OC2 | Neurogenetics

Non-invasive and viral-mediated silencing of mutant ataxin-3 alleviates motor and neuropathological deficits in a transgenic mouse model of Machado-Joseph disease

Nobre, Rui J.*[1]; Saraiva, Joana[1]; Fusco, Clelia[1]; Paixão, Susana[1]; Santana, Magda[1]; Sena-Esteves, Miguel[2]; Pereira de Almeida, Luis[1,3]

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Machado-Joseph disease (MJD) is the most common dominantly-inherited ataxia. It is associated with the expansion of a (CAG)n tract in the coding region of the MJD1/ATXN3 gene. This abnormal over-repetition is translated into an expanded polyglutamine tract within ataxin-3, conferring toxic properties to this protein and resulting in severe clinical features. Although there is no medical treatment, several preclinical studies have demonstrated that silencing mutant ataxin-3 expression using RNA interference (RNAi) is a promising therapeutic approach for MJD. Our group showed that intracranial injection of viral vectors targeting mutant ataxin-3 significantly decreases the severity of the neuropathological abnormalities in rodent models of MJD (Alves et al., 2008, 2010; Nóbrega et al., 2013). However, this is an invasive procedure, which is associated with potential adverse effects and a limited vector distribution in the brain.
The present study aimed to develop a non-invasive strategy to deliver RNA interference-based treatments to the brain by intravenous (iv) injection. For that, we used adeno-associated viral vector serotype 9 (AAV9), a vector that has a remarkable ability to bypass the blood-brain barrier (BBB) and transduce the central nervous system of mammals. AAV9 vectors encoding an artificial microRNA that targets the mutant form of ataxin-3 mRNA (AAV9-mirATAX3) were firstly generated. Their efficacy and specificity were tested in neuronal cell models and the therapeutic potential was then evaluated in a severely impaired transgenic mouse model of MJD. Mice were intravenously injected at postnatal (PN) day one (PN1); they were submitted to behavioral tests at 3 different ages and were sacrificed at PN95. We observed that AAV9-mirATAX3 vectors efficiently spread throughout the brain, transducing regions affected in MJD, such as the striatum, cerebellum, brainstem and spinal cord. Moreover, AAV9-mirATAX3's treatment reduced the number of protein aggregates and cerebellar neuropathology, leading to significant improvements in all behavioral tests.
Overall, this study provides compelling evidence that a single iv injection of AAV9-mirATAX3 at PN1 is able to transpose the BBB, silence mutant ataxin-3 and alleviate MJD motor phenotype. To our knowledge, this is the first time that a non-invasive viral-mediated strategy has significant impact on motor deficits in a polyglutamine disorder.

OC3 | Neurogenetics

Increased frequency of CNVs targeting genes that regulate exposure to toxicants in Autism Spectrum Disorder (ASD) – a role for gene environment interactions

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ASD is a neurodevelopmental disorder characterized by complex clinical presentation and multifactorial etiology. While rare genetic variants, including Copy Number Variants (CNV), are responsible for a substantial fraction of ASD etiology, pre-, peri- and post-natal exposure to environmental factors has also been implicated. A polygenic and multifactorial model is the prevalent hypothesis to explain the syndrome. Here we seek to identify ASD-associated genetic variants that may interact with environmental factors. Permeability barriers, such as placenta and blood-brain barrier, are crucial in limiting the exposure to toxicants, particularly during neurodevelopment, while detoxification is fundamental for removal of toxic substances from the organism. We thus examined whether CNVs deleting or duplicating detoxification and barrier genes are more frequent in ASD subjects (N=2157), genotyped by the Autism Genome Project GWAS, than in a control dataset (N=10355), available from the Database of Genomic Variants (DGV).

Of 491 selected genes involved in detoxification or permeability of the barriers for toxic substances, 240 (49%) were targeted by CNVs in ASD subjects. Of these, 51 genes (21%) were exclusively found in CNVs from ASD patients, with *CYP2D6* the most frequently targeted gene (in 16 ASD subjects, 0.74%), followed by *GAL3ST2*, *ARSF* and *TRIM64B*, targeted by CNVs found in 6, 5 and 4 ASD patients, respectively. From the remainder 189 genes included in CNVs from both patients and controls, 40 genes were significantly more frequent in CNVs from individuals with ASD compared with controls, after correction for multiple testing ($P<2.6x10-4$). Some of the more significant genes clustered in metabolic pathways (eg. CYP450 and UGT genes, encoding cytochromes P450 and UDP-glucuronosyltransferases) or in transport mechanisms (*ABCAB1*, *SLC2A3* and *SLC2A14*); others, like *COMT* and *SHANK2*, were previously implicated in ASD etiology. This work reinforces the hypothesis that interactions between environmental exposure and genetic variation may contribute to ASD. Some of the genes more frequently deleted/duplicated in ASD subjects are part of ubiquitous metabolic or transport pathways, suggesting overarching sensitivities to a wide variety of toxicants. Others, with specific targets, may identify the most damaging toxicants for genetically susceptible individuals, suggesting preventive and therapeutic measure.

OC4 | Cytogenetics and Genomics

Array-CGH as a tool in a clinical laboratory set- up: experience in 4000 samples

Ferreira, Susana I.*[1]; Pires, Luís M.[1]; Val, Mariana[1]; Jardim, Ana[1]; Mascarenhas, Alexandra[1]; Pinto, Marta[1]; Lavoura, Nuno[1]; Pais, Cláudia[1]; Paiva, Patrícia[1]; Simões, Lúcia[1]; Melo, Joana B[1,2,3]; Carreira, Isabel M [1,2,3]

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Microarray-based comparative genomic hybridization (array-CGH) allows the possibility to screen the whole genome at once and with high resolution. It is currently assumed that array-CGH should be the first genetic test offered to detect genomic imbalances in patients with intellectual disability(ID) with or without dysmorphisms, multiple congenital anomalies (MCA), learning difficulties and autism spectrum disorders (ASD). As array-CGH allows the detection of imbalances below the 5–10Mb resolution level of conventional cytogenetics, the average diagnostic yield can be up to 10% higher. Since 2010 we have analyzed more than 4000 samples by array-CGH, using Agilent 60K and 180K oligonucleotide platforms. The samples analyzed included prenatal samples, postnatal samples from index patients with ID, ASD, congenital anomalies, learning difficulties, from progenitors and relatives of patients with previously identified imbalances and also tumor samples.

In 40% of the postnatal samples from index patients, only benign Copy Number Variants(CNVs) were identified, without relevant imbalances that justified the clinical phenotype. In 60% of the patients an imbalance was identified corresponding either to a previously established syndrome or to genomic imbalances that justified the study of the progenitors to determine the inheritance pattern and to ascertain pathogenicity. In 109 patients, genomic imbalances corresponding to OMIM described syndromes were identified, 64% corresponding to deletion syndromes and 36% to duplication syndromes. As expected, the majority of the imbalances were de novo. In nearly 1400 patients, deletions and duplications not described as commons CNVs were identified, leading us to request the progenitors to determine inheritance. Not all the parents were available for study, but the ones studied revealed a high percentage of carrier status, difficulting the genotype-phenotype relationship since most of the parents are normal while the child is affected. In 282 of the prenatal samples, 65% had a normal result, in 24% an imbalance was identified, either a de novo causative imbalance or imbalances that proved to be familiar and in 11% an inconclusive result was obtained

Array-CGH is a powerful toll to detect genomic imbalances, with impact in the diagnostic management of patients with ID, ASD and MCA and of prenatal samples. It has clearly improved the diagnosis yield but has also posed challenges in the interpretation of the variants identified.

OC5 | Molecular Genetics

Sequence variation between 462 human individuals fine-tunes functional sites of RNA processing

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Recent advances in the cost-efficiency of sequencing technologies enabled the combined DNA- and RNA-sequencing of human individuals at the population-scale, making genome-wide investigations of the inter-individual genetic impact on gene expression viable. Employing mRNA-sequencing data from the Geuvadis Project and genome sequencing data from the 1000 Genomes Project we show that the computational analysis of DNA sequences around splice sites and poly-A signals is able to explain several observations in the phenotype data. In contrast to widespread assessments of statistically significant associations between DNA polymorphisms and quantitative traits, we developed a computational tool to pinpoint the molecular mechanisms by which genetic markers drive variation in RNA-processing, cataloguing and classifying alleles that change the affinity of core RNA elements to their recognizing factors. The *in silico* models we employ further suggest RNA editing can moonlight as a splicing-modulator, albeit less frequently than genomic sequence diversity. Beyond existing annotations, we demonstrate that the ultra-high resolution of RNA-Seq combined from 462 individuals also provides evidence for thousands of *bona fide* novel elements of RNA processing—alternative splice sites, introns, and cleavage sites—which are often rare and lowly expressed but in other characteristics similar to their annotated counterparts.

OC6 | Clinical Genetics

Broad multi-gene panel or whole exome sequencing in malformed fetuses reveals five definitive and one likely diagnoses in the first nine cases studied in prenatal setting.

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Introduction: In the last year, broad multi-gene panel or whole exome sequencing became available in the clinical practice at our country. Here we present the results from the first nine couples to which broad multi-gene panel or whole exome sequencing was offered in just our pre-natal diagnosis centre. **Methods**: After the diagnosis of sonographic anomaly and medical termination of pregnancy (8), or after foetal death with multiple anomalies confirmed at autopsy (1), a predefined panel of 4813 genes with known associated clinical phenotypes [Illumina Trusight One] (8) or a trio whole exome sequencing (WES) (1) were performed and interpreted, following a previous extensive and inconclusive evaluation, most notably chromosome arrays for comparative genomic hybridization. **Results**: It was possible to reach a definitive diagnosis in five cases: primary microcephaly-5 [MIM 608716, gene *ASPM*]; minicore myopathy [MIM 255320, gene *RYR1*]; microcephaly, seizures, and developmental delay [MIM 613402, gene *PNKP*]; hydrocephalus due to aqueductal stenosis [MIM 307000, gene *L1CAM*]; fetal akinesia deformation sequence [MIM 208150, gene *RAPSN*]; and a likely diagnosis in one case of Fanconi anemia [MIM 227645, genes *FANCC* and *FANCD2*]. None of those diagnoses was previously evoked by the prior available elements. The definite five diagnoses were clearly presented in the laboratory report. **Discussion**: The very high diagnostic yield achieved likely derives from cohort ascertainment: recurrence was present at four of the six families and severe post-natal life compatible pathology in the other two. In the three remaining families without a diagnosis, the phenotype was always associated with intra-uterine lethality, suggesting the causative gene was not yet described and trio WES or WGS may turn out to be a more appropriate test. The information was highly relevant to the five families with a definitive diagnosis: in three, diagnosis was established during a pregnancy and recurrence could be excluded; to the others the result was given at a pre-conception setting. At the moment our proposal is to restrain the use of broad multi-gen panels or trio WES to cases with recurrence or high severity and only if collaboration with a medical geneticist is assured. In a short future the use of these tests will increase and became part of the initial diagnostic approach.

OC7 | Cytogenetics and Genomics

Application of whole-genome Array CGH in prenatal diagnosis

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Chromosomal analysis has been the main approach for the detection of chromosomal abnormalities in prenatal diagnosis (PND), however it is being supplemented and in some cases, especially in cases with ultrasound anomalies, replaced by Array – Comparative Genomic Hybridization (array-CGH). Array–CGH has the advantage to provide higher resolution and the results can be available more quickly, when compared to conventional cytogenetics. In fetus with ultrasound abnormalities, array-CGH can identify imbalances in up to 6% of the fetus with a normal karyotype.

In the last 44 months a total of 4170 prenatal samples were received, including amniotic fluids, chorionic villus samples (CVS), cordocentesis and skin biopsies from products of conception. In 282 of those samples, array-CGH was requested being the indications for analysis: 1) ultrasound anomalies, 2) carrier progenitor of genomic imbalance, 3) clarification of conventional cytogenetic findings and 4) miscarriages. Aneuploidy screening was performed previously to array-CGH and in 22 samples an aneuploidy or triploidy was identified, cancelling array-CGH. Of the 260 samples that proceeded to array-CGH, 57.3% were amniotic fluids, 30% CVS, 10% skin biopsies and 2.7% cordocentesis. Fibroblast and CVS were the samples with the higher rate of inconclusive results in array-CGH, 27% and 19% respectively, mainly because of tissue contaminants in the DNA sample. In 65% of the samples a normal result was obtained, in 11% an inconclusive result was obtained and in 24% an imbalance was identified, either a *de novo* causative imbalance or imbalances that proved to be familiar.

Array-CGH is valuable not only to establish a diagnosis in samples with ultrasound anomalies but also to characterize cytogenetic findings, showing to be a good tool for genetic counselling. In our laboratory, array-CGH has been useful in: 1) the characterization of markers or derivative chromosomes identified in PND by conventional cytogenetics, 2) exclusion of imbalances in samples with apparently balanced translocations, 3) study of familiar imbalances from a carrier progenitor and 4) identification of imbalances in fetus with ultrasound anomalies, below the resolution level of cytogenetics. Array-CGH gives an increase in detection rate of unbalanced structural abnormalities in prenatal context. The use of array CGH in high-risk pregnancies in conjunction with the karyotype analysis seems to be the best strategy in prenatal diagnosis.

OC8 | Clinical Genetics

Clinical exome sequencing: the importance of re-analyzing unsolved cases

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Introduction

Whole exome sequencing is currently a widely used technique for diagnosis of disease-causing variants in the diagnostic context. Although WES allows the genetic explanation of many cases in the clinical context, the number of unsolved cases is still elevated. In the last years, the broader implementation of WES techniques has allowed the discovery of new disease associated genes almost daily. This brings forward the need for re-analyzing results of exome analysis regularly, in order to update the reports in the light of more recent data. The aim of this work was to identify the genetic cause of disease in a clinically heterogeneous group of pediatric patients and to evaluate the yield obtained in the re-analysis of older cases (from 3 years ago). Additionally, the efficacy of a software for variant prioritization (Exomiser) was also tested.

Patients and methods

In this project we studied a group of 201 patients using two different approaches: (I) the application of a virtual gene panel selected based on the patient's phenotype and, if negative, (II) the entire exome analysis. Sixty-three of the patients were previously studied between the year of 2013 and 2014 and were again re-analysed in 2016. The selection of the candidate genes was done using bibliography searches using OMIM and PubMed databases. For all the patients the Illumina platforms HiSeq2000 and HiSeq2500 after exome capture with Agilent SureSelect Human All Exome Capture kit. In the filtering variants with MAF above 0.5% were excluded. De novo, recessive homozygous and compound heterozygous and maternally transmitted variants in male patients were considered for analysis.

Results and Conclusion

We applied 12 different virtual gene panels to the 201 patients. Likely pathogenic variants were found in 28% of the cases. Of the 63 re-analyzed patients in 2016 only 35 remained unsolved. Previously unidentified likely pathogenic variants were found in MAGEL2, FBXL4, SLC6A9 and DNAJC21 genes, among others. This work highlights the importance of re-analyzing results of exome analysis on a regular basis.

OC9 | Clinical Genetics

Diagnosis of Mendelian Disorders Using a Comprehensive 4813 Genes NextGeneration Sequencing Panel – Review of 92 Cases

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Introduction: In the last years, the accurate choice in each patient between a (broad vs focused) gene-targeted panel or whole-exome sequencing (WES) and their respective interpretation constitute crucial challenges for the clinical geneticist. In this study, we sought to review our experience analysing ninety-two consecutive cases using a broad multi-gene panel. **Methods**: A predesigned next generation sequencing (NGS) panel for 4813 genes with known associated clinical phenotypes (Illumina Trusight One) was performed in selected patients with unknown genetic diagnosis despite previous workup. We analysed the laboratory reports which used the ACMG 2015 guidelines, clinically re-evaluated each patient and their medical records given that information, performed family studies when appropriate and confirmed or reclassified the variants. These variants were categorized in four groups: pathogenic or likely pathogenic variants in disease genes associated with the reported phenotype (Group 1), variants of uncertain significance in disease genes associated with the reported phenotype and variants in disease genes with a possible association with the reported phenotype (Group 2), secondary findings according to the ACMG 2013 recommendations (Group 3) and other variants not associated with the reported phenotype (Group 4). **Results**: Ninety-two index unrelated patients were tested. The laboratory reported a total of 171 variants which represent 1.9 variants per patient. The maximum number of variants included in two patient's report was 6 and in 13 patients no relevant variant was identified. After our classification, we concluded that 30 variants should be included in Group 1, 30 variants in Group 2, 3 variants in Group 3 and 108 in Group 4. A definitive diagnosis was achieved in 22 patients and a likely diagnosis in 5. Forty-nine patients remained without a diagnosis. In 16 the reclassification of variants is still under way (23 variants classified as Group 2 and 19 variants classified as Group 4). The overall diagnostic yield of this panel in this cohort was between 29.3% (considering negative results for the uncompleted cases) and 35.5% (excluding the uncompleted cases). **Discussion**: We will present a comparison with the few other studies in the literature which used the same or other broad multi-gene NGS panel and also WES. To exemplify the clinical utility of this test, a few cases with positive findings will be briefly described.

OC10 | Cancer Genetics

The relevance of rare CDH1 non-coding variants in HDGC syndrome: redefining CDH1 cis-regulatory elements

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Introduction: Hereditary Diffuse Gastric Cancer (HDGC) is a rare, highly penetrant syndrome with a mortality rate of >90%. Heritable coding *CDH1*/E-cadherin mutations explain <50% of HDGC families. Strikingly, 70% of *CDH1* mutation-negative families display germline *CDH1* monoallelic downregulation at RNA level. Also, regardless of harbouring germline *CDH1* coding mutations, 90% of HDGC tumours present aberrant E-cadherin expression. These observations raise the hypothesis that *CDH1* mutation-negative families may be explained by germline mutations within *CDH1* intronic cis-regulatory elements (CREs). We aimed at disclosing the landscape of intronic variation in HDGC and defining novel *CDH1* CREs.
Methods: The full *CDH1* locus of 200 *CDH1* mutation-negative probands was submitted to NGS. Bioinformatics analysis was used to prioritize rare noncoding variants (NCVs) and integrate them with annotated regulatory features to define putative CREs within *CDH1* introns. CREs putative regulatory function was investigated through in vitro and in vivo (zebrafish) reporter assays. CREs were deleted using CRISPR-Cas9 and its impact evaluated through allele-specific expression, qRT-PCR, immunofluorescence and western-blot analysis.
Results and Discussion: We defined 12 potential CREs within *CDH1* introns 1 and 2, based on clustering of NCVs and described/annotated regulatory features. 46 potentially deleterious NCVs overlapped defined CREs. CRE 1 (intron 2) and CRE11 (intron 1) were found to act as enhancers in vitro and in vivo (zebrafish) reporter assays. Point mutations within these CREs were shown to impair their enhancer function in vitro. CRE 1 and 11 CRISPR-Cas9-mediated deletion in human gastric cell lines lead to loss of the characteristic epithelial morphology, which was accompanied by monoallelic downregulation of *CDH1*, as well as to a significant decrease of total *CDH1* mRNA and E-cadherin protein expression. In conclusion, we were able to identify two CREs within *CDH1* introns that present enhancer function and seem crucial for *CDH1* mRNA and protein expression. These data, and the fact that several NCVs found in HDGC families cluster within these CRE sequences, support our hypothesis that germline NCVs may work as a *CDH1* inactivating mechanism. If further confirmed, these CREs should be integrated in the molecular screening approach currently offered to HDGC families.

OC11 | Cancer Genetics

Familial intestinal gastric cancer: search for a germline and somatic cause

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Introduction: Gastric cancer is the third cause of cancer-related mortality and the fifth most common cancer worldwide. The majority of the cases have sporadic nature, however 10% display familial aggregation. These include hereditary diffuse gastric cancer (HDGC), gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) and familial intestinal gastric cancer (FIGC). FIGC has an autosomal dominant inheritance pattern and is characterised by intestinal type adenocarcinoma without gastric polyposis, displaying common macroscopic features observed in sporadic gastric cancer. However, no inherited mutations have been yet described, and no genetic screening is available for FIGC patients. This underlies the importance of novel research in this area. The aim of this study is to identify germline mutations that may cause FIGC and to characterise the somatic 2nd hit in these genes which may lead to their inactivation in FIGC tumours. **Material and Methods**: 53 FIGC probands were screened with Illumina's TruSeq Custom Amplicon assay on the MiSeq platform, using blood and tumour DNA. The 55 selected genes for analysis have been implicated in upper gastrointestinal tract tumours. Some of these genes were a target of investigation in FIGC tumours of 2nd hit inactivation, such as 2nd mutation, loss of heterozygosity (LOH) and promoter methylation. **Results and Discussion**: In the 53 FIGC families fulfilling IGCLC criteria, 37 germline variants were found. These include 24 variants of unknown significance in APC, ATM, BRCA1, CASP10, CDH1, CTHC1, FAT4, ITIH2, MAP3K6, MSH2, MSH6, MSR1, MTUS1, SDHD and TGFBR2 genes. Three variants, classified as likely pathogenic, were found in BRCA2 and MAP3K6. Ten variants were classified as pathogenic in genes MSH6, MSR1, SDHB and SDHD. The 2nd hit analysis is still ongoing. In conclusion, we were able to identify a set of germline gene defects that may be the cause of FIGC in the families studied, being a step forward in the molecular diagnosis for this syndrome. This work is funded by: 1) FEDER/COMPETE, FCT/MEC/FEDER/PT2020 and FCT funds (projects "PEst-C/SAU/LA0003/2013"; project 007274 (UID/BIM/04293); 2) ON.2–O Novo Norte/FEDER/QREN (projects NORTE-07-0162-FEDER-000118 and NORTE-07-0162-FEDER-000067); 3) No Stomach for Cancer Foundation; 4) FCT Fellowships (SFRH/BPD/89764/2012 to PO; SFRH/BPD/86543/2012 to JC; SFRH/BPD/79499/2011 to HP).

OC12 | Cancer Genetics

Germline variants in homologous recombination (HR)-mediated DNA damage repair genes may contribute to increased colorectal cancer susceptibility in a subgroup of FCCTX families

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Introduction: Familial colorectal cancer type X (FCCTX) families are clinically defined by the Amsterdam criteria, the absence of germline mutations in mismatch repair (MMR) genes and the presence of microsatellite stable tumours. We have previously reported the presence of two distinct molecular entities amongst tumours from 15 FCCTX families: one (n=10) whose tumours presented frequent loss of heterozygosity in tumour suppressor genes (TSG+) and another (n=5) with tumours lacking this molecular feature (TSG-). Amongst TSG+ tumours, we found a subgroup (n=7) with a prevalence of *APC/KRAS* somatic mutations and *MGMT*/MMR methylation, and a second, where these features were almost absent. In the present study we aimed to characterize these distinct subgroups at the germline level by the analysis of a multigene panel of 94 genes associated to increased cancer risk. **Methods**: Next generation sequencing was performed using the TruSight Cancer panel, in a Miseq platform, in the 15 index patients previously studied. Large deletions were evaluated for all genes associated with hereditary colorectal cancer syndromes by MLPA. Likely pathogenic variants were confirmed by Sanger sequencing. **Results**: In 7/15 families, all TSG+, we found one or more likely pathogenic germline variants in genes encoding proteins involved in double strand breaks (DSB) associated DNA repair pathways, secondary to DNA damage response to genotoxic stress, particularly in homologous recombination (HR)-mediated DNA damage repair. Five of the seven families belong to the subgroup whose tumours presented frequent *KRAS* somatic mutation and/or *MGMT*/MMR gene methylation. In two of these families we have also detected a likely pathogenic missense mutation in *BMPR1A* gene and a deletion of *SMAD4* exons 5-8, respectively. **Discussion**: The cytotoxic effects of alkylating agents, if not repaired by MGMT and MMR system, will eventually lead to DNA double-strand breaks. The latter, together with defects in HR-DNA repair pathways, will result in elevated chromosomal/DNA breakage and genome instability, which are consistent with the mutation signature previously reported by us in the FCCTX TSG+ subgroup. Therefore, germline defects in HR-DNA repair genes, identified in the present study, may contribute to increase colorectal adenoma/carcinoma risk in a subgroup of FCCTX families with TSG+ tumors, carrying frequent *KRAS* mutations and/or *MGMT*/MMR gene methylation.

OC13 | Cancer Genetics

Gene expression profiling to predict clinical outcome of oral cavity carcinomas

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Purpose Oral squamous cell carcinoma (OSCC) is a frequent neoplasm with an aggressive and unpredictable biological behavior and consequently unfavorable prognosis. Genome-wide mRNA expression profiling has been currently used for the classification of solid tumors into relatively homogeneous groups, predicting its clinical outcome. However, there is no biomarker used clinically able to stratify OSCC in order to predict and improve the survival and management of these patients. In this study we analyzed the gene expression patterns of OSCC aiming at identifying distinct signatures with different clinical outcome. **Material & Methods** We performed gene expression microarray analyses in 48 OSCC patients. Both statistical and machine learning methods were used to identify gene expression signatures. Through an Artificial Neuronal Network (ANN) we identified a set of genes as possible biomarkers. Data were split into training and testing sets allowing to adjust the classifier model and to assess the predict accuracy of the signatures identified. **Results** From the 17 648 genes differently expressed in our cohort, we observed different gene expression signatures between tumor stages (I to IV), tumor location (tongue vs. floor of the mouth) and patients that develop relapses/metastases after treatment for primary tumor from those without recurrence. We identified a specific subset of genes whose expression highly correlates with the clinical phenotypes in study and allows predicting with reasonable confidence the development of relapse/metastasis. **Conclusion** We have identified a set of genes that might be used as a predictor of outcome in OSCC and may be useful for the development of novel diagnostic markers and for therapeutic modalities.

CASOS CLÍNICOS
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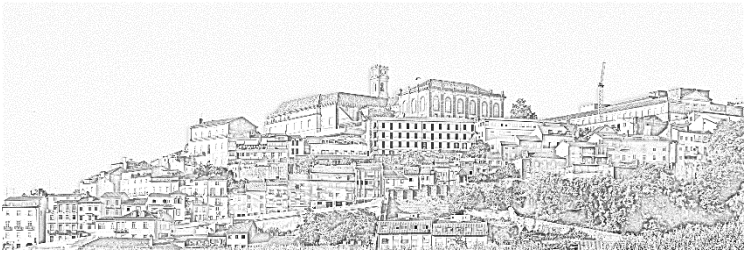

Session	Clinical Cases
Session I – Thursday, 10th November, 15:30 h	CC1 – CC6
Session II – Saturday, 12th November, 08:45 h	CC7 – CC13

CC1 | Clinical Genetics

Case Report of Oculoectodermal Syndrome due to a Mosaic KRAS Mutation

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Introduction
Oculoectodermal syndrome (OES) is a rare condition characterized by congenital scalp lesions and ocular dermoids. There may be additional features, such as non-ossifying fibromas and giant cell granulomas of the jaw. Only 27 cases of OES have been described in the literature. Most patients had normal cognition and normal neuroimaging. Recently, mosaic KRAS mutations were found to underlie OES. We report a case of OES with hemimegalencephaly and mild developmental delay in whom a mosaic KRAS mutation was found.

Clinical Report
A 12-months-old girl was referred for genetic evaluation because of epibulbar choristomas and ectodermal abnormalities. Her family history was unremarkable. The pregnancy was uneventful except for fetal ventricular extrasystoles. She was born by emergency caesarean section due to fetal bradicardia. At birth, her weight was 4125g (90th centile), her length was 54,5cm (95th centile), and her head circumference (HC) was 35,8cm (75th-90th centile). She had mild hypotonia, microphthalmia, coloboma and epibulbar choristomas, and right hemicranium alopecia. Physical examination at 12 months of age showed relative macrocrania with frontal bossing and HC of 45,5cm (75th centile), length of 72cm (25th centile), weight of 7,55kg (below 5th centile). Additional skin features were recognized consisting of multiple circumferential atrophic skin scars in an area of alopecia, and discrete areas of hyperpigmentation at the back of the neck following Blaschko's lines. At 2 years of age she was diagnosed with epilepsy. Head MRI was compatible with right side hemimegalencephaly. At 3 years of age, she had more extensive hyperpigmentation anomalies. Epilepsy was effectively controlled with lamotrigine and valproate, but she had mild developmental delay.
The typical skin and eye abnormalities led to the diagnosis of OES. Sequencing of KRAS in DNA extracted from the skin biopsy of a scalp lesion revealed the same heterozygous mutation, c.436G>A (p.Ala146Thr), previously reported in other OES patients.

Discussion
We report a case of OES with hemimegalencephaly and developmental delay, which are rare findings in this syndrome. Molecular characterization identified a mosaic KRAS mutation, confirming OES as a new mosaic RASopathy. KRAS is a proto-oncogene involved in diverse neoplastic processes, thus oncologic surveillance may be required for these patients. This case amplifies the clinical spectrum of OES and KRAS associated pathologies.

CC2 | Clinical Genetics

X-Linked Intellectual Disability Syndrome type Nascimento, caused by UBE2A mutations – report of two affected brothers and literature review

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Introduction:
Ten percent of cases of intellectual deficiency (ID) in boys are caused by abnormalities in genes located on the X chromosome. X-linked intellectual disability (XLID) includes more than 200 syndromes and 100 genes identified to date. Many of these conditions are not very specific but careful dysmorphological evaluation is needed as there is a growing number of rare recognizable syndromes. The present report constitutes the ninth family with UBE2A point mutations causing XLID type Nascimento (OMIM#300860).
Case reports:
We describe two affected brothers, with non-consanguineous healthy parents who performed multiple IVF (blocked tubes). The mother has no relevant family history and the father has a nephew with severe non syndromic ID. The older brother (5 year-old) was born prematurely at 31 weeks of gestational age and had a twin sib that died in uterus. He had delayed motor milestones and developed moderate-severe ID/developmental delay with significant speech problems and seizures. He had normal growth, bilateral sensorineural hearing loss, umbilical and bilateral inguinal hernias and multiple ENT infections. His brain MRI, at 3 years, was reported as normal. The 3 year-old brother presented with moderate developmental delay, significant motor and speech problems, no seizures, umbilical hernia, normal growth and normal hearing.
Both brothers had strikingly similar phenotypic features namely broad face, hypertelorism, synophoris, almond-shaped palpebral features, depressed nasal bridge, prominent columella, hypoplastic alae nasi, macrostomy, mild hypertrichosis/ hirsutism, brachydactyly, fetal pads, broad toes with onychodystrophy. Array-CGH did not reveal pathogenic CNV. Sanger sequencing of *UBE2A* identified the missense mutation c.326T>A (p.Ile109Lys), hemizygous in both affected brothers and heterozygous in the mother. This variant was not reported in the literature nor in ExAc or other databases. It affects a highly conserved amino acid and in silico analysis points out to likely pathogenicity. X-inactivation studies in the mother showed complete skewing.
Discussion:
XLID Syndrome type Nascimento is a rare but clinically very recognizable syndrome caused by *UBE2A* loss-of-function mutations. Our careful description and literature comparison confirmed previous findings: a homogeneous phenotype in all affected males and all carrier females are unaffected, showing a completely skewed X inactivation in blood.

CC3 | Clinical Genetics

An emerging XLID syndrome affecting females caused by DDX3X de novo variants: A case report

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Introduction: To date, more than 100 genes associated with recessive X-linked (XL) intellectual disability (ID) have been reported. Yet, the identification of XL conditions causing ID in females is limited. In 2015, DDX3X loss-of-function de novo variants were proposed as a frequent cause of ID (OMIM 300958, MRX102), accounting for 1-3% of unexplained ID in females. Missense mutations in DDX3X have also been found in males in rare families with an ID pattern compatible with recessive XL inheritance. It appears that DDX3X is dosage sensitive which, in the context of functional mosaic females and hemizygous males, could explain the observed gender differences. DDX3X is involved in many cellular processes, including cell cycle control, apoptosis and tumorigenesis. Recently, it was suggested that DDX3X modulates neurite development. Data collected from 40 affected females indicates DDX3X de novo mutations cause varying degrees of ID (mild to severe) with associated neurological abnormalities (including hypotonia and movement disorders). Behaviour problems (such as autism spectrum disorders, hyperactivity and aggression) have been reported in about half. Structural brain anomalies (namely corpus callosum hypoplasia and ventricular enlargement) are also common features. There is no consistent recognizable facial phenotype.

Clinical Report: The patient is a 5-year-old girl, only child of a healthy non-consanguineous couple with unremarkable family history. She was referred to us at 23 months for moderate developmental delay and axial hypotonia. On physical examination she presented brachycephaly, minor unspecific facial dysmorphisms, wide-based gait, stereotyped and repetitive behaviors. Array-CGH and FRAXA and MECP2 gene analyses were normal. Whole exome sequencing (WES) (trio approach) detected a novel heterozygous variant in DDX3X: c.1021T>C (p.Cys341Arg), which was not present in either parent.

Discussion: This DDX3X variant occurred de novo. It is located within the critical helicase ATP-binding domain, and it is predicted to be deleterious by in silico analysis. Also, the clinical presentation albeit unspecific is consistent with the main features described in previous patients.

WES is increasingly used in the etiological investigation of ID patients. This case illustrates its potential and clinical usefulness. Molecular diagnosis allowed proper genetic counseling, including informed reproductive options, and avoided unnecessary additional investigations.

CC4 | Clinical Genetics

X-linked intellectual disability caused by a novel PAK3 a mutation in a large pedigree

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Introduction: Ten percent of cases of intellectual deficiency (ID) in boys are caused by abnormalities in genes located on the X chromosome. X-linked ID (XLID) includes more than 200 phenotypes and 100 genes identified to date. Next-generation sequencing techniques significantly increased the diagnostic yield in XLID, especially in non-syndromic cases. We describe the seventh family with *PAK3* XLID.
Clinical report: We describe a large family with clear X-linked inheritance with 5 affected males, 4 of which were observed and had severe ID with almost absent speech, post-natal microcephaly, ataxia/wide-based walking, stereotypic movements, Angelman syndrome-like behavior (1/4), epilepsy (1/4), no significant dysmorphic features and MRI abnormalities, namely mild cerebellar atrophy. Of the observed females, at least one obligate heterozygous female had significant learning difficulties but without other features. Extensive previous conventional etiological was inconclusive and included: karyotype, subtelomeres FISH analysis, MLPA kit frequent microdeletions, 15q11.2 methylation studies, *FRAXA*, *FRAXE*, *UBE3A* and *SLC9A6* molecular analyses, ammonia, lactate, renal and liver function, hemoglobin H; TFTs; transferrin isoforms studies; plasma and urine amino acids and urine organic acids chromatography; purines studies. Two obligate female carriers had normal X-inactivation studies.
A detailed custom oligo-array CGH targeting the exonic regions of the X-chromosome (~500 bp resolution) failed to identify any pathogenic CNV. Subsequently, custom X-exome sequencing was performed in 3 affected males in a research setting. Only three variants were present in all 3 individuals, two known polimorphisms and the c.1287 G>A; p.M429I in *PAK3* gene, a known ID gene. It was confirmed by Sanger sequencing and segregation studies. This variant was considered likely pathogenic as it not previously reported in literature or any database; the amino acid residue affected is highly conserved and has a high GERP score; it is predicted to be disease causing by all in silico prediction tools used and is located in PAK3 kinase domain.
Discussion: Mutations in this gene were already described six families with XLID. The description of this large family expanded the phenotypic spectrum of PAK3 mutations as our patients have a more severe ID than described and additional features such as Angelman syndrome-like behavior and mild cerebellar atrophy were not previously described.

CC5 | Molecular Genetics

Comprehensive genomic studies decipher the classical Fragile-X phenotype in a female patient

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INTRODUCTION and AIM: Fragile X syndrome - the most common form of inherited intellectual disability (ID) in males – is caused by a methylated *FMR1* full mutation in the X chromosome. Around two thirds of females with the full mutation present some degree of cognitive, behavioural and/or social impairment, while full penetrance of the fragile-X phenotype is extremely rare. We present the case of an 11 month old female infant, referred for genetic testing due to global psychomotor developmental delay, attention deficit and hyperactive behavior without any specific dysmorphic features or relevant family history. Re-evaluation at 44 months showed persistence of the same problems and poor language skills. *FMR1* mutation screening revealed the presence of a methylated full mutation and a normal but inactive FMR1 allele, which led us to investigate this case further.

METHODS and RESULTS: The HUMARA assay, performed in blood leukocytes, revealed complete skewing of the X chromosome inactivation (XCI) pattern in the patient, whereas the mother and maternal grandmother (both *FMR1* pre-mutation carriers) had normal, random X-inactivation. This assay, as well as an *FMR1* -specific methylation PCR (mPCR), demonstrated that the paternally inherited normal allele was preferentially inactivated in the patient. *XIST* promoter screening was performed in an attempt to explain the XCI skewing, but no pathogenic variants were identified. Microarray analysis subsequently revealed a 439kb deletion in Xq28, encompassing several genes. According to the literature, similar deletions in this region are associated with an extreme XCI, are embryonic lethal in males and can cause high miscarriage rates in females but with normal cognitive function. These findings, together with further studies, now enable us to conclude that this is a *de novo* deletion that occurred on the paternally inherited X chromosome.

DISCUSSION: Although we were unable to exclude mosaicism or the presence of other X-linked recessive pathogenic variants in genes involved in the deletion, it is highly likely that the ID phenotype is the cumulative result of a methylated *FMR1* full mutation on the active X-chromosome and an XCI pattern skewed towards the other homologue carrying the 439kb deletion and the normal *FMR1* allele. This case shows how one may be mislead by incomplete studies and calls for reflection on the diagnostic protocol used for females with ID.

CC6 | Clinical Genetics

KBG syndrome: the experience of a regional Medical Genetics Unit

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Introduction: KBG syndrome is an autosomal dominant disease typically characterized by intellectual disability, behavioural abnormalities, short stature, and distinct craniofacial features, namely macrodontia of the upper central incisors. Other recognized features include heart defects, cervical ribs and other skeletal abnormalities, hearing loss, and seizures. It is caused by haploinsufficiency of *ANKRD11* gene resulting from either heterozygous loss-of-function variants or chromosomal rearrangements disrupting this gene. ANKRD11 protein is a member of a family of ankyrin repeat-containing cofactors that interacts with p160 nuclear receptor coactivators, thus inhibiting ligand-dependent transcriptional activation. Our purpose is to compare our KBG cases with those reported in the literature.
Methods: We report six families with nine confirmed KBG syndrome patients. Clinical characterization was performed, followed by whole genome oligonucleotide array-CGH and, in most cases, Sanger sequencing of *ANKRD11* gene or whole exome sequencing accompanied by targeted gene analysis.
Results: All of our patients have typical craniofacial features, but four patients (4/9) do not have short stature. Two patients (2/9) have microcephaly, a less commonly described feature, and another patient has an elevated IGF BP3, not previously reported in patients with KBG syndrome. Two patients (2/9) have a 16q24.3 deletion involving *ANKRD11* gene detected by array-CGH while the other patients (7/9) have a mutation either detected by Sanger sequencing (5/9) or by whole exome sequencing (2/9).
Discussion: Although the classical KBG syndrome phenotype is firmly established, whole exome sequencing is uncovering a significant number of more "atypical" cases that probably represent the milder forms of the spectrum and possibly the majority of cases. Features like learning difficulties / intellectual disability, behavioural abnormalities and macrodontia of the upper central incisors seem to be present in most cases described so far. However, as an increasingly number of patients is being diagnosed with KBG syndrome, rarer features are being identified. Their importance for KBG patient management is yet to be determined.

CC7 | Cytogenetics and Genomics

Identification of two new candidate genes OAF and PVRL1 for Peters anomaly and ectopia lentis

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Peters anomaly (PA) is a congenital defect of the anterior chamber of the eye. The aim of this study is identification of molecular alterations responsible for a syndromic form of Peters anomaly (PA), identified in an individual with an apparently balanced chromosome translocation t(11;18)(q23.3;q11.2)dn. Disruption of the human orthologue of the *Drosophila oaf* gene (*OAF*) by the 11q23.3 breakpoint results in reduced expression level of this transcriptional regulator. Additionally, expression of the cell adhesion protein *PVRL1*, a paralogue of *PVRL3* associated with congenital ocular defects, situated 500 kb upstream from 11q23.3 breakpoint is significantly increased. The 18q11.12 breakpoint is within the intergenic region between *CTAGE1* and *RBBP8*. Genomic imbalance that could contribute to the observed phenotype was excluded. Finally, analysis of mouse lens expression datasets suggests that *OAF* expression is significantly enriched in the lens from early stages of development through adulthood, whereas *PVRL1* is lens-enriched until E12.5 and then down-regulated. Our findings that mouse lens epithelium, that remains abnormally connected to the overlying cornea in PA, normally exhibits high *OAF* expression and low *PVRL1* expression, in contrast to the propositus who exhibits low *OAF* and high *PVRL1* expression offers further support that these are the molecular alterations responsible for this phenotype. Interaction data for *PVRL1* further supports this model. This two gene misregulation model may justify the absence of reported isolated mutations within these genes in unrelated PA patients. In conclusion, these findings suggest that disruption of *OAF* and misregulation of *PVRL1* likely contributes to the observed ocular phenotype.

CC8 | Clinical Genetics

Intermediate Autossomal Recessive Osteopetrosis- Long Term Follow Up on 3 Cases with CLCN7 mutations

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Osteopetroses are a heterogeneous group of increased bone density diseases that include infantile severe autosomal recessive (ARO), childhood intermediate autosomal recessive (IARO), and adult autosomal dominant (ADO type II) forms. They are associated with osteoclast dysfunction and more than 10 causative genes have been described. The intermediate forms are the less understood with limited information published. We describe 3 adults with *CLCN7* IARO, one novel case and the long-term follow-up on two previously reported patients.

Clinical Reports: Patient 1 is a 17 year-old adolescent with childhood onset of limited spine mobility, generalized osteosclerosis without fractures, mild intellectual disability (ID) and neuromuscular manifestations with persistent high levels of CK and LDH. *CLCN7* gene sequencing identified an intronic homozygous variant c.1798-14C>A. In silico splicing prediction studies and abolished CLCN7 transcription on leukocytes supported the pathogenicity of this variant.

Patient 2 was reported at 7 years of age, IARO diagnosis was raised through the investigation of a spontaneous femoral fracture and confirmed by the identification of the *CLCN7* homozygous variant c.608G>A (p.Gly203Asp). Re-evaluation at 25 years old revealed that he developed mild ID and anemia with necessary regular transfusional support, likely related to the progression of the skeletal problems.

Patient 3 was reported at 5 years old, IAOR diagnosis was raised during the radiological studies following a spontaneous humeral fracture and confirmed by the identification of the homozygous *CLCN7* variant c.1409C>T (p.Pro470Gln). Re-evaluation at 31 years-old revealed she developed mild intellectual disability; strabismus, bilateral optic atrophy; hearing impairment and tinnitus due to ossification of mastoid cells.

Discussion: Interesting, loss-of-function mutations in *CLCN7*, chloride channel protein 7, can cause all types of clinical forms mentioned above but there are no accurate genotype-phenotype correlations. Concerning patient 1, whose clinical picture is atypical due to neuromuscular manifestations, it is the first time that a splicing homozygous variant is described for IARO. All three patients had mild ID but showed distinct evolution of the phenotypes and related complication, potentially related with the type and location of the mutation and the predicted effect on CLC channels' function.

CC9 | Clinical Genetics

A new mutation in ADNP in a boy with Helsmoortel-Van der Aa syndrome

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Introduction: Helsmoortel-Van der Aa syndrome (HVAS) is a rare autosomal dominant syndrome characterized by intellectual disability (ID), autism spectrum disorder, behavioral problems, hypotonia, feeding difficulties, sleep disturbances, visual impairment and dysmorphic features. Heterozygous mutations in the *ADNP* gene were first clearly associated with the phenotype in 2014. Since then, only 25 cases have been reported, all caused by de novo mutations.
Clinical Report: The proband is a 3 year-old boy, referred to our Medical Genetics Department for mild global developmental delay, global hypotonia and dysmorphic features. On examination he presented with short stature; microcephaly; coarse facies with downslanted palpebral fissures, epicanthus, hypertelorism, broad nasal bridge and thin upper lip; small spaced teeth; wide thumbs; joint hyperlaxity; inverted nipples and mild hypertrichosis on the front and back. Pregnancy was complicated by intrauterine growth restriction and a positive biochemical prenatal aneuploidy screening. Fetal karyotype was performed, revealing a maternally inherited paracentric inversion of the X chromosome; we later confirmed its presence in his healthy maternal grandfather. There was a history of ankyloglossia, neonatal feeding difficulties, four lower airway tract infections in the first year, astigmatism, conductive deafness and sleep disturbance. Trio whole exome sequencing revealed the previously unreported de novo heterozygous variant c.2405C>T (p.Ser802Phe) in exon 5 of *ADNP*. The variant is classified as likely pathogenic and, considering the patient's phenotype, consistent with the diagnosis of HVAS.
Discussion: *ADNP* encodes a transcription factor which interacts with the BAF chromatin remodeling complex, playing a role in neuronal cell differentiation and maturation. The precise mechanism for the pathogenicity of *ADNP* mutations is not known, although they are believed to have a dominant-negative effect on the function of the BAF complex. Mutations in other genes involved in this complex have previously been implicated in syndromic and nonsyndromic ID, globally referred to as "BAFopathies" and classified within the group of chromatin remodeling defect disorders.

CC10 | Clinical Genetics

Beta-propeller protein-associated neurodegeneration (BPAN) in monozygotic twins due to a new mutation WDR45

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Introduction: Neurodegeneration with brain iron accumulation (NBIA) comprises a group of rare diseases with iron deposition in the basal ganglia. One form of NBIA was named BPAN (Beta-Propeller Associated Neurodegeneration) after demonstration of de novo mutations in WDR45 gene, located in chromossome Xp11.23. The patients have global developmental delay in early childhood that is essentially static, with slow motor and cognitive gains until adolescence or early adulthood. In young adulthood, affected individuals develop progressive dystonia, parkinsonism, extrapyramidal signs and dementia resulting in severe disability (MIM 300894).
Case report: Monozygotic female twins eight years old, the result of a pregnancy of 36 weeks with pre-eclampsia but without complications in the neonatal period; non-consanguineous parents and one brother of 15 years-old, all healthy. The twins were seen in our genetics unit due to severe global delay psychomotor development and behavioral alterations consistent with autism spectrum disorder. Since the last year there was a notion of global regression, that was more significant in one of the sisters that has drooling, incontinent bladder, aggressive behavior and stereotypies. The EEG showed multifocal paroxysmal activity. Brain Magnetic Resonance showed abundant iron deposition at the basal ganglia, mostly reaching the globus pallidus and substantia nigra, which results in hyposignal T2GE and SWI weights. Disease exome sequencing (Illumina TruSightOne; CGC Genetics) was performed in one of the sisters with identification of a probable pathogenic variant NM_007075.3: c.447_448del(p.Cys149 *) in heterozygosity at WDR45 gene. The heterozygozity was confirmed at the twin sister and excluded in both parents. To date, all reported affected individuals have been simplex cases. The majority are females suggesting that pathogenic variants are lethal in most males. The phenotypical differences between the twins may be explained by a different pattern of X chromosome inactivation.
Conclusions: We describe two twins with BPAN associated with a mutation not previously described in WDR45 gene.

CC11 | Clinical Genetics

2q31.1 deletion syndrome: report of three patients

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Introduction
Interstitial deletions encompassing 2q31.1 are rare with only about 20 patients reported in the literature. These deletions are responsible for a spectrum of clinical manifestations, including intellectual disability (ID), craniofacial dysmorphism, a variety of limb anomalies, and growth retardation. Additional features may be present depending on the size and location of the deletion, namely seizures, cleft palate, and anomalies of the eyes and genitalia. Some genotype-phenotype correlations have emerged.

Case Report
We present clinical and molecular findings in three patients with overlapping 2q31 deletions. The deletions were identified by *array*-CGH and range in size from 3,15 to 21,46Mb. Common features in our patients include intra-uterine growth retardation (3/3), hypotonia (3/3), feeding difficulties in the neonatal period (3/3), mild to moderate ID (3/3), and limb abnormalities comprising broad halluces (3/3), sandal gap (2/3) and cutaneous II/III syndactyly on both feet (3/3). Although no recognizable facial phenotype was apparent, they share some craniofacial dysmorphisms, most notably short palpebral fissures (2/3), thickened helices (3/3), and micrognathia (2/3). Cleft lip/palate, seizures, heart defects, visual problems, and genital anomalies were present in one patient each.

Discussion
The clinical phenotypes of our patients are concordant with the cases reported in the literature. Looking at the genes located in 2q31 some candidate genes have been proposed to account for different clinical features. The *HOXD* gene cluster plays a key role in embryonic limb development and is believed to be responsible for the variable limb phenotype in these patients. *RAPGEF4* and *CHN1* genes play a role in brain development. These genes were deleted in all three patients and appear to be at least partially causal for ID. Cleft palate might be *DLX1/DLX2* related. The latter encode homeobox transcription factors know to be involved in craniofacial development and were deleted only in the one patient presenting with cleft lip/palate. *Array*-CGH allows detection of small deletions with precise determination of the breakpoints and better delineation of the corresponding phenotypes. Over time this should improve genetic counseling.

CC12 | Molecular Genetics

A new neuromuscular disorder caused by defects in the activating signal cointegrator 1 complex: the second case with a loss-of-function variant in ASCC1

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Introduction

Our current research aims to identify new genetic causes in patients with undiagnosed neuromuscular diseases, including those with perinatal manifestation, using next-generation sequencing (NGS) technology.

Clinical Report

A healthy couple with no known consanguinity had one late intrauterine fetal death followed by two live births (female and male) with severe hypotonia, congenital bone fractures and lack of spontaneous respiratory movements. Both newborns died within the first few days of life.

Genetic testing of the female patient yielded normal results for karyotyping and for several genes related with spinal muscular atrophy (SMA), myotonic dystrophy and congenital myopathies. Whole-exome sequencing (WES) was performed, with variant filtering obeying a recessive disease model and a frequency below 0.1%, in candidate genes related firstly with congenital myopathies, then with all known primary muscle diseases and finally extended to other neuromuscular disorders. No plausible disease-causing variants were identified. Just recently, Knierim and collaborators (2016) described a severe prenatal form of SMA with respiratory distress and congenital bone fractures, in patients with recessive loss-of-function mutations in two genes: *TRIP4* (three families) and *ASCC1*(one family), both encoding subunits of the nuclear activating signal cointegrator 1 (ASC-1) complex. Based on these findings, we reassessed our WES data and identified a homozygous frameshift variant (c.157dupG, p.Glu53Glyfs*19) in the *ASCC1* gene. Sanger sequencing confirmed the patient's genotype and showed that the parents are heterozygotes.

Discussion

ASCC1 disease models are compromised in terms of axonal outgrowth, neuromuscular junction density and organization of the myotome. The ASC-1 complex, as a whole, is proposed to be a key player in muscle development having a role in late myogenesis and/or myotube growth. Since only one case has been documented worldwide, this report provides the definitive evidence that homozygous truncating variants in the *ASCC1* gene give rise to a severe neuromuscular disease, possibly within the SMA or primary muscle disease spectra. Ongoing research aims to further characterize this clinical entity and its underlying pathological basis

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P1 | Clinical Genetics

Next generation phenotyping complementing next generation sequencing: review of initial research studies

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Disclaimer: The authors are employees of FDNA

The role of objective facial analysis using automated facial recognition technology in making diagnoses following whole exome analysis (WES), has been shown in recent publications (Gripp et al. 2016; Mensah et al., 2106). Clinical phenotyping complementing next generation sequencing is reaching the level of next generation phenotyping (Hennekam, Biesecker, 2013) with the Facial Dysmorphology Novel Analysis (FDNA) technology. This technology is implemented through Face2Gene, a novel phenotyping tool that is offered gratis to clinicians and combines facial recognition algorithms with clinical feature annotation and anthropometric measurements, enabling detection of syndrome features from 2D facial photographs (Basel-Vanagaite et al.,2016; Gardiner et al.,2016). The results of several studies conducted on syndromes as diverse as Angelman Syndrome, Cornelia de Langue and 3MC show that the technology's detection rate is comparable with dysmorphology experts, further suggesting that a clinical application utilizing such technology may be a useful tool for healthcare professionals in clinical settings as well in gene variants prioritization.

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P2 | Clinical Genetics

Characterization of an E-cadherin-related common ancestor in Hereditary Diffuse Gastric Cancer families from the northern Portugal

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Introduction: Hereditary Diffuse Gastric Cancer (HDGC) is an autosomal dominant syndrome, caused by *CDH1* mutations, predisposing carriers for diffuse type gastric (DGC) and lobular breast cancer (LBC). A *CDH1*-associated founder effect was reported for Newfoundland HDGC families. We recently identified five seemingly unrelated families in Northern Portugal, carrying the c.1901C>T *CDH1*mutation. **Methods**: In five HDGC families we collected and analysed family history, clinical reports, performed c.1901C>T carrier tests and haplotype analysis (*CDH1* proximal and distal flanking markers in mutation carriers and affected vs non-carriers in each family). **Results and Discussion**: We collected 74 individuals from five 1901C>T *CDH1* positive families. 41/74 (55%) carried the mutation (age range: 14-82). We selected 28 individuals from at least 2 generations from each family branch for haplotype analysis. We identified a 2.650.596bp haplotype block shared exclusively by c.1901C>T carriers, indicating a common ancestor. Assuming these five families as a single one, we analysed their clinical data as a whole. From the 41 mutation carriers, 12 (29.3%) were affected by/died of DGC or LBC (age range: 18-62), and 10 (24.4%) revealed cancer foci in risk reduction gastrectomy (age range: 14-66). From the 12 patients affected by cancer, 3 (25.0%) developed LBC after 49, while 9 (75.0%) developed DGC from 18-35 years of age. The youngest carrier dying of DGC was an 18-year old male that did not report family history of HDGC. Predictive testing in his 14-year old sib revealed the *CDH1* mutation and cancer foci were identified in her risk reduction gastrectomy. Taken together our results show: 1) the definition of a founder effect helps identifying individuals at risk for predictive testing and disease prevention; 2) DGC penetrance is low by the age of 50 in 1901C>T *CDH1* mutation carriers; 3) given the early onset of DGC, genetic testing should be made available before 18 years old after proper genetic counselling; 4) risk reduction gastrectomy in carriers is lifesaving; 5) LBC MRI surveillance should be offered to mutation carriers. This is the first *CDH1* related founder effect described in Portugal, highlights the need for early genetic testing and DGC surveillance in young carriers of *CDH1* c.1901C>T mutation, and provide the rationale for prioritizing the screening of this mutation in HDGC families from Northern Portugal.

P3 | Clinical Genetics

Broad Multi-Gene Panel in 92 Portuguese patients – Analysis of Unsolicited and Secondary Findings

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Introduction: The return of results in clinical practice from broad next-generation sequencing (NGS) gene panels and whole-exome/genome sequencing has raised a growing number of controversial issues, especially concerning variants unrelated to the phenotype. In this study, we reviewed our experience in addressing secondary and unsolicited findings during the analysis of a broad multi-gene panel tested in ninety-two consecutive cases. **Methods**: A predesigned NGS panel for 4813 genes with known associated clinical phenotypes (Illumina TruSight One) was performed in selected patients with unknown genetic diagnosis. The unsolicited and secondary findings protocol included an opt-in option to get additional information. We analyzed the laboratory reports, that used the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines for variant interpretation, clinically re-evaluated each patient as well their medical records and performed family studies when appropriate. All variants were reassessed and categorized in four groups. The present study focused on the secondary findings (Group 3, according to ACMG 2013 recommendations) and all variants considered not to be associated with the reported phenotype after the mentioned re-evaluation (Group 4). **Results**: Ninety-two indexes unrelated patients were tested. The laboratory reported a total of 171 variants. Three variants (in 3 different cases) were reported and categorized in Group 3 and affected the following genes: *LDLR* (familial hypercholesterolemia), *VHL* (Von Hippel Lindau Syndrome) and *MSH2* (Lynch Syndrome). We reclassified 108 out of the 171 variants into group 4, of which 20 (18.5 %) were considered "pathogenic" or "likely pathogenic": 6 heterozygous variants (5.6 %) in dominant genes, 14 heterozygous variants (12.9 %) in recessive genes and none in X-linked genes. **Discussion**: The ACMG recommends the return of secondary findings classified as known or expected pathogenic variants in a set of 56 genes. In all the 3 secondary findings detected in our cohort, family history was retrospectively found to be compatible with those conditions, pointing out to the relevance of this protocol. Despite no additional search for unsolicited findings was performed, the laboratory reported a significant number of variants, especially heterozygous for recessive disorders, with potential clinically significance. Examples of particular cases will be presented and controversial questions raised.

P4 | Clinical Genetics

Discreditable inheritance: stigma and familial amyloid polyneuropathy ATTR Val30Met in north-western Portugal

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Familial amyloid polyneuropathy (FAP) ATTRVal30Met is a late-onset autosomal dominant disorder that leads to severe sensory-motor impairment. Such genetic conditions may result in feelings of stigmatisation, mainly because visible physical appearance (motor deficiency, caquexia) and its transmissibility to offspring. Research on stigma related to genetic inherited disorders is still scarce. We collected accounts of stigmatisation from patients affected by FAP living in the northwest coast, where the illness has been initially found and its largest cluster worldwide. Semi-structured interviews were conducted with 11 mutation carriers, recruited through the patients' association, and at different stages after presymptomatic testing. Qualitative thematic analysis of the interviews was undertaken.

Findings showed the influence of a discrediting social context in the enactment of stigma: FAP was perceived as a source of devaluation and social distance, and permeated by beliefs of contagion in the community. The transgenerational nature of this illness was felt as a source of rejection for courtship and mating, and of devalued reproductive worth. Decisions to have children seemed to be a target of implicit negative judgment. Dealing with stigma included restraint in talking about FAP, especially outside the family, the active confrontation of others, and social withdrawal. Participants also refer a reduction in stigma over the past few decades.

These findings may help to set out the social consequences of stigma towards this group, and to understand how stigma is experienced in other genetic disorders. We discuss the interactional nature of stigma with health literacy, individual biography and family dynamics, and with societal changes. Participants' perception of a reduction in the intensity of the stigmatisation may be explained by the recent availability of medical treatments and clinical trials, which has significantly decreased the burden on patients and affected families —as a result, some now see FAP as a potentially curable disease and, possibly, with less social stigma attached. Another possible reason may be that stigmatisation for health related issues, in general, has become less socially acceptable. How families deal with genetic information about predisposition to potentially stigmatising conditions is of importance to genetics healthcare professionals in assisting patients and relatives to effectively manage these issues.

P5 | Clinical Genetics

Some Lessons from Next generation Sequencing in Hypertrophic Cardiomyopathy

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Introduction: Sarcomeric hypertrophic cardiomyopathy (HCM) is the most common genetic cause of unexplained left ventricular hypertrophy and has no specific treatment. Sudden cardiac arrest (SCA) in young adults is frequently caused by inherited cardiac diseases, being HCM the most prevalent. Genetic testing is essential for the follow-up of survivors and family members.
Patients: Here we report on two interesting cases of HCM. First we describe an illustrative case of a female patient regularly followed during 25 years with a diagnosis of familial HCM and no identified sarcomeric mutations. Next generation sequencing analysis identified a novel pathogenic mutation in the GLA gene, leading to the uncovered diagnosis of multisystemic Anderson-Fabry disease (AFD) with the consequent implications for patient's treatment, prognosis and familial screening. The other case is a young survivor of SCA due to ventricular fibrillation with no structural heart disease. Genetic testing found a pathogenic mutation in SNC5A associated with reduction in the sodium currents, although Brugada syndrome could not be confirmed. Besides, we found a mutation in MYBPC3 with probability of pathogenicity. Both variants were identified in the healthy father showing negative provocative test for Brugada.
Conclusions: Next generation sequencing of panels of genes has become the routine method in genetic diagnostics of cardiomyopathies. This might help to undercover rare diseases, like AFD, now treatable. However, genotype-phenotype associations in inherited cardiac diseases should be interpreted with caution.

P6 | Clinical Genetics

Mutation screening of the EYA1, SIX1, and SIX5 genes in 31 portuguese families with branchiootorenal syndrome

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Introduction: Branchio-oto-renal (BOR) syndrome is an autosomal dominant disorder, characterized by high clinical variability, with an estimated prevalence of 1/40,000 individuals. Major features encompasse branchiogenic malformation, hearing loss and renal anomalies. BOR syndrome displays a high degree of genetic heterogeneity and three genes, *EYA1, SIX1* and *SIX5* have been reported as causative genes in BOR syndrome. The present study describes a casuistic analysis of 31 families with BOR spectrum which were fully analyzed for the 3 genes.
Methods: Thirty one Portuguese families, corresponding to a total of 38 patients that fulfilled criteria for BOR syndrome were genetically studied. Mutational screening of the complete *EYA1, SIX1* and *SIX5* genes was performed by direct sequencing analysis. MLPA (Multiplex Ligation-dependent Probe Amplification) technique was performed for *EYA1* gene to detect large deletions and duplications.
Results and Discussion: Among the studied families, three new variations were identified in three different families: one nucleotide change resulted in a truncated EYA1 protein on exon 11 of gene, *EYA1*, other on exon 18 of gene leading to an aminoacid change and a frameshift variation on DNA sequence on exon 2 of gene. Additionally, several sequence variations reported on NCBI database were identified, particularly in *EYA1* and *SIX5* genes, described as unknown significance variants. Familial genetic studies were performed to elucidate the clinical significance of these variants. The three novel variations detected on *EYA1* and *SIX1* genes were analysed by bioinformatic tools to predict their effects on functional proteins. Mutation Taster® and Poliphen-2® software pointed to the probably damaging effect of those variations on EYA1 and SIX1 proteins and being disease-causing mutations. As described on the literature, the expression of the disease varies widely from one family to another, and even among individuals of the same family, what makes the BOR syndrome ranging in a large phenotypic spectrum. The analysis of these results pointed to wide expression variability of the branchio-oto-renal spectrum and to the putative involvement of other genes, not yet identified, contributing to the disease.

P7 | Clinical Genetics

Upper limb phocomelia: a prenatal case of TAR syndrome diagnosed by array CGH

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Introduction
Upper limb reduction defects detected in the prenatal period, particularly defects of the radial axis, constitute a true diagnostic challenge for the obstetrician and geneticist. Here, we present the case of a fetus with upper limb phocomelia detected on routine obstetric ultrasound. Array-CGH allowed the diagnosis of Thrombocytopenia-Absent Radius (TAR) syndrome, an autosomal recessive disorder (OMIM #274000) caused by biallelic mutations in the RBM8A gene. The aim of this report is to describe the ultrasonographic, pathologic and molecular findings in a fetus with TAR syndrome, and to illustrate the contribution of array-CGH to the etiological investigation of fetal upper limb reduction defects.

Clinical Report
A healthy, non consanguineous couple was referred to our Medical Genetics Department after pregnancy termination at 20 weeks of gestation for severe upper limb bilateral phocomelia detected in the routine second trimester ultrasound. Fetal autopsy showed severe shortening of the arms and forearms, five normal fingers bilaterally, minor facial dismorphisms and no internal organ malformation. The fetal skeletal survey confirmed the absence of the radii, ulnae and humeri. Fetal karyotyping (performed earlier in the pregnancy for advanced maternal age) had been normal, 46,XY. Array-CGH revealed an interstitial 357.52 kb deletion in 1q21 including the RBM8A gene. Subsequent Sanger sequencing of this gene identified a hypomorphic mutant allele, c.-21G>A (rs139428292), confirming the diagnosis of TAR syndrome. Parental segregation analysis concluded that the 1q21 deletion was inherited from the mother and the mutant RBM8A allele from the father.

Discussion
Defects in the radial axis are associated with several syndromic diagnoses, whose differential diagnosis may be difficult, particularly in the prenatal period. In TAR syndrome these defects are always bilateral with presence of both thumbs, and can be severe, as in the present case. In the post-natal period, thrombocytopenia is present in almost all affected patients and in most manifests during the first weeks of life. Array-CGH analysis led to the diagnosis of TAR syndrome in this case, showing that it should be considered a first-line evaluation in fetal malformations. The couple was counseled about recurrence risks (25%) and the possibility of preimplantation genetic diagnosis or molecular prenatal diagnosis.

P8 | Clinical Genetics

Marfan Syndrome: A familial case with unexpected molecular findings

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Introduction
Marfan Syndrome (MFS) is an inherited autosomal dominant connective tissue disorder, involving primarily the skeletal, ocular and cardiovascular systems with a high degree of clinical variability. The diagnosis is established according to the revised Ghent nosology. Heterozygous mutations in *FBN1* gene encoding fibrillin-1, an extracellular matrix protein, are found in the majority of patients with MFS. Only a few cases of homozygous and compound heterozygous *FBN1* mutations are found in the literature.

Clinical Report
Two brothers were referred to our Genetics Department with suspected MFS. A clinical diagnosis of MFS was made in the 13-year-old younger brother according to Ghent criteria (positive systemic score and subluxation of both lenses; normal cardiac ultrasound). The older brother, aged 17, was only positive for systemic criteria, including severe pectus excavatum waiting corrective surgery. He had an innocent systolic murmur and no ophthalmological abnormalities. The parents were also observed: the mother presented MFS stigmata and the father was negative for features of MFS. Molecular analysis of FBN1 identified two variants in both brothers, namely c.5443G>A (inherited from the mother) and c.4337-2A>G (inherited from the father).

Discussion
The c.5443G>A (p.Gly1815Ser) variant in exon 45 of *FBN1* is classified as "likely pathogenic" in ClinVar. In silico analysis predicts a damaging effect on protein structure/function, and mutations in nearby residues have been reported in association with MFS. The c.4337-2A>G variant is classified as "pathogenic" in ClinVar, it destroys the canonical splice acceptor site in intron 35 and is predicted to cause abnormal mRNA splicing. Other splice site mutations in *FBN1* have been reported in MFS. Both variants are absent in population databases (ExAC and dbSNP) supporting the idea that they are not common variants in the population. Thus, we conclude both brothers have two *FBN1* pathogenic mutations in trans. Nine cases of compound-heterozygous *FBN1* mutations associated with MFS have been documented in the literature. Five of them presented a mild phenotype that can be explained by incomplete penetrance. These rare events alter genetic counseling and should not be overlooked neither in the early and very severe cases nor in the classic form of MFS. Thorough ascertainment of gene variants is essential and poses a challenge to the clinical geneticist

P9 | Clinical Genetics

Myhre Syndrome: 2 Clinical Cases

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Introduction: Myhre syndrome (MS) is characterized by intellectual disability of variable degree, short stature, typical facial dysmorphism, thickened skin, joint limitation, striking muscular build, laryngotracheal anomalies and a recently appreciated spectrum of cardiovascular anomalies. It is an autosomal dominant condition caused by specific missense gain-of-function heterozygous mutations in *SMAD4* gene, leading to a dysregulation of the TGFB pathway. We present 2 cases of typical MS with molecular confirmation and compare with the literature.

Clinical Report:

Patient 1: 11 year-old girl who was referred to our clinic with short stature, precocious puberty with menarche at 10 y.o. and hirsutism. She had mild learning difficulties and ADHD, history of "tip-toe" walking, astigmatism, abnormal voice and prolonged episodes of severe and persistent dry cough. She had peculiar facial features, brachydactyly, evident muscular built and limited joint mobility. This clinical picture was suggestive of MS. *SMAD4* hotspot targeted sequencing identified the pathogenic variant c.1486C>T(p.Arg496Cys). This variant is the second most common described in MS patients. Cardiac and pneumology evaluation were normal at this age.

Patient 2: 6 year-old girl with intellectual disability, astigmatism, and left ventricular non-compactation cardiomyopathy. At physical examination she present with short stature, low weight, microcephaly, facial dysmorphisms and brachydactyly. After an extensive study, suspicion of an acromelic dysplasia was raised and a commercial nonspecific 4,813 genes NGS panel (Trusight One, Illumina) was performed. MS diagnosis was established by the identification of the *SMAD4* heterozygous variant c.1499T>C(p.Ile500Thr), the most frequently described in this syndrome.

Discussion: MS can be distinguished from all the other acromelic dysplasias (Weill–Marchesani syndrome, geleophysic and acromicric dysplasias) by facial features, the presence of developmental delay, deafness, distinct skeleton features and inheritance pattern. All the mutations described are *de novo*; the risk of recurrence is inferior to 1%. An early diagnosis is not only important for a precise genetic counseling but mainly for an appropriate follow-up by a multidisciplinary expert team. The long-term follow up of these patients shows that these conditions are progressive with life threatening complications, often requiring respiratory and cardiac surveillance.

P10 | Clinical Genetics

FADS: Key Signals For a Specific Prenatal Diagnosis- Report of 3 Cases

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Introduction: Fetal akinesia deformation sequence (FADS) is a rare condition first defined by Moessinger in 1983, who proposed that decreased movements in utero lead to a predictable series of secondary anomalies. It is characterized fetal akinesia, IUGR, craniofacial features, arthrogryposis, polyhydramnios and severe pulmonary hypoplasia (required for diagnosis, to distinguish from arthrogryposis multiplex congenita- a benign condition). There are many pathogenic mechanisms for FADS, including monogenic diseases, maternal illness (myasthenia gravis), teratogenic exposures (maternal cocaine abuse), trauma, thrombophilia and CMV.
Clinical Report: We report and summarize 3 cases from non-consanguineous healthy couples who showed specific alterations in the US and in which fetal autopsy helped recognizing FADS.
Case 1: presented at 22w+4d with recurrent non immune fetal hydrops, decrease mobility and arthrogryposis. Fetal autopsy revealed specific craniofacial features and pulmonary hypoplasia. Whole exome sequence identified the variant c.1029_1045del (p.Glu344Cysfs*127) homozygous in *RAPSN* gene.
Case 2: presented at 31w+1d with polyhydramnios, decreased mobility and generalized subcutaneous edema. Fetal autopsy showed specific craniofacial features, arthrogryposis and pulmonary hypoplasia. NGS gene panel (Trusight One, Illumina) was performed and compound heterozigoty c.9262 G>A (p.Val3088Met) e c.13639 G>A (p.Val4547Met) was identified in *RYR1* gene.
Case 3: presented at 29w+1d with polyhydramnios, fetal hydrops, decrease mobility and abnormal posture of the limbs. Autopsy showed generalized arthrogryposis, severe pulmonary hypoplasia, but no craniofacial features. NGS gene panel (Trusight One, Illumina) was performed and pathogenic variant was detected in heterozigoty in *NEB* gene. Study of chromosomal rearrangements is in course.
Discussion: Lack of fetal movement produces a recognizable sequence of deformations; prenatal US diagnosis is possible, as early as 12 weeks. Depending on the pathogenic mechanism and type of mutation the phenotype will be more or less severe, appearing earlier or later in US. Most cases die in utero, at birth, or in new born period. Because of heterogeneous causes it is difficult to find the right diagnosis. Next generations sequence panels can be a particularly valuable tool in these cases, allowing a precise diagnosis, accurate genetic counseling and early detection in future pregnancies.

P11 | Clinical Genetics

Complex Syndrome of bone marrow failure and hepathic chirrosis due to mutation in TERC gene. The importance of an Exome Panel as a diagnostic tool.

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Introduction : Dyskeratosis Congenita (DC) is a multisystemic genetic disorder with autossomal dominant inheritance, characterized by cutaneous abnormalities, bone marrow failure (BMF) and an increased predisposition to cancer.
Disease penetrance is highly variable as well as clinical expression. Mutations in the essential telomerase genes TERC and TERT have been described as responsible for this disease. The primary cause of mortality in these patients is aplastic anemia (AA). A subset of patients with BMF, pulmonary and/or hepatic fibrosis and absence or very attenuated typical clinical features of DC also carries mutations in these genes. In this group, germline mutations in TERT and TERC genes account for 8-15% of familial AA and 1-3% of sporadic cases.
Clinical report : Female caucasian 26 year-old patient with medullar aplasia diagnosed since 4 years of age. Patient´s mother had history of medullar aplasia also. This patient was submitted to several clinical studies to investigate the BMF origin. In 2016 she was admitted at hospital due to evolution of pancitopenia and liver fibrosis. Immunosuppression therapy has been tried without success. Early piebaldism (white hair) was noticed.
Results and Discussion : The karyotype of this patient was normal. We performed exome panel on a NextSeq platform from Ilumina with 9 relevant genes (CTC1, DKC1, TERC, TERT, TINF2, NHP2, NOP10, WRAP53, RTEL1).
A heterozygous variant in TERC gene, nt.143G>T was detected (NR_001566.1). This variant is not described in the literature and no information is listed in the dbSNP database about its frequency. However, a pathogenic variant was detected at the same position nt.143G>A in two families: one with DC and the other with AA. The patient clinical information as well as the evidence of literature cases, allow us to consider this mutation as likely pathogenic.
The exome panel has proved to be very helpful to establish the definitive diagnosis and helps genetic counseling. The study of telomere length measurement seems not to be very helpful in these patients diagnosis.
As described before, immunosuppression therapy seems not be efficient in these patients.
Attention must be paied by clinicians to this special subset of patients by its specific characteristics. This entity behaves more like a specific subgroup with an atypical mixed clinical spectrum picture of DC and AA (not showing all the clinical signs).

P12 | Clinical Genetics

Neurofibromatosis type 1: Clinical report of three cases of NF1 gene deletion

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Introduction: Neurofibromatosis type 1 (NF1; OMIM 162200) is the most common neurocutaneous genetic disease. The degree of severity and the presence of complications are often unpredictable. 5% are due to 17q11 microdeletions encompassing the whole NF1 gene. Clinical presentation differs from the phenotype associated with intragenic NF1 mutations in that it is usually more severe with early onset of cutaneous neurofibromas, higher frequency of cognitive impairment and greater risk of tumor malignization. Childhood overgrowth, large hands and feet, and a Weaver-like appearance have been reported in these patients. Heart defects, macrodactyly, hemihypertrophy and vascular anomalies also appear to be more prevalent.
Clinical Report: We describe 3 independent de novo cases of NF1 gene deletion, all referred to the Genetics Department due to café au lait macules and intellectual disability. Family history was unremarkable. **Patient 1:** A 9-year-old girl presented, besides café au lait spots (>6; >15 mm) and inguinal and axillary freckling, overgrowth, coarse face, macrodactyly of the 3rd finger on the right hand and asymmetrical lower limbs. **Patient 2:** A 9-year-old girl was referred at 15 months for features resembling Weaver syndrome, namely overgrowth, coarse face, broad forehead, palpebral ptosis, epicanthus, retrognathia, large ears, pectus carinatum, and large hands and feet. On follow-up she developed an extensive plexiform neurofibroma on the right leg which is currently under regular radiological surveillance. **Patient 3:** A 10-year-old girl presented with overgrowth, café au lait spots (>6), and inguinal and axillary freckling. Cardiac evaluation was normal in all three.
MLPA for the NF1 gene identified a heterozygous whole-gene deletion in the 3 cases.
Discussion: A recognizable phenotype different from classical NF1 is typical of 17q11 microdeletions encompassing the NF1 gene, and is the result of haploinsufficiency of contiguous genes in the deletion interval. This phenotype is characterized by overgrowth, intellectual disability, and a distinctive Weaver-like face. Thus, when such features are present together with NF1 cutaneous stigmata, it is important to consider NF1 gene deletions as a possible cause. Early diagnosis allows proper counselling about long term prognosis and medical surveillance, which is particularly relevant since these patients have a more severe presentation and higher risk of malignancies.

P13 | Clinical Genetics

Intellectual disability X-linked associated to IL1RAPL1 gene (Xp21.2)

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Case report: The index patient was referred to our Genetic Unit because of microcephaly, pre-natal onset, intellectual disability and dysmorphic features. He was 12 years-old boy, first children of non-consanguineous parents and he has younger sister, all healthy. He was the result of a pregnancy of 38 weeks with mild arterial hypertension. At birth, it was possible to confirm the presence of microcephaly and he had some feeding difficulties in first years of life.
Karyotype analysis was performed by routine GTG banding technique in peripheral blood lymphocytes (46,XY, N); FISH 22q11.2 (46,XY.ish22q11.2(D22S75x2);N); seric and urinary amino acids (N); chromossomal breaks study (N); fatty acids test (N); MRI encephalic (N) and array-CGH 180K.
Chromossomal study by Array-CGH (180K) identified a X(p21.2) gain that parcially involves IL1RAPL1 gene. MLPA was performed to confirm the duplication of 9 to 11 exons of IL1RAPL1 gene.
Posteriorly, Array-CGH was performed in his parents; the same duplication was identified in his mother. Maternal grandparents was studied and the same duplication was identified in maternal grandmother. Both woman are healthy and without cognitive impairment. Deletions in this gene are reported associated to intellectual disability, behavioral alterations and dismorphic features (Franek et al. , Am J Med Genet A 2011, 155:1109-1114; Barone et al, Am J Med Genet A 2013, 161:1381-1385).
Conclusions: Our case compares with the ones already published with the diagnosis of MRX21 [MIM 300143].

P14 | Clinical Genetics

De novo TBL1XR1 deletion at 3(q26.32) in a boy with global developmental delay, growth retardation and dysmorphisms – 3rd reported case

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Introduction: Array-Comparative Genomic Hybridization (array-CGH) has the power to study the whole genome with a higher resolution, detecting imbalances that can range from single gene or even exon imbalances to chromosome segments or entire aneuploidies. In patients with a specific phenotype, the identification of a single gene imbalance, namely a deletion, allows the identification of disease-related genes and genotype-phenotype correlations. *TBL1XR1* mutations have been reported to be associated with autism, autosomal dominant mental retardation (OMIM: 616944) and Pierpont syndrome (OMIM: 602342). Only two cases with gene deletions have been reported: a simplex case with mild developmental delay (DD) and a 1.6Mb *de novo* deletion at 3q26.31q26.32; and a familial case (mother and daughter) with moderate intellectual disability (ID) and facial dysmorphisms with a 708 kb deletion at 3q26.32.
Clinical Report: We report a 5 ½ year old male with global DD, growth retardation and dysmorphisms with a 3q26.32 *de novo* deletion, being *TBL1XR1*the only known coding gene within the deletion. *TBL1XR1* encodes a ubiquitously expressed protein that localizes to the nucleus and plays a role in transcription mediated by nuclear receptors. *TBL1XR1*mutations have also been described in autistic patients with ID. In Decipher Database, deletions encompassing *TBL1XR1* are reported in two patients: one with a *de novo* deletion and autism, hearing impairment and spotty hyperpigmentation, and the other with a partial *de novo* deletion of the gene but with other genomic imbalances.
Discussion: The fact that the gene presents a high haploinsufficiency score, together with the report of patients with ID and *de novo TBL1XR1* deletions, supports its involvement in human disease.

P15 | Clinical Genetics

Subtelomeric rearrangements: presentation of 17 probands with emphasis on familial cases

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Introduction
Subtelomeric regions are gene-rich chromosomal regions with a highly repetitive structure, frequently involved in chromosomal rearrangements. Cryptic subtelomeric abnormalities are well known causes of intellectual disability, syndromic or isolated, being responsible for 3-16% of cases. Karyotype with FISH, MLPA and array-CGH have been used as routine studies for identifying these patients.
These abnormalities may be de novo or inherited. An imbalanced rearrangement may be inherited from an affected parent or from a healthy carrier of a balanced chromosomal. An accurate family history may show important clues for a possible familial rearrangement.
The aim of this work was to characterize the patients from our Medical Genetics Center, from both clinical and cytogenetic perspectives, in whom a deletion and a duplication on subtelomeric regions were found.
Methods
Our study included 17 probands followed at our Medical Genetics Center, from 1998 until 2016, who have simultaneously a deletion and a duplication on the chromosomal subtelomeric regions. We describe gender, age at first consultation, somatometry, craniofacial and other dysmorphisms or congenital anomalies, family history, cytogenetic findings in patients and parents.
Results
We describe 17 probands (6 males and 11 females) from 15 families that presented with intellectual disability and facial dysmorphisms. In 4 families, the parental clinical and cytogenetic study was not possible. Three were de novo events, 7 were inherited from a balanced cryptic rearrangement in one of the parents and 3 probands inherited the anomaly from a similarly affected parent. Two cases were diagnosed by array-CGH and the others by MLPA/FISH.
Discussion
With this work, the authors intended to show the importance of parental cytogenetic analysis in order to recognizing potential cytogenetic rearrangements. Only with this complete characterization of patients' genotype, it is possible to offer an accurate genetic counselling for family members, which is fundamental in these cases, given the high recurrence risk of abnormalities in future pregnancies.

P16 | Clinical Genetics

Intrafamilial phenotypic variability of 17q12 duplication syndrome

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Introduction: 17q12 duplication syndrome is a recurrent contiguous gene duplication syndrome with reduced penetrance and marked phenotypic variability. Cognition may be normal or range from mild to severe intellectual disability (ID). Other possible common features include hypotonia, speech delay, behavioral abnormalities (including aggression), neuropsychiatric disorders, such as autism spectrum disorder and schizophrenia, epilepsy, ophthalmologic anomalies and (although individually rare) renal and cardiac malformations. Dysmorphic features are minor and inconsistent, but microcephaly may be present in up to 50%. 90% of reported cases are inherited from normal or minimally affected parents.
Clinical Report: The proband is an 8 year-old boy, referred to our Medical Genetics Department for global developmental delay, most prominently language delay and speech difficulties, attention deficit/hyperactivity disorder (ADHD) and dysmorphic features. On examination he presented with relative microcephaly, upslanted palpebral fissures, long and smooth philtrum and thin upper lip. He had left esotropia, astigmatism and a history of episodes characterized by loss of muscular tonus and consciousness, with normal cardiac and neurologic evaluations. Array-CGH revealed a 1.39 Mb duplication in 17q12. His mother, who was later confirmed to have the same duplication, suffers from depressive disorder and has a history of learning difficulties, as is also the case for her two brothers, who have not been tested. The proband's sister has ADHD and dyslexia; array-CGH is undergoing to see if she inherited the same copy number variation (CNV).
Discussion: This family illustrates the phenotypic variability of 17q12 duplication syndrome and alerts to the problems arising in genetic counseling from this kind of CNV, namely regarding recurrence risk and the option for prenatal diagnosis. As more patients with the same genotype are reported, the phenotype and penetrance can be more accurately defined to help overcome these difficulties.

P17 | Clinical Genetics

Tetrassomia Parcial do Cromossoma 22 - Um Caso Com Manifestações "Floridas"

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Introdução / Descrição do Caso

Recém-nascido de termo, sexo masculino, transferido em D2 por malformação ano-retal (MAR) e suspeita de drenagem venosa anómala pulmonar total (DVAPT). Na admissão apresentava distensão abdominal, fosseta pré-auricular esquerda e coloboma do segmento posterior à direita. A ecografia abdominal revelou hidronefrose à direita. Submetido a colostomia sigmoideia alta por MAR "alta" com fístula reto-uretral em D3. Após confirmação foi efetuada correção cirúrgica da DVAPT em D26. No pós-operatório apresentou quilotórax à esquerda, pneumotórax à direita e trombose da veia jugular interna esquerda. A suspeita clínica de tetrassomia parcial do cr22 (síndrome de "Cat-Eye") como diagnóstico foi confirmada por array CGH e cariótipo - 2 cromossomas 22 com 2 centrómeros, 2 braços curtos e duplicação parcial do braço longo (22q11.2). Alta para o domicílio em D69 de vida, com seguimento multidisciplinar e indicação para correção ulterior definitiva da MAR.

Comentários/Conclusões

A síndrome de "Cat-Eye" é uma cromossomopatia que, sendo rara (incidência entre 1:50,000 – 1:150,000), apresenta fenótipos clínicos diversos. Com cerca de 100 doentes publicados, menos de 10% apresentam as 2 características clínicas major – MAR e coloboma ocular. A presença concomitante de anomalia craniofacial e malformações congénitas nefrourológica e cardíaca torna este caso ainda mais raro. A maioria dos doentes apresenta potencial cognitivo normal ou ligeiramente abaixo do esperado para a faixa etária, estando o prognóstico sobretudo dependente da gravidade das comorbilidades (cardíacas, urológicas e gastrointestinais). Os autores pretendem destacar a importância da abordagem multidisciplinar de uma síndrome rara e complexa, realçando a associação de várias malformações num único doente.

P18 | Clinical Genetics

An unexpected result in a balanced translocation detected in Prenatal Diagnosis

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INTRODUTION: Translocations are relatively uncommon in prenatal diagnosis rounding about 1 in 2000 amniocenteses. Conventional cytogenetic is the most accurate technique to detect them. Currently, Array Comparative Genomic Hybridization (aCGH) techniques are very useful to complement the diagnosis, since they can identify chromosome imbalances that are too small to be detected by conventional cytogenetics.
The authors present a case with two distinct genetic findings one detected by conventional cytogenetics and the other by aCGH.
CLINICAL REPORT: 47-year-old pregnant woman referred to prenatal diagnosis due to maternal age. It was the first pregnancy of a non-consanguineous couple with no familial or personal story of anomalies. The woman had two children and the men four girls of a previews marriage. Cytogenetics analysis revealed a balanced translocation involved the short arm of chromosome 1 and the long arm of chromosome 10. The mother´s karyotype was normal. aCGH confirmed the balanced translocation however it revealed a deletion in Xp22.31 region. The segment deleted had 1,680 Mbp and involved the genes HDHD1, MIR4767, STS, VCX, PNPLA4, MIR651. The karyotype was 46,XY,t(1;10)(p32;q26).arr Xp22.31(6,455,151-8,135,560)x0.
DISCUSSION: The case described presented two unrelated findings, a balanced translocation identified by conventional cytogenetic and a microdeletion on Xp22.31 revealed by aCGH. Together, these techniques enable a correct diagnosis and therefore a more accurate genetic counseling.
The development of new techniques, such as array Comparative Genomic Hybridization (aCGH), has increased the resolution of novel or rare microdeletions/microduplications, but chromosome analysis remains the gold to delineate chromosomal structural rearrangements.

P19 | Clinical Genetics

Partial duplication of 8p: a rare event

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INTRODUTION: The partial duplication of the short (p) arm of chromosome 8 is a rare syndrome. Clinical manifestations vary from healthy to several degrees of mental retardation, multiple congenital anomalies - like hypotonia, heart defects, brain malformations (Dandy-Walker syndrome, dilation of the third ventricle and agenesis of the corpus callosum) and facial dysmorfism.
The authors presented a case of partial trisomy 8p.
CLINICAL REPORT: 12 years old girl with mental retardation and agenesis of the corpus callosum. Cytogenetics analysis revealed extra material on the short arm of chromosome 8. Parents karyotype were normal. Fluorescence in situ hybridization (FISH) technique identified the extra material as chromosome 8. Array Comparative Genomic Hybridization (aCGH) technique revealed a duplication of 8p11.23 to 8p11.1. The segment duplicated had 6.4 Mbp and involved 62 genes. The karyotype was 46,XX,dup(8)(p11.23p11.1). arr 8p11.23p11.1 (37,348,105-43,754,516)x3.
DISCUSSION: The present case has a duplication of 8p11.23p11.1 region. To our knowledge this is the first case involving only this region. The others cases described involved a small extra chromosome 8 with a larger duplicated segment. The phenotypic characteristic observed in the girl included mental retardation and agenesis of the corpus callosum, which are consistent with partial trisomy 8p phenotype. Every new case of a rare chromosomal alteration should be reported in order to obtain a more precise genotype/ phenotype correlation, improving risk evaluation and genetic counselling.

P20 | Clinical Genetics

Prenatal Diagnosis of terminal 4q deletions: a rare condition and a challenge for genetic counselling

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Introduction:
The 4q- syndrome, a well known but rare chromosomal disorder, involves deletions in the long arm of chromosome 4, including interstitial and terminal deletions. The wide range of phenotypic findings and the severity of the anomalies depend mainly on the size of the deletion and of the breakpoint location. Small terminal deletions at 4q35 have been described in apparently normal individuals, but also in affected patients that may show a wide spectrum of clinical manifestations, from minor dysmorphic features, behavior and psychiatric symptoms to intellectual disability.

Clinical Reports:
We present two prenatal cases with fetus showing 4qter deletions: Case 1: A 35-year-old pregnant woman who performed amniocentesis at 17w due to positive serum screening. The fetal karyotype revealed a 4q35 deletion, confirmed by FISH analysis and further confirmed by CGH array as a ~6.6 Mb deletion, later identified as maternally inherited.
The fetal autopsy confirmed: craniofacial, brain, kidney and intestinal anomalies. Case 2: A 30-year-old pregnant woman who performed amniocentesis at 17w due to positive serum screening. A de novo 4q35 deletion was identified by cytogenetic and with an estimated size of ~5.8 Mb by CGH array.
Fetal autopsy is in progress.

Discussion:
We present two prenatal diagnosis cases with a 4q35 deletion detected by conventional cytogenetic analysis, one of which was maternally inherited and the other was a "de novo" case. The genotype-phenotype correlation of 4q deletion syndrome rises a challenge to genetic counsellors, specially in prenatal cases with small terminal deletions. This is a rare condition, with incomplete penetrance and a wide clinical spectrum of anomalies. The size of the deletion was almost the same and was classified as VOUS (variant of uncertain significance) in the array study. After genetic counseling and by parental request, both pregnancies were terminated.
High resolution ultrasound scans, as well as CGH arrays characterization of the deletion size, are tools that perhaps can better help to establish the risk of an affected fetus, even in familial cases.

P21 | Clinical Genetics

Genomic profile of 8p copy number variants detected by array-CGH in patients with intellectual disability

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The short arm of chromosome 8 (8p) spans about 44 million base pairs and contains near 500 annotated genes. Point mutations in more than 50 genes on 8p have been associated with various genetic disorders. 8p region is structurally complex because of the existence of two olfactory gene clusters (REPD and REPP) flanking a 5Mb region of 8p23.1, several low copy repeats (LCRs) and common inversion polymorphisms making this region prone to recurrent genomic rearrangements that include: 8p23.1 deletions or duplications, 8p23.1 paracentric inversions, pericentric inversions, 8p translocations, 8p interstitial inverted duplication with associated terminal deletion and different types of supernumerary chromosome 8 involving 8p23.1. In addition to these 8p pathogenic imbalances, others have been reported as copy number variants (CNVs) without apparent clinical significance.

In the last decade, array-CGH (Array-Comparative Genomic Hybridization) has increased the diagnostic yield in patients with intellectual disability (ID), autism spectrum disorders (ASD) and multiple congenital anomalies.

We evaluated about 1500 patients with ID and/or ASD by Agilent 180K oligonucleotide array-CGH, and found in our cohort, 15 patients with 8p imbalances (6 deletions, 8 duplications and one inverted duplication). Single gene deletions were observed in 3 patients and larger interstitial deletions in 2 patients (one with a 3,4Mb 8p21.2 deletion and the other with two adjacent deletions in 8p23.1 and in 8p23.1p21.3). Interstitial duplications were identified in 8 patients, including a 6,3Mb 8p21.3p21.1 duplication, a 960Kb 8p23.1 duplication, a 274Kb 8p22 duplication, 4 patients with a small 160Kb duplication inherited from healthy parents classified as a CNV of unknown clinical significance and one patient with a single gene duplication. In brief, our results are in accordance with published data suggesting existence of multiple hotspots in 8p causing complex rearrangements and high diversity of phenotypes.

P22 | Cytogenetics and Genomics

Karyotyping and Y-chromosome microdeletion testing using MLPA: prospective study in idiopathic cases of azoospermia and severe oligozoospermia

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Introduction: Infertility is a condition that may be defined as the inability to achieve conception or take a pregnancy to live birth; it affects about 15% of couples and may have many different causes, either for male or female factors. Approximately half of the infertility registered cases may be attributed to male causes, azoospermia and severe oligozoospermia being the main manifestations in the latter. Abnormalities of the sex chromosomes account for about 6% of male infertility; Klinefelter Syndrome (47,XXY) being the most common aneuploidy and Y chromosome microdeletions are estimated to be detected in approximately 10% to 15% of men with nonobstructive azoospermia or severe oligospermia. In recent decades, several reports have associated partial deletions of azoospermia factor regions (AZFa, AZFb and AZFc) present on Yq11.2 with spermatogenetic failure and male infertility, particularly with idiopathic azoospermia and severe oligozoospermia (sperm concentrations <5 x 106/mL). The main objective of this study was to investigate the presence of structural chromosomal abnormalities by classical cytogenetics methods and AZF microdeletions by Multiplex Ligation-dependent Probe Amplification (MLPA®).
Materials and Methods: The authors have analyzed a total of 40 individuals (26 severe oligozoospermia and 14 azoospermia), between 01.01.2015 and 31.05.2016. All DNA samples were analyzed for AZF region chromosome Y-microdeletions by MLPA (SALSA® MLPA® kit probemix P360-A1 Y-chromosome microdeletions). Results: One case showed a 46,XX chromosomal constitution, the other 39 were normal (46,XY). Two cases (5%) showed large amplifications of two probes in the AZFa region, six samples (15%) revealed partial deletions in the AZFb-c regions and two samples (5%) revealed a large amplification of two probes in 12q14.2 (OMIM #613993-Spermatogenic failure).
Discussion: With this study the authors demonstrate both the usefulness of routine cytogenetic tests and the use of MLPA for Y chromosome microdeletions research in infertile couples. Also it allows us to consider that MLPA may be a first approach for male infertility research investigation and screening. The analysis of partial AZFa, AZFb or AZFc microdeletions is important for the study of infertility in other male members of their families and the provision of appropriate genetic counselling.

P23 | Cytogenetics and Genomics

Prenatal diagnosis of de novo apparently balanced chromosome rearrangements

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De novo apparently balanced chromosome rearrangements (dnABCRs) are detected in approximately 1:8001 to 1:10002 prenatal tests. Whenever a dnABCR is identified, the outcome for the fetus is difficult to predict because there is no evidence for a normal phenotype in a carrier parent and the rearrangement may not be truly balanced. Phenotypic abnormalities observed in the patients with dnABCRs can be due to 1) submicroscopic deletions or duplications near the breakpoints, 2) submicroscopic deletions or duplications unrelated to the ABCR, 3) gene disruption at the breakpoints, 4) a position effect, 5) uniparental disomy, or 6) another unidentified genetic or environmental factor. We report on five recent prenatal cases carrying a dnABCRs detected with conventional karyotyping. Three of the cases had reciprocal translocations, one had a pericentric inversion and another presented both an inversion and a translocation. Array-CGH was performed to search for genomic imbalances that were revealed in two of the five cases. Only one of the imbalances identified was near one of the breakpoints and all the imbalances detected were *de novo*.
Interpretation of dnABCRs in a prenatal setting and the ensuing genetic counseling is challenging due to the limited resolution of routine cytogenetics. The cases presented provide further evidence that in case of prenatally detected dnABCRs, array-CGH is useful not only in identifying the genomic imbalances at the breakpoints, but also in detecting unexpectedly complex rearrangements in other chromosomes. Seeing as the increased risk for phenotypic anomalies has been mainly attributed to the gain or loss of genetic material, the diagnosis of these imbalances is paramount and, therefore, in prenatal diagnosis, array-CGH should always be proposed to fetuses with dnABCRs, even if not associated to malformations, so that an accurate risk assessment can be provided.

P24 | Cytogenetics and Genomics

Two cases of rare pericentric inv(22) cytogenetically detected at prenatal diagnose: its characterization and implications on the phenotype

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Inversions are frequently innocuous. Nevertheless, rarely, it may induce a submicroscopic alteration, with a locus or a small number of loci being disrupted or removed. Excluding those involving the heterochromatic regions of chromosomes 1, 9, 16 and Y and others considered harmless variants, inversions are uncommon rearrangements, with a frequency range from about 0,12% to 0,7% (pericentric) and 0,1% to 0,5% (paracentric).

We report two cytogenetically identical pericentric inversions on chromosome 22(p13q11.21), prenatally detected, inherited from apparently normal parents. To guarantee the integrity of proximal region on chromosome 22, including the critical region for DiGeorge syndrome, MLPA (P250) was performed. Family 1 showed a normal result but in Family 2 a small deletion in 22q11.21 of about 600Kb was found. This deletion, as well as the pericentric inversion was inherited from the father. Inversions of chromosome 22 are rare with only 8 cases reported, all of them involving a larger segment of chromosome 22, with the breakpoint in 22q in a more distal position than it is in our two cases.

The deletion identified in Family 2 is an atypical deletion in the distal part of the "22q11.2 microdeletion syndrome" critical region and shows a great inter- and intrafamilial phenotypic variability, with some patients reported in Decipher database with deletions that overlap the one we report.

The inv(22) seems to be a rare event. However, this may be due to an under diagnosis considering the difficulty in identifying the recombinant chromosome by conventional cytogenetic. The accurate characterization of these alterations requires the use of molecular techniques.

The two inv(22) we report were inherited from apparently normal parents. Even in those situations the integrity of the 22q11.2 region must be guaranteed, since it contains a cluster of LCR that mediates non-allelic homologous recombination that results in deletions/ duplications in the region.

P25 | Cytogenetics and Genomics

Interstitial deletion on chromosome 14q in prenatal diagnosis

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A limited number of prenatal diagnosis (PND) cases have reported interstitial deletions of the long arm of chromosome 14 involving the 14q31-32 region. Those cases presented cardiac anomalies, urogenital anomalies, congenital diaphragmatic hernia, and mild pyelectasis. We report the PND of a 33-year-old pregnant woman, who underwent chorionic villus sampling at 12 weeks of gestation after a positive combined 1st trimester screen. The karyotype revealed a 14q interstitial deletion. Amniocentesis was performed at 18 weeks of gestation to confirm the deletion and to exclude a confined placental mosaicism and a microarray analysis was performed in order to accurately define the deletion breakpoints. Cytogenetics analysis revealed a karyotype 46,XY,del(14)(q31q32.2)dn. Microarray analysis allowed to redefined the breakpoints accurate localization and the identification of a ~21Mb deletion (arr[hg19] 14q31.1q32.31(79917376_101568230)x1). At 18 weeks of gestation the fetus presented abnormal fetal biometric parameters (occipitofrontal diameter, cephalic perimeter and abdominal circumference) on ultrasound. After counseling the couple opted for pregnancy termination. The postmorten analysis presented decreased biometry, low weight and low fetal size, facial dysmorphism, clinodactyly, club foot, overlapping fingers and short penis. In internal habitus he presented thymus hypoplasia, bladder hypoplasia, and horseshoe kidneys. The genotype-phenotype correlation in PND pure del(14q) cases is not well established. Furthermore, to our knowledge, del(14q) had not been reported so early in the gestation yet. In this case the positive 1st trimester screen was related to the inverted ductus venosus and low PAPP-A value. The urogenital anomalies (as horseshoe kidneys) and biometry anomalies are described in the literature. However, to our knowledge, some features of the present case were not seen in other reported cases, for instance clinodactyly, club foot, overlapping fingers, thymus hypoplasia and bladder hypoplasia. Other reports described cardiac and cerebral anomalies, diaphragmatic hernia, and also UPD(14)like phenotypes, which are possibly liked to the 14q32 imprinted region. The establishment of a phenotype-genotype correlation is difficult given the size of the deletion, which includes a large number of genes in distinct regions. Nevertheless, this work contributes to a better identification of additional features associated to del(14q) that can be present in PND.

P26 | Cytogenetics and Genomics

Autism Spectrum Disorders –clinical usefulness of aCGH

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Introduction: Autism spectrum disorders (ASD) are neurodevelopmental conditions showing extreme genetic heterogeneity. Array CGH offers superior sensitivity for identification of submicroscopic copy number variants (CNV) and it is advocated to be the first tier genetic testing for patients with ASD. The aim of this study was to correlate pathogenic CNVs or potencially pathogenic CNVs with clinical features in syndromic and non-syndromic ASD patients. Additionally, we would like to identify which variants of uncertain significance (VOUS), taking into account their gene contents or localization, should be revised from time to time.
Methods: Agilent 4x180K microarrays and cytogenomics 4.0.2.21 software were applied for the study of ASD patients. From 173 patiens two main groups were established: ASD isolated patients (non-syndromic) (95) and ASD patients with additional features (syndromic) (78).
Results: We identified a total of 31 pathogenic or potentially pathogenic CNVs plus 33 VOUS. Within these, 16 pathogenic CNVs plus 11 VOUS were found in non-syndromic ASD patients and 15 pathogenic CNVs plus 22 VOUS in syndromic ASD patients. In 137 patients (79 and 58 with non-syndromic and syndromic, respectively) no pathogenic CNVs were identified but 215 VOUS were present. The pathogenic or potentially pathogenic CNVs sizes in ASD patients ranged between 18 Kb and 3Mb in different genomic regions from different chromosomes. The 22q11.21 duplication was present in at least 3 patients with syndromic ASD.
Discussion: In our study, we are able to identify 19% of ASD patients with pathogenic or potentially pathogenic CNVs. The majority were private CNVs but we identified 3 patients with a similar duplication on 22q11.21 presenting ASD and macrocephaly. We would like to highlight the clinical usefulness of aCGH as a first-tier test in evaluation of syndromic and non-syndromic ASD patients. It is highly recommended to perform the confirmatory study of all pathogenic/potentially CNVs and also for some VOUS in the progenitors, using aCGH or other techniques such as Q-PCR or MLPA. Patients with no pathogenic/potentially CNVs should be further analysed by an ASD genes panel.

P27 | Cytogenetics and Genomics

A new case of 8p23.1 deletion syndrome with congenital heart defects not associated with GATA4 haploinsufficiency

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Deletions of 8p23.1 chromosome region are rare, over 50 cases of interstitial or terminal 8p23.1 have been reported, detected by conventional cytogenetics or by molecular techniques. The major clinical picture of the 8p23.1 deletion syndrome include congenital heart malformations, microcephaly, pre and postnatal growth retardation, craniofacial abnormalities, mild intellectual deficit and a characteristic hyperactive behavior. The patient reported is a 11-day-old male with transposition of the great arteries and microcephaly, referred for high resolution conventional cytogenetic analysis, performed in peripheral blood lymphocytes, that revealed a deletion del(8)(p22→pter). Oligoarray-CGH redefined this result to two interstitial deletions described as 46,XY,del(8)(p21.3p23.1).arr 8p23.1(7,169,490-10,144,656)x1, 8p23.1p21.3(11,967,805-22,650,199)x1. The mother had a normal karyotype and the father was unavailable to study. Of the two deletions found in our patient, the more relevant to the phenotype seemed to be the distal deletion at 8p23.1(7,169,490-10,144,656). As this deletion is located between two previously described LCRs, REP and REPP, an abnormal recombination of mispaired copies of the repeated sequences is the most probable mechanism underlying this kind of deletions. However, in the present case the origin could be FoSTeS (Fork Stalling and Template Switching), a more complex process occurring during replication that could explain the two deletions. The distal deletion partially overlaps the region thought to be involved in heart defects, but without involvement of *GATA4* and *SOX7* genes. The present report not only supports the hypothesis that other genes besides *GATA4* and *SOX7* are important in heart development but also narrows the minimal critical region to 1,8 Mb (Ch8:7,169,490-8,925,021, hg19) for the major features of 8p23.1 deletion syndrome.

P28 | Cytogenetics and Genomics

Rare familial (Xq;Yq) translocation in three generations detected at prenatal diagnosis

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Introduction: Homologous sequences located in the pseudoautosomal regions of both chromosomes X and Y allows their pairing at meiosis. Occasional errors in the process of crossing over can lead to translocations between X and Y. X/Y translocations are rare rearrangements in humans and most of the cases reported in the literature involve Xp and Yq segments. We describe a prenatal diagnosis case in which the transmission of a derivative X chromosome with additional Y material, in 3 generations, derived from a Xq/Yq familial translocation, was detected. **Methods:** The amniotic fluid karyotype request of a 37-year-old pregnant woman was referred to our lab after a 23 weeks gestation, due to a positive first trimester maternal serum screening and increased nuchal translucency. Aneuploidy screening (Multiplex-PCR), karyotype analysis and FISH studies were performed. The initial results justified further investigations to enable chromosomal characterization.
Results: Rapid detection of common aneuploidies by quantitative multiplex-PCR confirmed absence of aneuploidies in a female fetus. Conventional fetal karyotype showed two X chromosomes, one of them with additional material at Xqter. DA/DAPI banding revealed a strong positive signal at the end of the der(X), suggesting the presence of Yq material. Revision of the PCR result confirmed absence of SRY gene (Yp11.3). FISH analysis revealed only one signal for the Xq/Yq subtelomeric region in the terminal region of the der(X). No Y-cen hybdridization signal was detected. Cytogenetic analysis of the couple and husband´s mother revealed that the fetal der(X) was paternally inherited, who inherited the rearrangement from his mother. The fetus karyotype was described as 46,X,der(X)t(X;Y)(q28;q11.2)pat.
Discussion: Phenotypic consequences of X/Y translocations depend mainly on the breakpoint location in both chromosomes. Only a few cases of Xq/Yq translocations are reported in the literature. In the present case/family, classical and molecular cytogenetic techniques allowed us to confirm that the derivative chromosome was composed by X and Y chromosome material, with breakpoints in the long arms of both chromosomes. The X-chromosome breakpoint indicates that only the subtelomere Xq region was lost, not involving important X gene regions (FMR1, etc.). Furthermore, the normal phenotype of the grandmother and father led us to the prediction that the female fetus would be normal.

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Novel Deletions and Unusual Genetic Mechanisms Underlying Alphathalassemia

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Hemoglobin (Hb) is a protein responsible for oxygen transportation from lungs to the entire body. It is composed by four globular subunits - the globins - each with a central core containing a heme molecule. Globins are encoded by the α- and β-globin gene clusters located at 16p13.3 and 11p15.5, respectively. The pattern of globin genes expression during development is precisely controlled by the interaction of cis-regulatory genomic regions (located in close proximity to and far from genes) with trans-activating/silencing factors within permissive chromatin domains. In fact, approximately 25-65 kb upstream of the α-globin genes there are four multispecies conserved sequences (MCS-R1 to R4) which are critical for the expression regulation of the downstream globin genes. The main objectives of this work were to characterize the molecular lesions underlying eight unusual cases of α-thalassemia or Hb H disease, and to understand their origin and functional consequences. Deletions were detected by Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA MLPA P140B HBA kit (MCR-Holland). Additionally, specifically designed synthetic MLPA probes, as well as Gap-PCR and Sanger sequencing were performed for fine deletion breakpoint mapping. We have found seven different deletions (ranging from 3.3 to ≈323 kb), four of them not previously described. The four largest deletions removed all the α-globin genes, whereas the other three deletions removed one or more of the distal regulatory elements keeping the globin genes structurally intact. In one case, only the MCS-R2 (also known as HS-40) was removed and replaced by a 39 nt DNA fragment possibly resulting from a complex rearrangement that introduces new pieces of DNA (probably from Chrs. 3 and 7) bridging the two deletion breakpoints. In the remaining case, no deletion was found and the patient revealed to be a very unusual case of acquired alpha-thalassemia-myelodysplastic syndrome. It is important to detect individuals with this type of uncommon deletions as there is a 25% risk of having a child with Hb Bart's hydrops fetalis or Hb H disease if their partner is a carrier of an α^o-thal or α+-thal allele, respectively. Moreover, further investigation is currently being developed on one of these natural mutants which is bringing new insights into the long-range regulation mechanism of the globin gene expression and to the pathophysiology of the α-thalassemia.

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Study of copy number variation by MLPA in retinal angiomatous proliferation: pilot study

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Introduction: Age-related Macular Degeneration (AMD) is a late-onset multifactorial disease characterized by a progressive destruction of the retina's central region, the macula. Usually AMD starts by early asymptomatic stages, but can progress to the late blinding forms of the disease – geographic atrophy and choroidal neovascularization (NV). Among the last, stands out retinal angiomatous proliferation (RAP), a NV subtype characterized by the growth of abnormal vessels beginning in the retina, which presents the most reserved prognosis. AMD's etiology is multifactorial, combining genetic factors, which account for up to 70% of the reported cases, and non-genetic risk factors, such as smoking and ethnicity. Most genetic alterations associated with AMD incidence and progression 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 copy number variation within the genes most commonly associated with AMD in patients with RAP. Taking advantage of the characteristics of multiplex ligation probe amplification (MLPA) technique, in which a SALSA probemix (MRC-Holland) was adapted to this project and was used to evaluate 99 samples: 32 RAP patients and 67 controls. **Results and Discussion**: Of all evaluated genes and considering the studied cohort, the only one with significant differences between RAP and controls was *ARMS2* SNP A69S rs10490924 ($p \leq 0.001$). The presence of this genetic variation is therefore a potential biomarker of RAP development prognosis, as what has been previously reported. Whereas, copy number variation in *CFHR3* and *CFHR1* as well as SNPs in *CFH* and *C2* were also evaluated but did not show much implication with RAP. Considering the multifactorial nature of AMD's development, having two or more risk factors seems to influence RAP's development since the vast majority of RAP samples present 2 or more risk factors (78.1%) whereas they are present in only 50.7% of controls ($p=0.012$). **Conclusion**: These preliminary but promising results contribute to a better understanding of RAP's genetic background being one further step towards a new diagnostic approach of this AMD subtype and ultimately, to prediction of this AMD's subtype development.

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Primary results of the NGS panels protocol between CGMJM/CHP and UDTECM/CHUSC

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Introduction Next Generation Sequencing (NGS) increased enormously the capacity of DNA sequence analysis. This achievement has been applied to clinical purposes on various ways, from simultaneous analysis of gene panels related to disease/clinical presentation to whole genome sequencing. Centro de Genética Médica Jacinto Magalhães have colaborated since 2010 with UDTECM/CHUSC on implementation and validation of gene panels to be analysed by NGS. In 2015 an institutional protocol was signed allowing Centro Hospitalar do Porto to obtain access to NGS panel analysis applied to diagnostics, provided by UDTECM/CHUSC. **Methods** 118 patients, from CHP and other portuguese hospitals - mainly CHLN and CHUC - have completed NGS panels study. Neuromuscular and epileptic encephalopathies panels had been the most requested (32 and 26 requests, respectively), followed by mithocondrial diseases (16 requests), mental retardation (14 requests) and cerebral morphogenesis defects (9 requests). **Results** The results obtained in the studies are presented, with special focus on the detection of large deletions, often missed by this approach, and the determination of mosaicism. In several studies it was possible to clearly find the genetic cause of the disease, namely in neuromuscular and epileptic encephalopathies panels, but many were not conclusive, mostly when it concerns to mithocondrial diseases and mental retardation panels. Some studies revealed variants of uncertain significance and others found only one heterozygous causal variation in genes presenting autosomal recessive inheritance. In both situations, family studies and clinicians feedback were important to evaluate if and how the affected genes are suitable of causing the phenotypes. **Discussion** The NGS gene panels approach has proven to be a helpful and cost effective tool in diagnostic situations. The gain in information concerning copy number, compared to usual NGS results, is of great importance, as this was a pitfall "inherited" from Sanger sequencing. Some interesting results came up, as multiple genes affected by causal variants, suggesting overlapped diseases that could explain some unusual phenotypes, but also identification of causal variations in genes that were not the main suspects, proving the advantage of multiple gene analysis. As final remark, to notice the importance of extensive information about the patients and laboratory-clinician crosstalk as a mean to achieve the most comprehensive results.

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Preimplantation Genetics Diagnosis for different pathologies

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Introduction: The aim of this study is to present the results obtained for Preimplantation Genetics Diagnosis (PGD) performed for Spinal Muscular Atrophy (SMA), Machado-Joseph Disease (MJD), Huntington Disease (HD) and Steinert Myotonic Dystrophy (DM1). **Methods**: Embryos from intracytoplasmatic sperm injection (ICSI) were biopsied at day 3 of development and two cells per embryo were analyzed independently using fluorescent multiplex PCR. Nine SMA carrier's couples performed 15 PGD cycles (4 couples have done one cycle, 4 repeated the treatment cycle once, and 1 couple repeated the treatment cycle twice) with 85 embryos analyzed; For MJD, 6 couples (3 female affected and 3 male affected) performed 7 cycles (1 couple repeated the treatment once) and a total of 33 embryos were analyzed; For HD, 13 couples (7 female affected and 6 male affected) performed 16 cycles (3 couples repeated the treatment once) and 88 embryos were analyzed. For DM1, 7 couples (6 female affected and 1 male affected) performed 9 PGD cycles (2 couples repeated the treatment once) and overall 62 embryos were analyzed. **Results and Discussion**: For SMA, 77 out of 85 embryos were amplified (90,6%): 52 genetically transferable, 23 embryos with SMA, and 2 embryos with a monoallelic component. From the 52 genetically transferable, 21 were transferred to the maternal uterus resulting in 3 biochemical pregnancies (one ongoing). For MJD, 31 out of 33 embryos amplified (93.9%): 12 embryos were genetically transferable and 17 embryos were carriers of the CAG expansion, and 2 showed either monoallelic or triallelic components. Six embryos were transferred resulting in a term twin pregnancy. For HD, 83 out of 88 embryos amplified (94.3%): 28 embryos without HD, 46 embryos with HD, 4 showed either monoallelic or triallelic components and for 5 embryos it was not possible to obtain a diagnosis. Thirteen embryos were transferred resulting in 3 pregnancies (1 ectopic and 2 term pregnancies). For DM1, 59 out of 62 embryos were amplified (95.2%): 22 embryos had 2 normal alleles and 26 embryos had one allele with CTG expansion, 8 showed either monoallelic or triallelic components and for 3 embryos it was not possible to obtain a diagnosis. Twelve embryos were transferred, resulting in 4 biochemical pregnancies (one ongoing and one term pregnancy). PGD is a well established procedure allowing the birth of unaffected children.

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Regulation of the human erythropoietin expression via an upstream open reading frame in cardiac tissue

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Cellular stress activates an integrated stress response, which includes rapid changes in global and gene-specific mRNA translation. Translational regulation of specific transcripts mostly occurs at translation initiation and is mediated via different cis-acting elements present in the mRNA 5' untranslated region (5'UTR); these elements include upstream open reading frames (uORFs). uORFs modulate translation of the main ORF by decreasing the number and/or efficiency of scanning ribosomes to reinitiate at the start codon of the main ORF; thus, uORFs are major regulators of gene expression. In its classical hormonal role, human erythropoietin (EPO) is a glycoprotein synthesized and released mainly from the kidney, which has a key role in hematopoiesis. However, recent studies have revealed that EPO is a multifunctional molecule produced and utilized by many tissues that rapidly responds to different cell stress stimuli and tissue injuries. The 5'UTR sequence of the human EPO mRNA has one uORF with 14 codons, which is conserved among different species, indicating its potential role in translational regulation. Indeed, we have recently shown that translation of human EPO mRNA is regulated by its uORF in response to hypoxia in HeLa cells [Barbosa & Romão (2014). RNA. 20(5):594-608]. To test whether EPO expression is translationally regulated in response to ischemia in cardiac tissue, reporter constructs containing the normal or mutant EPO 5'UTR fused to the Firefly luciferase cistron were expressed in H9c2 (heart/myocardium myoblasts) and C2C12 (muscle myoblasts) cell lines. Luminometry assays revealed that the EPO uORF represses translation of the main ORF in both cell lines. Under chemical ischemia, EPO uORF-mediated translation repression is specifically released in muscle cells. In response to hypoxia, translational derepression occurs in both cell lines. We are currently exploring additional mechanisms through which EPO cardioprotection effects are regulated at the translational level.

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Investigation of OXPHOS complexes' assembly in four LHON patients - Heterogeneity of genetic background

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Introduction: Mitochondrial cytopathies comprise a heterogeneous group of multisystem disorders related to mitochondrial dysfunction and ATP underproduction. Since mitochondrial respiratory chain (MRC) subunits are encoded by both nuclear (nDNA) and mitochondrial (mtDNA) DNA, the pathology may be caused by diverse types of mutations in one or both genomes. Leber's hereditary optic neuropathy (LHON) is one of the most common mitochondrial cytopathies. It is mainly characterized by degeneration of retinal ganglion cells, causing blindness in young males. It is estimated that ~70% of LHON cases are due to the point mutation m.11778G>A that encodes the ND4 Complex I (CI) subunit. Therefore, it is expected that nDNA alterations have a synergistic role with mtDNA mutations, causing a severe biochemical defect. Moreover, it is possible that modifications in nDNA coding for mitochondrial factors of major importance to the assembly, stability and maintenance of the MRC, lead to an imbalance in oxidative phosphorylation (OXPHOS)-dependent energy production, compromising mitochondrial function. The assembly factors, despite not being part of MRC structure, play a crucial role in the correct complexes assembly for an adequate energy production. **Objective**: Thus, the present study aims to clarify the role of MRC assembly in LHON pathology, in a cohort of four (P1-4) patients, related to the presence (P1, P2) and absence (P3, P4) of the m.11778G>A mutation. **Methods**: The DNA samples were analysed using Sanger and Next generation sequencing. The spectrophotometric bioanalytical evaluation of MRC activities and BN-PAGE followed by western blotting, were performed in skin biopsy derived cell/mitochondrial extracts, in order to determine whether MRC complexes are structurally and functionally impaired. **Results and Discussion**: The results showed that the CI activity was highly reduced in P1 and P2. The analysis of the MRC complexes' assembly status allowed to identify impaired CI assembly in P1 and P4. Furthermore, P4 also showed a decrease in CV assembly. The genetic analysis of the assembly factors revealed a promising sequence variation, under characterization, in P1. **Conclusion**: The present results are promising and suggest that impairment of the MRC assembly should be considered in LHON, although there are other factors to be taken into account in the pathological mechanism underlying the disease.

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Identification of TEK gene as a candidate for primary congenital glaucoma

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Introduction: Primary congenital glaucoma (PCG) is a rare but severe autosomal recessive disease characterized by high intraocular pressure during neonatal or early infantile period. If not detected and treated early, corneal opacification, optic nerve damage, or amblyopia may cause permanent vision loss. Mutations in the CYP1B1 gene are the major cause of PCG. However, there is a group of PCG patients who do not have CYP1B1 mutations. The aim of the study was to identify novel genes that may be causative of PCG in patients negative for CYP1B1 mutations. We report on a novel mutation in a new PCG candidate gene.

Methods: DNA was extracted from a blood sample of a PCG patient with unilateral glaucoma. First, mutations were studied in CYP1B1 gene coding region, UTRs, introns, promoter, and enhancer, by Sanger sequencing. Following this step, the whole exome was sequenced with AmpliSeq™ Technology in Ion Proton™. After annotation with ANNOVAR, rare variants with predicted functional impact were selected. Variants were first searched in LTPB2, MYOC, FOXC1, and PIXT2 genes, already associated with PCG. The second approach considered the autosomal recessive model. Finally, novel high functional impact variants mutations were considered.

Results: The PCG patient was negative for mutations in CYP1B1, LTPB2, MYOC, FOXC1, and PIXT2 genes. Similarly, there were no relevant candidate genes for the autosomal recessive model. From the selection of novel and with predicted high functional impact variants one good candidate was identified: the missense p.V188G mutation in TEK gene. SIFT, PolyPhen, MutationTaster, and CADD predicted this novel variant as pathogenic. The heterozygous mutation is located in the Ig2 extracellular domain of the protein and is predicted to disturb the protein, according to FoldX.

Discussion: We found a promising novel mutation in the PCG candidate gene TEK. Transgenic mice without a functional TEK gene were reported to have a phenotype similar to PCG. Another recent study identified TEK mutations in PCG patients in an autosomal dominant model with incomplete penetrance. Experimental functional validation of p.V188G is currently being conducted and may add to this novel PCG disease model. We consider that whole exome sequencing is a good technology to identify candidate genes for PCG, including with a different inheritance model from the one initially predicted.

P36 | Molecular Genetics

PNKP mutations are a frequent cause of ataxia with oculomotor apraxia in Portugal

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Introduction: Ataxia with oculomotor apraxia type 4 (AOA4) is an autosomal recessive (AR) disorder caused by mutations in the *PNKP* (polynucleotide kinase 3'-phosphatase) gene. AOA4 is a progressive, complex movement disorder that includes ataxia, dystonia, eye movement abnormalities, polyneuropathy and varying degrees of cognitive impairment. Mutations in *PNKP* were originally found to cause early infantile epileptic encephalopathy, characterized by microcephaly, seizures, and developmental delay. Here we describe 4 Portuguese patients with *PNKP* mutations presenting with an AOA4 phenotype. Our aim was to establish the molecular diagnosis, by screening the 16 exons of *PNKP*, in patients with clinical indication.
Methods: Mutation screening was performed by PCR amplification of all coding and flanking regions, followed by bidirectional Sanger sequencing. Results: By molecular analysis, we identified four different mutations, one missense, one insertion/deletion and two deletions. Mutations detected: two brothers homozygous for the c.1123G>T; p.Gly375Trp mutation; two siblings compound heterozygous for c.1221_1223del; p.Thr408del and c.1315_1330delinsGGGGACG; p.Arg439_Pro444delinsGlyAspAla); one case compound heterozygous for c.1123G>T; p.Gly375Trp and c.1221_1223del; p.Thr408del and another case harboring the c.1123G>T; p.Gly375Trp and c.1510del; p.Arg504Glyfs*?. This last mutation has not been previously described.
Discussion: In this study, we have identified homozygous or compound heterozygous *PNKP* mutations in four of the 13 Portuguese families we studied (a total of 6 affected individuals), suggesting that, in Portugal, mutations in *PNKP* are the most frequent cause of AOA. Mutation screening in these genes can be invaluable to confirm and establish an early diagnosis, and to allow proper genetic counselling, including the offer of prenatal diagnosis, whenever appropriate.

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The titanic (TTN) landscape of cardiac and skeletal muscle diseases.

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Introduction

The *TTN* gene, with the largest ORF in the human genome (363 exons in the canonical isoform), encodes the major component of the sarcomere: the gigantic protein Titin (3.9 MDa). Its function in skeletal and cardiac muscle goes beyond the structural or mechanical roles, and includes development and regulatory signaling functions modulated by mechanical stress on the cytoskeleton. Owing in great part to the use of NGS technology, Titin is rising as a major cause of muscle diseases. At least 11 different conditions are now known to comprise the multi-faceted titinopathies. We describe the phenotypic and genetic heterogeneity of a patient cohort with *TTN* variants, studied in our centre.

Methods

Mutation screening was performed by NGS (gene panel targeted resequencing) and/or Sanger sequencing. Expression studies at the mRNA or protein level (by cDNA analysis and/or western-blot techniques) were carried out to characterize new variants. Segregation analysis was performed in some families. In all, clinical and histological data was collated from 13 patients with pathogenic/probably pathogenic variants in the *TTN* gene (the majority of which have not been reported in the literature or in databases).

Results

Within our patient cohort, one case presented a congenital myopathy with multicores and fatal dilated cardiomyopathy. The remainder had different and exclusively skeletal muscle phenotypes: one family (5 patients) and one sporadic case were diagnosed with HMERF (hereditary myopathy with early respiratory failure), and two unrelated patients had a recessive centronuclear congenital myopathy. The remaining four patients presented an infantile or childhood onset myopathy which is being tentatively correlated with the respective detected variants.

Discussion

The diversity of *TTN* variants and their underlying pathophysiological mechanisms, the broad clinical phenotypes with different transmission patterns, and the relatively high frequency of variants with unknown clinical significance (including truncating changes) often found in healthy individuals, makes the titinopathies one of the most challenging group of diseases to interpret in the clinical context. In order to manage this, there is a need to employ new strategies for variant interpretation and validation (including *TTN*-specific bioinformatic tools, variant databases and functional assays) and, most importantly, a truly multidisciplinary effort is mandatory.

P38 | Molecular Genetics

Novel mutations in the PNPLA6 gene in Gordon Holmes syndrome

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Introduction
Gordon Holmes syndrome (GHS) is an autosomal recessive disorder characterized by cerebellar ataxia and hypogonadotropic hypogonadism. Recently, mutations in the *PNPLA6* gene were associated with this syndrome. Beyond this syndrome, *PNPLA6* gene was related with other disorders such as hereditary spastic paraplegia type 39 (SPG39) and Boucher-Neuhauser syndrome. The *PNPLA6* gene encodes an enzyme that catalyzes the conversion reaction of membrane phosphatidylcholine into its constituent fatty acids and glycerophosphocholine.
Clinical report
We have ascertained a woman of 46 years with clinical history of cognitive impairment that around 25 years old developed postural tremor of the hands, imbalance and falls. This patient is from a family with no consanguinity, 8 brothers and known family history in 2 sisters of cognitive impairment, tremor and ataxia, in which had been identified an intermediate allele for fragile X tremor/ataxia syndrome (51 repeats). The patient had, like her two sisters, a primary amenorrhea whose research confirmed that it was a hypogonadotropic hypogonadism. Neurological examination revealed an ataxia associated with extrapyramidal syndrome (tremor and dystonia) and brain MRI showed marked cerebellar atrophy involving the hemispheres and the cerebellar vermis. In the index case was excluded the presence of the intermediate allele for fragile X.
We have performed *PNPLA6* mutation analysis, by PCR amplification of all exons and flanking intronic regions, followed by direct bi-directional sequencing.
Discussion
This study identified two mutations in *PNPLA6* in compound heterozygosity. Both mutations were novel: a missense mutation that replaces a glutamate for a glutamine at position 802 and a nonsense mutation that substitutes an arginine for a stop codon at position 1361. The family investigation confirmed the segregation of this phenotype with the mutations identified in *PNPLA6* gene and that the intermediate allele fragile X was asymptomatic.
We have confirmed the diagnosis in this family and expanded the *PNPLA6* mutational spectrum. Molecular confirmation of the clinical diagnosis in patients with hypogonadotropic hypogonadism allows proper genetic counselling to patients and their relatives.

P39 | Molecular Genetics

Diagnosis of LAMB3 associated epidermolysis bullosa of a deceased child through the NGS study of a carrier parent

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Epidermolysis bullosa is a skin disorder that can be significantly severe with extensive blistering of difficult managing. The disease is genetically heterogeneous and usually detailed histological classification is not available. A consanguineous couple was being followed by a clinical geneticist in a preconceptional context because their first daughter died with epidermolysis bullosa.

The DNA available from the child isolated from a dried blood spot was insufficient to perform a 13 genes NGS panel. To overcome this we use a DNA sample from the mother assuming that she would be a carrier for an autosomal recessive form of epidermolysis bullosa. A heterozygous frameshift mutation was found in *LAMB3* (c.31dup; p.Leu11Profs*43). This mutation was then screened for in the father's sample who was also heterozygous. Finally, we also confirmed that the child was an homozygote for this mutation, confirming the diagnosis.

This approach allowed us not only to establish the molecular diagnosis of the child but also to offer prenatal diagnosis (PND) to this couple in future pregnancies. This case illustrates that in some specific scenarios (like this of a consanguineous couple with an affected child that was already deceased with a probable recessive disorder) we can identify an etiology by studying the healthy parents.

P40 | Molecular Genetics

OCRL gene alterations profile in families with Lowe Syndrome and Dent Disease 2 from the Portuguese population

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Introduction: The Lowe oculocerebrorenal syndrome (OCRL; OMIM#309000) is a rare X-linked multi-systemic disorder characterized by mental retardation, congenital cataract and generalized aminoaciduria. Both, the age of onset and the severity of the renal tubular dysfunction are variable. Lowe syndrome is caused by markedly reduced activity of an inositol polyphosphate 5-phosphatase OCRL, encoded by *OCRL*. Defects in the *OCRL* also leads to Dent disease 2 (OMIM#300555), an X-linked renal tubulopathy. The OCRL gene encodes a ~5.8 kb transcript (23 coding exons), with a gene product of 105-kD. Similar to other X-linked recessive disorders, these syndromes are also characterized by mutational heterogeneity, a large number of so called familial alterations. Nearly 250 different *OCRL* alterations have been reported, mainly missense or nonsense. Splicing alterations, small indels and gross deletions or insertions have also been reported.
Subjects and Methods: The entire coding region and flanking intronic sequences of the *OCRL* were analysed in a total of 21 proband with clinical diagnosis of Lowe syndrome and two probands with Dent disease. Genomic DNA was extracted from peripheral blood samples. Molecular analysis was performed by either PCR-DHPLC or PCR-SSCP analysis followed by Sanger sequencing, or directly by Sanger sequencing of the PCR products. Whenever possible, familial segregation analysis of the mutations was performed.
Results and Discussion: We were able to identify a total of 17 unique *OCRL* alterations in probands with Lowe syndrome, namely six missense, six small insertions or deletions, three splicing and a nonsense alteration. A gross deletion of 1,252 bp, starting at intron 21 of the gene, was also identified. Additionally, a synonymous alteration of the last nucleotide of exon 16 of *OCRL* was identified in a patient with Dent disease. *In silico* analysis indicates that this alteration most likely results in altered splicing of the *OCRL* pre-mRNA. The type of *OCRL* alterations and its distribution are in accordance with the reported in other populations. This study contributes to the elucidation of the molecular basis of Lowe Syndrome and Dent Disease 2 in the Portuguese population, furthermore posibiliting carrier detection and prenatal diagnosis in these families.

P41 | Molecular Genetics

Update of The Molecular Study of Maturity Onset Diabetes of the Young (Mody) in Portugal

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Introduction: Maturity onset diabetes in the young (MODY) is a heterogeneous autosomal dominant form of diabetes mellitus with typical onset before age 25 and a primary defect in pancreatic beta-cell function. Patients with MODY may erroneously be classified as having type1 diabetes or type2 diabetes and MODY is thought to explain about 2% of all diabetes but its true prevalence in many populations is still not clear. It is estimated that Portugal has 600.000 diabetic patients and about 12.000 could be MODY. MODY2 patients have mild, asymptomatic, and stable hyperglycaemia that is present from birth. In contrast, patients with MODY3 have a progressive defect in insulin secretion frequently resulting in severe and progressive hyperglycaemia in adult life. MODY2 and MODY3 are the most common forms in Europe. The different MODY types can only be determined by molecular diagnosis. A genetic diagnosis often changes patient management, since patients with GCK mutations rarely require pharmacological treatment and HNF1A/4A mutation carriers are sensitive to sulfonylurea. The aim of this work was to characterize the MODY gene defect associated to each patient to improve patient management.
Methods: A total of 54 index cases with clinical diagnosis of MODY and relatives were received. The molecular studies were performed using direct sequencing and MLPA techniques for GCK (MODY2), HNF1A (MODY3), HNF4A (MODY1) and HNF1B (MODY5) genes.
Results and Discussion: The molecular study has been performed for 46 index patients and until now 10 patients were found to have a putative pathogenic variant in GCK, 7 in HNF1A and 3 have a full deletion of HNF1B. No mutations were found in HNF4. A total of 15 different variants were found, 2 of these have not been described before. Molecular genetic testing is important because confirms a diagnosis of monogenic diabetes, predicts clinical course, determines treatment according to their condition minimising the effects of the disorder, and defines risk for relatives.

P42 | Molecular Genetics

Use of FMR1 gene promoter methylation percentage to infer the X-chromosome inactivation pattern

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INTRODUCTION. The human androgen-receptor (*AR*) gene contains a trinucleotide repeat (CAG) in the first exon and two methylation sensitive endonuclease sites located 100 bp upstream. It is commonly accepted that the methylation status of this locus correlates well with the inactivation pattern of the X-chromosome (XCI). Results obtained resorting to PCR based *FMR1*-CGG repeat length and methylation evaluation were compared with those previously observed using the gold-standard strategy for determining XCI skewing percentage, commonly known as human androgen-receptor assay (HUMARA). Our goal is to assess the use of *FMR1* gene promoter methylation percentage to infer the X-chromosome inactivation pattern.
METHODS. To analyze the *FMR1*- locus, the Asuragen AmplideX kit was applied: gDNA (obtained from blood) was submitted to PCR, after digestion with *Hha*I and amplified with a HEX-labeled primer set, and the undigested aliquot amplified with a 6-FAM-labeled primer. Ratios of HEX to 6-FAM peak areas were used to calculate the percentage XCI of each homologue. This strategy was validated by comparing the XCI pattern of 20 female samples, using both the Asuragen methodology and the standard HUMARA, as previously published.
RESULTS. Values between 50:50 and 80:20 reflect a random pattern of inactivation. The validation cohort included 3 female samples showing complete skewing (0:100 – 10:90), 10 female samples with a random XCI pattern (around 50:50 – 20:80), 2 uninformative for the *AR* CAG repeat (homoallelic) and 5 with a borderline result (20:80 - 10:90). Referrals included: X-autosome translocation carriers (n=3), Fragile-X patients (n=5), carriers of different X-linked recessive pathogenic variants (n=5), a microarray deletion carrier and a 47,XXX case. Overall, the Asuragen method yielded XCI skewing percentages that were lower when compared to those obtained by the HUMARA. We attributed this fact to the higher frequency of carriers with alleles differing by a single CGG repeat in the *FMR1*locus.
CONCLUSION. *FMR1*-CGG repeat methylation pattern results corroborated those previously obtained with HUMARA and uses a significantly less amount of gDNA. These results, which demonstrate that the Asuragen methylation assay can be used to infer the X-chromosome inactivation pattern, suggest that this is a simple alternative to the HUMARA particularly when non-informative or critical samples are being analyzed.

P43 | Molecular Genetics

Diagnosis of Charcot-Marie-Tooth type 4 by next generation sequencing

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Introduction: Charcot-Marie-Tooth (CMT), also known as hereditary motor and sensory neuropathy, is a very heterogeneous group of genetic disorders that affect the peripheral nervous system with different modes of inheritance. The clinical presentations can be variable but the main features include slowly progressive neuropathy of the limbs which result in muscle weakness and atrophy. The onset ranges from early childhood to adulthood. CMT is subdivided into two main groups: demyelinating CMT1 and axonal CMT2. Until now, mutations in more than 40 genes have been described as causative of CMT. CMT4 distinguishes from the other CMT forms by the autosomal recessive inheritance, and a phenotype characterized by the typical CMT features of distal muscle weakness and atrophy associated with sensory loss and, frequently, pes cavus foot deformity.
Clinical cases: The first patient presented congenital hypomyelinating neuropathy with distal muscle weakness and decreased osteotendinous reflexes. The second patient showed distal lower limb weakness and sensory symptoms such as paresthesias; EMG suggested a multifocal asymmetric sensory-motor primary demyelinating neuropathy with conduction block. We have developed and validated a next generation sequencing (NGS) gene panel that includes 11 genes known to cause CMT4. Mutation screening was performed by multiplex amplification followed by NGS using the Ion Torrent PGM. Data analysis was performed using JSI SeqNext.
Results: The molecular study of these two patients allowed the identification of two heterozygous mutations in the FGD4 gene in the first patient: c.823C>T (p.Arg275*) and c.2005C>T (p.Gln669*); the first mutation was already known whereas the second is novel. The second patient was also a compound heterozygous but for mutations in the FIG4 gene: a T-to-C transversion at nucleotide 122, which results in a missense mutation (p.Ile41Thr) and a duplication of nucleotide 500, which results in a nonsense mutation (p.Tyr167*). Contrary to the c.500dup mutation that was reported for the first time, the c.122T>C mutation was already described. In both cases, the study of the parents allowed us to confirm that the mutations were located in different chromosomes.
Discussion: NGS methodology allows us to study a large number of genes simultaneously and the larger the panel, higher the diagnosis probability. The establishment of the molecular diagnosis allows proper genetic counselling to patients and their relatives.

P44 | Molecular Genetics

Beneficial effect of creatine-supplemented diet in a mouse model of MachadoJoseph disease

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Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder caused by the expansion of a polyglutamine tract (polyQ) in the C-terminus of the ATXN3 gene product, ataxin-3. Mitochondrial dysfunction has been implicated in several neurodegenerative diseases. Creatine kinase and its substrates (creatine and phosphocreatine) are known as cellular energy buffers. Creatine administration increases brain concentrations of phosphocreatine and an inactivation of the mitochondrial permeability pore, which exerts protective effects in the brain. In this study we performed two pre-clinical trials – PCT1 and PCT2 - using the CMVMJD135 mouse model of MJD (groups of animals with a 133 and 139 CAG repeat mean respectively), to which creatine 2% supplemented food was provided either for 19 (PCT1) or 29 (PCT2) weeks. Oral administration of creatine led to an overall improvement in the motor phenotype of CMVMJD135 mice on both trials. Interestingly, in PCT1, with shorter creatine treatment duration but with less disease severity, the muscular strength deficits of the CMVMJD135 were improved, while in PCT2, corresponding to a longer treatment but a high severity disease condition those improvements were not so evident. Creatine-treated animals did, however, show improvement in both trials in motor coordination, limb strength and gait quality, as well as in other neurological parameters such as tremors and clasping. Creatine chronic treatment delayed the onset of several symptoms and, in some cases, completely abolished the appearance of the phenotype. Furthermore, creatine treatment showed to be neuroprotective by increasing the calbindin staining in the Purkinje cell layer of the cerebellum and reducing the astrogliosis in the brainstem of the CMVMJD135 mice. The present findings support creatine supplementation as a useful strategy to slow the progression of MJD.

P45 | Molecular Genetics

Influence of variants in ATXN3L, JOSD1 and JOSD2 on the age-at-onset of Machado-Joseph disease

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Machado-Joseph disease (MJD) proteases are a subfamily of deubiquitinating (DUB) enzymes that include ataxin-3 (ATXN3), ataxin-3 like (ATXN3L), the Josephin domain-containing 1 (JOSD1) and 2 (JOSD2). ATXN3 plays an important role in the proteasome degradation pathway, but is better known due to its polyglutamine region in the C-terminal, which is expanded above 61 glutamines in patients affected with MJD, a dominant neurological disease. Given the pleomorphism of MJD, the normal *ATXN3* allele has been one of the first suggested modifiers to influence the age-at-onset (AO; age of appearance of first symptoms). Indeed, several lines of evidence support such hypothesis and indicate a partial loss-of-function mechanism for MJD pathogenesis since: a protective role of the wild-type ATXN3 in MJD neurotoxicity has been observed in a fly model; patients homozygous for the expansion usually present a more severe phenotype; and an enhanced stress response is observed in ataxin-3 knockout models.
The DUB activity and residue similarity in the catalytic triad of all members of this subfamily led us to hypothesize that they may compensate for the decreased levels of wild-type ATXN3 in MJD by exerting similar functions. Therefore, our aim was to assess the possible role of *ATXN3L*, *JOSD1* and *JOSD2* as modifiers of the MJD clinical presentation. We optimized multiplex reactions to sequence regulatory and exonic regions of these candidate genes and searched for genetic variants that might explain part of the AO variability in MJD. We identified 16 variants in our cohort of 100 Portuguese MJD families. Analyses of covariance have shown that five of these variants (rs16999010 in *ATXN3L*; COSM3713736 in *JOSD1*; and rs550589305, rs141848929 and rs796424878 in *JOSD2*) contributed, together, to explain 8.4% of the remaining AO variability in this cohort, after removing the known effect of the (CAG)n expansion size. The cumulative influence of this set of SNPs on MJD led us to design a new and more complex framework for the linear regression AO / expanded (CAG)n. Next, we will extend this variant screening to a larger cohort of patients and to other populations; the most promising variants, after statistical analyses, will be selected for functional assays in order to elucidate the underlying mechanisms that might explain their influence on the pathogenesis of MJD.

P46 | Neurogenetics

Twenty years' experience with a protocol of presymptomatic testing for late-onset neurological diseases

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A national programme of genetic counselling and presymptomatic testing for late-onset neurological diseases was begun in 1995, at five genetic services in Portugal. It was accessible to adults at-risk for Machado-Joseph disease, and then extended to other hereditary ataxias, Huntington disease and familial amyloid polyneuropathy (FAP) ATTR Val30Met, following a multidisciplinary approach. We aim now at describing the consultands' profile, for a better understanding of the population seeking our services, and to reflect on the protocol we have been following for 20 years, and its challenges and modifications needed.

We reviewed 1,446 records of consultands who requested presymptomatic testing at our centre. Their social-demographic profile was similar to those in other programs. Mean age at the time of testing was 30.7 years; females (56.0%) predominated; 60.9% consultands had no offspring; and 56.9% were non-carriers. Contrarily to other reports, however, withdrawal before results disclosure was only about 15%. Among the motivations for uptake of presymptomatic testing, the most common were relieving uncertainty (41.7%), preparing for disease-onset (23.2%), family planning (23.2%) and informing offspring (18.0%).

Our practice along these 20 years pointed out to the need for harmonizing national registers of counselling sessions, as well as to the relevance of a more qualitative approach to document consultands' experiences. We discuss changes made to the protocol along these years. For a better understanding of presymptomatic testing, as we have been conducting it until now, we need tools for quality assessment of counselling practice, to address family-related features surrounding uptake of testing, as well as studies of at-risk relatives who decide not to be tested.

P47 | Neurogenetics

Genetic modifiers of age-at-onset variability in FAP ATTRV30M: the role of complement C1q genes

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Introduction: Familial amyloid polyneuropathy (FAP ATTRV30M) is an autosomal dominant systemic amyloidosis caused by a point mutation in the TTR gene (chr18q12.1) and the most frequent disease causing variant in Portugal is the V30M. FAP ATTRV30M shows a wide variation in age-at-onset (AO) [19-82 years, in the Portuguese population], including within some families, offspring often showing anticipation in AO when compared to their parents. Our aim was unravel if candidate genes associated with TTR pathways, as C1QA and C1QC, might act as genetic modifiers of AO in FAP ATTRV30M.
Methods: We genotyped 267 patients, from 117 families by automated bidirectional sequencing. For the statistical analysis, the SPSS Statistics software (v.23) was used. We performed intensive in silico analyses, using various softwares to assess miRNAs, splicing sites and transcription factor binding sites (TFBS) alterations. Additionally, we performed a Multifactor Dimensionality Reduction (MDR) analysis to search for gene-gene interactions.
Results: We found 2 statistically significant variants for C1QA the GA genotype ($p<0.001$) of rs201693493 and the CT genotype ($p<0.001$) of rs149050968 were associated with later AO (> 50 years) (increasing the mean AO in 16 and 10 years, respectively). In silico analysis demonstrated that rs201693493 may alter the splicing activity. Regarding C1QC, we found 4 statistically significant variants: the GA genotype ($p=0.003$) of rs2935537, the CT/TT genotype ($p=0.037$) of rs15940, the GA genotype ($p<0.001$) of rs201241346 and the GA genotype ($p<0.001$) of rs200952686. The first three variants were associated with earlier AO (≤ 40 years), and this variation correspond to a decrease in mean AO to 5 and 11 years, while the last one was associated with late-onset, leading to an increase of 32 years in mean AO. Additionally, in silico analysis showed that rs2935537 may change the binding of NERF1a factor.
Discussion: These findings revealed that some variants in C1QA and C1QC were associated with late-onset and they can have a protective role in FAP ATTRV30M patients. On the other hand, three variants in C1QC were associated with an early-onset acting as risk factors for FAP ATTRV30M carriers. A strong interaction between C1QA and C1QC was found. Therefore, our results are important to understand the differences in AO between family members, with possible implications in genetic counselling, as well as in development of new therapeutic strategies.

P48 | Neurogenetics

Validating NGS pipelines for Neurogenetics using the Genome in a Bottle goldstandard reference material (NA12878)

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Introduction

Next-generation sequencing (NGS) is becoming a standard in clinical laboratories and is particularly helpful in genetically heterogeneous disorders. CGPP is a leading expert in the field of molecular genetic testing, the only ISO15189 accredited laboratory in Portugal for testing neurological disorders and our diagnostics routine includes the use of NGS. The validation process of NGS protocols comprises several challenging steps and only few guidelines have been established to harmonize the validation methodology. Therefore, each laboratory must tailor their approach to tackle specific challenges.

Methods

Our approach to genetic diagnostics integrates targeted sequencing, using homemade custom gene panels, and virtual gene panels, using Whole Exome Sequencing (WES). For targeted sequencing (Ion Torrent PGM), variants were obtained from data analysed using JSI SeqNext. For WES virtual panels (Illumina HiSeq 4000), variants were identified using a custom pipeline, based on BWA for alignment (GRCh37), GATK HaplotypeCaller for variant calling and Ensembl VEP, GEMINI and Alamut for annotation.

To validate these pipelines, we sequenced the Genome in a Bottle (GiaB) DNA from Coriell (NA12878), proposed as a reference material by the National Institute of Standards and Technology, and compared the variants found using our methodology with the set of high-confidence variants made available by the GiaB consortium, using four neurological disorder gene panels: hereditary ataxias (144 genes), epileptic encephalopathies (45 genes), hereditary spastic paraplegia (117 genes) and dementia (26 genes).

Results and Discussion

For the ataxia and epileptic encephalopathies panels, we successfully identified all 170 and 41 variants present in the GiaB high-confidence calls (100% sensitivity). For spastic paraplegia, we called 99 variants out of 100 (99% sensitivity) while for the dementias panel we identified 16 variants but missed 4 (80% sensitivity). In both cases the missed variants were located in low or non-covered regions. Sanger sequencing of these low covered regions is routinely performed, allowing our overall diagnostic sensitivity for these panels to reach 100%.

We present here an established, tested, optimised and validated NGS workflow with high sensitivity currently in use in our diagnostic routine, allowing physicians and genetic counsellors to focus on the patients and their disorders, instead of the technical aspects of genetic testing.

P49 | Cancer Genetics

Regulation of Alternative Splicing by Signal Transduction Pathways: Lessons from Studying Variant RAC1b in Colon Cancer

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Introduction
Alternative pre-mRNA splicing contributes significantly to both post-transcriptional regulation of gene expression and proteome diversity. While hereditary genomic mutations cause aberrant splicing in individual genes, somatic diseases such as cancer are characterized by more wide-spread changes in splicing patterns. These patterns can be affected by the presence of mutant splicing factors, epigenetic chromatin modulation, or in response to cellular signaling. Here we use the paradigmatic splice variant RAC1b to explore how signaling pathways are involved in the deregulation of alternative splicing in cell representing serrated colorectal tumors with mutations in the BRAF gene.
Materials and Methods
HT29 colorectal cells harbor one mutant BRAF-V600E allele and overexpress RAC1b. Cells were transfected with shRNA vectors directed against target candidate protein kinase transcripts and their effects on RAC1b levels analyzed 24 h later by Western Blot and qRT-PCR. Treatment of cells with kinase inhibitors or anti-inflammatory drugs was performed 24 h prior to cell lysis.
Results and Discussion
The presence of protein kinase BRAF-V600E was insufficient to change alternative splicing of RAC1b. Therefore, 20 candidate splicing-regulatory protein kinase genes were depleted by RNAi in HT29 cells and two kinases, SRPK1 and GSK3β, were found required to sustain RAC1b splicing levels. Both kinases were shown to act upon the phosphorylation level of splicing factor SRSF1, known to bind a splice-enhancing element in the alternative exon included in RAC1b. Reduced SRSF1 phosphorylation led to its reduced nuclear translocation and a concomitant reduction in RAC1b alternative splicing. GSK3β was further found to be a target of the anti-inflammatory drug ibuprofen. Mechanistic studies in HT29 cells revealed that ibuprofen but not aspirin, promoted a specific reduction in RAC1b splicing through an inhibitory phosphorylation of GSK3β. The reduction in RAC1b could be rescued when SRPK1 or SRSF1 were overexpressed in ibuprofen-treated cells, revealing the existence of a splicing-regulatory pathway. Together, our results indicate that alternative splicing in cancer cells can be deregulated through signal transduction pathways, e.g. in response to inflammatory stimuli. The data also identify a specific action of ibuprofen on alternative splicing of RAC1b and suggest it may be beneficial in treating patients with the subtype of BRAF-mutated serrated CRC.

P50 | Cancer Genetics

One patient and two synchronous tongue tumors: how genes help to discriminate bilateral carcinomas?

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Oral cavity cancer is ranked in the top 10 leading cause of deaths in males worldwide. Tongue cancer is considered the most aggressive tumor and with the worst prognosis of all oral cavity neoplasm, presenting a biologically different entity when compared with other oral tumors. Tongue tumors are usually located in the lateral border of the tongue and are often related to the use of tobacco and alcohol. We herein describe an 88-year-old woman diagnosed with synchronous bilateral tongue carcinoma. This woman did not present the traditional risk factors related to oral cancer - alcohol, tobacco and presence of human papiloma virus (HPV). This patient was submitted to surgery. Six months later was diagnosed with cervical metastasis and in the following two months died. Copy number alterations and methylation status of these two tumors were analyzed. In both tumors we identified several molecular traits similar to those described for tumors of the oral cavity and specific genomic and epigenetic signatures for each of these two tumors were observed allowing its molecular discrimination. This case shows the molecular heterogeneity of oral cavity tumors even in the same patient and anatomic site.

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RETINOBLASTOMA: Experience of the Portuguese Reference Center for ocular tumors in children

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INTRODUCTION: Retinoblastoma (RB; MIM#180200) is a malignant tumor of the developing retina in children, usually affecting children under the age of five years. This tumour occurs in cells that have cancer-predisposing mutations in both copies of RB1 gene and may be unilateral, bilateral or trilateral. The clinical diagnosis is established by eye examination under anaesthesia. Imaging studies can be used to support the diagnosis and stage the tumor. RB1 is the only gene known to be associated with retinoblastoma.
METHODS: Clinical data and molecular genetic results of the RB1 gene (sequencing and MLPA) were reviewed from patients with a diagnosis of retinoblastoma and their relatives referred to a medical genetics appointment at Hospital Pediátrico in the last 5 years.
RESULTS: The authors report on 29 cases with Retinoblastoma from 26 families (18 unilateral, 10 bilateral, one trilateral); 12 females and 17 males; the age of diagnosis in bilateral cases was between 1 and 24 months; in unilateral cases, between age 4 and 48 months; in the unique trilateral case, the diagnosis was made in the first month of life. Molecular genetic testing identified pathogenic variants in RB1 gene in 12 affected individuals: five cases of unilateral Rb (31,25%); six cases of bilateral Rb (75%) and one case of trilateral Rb (100%).Genetic analysis was performed in 31 healthy relatives and in three relatives with Retinoblastoma, from seven families; family mutation was found in 9 healthy relatives (29,03%) and it was confirmed in all three affected relatives. Nine different mutations was found, four mutations not described in the literature. Four studies are still ongoing: 2 unilateral cases and 2 bilateral cases.
DISCUSSION: Twelve of twenty-one individuals with germline mutation developed Retinoblastoma (57,14%). This is below the 90% penetrance suggested by literature. This difference can be explained by mutations associated with low penetrance as occurred in the two splicing mutations that were found in the 9 healthy individuals. Identification of RB1 pathogenic variants was possible in 75% of bilateral cases (90-97% in literature); 100% in trilateral and 31,25% in unilateral (15% in literature). The carriers identification is very important for the reproductive options - Prenatal Diagnosis and Preimplantation Genetics Diagnosis - and for the early diagnosis in children at risk.

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Oxidative Stress and DNA Repair Genetic Variants as Prognostic and Therapeutic Biomarkers in Chronic Myeloid Leukemia

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Oxidative stress (OS), resulting from an imbalance between Reactive Oxygen Species (ROS) production and antioxidant defenses, contributes to cell damage and ineffective hematopoiesis. Chronic myeloid leukemia (CML) is a clonal neoplastic disease associated with the reciprocal translocation t(9;22), encoding the BCR-ABL1 oncogene. BCR-ABL protein induces, among other mechanisms, production of reactive oxygen species (ROS). The antioxidant enzymes superoxide dismutases (SOD) and catalase (CAT), as well as DNA repair enzymes, such as OGG1, are important cell defenses against OS. SNPs in genes that codify these enzymes may reduce the protection against OS, influencing CML development and therapeutic response. Here we investigate the influence of SNPs in genes related with OS (CAT, GPX1, MPO, SOD1, SOD2, NFE2L2) and DNA repair (OGG1, NEIL1, XRCC1) in the development of BCR-ABL1 mutations, in therapy response and in overall survival of CML patients. Seventy-five CML patients were enrolled in this study. SNPs of CAT (rs1001179), GPX1 (rs1050450), MPO (rs2333227), SOD1 (rs2070424), SOD2 (rs4880), OGG1 (rs1052133), NEIL1 (rs5745920), XRCC1 (rs1799782) and NFE2L2 (rs13001694) were genotyped by tetra-primer-ARMS-PCR. OS (ROS/total antioxidant ratio) and DNA damage (8-hydroxy-2'-deoxyguanosine) were measured using commercial kits. Statistical analysis was carried out by variance analysis, $\chi 2$ test and Fisher exact test ($p<0.05$). Our results show that SOD2 genotype influence mutation status of BCR-ABL1 (CC genotype: OD 9.25, IC95% 1.24-18.82; p=0,007), being the CC genotype associated with higher OS levels. On the other hand, patients with MPO GG and AG genotypes have a high rate of sub-optimal response to tyrosine kinase inibitors (TKIs; OD 4.92, IC95% 1.24-9.10; p=0,043), and these genotypes were also associated with higher oxidative stress levels. Moreover, the overall survival of CML patients can be influenced by NEIL1 [CML patients with CT genotype had lower survival (166±5 months) than patients with CC and TT genotypes (204±6 months; p=0.041)] and NFE2L2 [CML patients with TT genotype had lower survival (88±7 months) than patients with CT and TT genotypes (216±8 months; p=0.003)]. Our results show that SNPs in OS and DNA repair related genes influence the prognosis of CML patients, their response to TKI treatment and the development of BCR-ABL1 mutations, through OS and DNA damage modulation, suggesting their potential as prognostic and therapeutic biomarkers.

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A multi-platform approach for a thorough (cyto)genomics characterization of a metastatic OSCC cell line

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Oral squamous cell carcinoma (OSCC) is one of the most common tumors worldwide and presents a high mortality. OSCC arises from genetic and epigenetic alterations in cellular and molecular pathways in the stratified squamous epithelium. The five-year survival rate for these patients remains low, around 50-60%, and one of the factors with the highest impact is the development of metastasis to cervical lymph nodes or distant organs. Here, we present a comprehensive characterization of a metastatic cell line.

HSC-3 cell line is derived from a primary tongue tumor that presented lymph nodes metastasis. Its characterization was assessed by karyotyping, multicolor Fluorescence In Situ Hybridization (mFISH) and array Comparative Genomic Hybridization (aCGH), which allowed the identification and characterization of structural and numerical aberrations.

Cytogenetic techniques allowed the characterization of HSC-3 as a near-triploid cell line (~61 chromosomes) and the identification of derivatives and centromeric/near-centromeric translocations as the most common aberrations. The majority of copy number variations observed in HSC-3 were gains: 1, 3q, 5p, 7p, 8q, 9q, 10, 11p, 11q13, 12, 13, 14, 17, 18p, 20, Yp and Xq. Copy number loss were less frequent, being the largest detected at chromosome 18. Furthermore, aCGH allowed the establishment of possible breakpoints associated to the aberrations often detected by the cytogenetic techniques.

HSC-3 represents a metastatic cell line, as such we found several aberrations that were previously related with the development of metastasis, advanced clinical stage and worse prognosis. Additionally, our cytogenetic analysis demonstrated that HSC-3 alterations are similar to the ones of primary tumors, supporting the use of commercial cell lines as good in vitro models for OSCC.

Overall, the application of these three different techniques enabled a thorough characterization of this cell line, highlighted several aberrations associated with clinic aspects. This allows a better choice of *in vitro* models and a more adequate result's interpretation, becoming helpful for clinical research and towards future personalized medicine for OSCC patients.

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Genomic predictors of the different survival rates in the head and neck carcinoma

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Introduction: Head and Neck Squamous Cell Carcinoma (HNSCC) is a neoplasia that appears on the upper aero-digestive tract, including the oral cavity, oral pharynx, larynx, hypopharynx and tongue and is the 6th most common tumour in the world. The development of this carcinoma results of the gradual build-up of changes and genetic/epigenetic instability that lead to carcinogenesis and tumor progression. Regarding the advances on the diagnosis technologies and types of treatment, these tumours are still diagnosed in advanced stages without an improve survival rates. The main goal of this study is to answer the following question: Are the different survival rates of patients with HNSCC associated to the presence of tumors with different genomic signatures? **Methods:** Results from array-Comparative Genomic Hybridization (aCGH) technique, that refer to Copy Number Variation (CNV) of genetic material, jointly with the clinic/pathologic information of the 104 HNSCC patients, were analyzed using the software platforms Matlab R2014a, R3.1.2 and SPSS. **Results and Discussion:** The association of "variable dependent" on the presence of metastasis connected with the aCGH data was verified and the minimum common regions more frequently altered in the chromosomes were identified. It was also possible to check the efficiency of two classifiers, Random Forest and SVM, to classify data. Eleven chromosomic regions clinically relevant were identified, being the amplification of regions 11q13.5-q14.1, 22q11.22-q11.23, 3p14.3-p14.2 and 11p14.1-p13 and deletion of regions 22q11.22-q11.23, 6q16.1-q16.3, 17q21.31-q21.32, 3p14.3-p14.2 and 3q26.31-q26.33 related with a worse prognosis and a lower survival rate; and the amplification of regions 17q21.31-q21.32 and 1q21.1-q21.2 related with a better prognosis. Potential biomarkers/predictors for HNSCC were identified.

P55 | Cancer Genetics

A Novel Approach for Screening the Oral Cavity and its Capability to Detect Oral Squamous Cell Carcinoma

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Introduction : Oral squamous cell carcinoma (OSCC) represents more than 90% of the oral cancers. Despites of scientific progress and the upgrading in therapeutic approaches, the five-year survival rate of OSCC remains low, mainly due to advanced stage of diagnosis and frequent development of loco-regional recurrences and/or metastasis. The establishment of genetic and epigenetic biomarkers and the use of minimal-invasive diagnostic techniques are essential to accomplish the early detection of OSCC and to follow up the patients in order to predict the disease progression. Therefore, this study aimed to evaluate the accuracy of a non-invasive method in the detection of genetic and epigenetic alterations characteristics of OSCC. **Methods** : Tumour cell, acquired by scraping the tumour surface, and tumour tissue samples were collected from 59 OSSC patients. Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) was conducted to screen copy number variation (CNV) and DNA methylation patterns in 41 and 27 tumour suppressor genes, respectively. The agreement between the tumour tissue samples and exfoliated cells results was determined using the Statistical Package for Social Sciences (SPSS) software. **Results**: Regarding CNV, it was found agreement in 22 (57.9%) of the 38 genes studied, specifically: *TP73, MSH6, RARβ, CASR, APC, ESR1, CFTR, CDKN2A, PAX5, CREM, KLLN, PTEN, MGMT, PAX6, WT1, CADM1, RB1, PYCARD, TP53, PMP22, KLK3, GATA5*. Concerning methylation status, the results showed agreement in 18 (72.0%) of the 25 genes analysed, specifically: *TP73, VHL, RARβ, ESR1, CDKN2A, PAX5, MGMT, PAX6, WT1, GSTP1, CADM1, CHFR, RB1, THBS, PYCARD, CDH13, STK11, GATA5*. Additionally, it was also found other genes with values closed to statistical significance, which can be reached by increasing the cohort. **Conclusion**: The results were truly promising since it is found a high agreement between non-invasive samples and tissue samples. This is a huge step in an attempt of validate this non-invasive methodology for screening the oral cavity and to follow up the patients diagnosed with OSCC.

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Determination of Predictors for Survival in Head and Neck Squamous Cell Carcinoma from a TCGA Database Cohort

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Introduction: Head and Neck Cancer (HNC) refers to a group of biologically similar malignancies arising in the upper aerodigestive tract. Approximately 95% of these cancers are Squamous Cell Carcinomas (SCC). Head and Neck Squamous Cell Carcinomas (HNSCC) exhibit extremely malignant phenotypes with frequent surrounding tissue invasion and distant metastasis presenting a 50% five-year survival rate. The detection of these tumors in early stages and the identification of associated characteristics with the prediction of clinical progression, remain challenging matters in clinical practice.
Methods: In this study, bioinformatical tools were applied to characterize the genomic profile of HNSCC using copy number data from 528 HNSCC patients extracted from The Cancer Genome Atlas (TCGA). These data were analyzed with MATLAB and SPSS.
Results: This study identified several genomic alterations consistent with the changes described in literature as being associated to HNSCC. Chromosomes 3, 5, 8, 9 and 11 were the ones that registered copy number alterations in a higher number of patients. In particular, the most frequently amplified regions were located at 8q24.21, 3q22.23, 5p15.33 and 11q13.3 and the most deleted regions were located at 3p21.2, 9p21.3, 8p22.3 and 11q23.2. Certain genes with the possibility of being biomarkers for prognosis were identified. For example, the deletion of one of those genes was found to be statistically significant for the risk of death of HNSCC patients, with 50% of patients that did not present deletion of the gene's loci surviving almost three years over those who did, conferring it the possibility of application in a clinical context.
Discussion: With this approach we were able to identify the most altered chromosomic regions in a broad heterogeneous population of HNSCC patients as well as possible biomarkers for the different survival rates observed within this cohort, only possible to achieve thanks to the establishment of a correlation between genomic and clinical information. These results are encouraging and may contribute to the development of clinical solutions ultimately seeking to reduce the number of deaths caused by this cancer.

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Relationship Between DNA Copy Number Aberrations and Chromosomal Rearrangements in Primary Culture Cells of Oral Squamous Cell Carcinoma – Preliminary Data

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Introduction: Despite technological and therapeutic advances, oral squamous cell carcinoma (OSCC) is diagnosed in late stage and the 5-year survival rate has not improved much. OSCC results from a multistep process in which genomic alterations play a major role. One of the main challenges is to identify cancer biomarkers that will help to improve early diagnosis and survival rates. Conventional and molecular cytogenetics analyses of tumour cell lines have 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. With this study we aimed to characterize the relationship between DNA copy number aberrations and chromosomal rearrangements in OSCC primary culture cells.

Methods: Primary culture cells lines were established from surgically resected samples obtained from different patients diagnosed with OSCC. The characterization was assessed by karyotype and array comparative genomic hybridization (aCGH) using sex-matched healthy controls.

Results and Discussion: The cytogenetic results showed complex karyotypes with several numerical and structural alterations. These cells seem to be near-triploid, with at least 63 chromosomes each. Several chromosomal rearrangements were identified, including i(5)(q10), i(9)(q10) and del(9), which are common aberrations of the carcinogenesis process. Cells with such alterations showed, by aCGH, gains of the entire chromosome arm of 5p and 9q as well as partial deletion of 9p. In addition, these alterations were also observed in commercial OSCC cell lines fully characterized in our labs. Moreover, aCGH detected genomic imbalances in almost all chromosomes, being chromosomes 3, 5, 8, 9, 18 and X the most frequently altered, in which are mapped important genes for oral carcinogenesis process.

The cytogenetic characterization and aCGH analysis revealed genomic imbalances described for OSCC. We report a relationship between genomic imbalances and cytogenetic rearrangements. These preliminary findings have been useful to define the genetic profile of OSCC and to attempt a correlation with the stage of the disease. In the future, these individual characteristics could help in the diagnosis and prognosis. It could also provide a research basis for pharmacogenomics studies and consequently for the development of new therapeutics targets for each patient.

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Cholangiocarcinoma cell lines: Extrahepatic and Intrahepatic cell lines characterization and comparison

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Introduction: Cholangiocarcinoma (CCA) 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. It 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 (10-25%). The diagnosis of CCA is not easy to obtain so the prognosis is usually poor, with an incidence and mortality rate very close to each other. Commercial cell lines are one of the most used models in biomedical studies, so its genetic characterization is very important to obtain more information about the tumors. The aim of this study was to perform a genetic characterization of two CCA cell lines, one extrahepatic and other intrahepatic. **Methods**: The genetic characterization was performed by conventional cytogenetics and by array Comparative Genomic Hybridization (aCGH). **Results and discussion**: The results obtained revealed the presence of several chromosomal aberrations in both extrahepatic and intrahepatic cell lines. Some of the alterations are common to both cell lines, as loss of 18, 6q, 13q and gain of 1q, 3q, 17q, 20q and 5p. On the other hand, some results were opposite to each other, as loss of 16 chromosome in intrahepatic cell line and gain of it in extrahepatic cell line and loss of X chromosome in extrahepatic cell line and gain of that chromosome in intrahepatic cell line. Furthermore can also be seen that in extrahepatic cell line there are loss of 4q, 9p and most of 9q, 11p, 17p and 19p and partial gain of 1p, 2p, 3p and 5q. In the other cell line there are loss of 8p and 10p and gain of 5q, 8q, 11q, 7p, 12p, 20p and almost total gain of Y chromosome. Regarding the analysis by conventional cytogenetics, both cell lines seem to be near-triploid with about 70 chromosomes and present also several structural aberrations. In extrahepatic cell line is common the presence of isochromosomes 1, 5, 15 and 17 and the deletion of 11p and the intrahepatic cell line presents several derivative chromosomes resulting from translocations, such as t(1;6), t(11;14) and t(14;17). The characterization of these cell lines is very promising, and opens many doors for the study of such tumors, in particular for studies in the area of pharmacogenomics.

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A case report of Lynch Syndrome due to 3´ end EPCAM gene germline deletion

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Introduction: Lynch Syndrome (LS) is one of the most common cancer susceptibility syndromes and is caused by inactivating germline mutations in the mismatch repair (MMR) system genes followed by a second somatic event which inactivates the remaining functional mismatch repair gene allele. LS is characterized by an early onset of colorectal cancer and increased risk for the occurrence of several extra-colonic malignancies, in particular endometrial cancer.
It was recently shown that germline deletions involving the last exons (8 and 9) of EPCAM gene, located approximately 16-kb upstream to MSH2 gene, may silence its neighbouring gene MSH2 with subsequent loss of MSH2 expression from the affected allele, by a mechanism known as promoter hypermethylation. This epigenetic inactivation seems to be effective only in tissues in which EPCAM gene is expressed. The EPCAM gene codes for the epithelial cell adhesion molecule protein, which is expressed in all normal epithelial cells as well as in carcinoma tumours.
Clinical report : We report an index female case of LS, from a family with several other cases of malignancies. This female had gastric, colorectal, small bowel and thyroid cancers. Large rearrangements were analyzed in peripheral blood sample for MLH1, MSH2 and MSH6 genes using MLPA (Multiplex Ligation – dependent Probe Amplification) (kits P072-C1 and P003-C1) according to the manufacturer's recommended protocol. In these MLPA kits the probes for exons 8 (only in kit P072-C1) and 9 of EPCAM gene were deleted. A subsequent test using MS (Methylation Specific)-MLPA for MMR genes (kit ME011-B3) was performed in peripheral blood and showed no hypermethylation of MSH2 promoter.
Discussion : The 3´ end EPCAM germline deletion detected in this patient is described in literature and is consistent with the status of the patient and may explain the several cases of colon / extra-colonic malignancies in this family. The detection of this germline deletion allows genetic counselling for all the first grade relatives.
As described in literature, the expression of EPCAM gene is high in epithelial tissues, among which are the main target tissues in LS and that could be the explanation for absence of hypermethylation of MSH2 in peripheral blood.
Further MLPA and MS-MLPA studies in DNA isolated from paraffin-embedded tumour tissue are in progress.

P60 | Quality Control and Public Services

A new portuguese instrument for quality assessment of genetic counselling practice: the contribution of national genetics healthcare services.

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Introduction
The importance of appropriate genetic counselling when offering genetic testing has been consensual. Although there are some guidelines for genetic counselling practice the tools and measures for quality assessment still insufficient. Recent national studies on consultands and professionals views, as well as a European study on this matter highlighted the need of the development of effective assessment instruments and quality indicators that support them. We present here the methodological design and preliminary results of a new Portuguese instrument for assessing the quality of genetic counselling practice at genetics healthcare services.

Methodology
After identifying a first group of 74 items resulting from previous national studies and literature, the instrument was submitted to a pre- test validation with five national experts who assessed their clarity, format and content, appropriateness, usefulness and relevance.

Results
Based on these procedures two main dimensions were outlined: (1) related to the effects of genetic counselling on consultands and (2) about the quality of the process itself. The first dimension include items that explore consultands motivations and expectations; familial and individual understanding of genetic risk; the decision making process as well as the consultand's emotional reactions. The dimension that refers to the quality of the process includes items about professional skills and model of practice, about the quality of engagement, empathy, respect for consultands autonomy and the existence of a reflective practice by the professional, among others.

Discussion
Soon the instrument shall be subjected to a statistical analysis of items testing the accuracy and value of the information collected, which will allow the selection of the best items. The process of psychometric validation should be completed next December and is expected to have the tool already available in early 2017. It is expected that this instrument can be useful both, in assessing the quality and effectiveness of the genetic counselling practice and monitoring the improvement of national genetic healthcare services.

P61 | Quality Control and Public Services

Eleven years experience of being under external quality assessment for the molecular genetic diagnosis of hereditary haemochromatosis HFE-associated and accreditation under ISO 15189

Loureiro P*[1]; Gomes S[1]; Torgal H[2]; Isidro G[3]; Gonçalves J[1,4]

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Laboratory quality is continuously present in our daily practice of molecular diagnosis in human genetics. Our participation in external quality assessment (EQA), specially with EMQN, gave us the opportunity to improve the reports of our genetic tests, to compare our performance/scores with other European laboratories, and to be permanently updated with the most recent recommendations for best practice in human molecular diagnosis.
Since 2005, our lab has been participating in the EMQN external quality assessment program for the molecular diagnosis of hereditary haemochromatosis HFE-associated (HH-HFE). Since then, our score regarding the genotyping category has achieved every year the highest marks (2.0), while for the interpretation category, in two years, the mean score was 1.75. These scores lead us to improve the "interpretation section" of our reports in order to give the best result to the physicians and patients. In addition of a correct genotyping, the report "interpretation section" is particularly important, it should include the main suggestions for the best patient's clinical management: i) immediate impact of the result for the patient, ii) patient's clinical follow-up and other diagnostic options, iii) long term-impact (specially in predictive tests), iv) relevance of the result for relatives and, v) recommendations of genetic counselling.
In accordance with OCDE disease specific guidelines for quality assurance in molecular genetic testing, and with the requirements of ISO 15189, in 2014 we were the first Portuguese laboratory accredited by IPAC, for HH-HFE - variants p.H63D and p.C282Y, and other genetic tests (http://www.ipac.pt/pesquisa/ ficha_15189.asp?id=E0015). Accreditation under the International Standard ISO 15189 is challenging but contributes to introduce improvements in our current practice because it comprises "management requirements" (e.g. quality management system, external services and supplies, preventive and corrective actions, control of records) and "technical requirements" (e.g. accommodation and environmental conditions, laboratory equipment, reagents and consumables, training and qualifications of personnel, examination processes, results reporting).
Accreditation enhances laboratory quality at different levels, gives credibility, competency and confidence, but primarily contributes to a better patient's clinical diagnosis reducing the turnaround time, patient management and treatment, and genetic counselling

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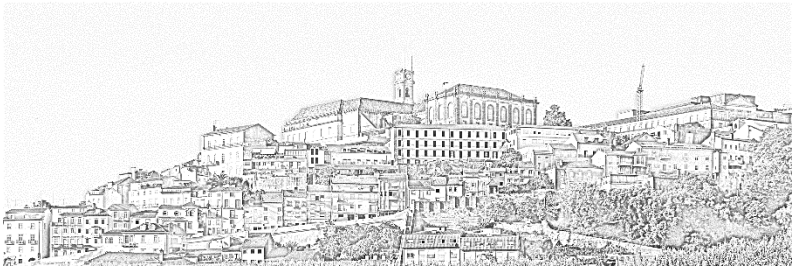

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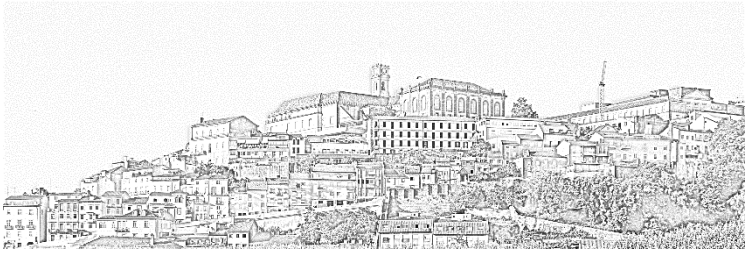

Thursday, 10th November

Dinner – Free night. No official reservations. However, we invite everyone to come to the historic center, **Almedina e Escadaria do Quebra-Costas**, were we will have dinner with our speakers. You can either make a dinner reservation there or join us later for a drink.

Restaurants suggestions:

- Tapas nas costas
  Rua de Quebra Costas 19, Coimbra 3000-340, Portugal

- Fangas
  Rua de Fernandes Thomas 45-49, 3000-168 Coimbra

- Arcada
  Rua Fernandes Tomás, 91, Coimbra 3000-266, Portugal

- Zé Neto
  R. das Azeiteiras 8, 3000 Coimbra

- Maria Portuguesa
  Rua Joaquim António de Aguiar, N°128, 3000-230 Coimbra

Friday, 11th November

Conference Dinner – 20:00h

Restaurant Loggia

Machado de Castro Museum
Largo Dr. José Rodrigues – 3000-236 Coimbra

Price: Participant: 15€*
Accompanying person:22€

* This year SPGH subsidizes part of the conference dinner price

Localization of Loggia.

Saturday, 12th November

***Brunch* com Ciência – 13:30 h**

Cafetaria do Museu da Ciência
Laboratorio Chimico, Largo Marquês de Pombal, 3000-272 Coimbra
GPS: 40°12'37"N, 8°25'25"W

Price: 14,5€

Coimbra Património Mundial – 15:00 h

Guided Tour, including Biblioteca Joanina e Pátio das Escolas

Price: 12€

Registration for all activities is required until 10th November.

NOTAS
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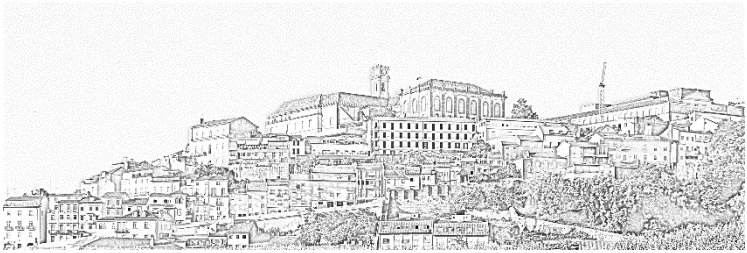

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