SOCIEDADE PORTUGUESA DE GENÉTICA HUMANA

22ª

Reunião anual

CIM FMUP
Porto

15-17 NOV, 2018

Caros colegas;

É com enorme satisfação que vos damos as boas vindas ao Porto, que este ano acolhe a Reunião Anual da Sociedade Portuguesa de Genética Humana, já na sua 22ª edição.

À semelhança do que aconteceu em reuniões anteriores, houve a preocupação de trazer à discussão as recentes inovações na área da genética, com destaque para alguns exemplos de regulação e desregulação epigenética, para o papel revolucionário da imunoterapia no tratamento do cancro e para novos avanços no diagnóstico etiológico e na terapia das doenças neurológicas. A edição do genoma é outro tópico que está na ordem do dia, e as implicações éticas serão tema de discussão na sessão de Bioética.

Os oradores convidados são cientistas de renome cuja presença muito nos honra, e o seu contributo será, sem dúvida, muito enriquecedor. O sucesso da reunião, porém, depende da participação ativa e interessada de todos os presentes; desfrutem desta oportunidade excecional para aquisição de novos conhecimentos!

Agradecemos à Comissão Científica por, mais uma vez e de forma exemplar, ter contribuído para a elaboração do programa, seleção dos resumos e escolha dos trabalhos premiados. Estão de parabéns os elementos da Comissão Local, que foram incansáveis em todo o processo de organização do evento. Não podemos esquecer as empresas que apoiaram esta reunião da SPGH de diversas formas, incluindo via o patrocínio de palestrantes e simpósios para apresentação de novidades tecnológicas.

Na vertente social da reunião teremos a visita guiada, seguida do jantar, numas caves do Vinho do Porto. É um programa quase obrigatório para quem visita esta cidade. E estamos em Novembro; muitos concordarão que o tempo convida a um cálice de vinho do Porto.

Um brinde à nossa Sociedade!

A Comissão Organizadora,

Rosário Santos, João Silva, Rosário Pinto leite

Dear colleagues;

It is with great pleasure that we welcome you to Porto, the host city of this year's Annual Meeting of the Portuguese Society of Human Genetics, which is now into its 22nd edition.

In line with previous meetings, efforts were made to bring to discussion recent innovations in the field of human genetics, with the spotlight on examples of epigenetic regulation and dysregulation, the revolutionary role of immunotherapy in cancer treatment, and the new advances in etiologic diagnosis and therapy of neurological disorders. Genome editing is yet another topic on the current scientific agenda, and its ethical implications will be addressed in the Bioethics session.

Our guest speakers are reknown scientists, who honour us with their presence, and their contributions will undoubtedly enrich the meeting. Ultimately, however, the success of the meeting depends on the interest and active participation of all attendees; do make the most of this exceptional opportunity for enlightment!

We thank the Scientific Committee for once again contributing towards elaborating the programme, selecting submitted Abstracts and electing the award-winning presentations. The members of the Local Organizing Committee are to be congratulated for their tireless work throughout all preparative stages of the event. Our gratitude extends to the sponsors, who supported this SPGH meeting in various ways, including sponsorship of guest speakers and symposia for the presentation of new technologies.

The social component of the meeting consists of a guided tour, followed by dinner, at one of the Port Wine cellars. It is an almost obligatory programme for those who visit this city. And it's November; many would agree that the weather calls for a glass of Port wine.

A toast to our Society!

The Organizing Committee,

Rosário Santos, João Silva, Rosário Pinto Leite

COMISSÃO ORGANIZADORA | **ORGANIZING COMMITTEE**

Rosário Santos
João Silva
Rosário Pinto Leite

COMISSÃO LOCAL | **LOCAL ORGANIZING COMMITTEE**

Emília Vieira
Paula Jorge
Paula Machado

COMISSÃO CIENTÍFICA | **SCIENTIFIC COMMITTEE**

Rosário Santos
Luísa Romão
Isabel Marques Carreira
Carla Oliveira (Coordenadora)
Ana Berta Sousa
Joana B. Melo
Lina Ramos
Manuel Teixeira
Sofia Dória
Susana Fernandes
José Carlos Machado
Sérgio Bernardo de Sousa

SECRETARIADO | SECRETARIAT

DIVENTOS
Congressos e Eventos, Lda.

Apoios Comerciais | Sponsors

A SPGH agradece às seguintes empresas o apoio financeiro ao evento:
SPGH thanks the following companies for sponsoring the event:

Diamante Plus

AstraZeneca · ThermoFisher Scientific · Biogen · Agilent · SOQUÍMICA

Diamante

BIOMARIN · I.L.C. - Instrumentos de Laboratório e Científicos, Lda.

Platina

DIAGNÓSTICA LONGWOOD · SYNLAB

Ouro

CGC genetics · Elsie · Germano de Sousa Centro de Medicina Laboratorial · Pfizer · Roche

Prata

A. Menarini diagnostics · Biocartis · Genetyca-ICM · nzytech · Rofer · Sarstedt · VWR

Bronze

Bioportugal · CeGaT · IBMC · CGPP · Citomed · GenomePT · PTC Therapeutics · Sanofi Genzyme · Sistemas Genómicos · Werfen Diagnostic Solutions for Life

Parcerias Científicas e Institucionais | Scientific and Institutional Partners

A SPGH agradece às seguintes organizações o apoio científico /institucional:
SPGH thanks the following organizations for the scientific/institutional support:

Luso-American Development Foundation · U.Porto FMUP Faculdade de Medicina Universidade do Porto · Ordem dos Médicos · Ordem dos Biólogos · anbioq · Sociedade Portuguesa de Neurologia · Sociedade Portuguesa de Neuropediatria · spdm Sociedade Portuguesa de Doenças Metabólicas · IPOPORTO · IPO Coimbra · Liga Portuguesa Contra o Cancro

ÍNDICE | INDEX

PROGRAMA CIENTÍFICO | SCIENTIFIC PROGRAMME

THURSDAY 15TH NOVEMBER

09:00h **REGISTRATION**

10:00h **SPGH CLUB MEETINGS** (Concurrent)

MOLECULAR AND CYTOGENETICS CLUB (Room 3)

Pitfalls in genomic analysis

Chairs: Paula Jorge; Cristina Candeias

DEB test for diagnosis/exclusion of Fanconi Anemia (FA): WHAT IF the test excludes FA but chromosome instability is significantly increased compared with controls?
Beatriz Porto (Laboratório de Citogenética, ICBAS, UP, Porto)

DSP 30 e IL2 na Leucemia linfocítica crónica
Marta Souto (Laboratório de Citogenética, H. São Pedro de Vila Real, CHTAD, Vila real)

NGS panels and hereditary cancer
Natália Salgueiro (GDPN/SYNLAB Porto)

Dilemas em pré-natal. Apresentação de dois casos: "mosaicismo" e "imprinting"
Vera Lima (Serviço de Genética, Deptº de Patologia, FMUP, Porto)

Inglourious Ring(16)
Bárbara Marques (Laboratório de Citogenética, Deptº de Genética, INSARJ, Lisboa)

Dilemas em arrayCGH
Paula Rendeiro (Laboratório de Citogenética, CGC Genetics)

Pseudogenes: a threat in WES diagnostics
Isabel Alonso (CGPP e UnIGENe, IBMC, i3S, Porto)

Ceroidolipofuscinose neuronal: mutação no gene CLN3 em "homozigotia" detectada em WES
Rita Cerqueira (Laboratório de Diagnóstico Molecular e Genómica Clínica, CGC Genetics)

Pitfalls in Next Generation Sequencing
José Luís Costa (IPATIMUP, Porto)

Duplicação e deleção intersticial em array? A FISH resolve!
Cristina Candeias (Unidade de Citogenética, CGMJM, CHUP, Porto)

DYSMORPHOLOGY AND CLINICAL GENETICS CLUB (Room 4)

Chairs: Gabriela Soares; Renata Oliveira; Cláudia Reis

10:00h *Blefarofimose, ptose e outras anomalias palpebrais: revisão do tema e apresentação de casos com diagnóstico*
Ana Rita Soares; **Teresa Saraiva** (S. Genética Médica, CGMJM, CHUP)

10:40h Apresentação e discussão de casos de outros Serviços, subordinados ao tema

12:00h Apresentação e discussão de outros casos, com ou sem diagnóstico

12:30h **SATELLITE MEETING: EUROPEAN REFERENCE NETWORKS IN PORTUGAL** (Room 3)

Panel Discussion: ***What are ERNs and what is in there for me?***
Chairs: Carla Oliveira; Sérgio Sousa

12:30h *What are ERNs and why were they created?*
Carmo Fonseca (Leader, Working Group for Implementation of ERNs in Portugal)

12:45h *Spreading the knowledge at the National level*
Carla Oliveira (National Coordinator GENTURIS ERN; Representative of P.CCC)

12:50h *ERN on bone disorders (ERN BOND) and ERN on congenital malformations and rare intellectual disability (ERN ITHACA)*
Sérgio Sousa (National Coordinator BOND ERN; Representative of CHUC, Coimbra)

13:00h *European Reference Network on genetic tumour risk syndromes (ERN GENTURIS)*
Carla Oliveira (National Coordinator GENTURIS ERN; Representative of P.CCC)

13:05h *The large ERN-collaborative project - Solve-RD*
Carla Oliveira

14:00h **OPENING & WELCOME**
Rosário Santos; João Silva; Rosário Pinto Leite

14:15h **KEYNOTE LECTURE**
Chair: João Silva

The Genetic Landscape of ID
Anita Rauch (Inst. of Medical Genetics, Zurich)

15:00h **EPIGENETICS**
Chairs: Carla Oliveira; Sofia Dória

The role of Imprinting in Cancer
David Monk (Inst. d'Investigació Biomédica, Barcelona)

Imprinting errors in spermatogenic cells of infertile patients
Joana Marques (Dept. Patologia, HSJ, Porto)

Establishing the human epigenome in development and pluripotency
Peter Rugg-Gunn (Babraham Institute, Univ. Cambridge)

16:30h **COFFEE-BREAK / POSTER VIEWING**

16:45h **POSTER HIGHLIGHTS:** Speed talks **(P1-P11)** (AUDITORIUM)

17:30h **SELECTED ORAL PRESENTATIONS (I) _ BASIC RESEARCH (OP1-OP5)**
Chairs: Joana Barbosa de Melo; Susana Fernandes

18:30h **SPGH GENERAL ASSEMBLY**

FRIDAY 16TH NOVEMBER

08:45h **SELECTED ORAL PRESENTATIONS (II) _ CLINICAL RESEARCH (OP6-OP10)**
Chairs: Isabel Marques; Paula Jorge

09:45h ***MEET THE EXPERT*: CLN2, A FORM OF BATTEN DISEASE** (SPONSORED BY BIOMARIN)
Chair: Manuela Santos

An algorithm for CLN2 Diagnosis
Miguel Leão (Dept. Genética Humana, HSJ, Porto)

CLN2 disease- from early diagnosis to early intervention
Eva Wibbeler (Dept. Paediatrics, UMC Hamburg-Eppendorf, Hamburg)

10:30h **COFFEE-BREAK / POSTER VIEWING**

10:45h **POSTER DISCUSSIONS** (e-Posters)

11:15h **IMMUNOTHERAPY AND GENETICS**
Chair: José Carlos Machado

Harnessing the immunotherapy revolution for the treatment of colorectal cancer: neo-antigens and beyond
Noel de Miranda (Leiden Univ. Medical Center, Leiden)

The genetic basis of response to checkpoint inhibitors
Vassiliki Kotoula (AUTH Medical School, Thessaloniki) (SPONSORED BY ASTRAZENECA)

CAR-T for the treatment of T cell malignancies
John F. DiPersio (Alvin J. Siteman Cancer Center / Div. Oncology, Washington Univ. Medical School) (SPONSORED BY FLAD)

13:00h **LUNCH BREAK**

14:00h **NEUROGENETICS AND THERAPY**
Chairs: Jorge Oliveira; Luísa Romão

Towards modifying disease progression and onset in Huntington's Disease and other genetic neurodegenerative disorders: hopes and challenges
Bernhard Landwehrmeyer (Dept. Neurology, Univ. of Ulm)

LRP10: a novel player in late-onset inherited synucleinopathies
Wim Mandemakers (Erusmus Medical Center, Rotterdam)

Fluid Biomarkers in Neurodegenerative Diseases
Luís Maia (Dept. Neurologia, CHUP, Porto)

Advanced therapies in SMA: beyond the clinical trials
Eduardo Tizzano (H. Valle Hebron, Barcelona) (SPONSORED BY BIOGEN)

16.15h **COFFEE-BREAK / POSTER VIEWING**

16:30h **POSTER DISCUSSIONS** (e-Posters)

16:45h **CORPORATE SYMPOSIUM _ AGILENT / SOQUÍMICA**
A clinical case: From the sample to the analysis on the NGS Workflow
Alba Mota; **Ivan Lesende**

17:15h **CORPORATE SYMPOSIUM _ 10X GENOMICS / ILC LDA.**
10x Genomics technology in Human Genetics: from bulk long range sequencing to Copy Number variation in Single Cells
Hannes Arnold

17:45h **KEYNOTE LECTURE**
Chair: Sérgio Sousa

The genetics of cognitive epigenetics
Hans van Bokhoven (Radboud Univ. Medical Center / Donders Inst. for Brain, Cognition & Behaviour, Nijmegen)

20:00h **CONGRESS DINNER**

SATURDAY 17TH NOVEMBER

09:00h **SELECTED ORAL PRESENTATIONS (III) _ CLINICAL CASE REPORTS (OP11-OP18)**
Chairs: Ana Berta Sousa; Lina Ramos

10:00h **BIOETHICS DEBATE** (SPGH BIOETHICS COMMITTEE)
GENOME EDITING – WHAT, WHY AND HOW? THE ETHICAL ISSUES
Chair: Heloísa Santos

CRISPR-Cas 9 – Method and potential applications
André Travessa (S. Genética, Hospital de Santa Maria)

Genome editing - from benefits to ethical limits
Heloísa Santos (SPGH Bioethics Com. / S. Genética, HSM)

Current Portuguese legislation and ethical guidelines
André Pereira (SPGH Bioethics Com. / Fac. Law, U. Coimbra)

11:00h **COFFEE-BREAK / POSTER VIEWING**

11:30h **KEYNOTE LECTURE**
Chair: José Luís Costa

Unlocking the diagnostic potential for circulating DNA
Y.M. Dennis Lo (Fac. of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China) (SPONSORED BY ALFAGENE)

12:15h ***SPGH AWARD* LECTURE**
Chair: Carla Oliveira

A Pentanucleotide ATTTC Repeat Insertion in the Non-coding Region of DAB1, Mapping to SCA37, Causes Spinocerebellar Ataxia
Joana R. Loureiro,***Ana I. Seixas**,* *et al*.
(Am J Hum Genet 2017; 101(1):87–103) *equal contributors

12:30h **SPGH AWARDS CERIMONY**

12:45h **CLOSING SESSION**

ORADORES CONVIDADOS | GUEST SPEAKERS

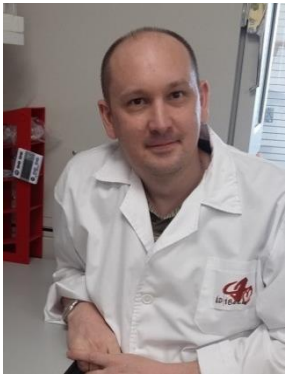
Anita Rauch, MD, PhD

Full Professor of Medical Genetics,
Director, Institute of Medical Genetics
University of Zurich
Schlieren, Switzerland

Anita Rauch is Professor of Medical Genetics at the University of Zurich. In 2009 she was appointed as director of the Institute of Medical Genetics in Zurich and heads the University's diagnostic genetic laboratory and outpatient clinic. She is a member of the research counsel of the Swiss National Science Foundation, a member of the Leopoldina, and a board member of the Swiss Academy of Medical Sciences as well as of the Swiss Society of Medical Genetics.

Her current research interests are reflected by steering board memberships in the Swiss Personalized Health Network and the Rare Disease Initiative Zurich (radiz). She uses high-throughput sequencing methods to systematically evaluate the genetic landscape of neurodevelopmental disorders, as well as a variety of essays and models including hiPSC-derived neurons to investigate genotype-phenotype correlation.

Her routes are in Germany, where she studied Medicine and performed specialized training in Human Genetics at the Universities of Regensburg and Erlangen-Nürnberg. In her early career she contributed important studies in genotype-phenotype correlation in a variety of chromosomal and microdeletion syndromes, especially concerning the 22q11.2 microdeletion syndrome. Her group also identified the genetic etiology of a variety of rare disorders, amongst others of primordial dwarfism type MOPDII.



David Monk, PhD, MSc, BSc(Hons)

Principal Investigator,
Imprinting and Cancer Group
Cancer Epigenetics and Biology Program,
Bellvitge Institute for Biomedical Research (IDIBELL),
Barcelona, Spain

David Monk obtained his PhD in 2001 from Imperial College London for cytogenetically defining the Chr7 critical region for the imprinting disorder Silver-Russell syndrome. This was followed by several post-doctoral projects characterizing epigenetic regulation in both mouse and humans, including a multicenter MRC funded position at MRC Harwell, Oxford, The Babraham Institute, Cambridge and Institute of Reproductive and Developmental Biology (IRDB), Imperial College London. After completing his final post-doc position at the Institute of Child Health (ICH), University College London, Dr Monk moved to the Cancer Epigenetic and Biology Program (PEBC), Bellvitge Institute for Biomedical Research (IDIBELL) in Barcelona where he was awarded a Ramòn y Cajal career development fellowship to establish his own research group. His teams' research is centered on understanding epigenetic mechanisms relevant to human diseases, focusing on the application of high-throughput genome technologies to analyze genomic imprinting. In particular, his studies have addressed the role of these epigenetically regulated transcripts in imprinting disorders, as well as in common morbidities, including fetal growth, fertility and cancer. Recently, his group has utilized methyl-seq to characterized methylation in human gametes and pre-implantation embryos, resulting in the identification of novel transient imprinted DMRs and high-throughput methylation arrays to study cancer. In addition to his research activities, Dr Monk was involved in the European COST network for Human Congenital Imprinting Disorders (EUCID; 2015-2017), being the coordinator of the Molecular Biology working group. To date he has over 100 publications.

Joana Marques, PhD, BSc(Hons)

Assistant Researcher
(FCT Starting Grant Researcher)
Genetics Unit, Department of Pathology,
Faculty of Medicine, University of Porto
Porto, Portugal

Ever since Dr. Marques graduated in Biology in 2001, she has been pursuing a career in research with a central theme on epigenetics. During her PhD, she focused on the mechanism of genomic imprinting in human spermatogenesis, describing for the first time the occurrence of imprinting errors in patients with defective spermatogenesis (Marques et al., Lancet 2004; Mol Hum Reprod 2008; Fertil Steril 2010; Andrology 2017).

After completing her PhD, in 2009, she moved to the lab of Prof. Wolf Reik at the Babraham Institute in Cambridge, UK, to work on the epigenetic regulation of pluripotency. During her postdoc, she developed a stable and inducible RNAi knockdown system to test the function of TET enzymes in mouse embryonic stem cells (Ficz et al., Nature 2011). She also analysed the expression of the TET family in oocytes, zygotes and early embryonic developmental stages (Wossidlo et al., Nat Commun 2011).

From 2012 to 2016, she worked at the Life and Health Sciences Research Institute (ICVS), University of Minho, focusing on the role of TET enzymes in neural differentiation. In August 2016 she moved back to the Faculty of Medicine of Porto to resume her original field of research, namely the epigenetic regulation of gametogenesis and embryogenesis.

Peter Rugg-Gunn, PhD

Group Leader, Epigenetics Programme
Babraham Institute, Cambridge, UK
Associate Principle Investigator
Wellcome-MRC Cambridge Stem Cell Institute,
Cambridge, United Kingdom

Peter Rugg-Gunn, Ph.D., is a Group Leader within the Epigenetics Programme at the Babraham Institute in Cambridge, UK. His research interests centre on understanding how the epigenome is established during human development and how this affects cell fate decisions in pluripotent stem cells and in embryos. His current work is focused on the mechanisms of epigenetic programming and reprogramming as human cells transition between pluripotent states.

Peter's interest in the epigenetic regulation of stem cells was sparked during his Ph.D. with Roger Pedersen at the University of Cambridge. Here, Peter investigated the status of imprinted genes as a means of assessing the epigenetic stability of human embryonic stem cells. Peter subsequently moved to Toronto for his post-doctoral training with Janet Rossant at The Hospital for Sick Children. His research identified lineage-specific epigenetic differences that are established during mouse development. Peter was then awarded a Wellcome Trust Research Career Development Fellowship in 2011, which he undertook at the Babraham Institute. He was awarded tenure in 2017.

Miguel Leão, MD, PhD

Clinical Geneticist,
Coordinator of the Neurogenetics Unit,
Department of Genetics
S. João Univ. Hospital Center
Porto, Portugal

Dr. Miguel Leão is the Coordinator of the Neurogenetics Unit of Hospital de S. João, in Porto, since 2012. He graduated in Medicine in 1984, at the Faculty of Medicine of Porto (FMUP) and is Board Certified by the Portuguese Medical Association (OM - Ordem dos Médicos) in Neurology (1995), Medical Genetics (1998) and Paediatric Neurology (2001).

Dr. Miguel Leão is currently a Visiting Professor of Medical Genetics at the FMUP since 2009, but has been since early in his career Guest Assistant of Medical Genetics (FMUP, 1982-1994), Genetics (FCNAUP, 1987-1991) and Legal Medicine (UCP, 1987-1996).

Besides his Clinical and Academic career, Dr. Miguel Leão has a long history of involvement in the OM at various levels, having held various posts including Board Secretary ((1990-1992) and President (1999-2004) of the Northern Regional Council of the OM.

Dr. Miguel Leão has authored or co-authored more than 20 PubMed indexed publications and has been Scientific Advisor of collaboration protocols with different institutions, as well as member of several National and Governmental Scientific and Technical Committees.

Eva Wibbeler, MD, PhD

Paediatrician,
University Medical Centre Hamburg-Eppendorf,
Hamburg, Germany

Eva Wibbeler is a Paediatrician at the University Medical Centre Hamburg-Eppendorf Children's Hospital, Germany. Under the supervision of Dr Angela Schulz, she has worked at the hospital in the Specialty Clinic for Degenerative Brain Disorders in Children and Adolescents, a centre of excellence for NCL Disorders, since 2016.

Dr Wibbeler received her medical degree from the University of Essen in 2012. Between 2010 and 2016, her research focused on neuromuscular diseases. Her PhD explored the long-term clinical aspects and genetic background of congenital myasthenic syndrome, which she completed in 2016.

Dr Wibbeler is a member of the clinical research group for neurodegenerative brain diseases in children at the International Centre of Lysosomal Storage Disorders. She is also a sub-investigator for clinical trials investigating intracerebroventricular ERT in patients with CLN2, as well as patients with MPS.

Noel de Miranda, PhD

Assistant Professor
Head of the Immunogenomics group
Department of Pathology,
Leiden University Medical Center
Leiden, The Netherlands

The Immunogenomics group has been focusing on the design of neo-antigen targeted therapies to trigger immune responses in cancers that are traditionally viewed as non-immunogenic such as mismatch repair-proficient colorectal cancers. This research strongly relies on the application of next-generation sequencing technologies for the characterization of cancer somatic mutations in a personalized setting. Furthermore, we also apply deep immunophenotyping technologies such as imaging and single-cell mass cytometry as well as single-cell RNA sequencing to disentangle the complexity of the cancer immune microenvironment and for the discovery of immune cell subsets with anti-cancer properties.

Noel de Miranda obtained his PhD in colorectal cancer immunity at the Leiden University (The Netherlands). Between 2010 and 2013 he was a postdoctoral fellow at Karolinska Institutet where he applied next-generation sequencing technologies for the characterization of genetic alterations in diffuse large B-cell lymphomas. Since 2015 he is the head of the Immunogenomics group at the department of Pathology of the Leiden University Medical Center. He is recipient of a Fight Colorectal Cancer-Michael's Mission-AACR fellowship, a VENI award (Dutch Organization for Scientific Research), and a Dutch Cancer Society Young Investigator Award.

Vassiliki Kotoula, MD, PhD

Department of Pathology,
School of Medicine, Aristotle University of Thessaloniki (AUTH),
Laboratory of Molecular Oncology, Hellenic Foundation for Cancer Research (HeFCR)
/ Hellenic Cooperative Oncology Group (HeCOG) / AUTH
Thessaloniki, Greece

Vassiliki Kotoula is associate professor in Pathology specialized in Molecular Pathology. She received her M.D. from AUTH (1982), her Ph.D on experimental pathology and cancer prophylaxis from AUTH (1992). She is a certified Cytopathologist. She trained in basic research and applied molecular biology and genetics at the Dept. of Pathology, NCI, NIH, Bethesda, MD (Pediatric Pathology lab, 1995-1997). In 1998, she developed a laboratory for tissue molecular diagnostics within the Dept. of Pathology, AUTH, which she continues to supervise, performing all major molecular tests on routine histologic material for research and diagnosis (certified for KRAS and EGFR mutation testing). In 2009, in collaboration with HeFCR and HeCOG (non-profit organizations in Greece promoting clinical and translational cancer research), she developed and has since supported and scientifically supervised the Laboratory of Molecular Oncology (MOL), within the Dept of Pathology, AUTH, where she devoted most of her time and efforts. In 2013, she introduced NGS with multigene panels on FFPE tissues in MOL. To date, MOL has genotyped and analyzed more than 5000 tumors along with histology and patient data from the repository and databases by HeCOG. The central idea behind all her efforts is bridging knowledge gaps between pathology, clinical practice, molecular biology and genetics. Her major field of interest is translational cancer research in cancer genetics and genomics for tumor typing in the context of personalized / precision medicine. As a pathologist, she is interested in studying genetic and phenotypic alterations in tumors and microenvironment, including tumor immune infiltrates. In this context, she has designed and conducted numerous studies and developed assays for the application of molecular methods on FFPE tissues / matched germline, and interpretation of results. At the academic level, in the last 20 years, she has been actively involved instructing issues on molecular pathology and cancer genetics to pathologists and clinicians.

John F. DiPersio, MD, PhD

Virginia E. and Samuel J. Golman Professor in Medicine
Chief, Division of Oncology,
Washington Univ. School of Medicine, St. Louis
Deputy Director,
Alvin J. Siteman Cancer Center
Professor of Medicine, Pathology & Immunology
St. Louis, MO, USA

Dr. DiPersio's research focuses on fundamental and translational aspects of leukemia and stem cell biology. These studies include identification of genetic abnormalities in human leukemias, understanding processes involving stem cell and leukemia cell trafficking, and clinical and translational programs in both leukemia/myelodysplastic syndrome and stem cell transplantation.

Dr. DiPersio is President of American Society of Blood and Marrow Transplantation, a member of the Board of Scientific Counselors (Clinical Science and Epidemiology) of the National Cancer Institute, an elected member of American Society for Clinical Investigation and American Academy of Physicians (AAP), previously Chair of the American Society of Hematology (ASH) Scientific Committee on Hematopoiesis and the 2013 recipient of the Daniel P. Schuster Distinguished Translational Investigator Award from Washington University, the 19th Annual AACR Joseph H. Burchenal Memorial Award for Outstanding Achievement in Clinical Cancer Research in 2014 and the 2014 recipient of the American Society of Hematology Mentor Award for Clinical Investigations. He has authored or co-authored more than 300 publications and over 60 invited reviews and book chapters.

Dr. DiPersio received his M.D. and Ph.D. from the University of Rochester and his B.A. in Biology from Williams College. He completed an internship and residency at Parkland Memorial Hospital and The University of Texas Southwestern Medical Center in Dallas. After serving as chief resident at Parkland Memorial Hospital, Dr. DiPersio completed a fellowship in the Division of Hematology/Oncology at the University of California, Los Angeles (UCLA) where he stayed on as an Assistant Professor before moving to the University of Rochester and then four years later to Washington University.

G. Bernhard Landwehrmeyer, MD, PhD, FRCP

Professor of Neurology,
Ulm University Hospital,
Ulm, Germany

G. Bernhard Landwehrmeyer, MD, FRCP is Full Professor of Neurology at Ulm University Hospital, Department of Neurology, where the Central Coordination of the European Huntington's Disease Network (EHDN) is situated.

In 2004 he was instrumental in founding EHDN and served as chairman of the Executive Committee until 2014. EHDN serves as a platform for professionals, people affected by Huntington's disease (HD), and their relatives to facilitate working together throughout Europe and conducts large prospective natural history studies in HD, e.g. the REGISTRY study. EHDN and REGISTRY is generously funded by the CHDI Foundation (USA).

Professor Landwehrmeyer received his MD degree and Doctoral Degree from the Albert-Ludwigs-University, Freiburg. He was trained at the Royal Victoria Hospital, Queen's University, Belfast, at the Kantonsspital, Basel and worked as a post-Doc from 1993 -1996 at MGH, Harvard Medical School, Boston. From 1995–1999, he was staff member at Albert-Ludwigs-University (Departments of Neurology and Psychiatry). In 1999 he received his Board Certification in Neurology and a year later the Venia Legendi and full Professorship ('C3') in Neurology at the University of Ulm. He served as Principal Investigator in numerous HD trials and is Global PI of the CHDI-sponsored Enroll-HD study - a prospective longitudinal observational study on HD and a clinical research platform with a worldwide reach that annually collects phenotypical clinical data and biomaterials.

Wim Mandemakers, PhD

Senior Scientist,
Department of Clinical Genetics,
Erasmus Medical Center,
Rotterdam, The Netherlands

After obtaining his PhD at Erasmus MC, Rotterdam, the Netherlands, and postdoctoral periods in the Labs of Dr. Ben Barres at Stanford University, USA and Dr. Bart de Strooper at KUL/VIB in Leuven, Belgium, Wim Mandemakers is currently a senior scientist at the Clinical Genetics department at Erasmus MC, Rotterdam, the Netherlands.

In close collaboration with Dr. Vincenzo Bonifati, the main goal of his research group is identification and functional characterization of genetic mutations in heritable forms of Parkinson's disease (PD) and other movement disorders. He uses patient derived induced pluripotent stem cells (iPS) that are differentiated into various brain cell types as a model system to study PD. His work on PD has contributed to insight into the molecular mechanisms that lead to pathology in PD, in particular the role of LRRK2 in synaptic vesicle recycling.

Wim Mandemakers received several honors and awards, including a Marie Curie Intra European Fellowship. He is also a member of the editorial board of Parkinsonism & Related Disorders. His work has been published in many peer-reviewed journals, including Science, EMBO journal, Neuron, Nature Neuroscience, Annals of Neurology. Most recently, work from Dr. Mandemakers and Dr. Bonifati and collaborators was published in Lancet Neurology, describing the identification of LRP10 as a novel gene in late-onset inherited synucleinopathies.

Luís F. Maia, MD, PhD

Clinical Neurologist,
Centro Hospitalar Universitário do Porto (CHUP)
Auxiliary Professor,
Instituto de Ciências Biomédicas, U. Porto
Invited Researcher,
Instituto de Biologia Molecular e Celular - i3S
Porto, Portugal

Dr. Luis Maia has been involved in clinical and translational neuroscience research since the beginning of his career: during his neurology residency together with Prof. Manuel Correia (Centro Hospitalar Porto, Portugal) unravelled new clinical phenotypes of Cerebral Amyloid Angiopathy; as a research fellow at Prof. Cheryl Wellington lab (University of British Columbia, Vancouver Canada) worked on Alzheimer´s Disease mouse models; and with Dr. Howard Feldman (Vancouver General Hospital and University of British Columbia, Vancouver, Canada) on new imaging biomarkers of Cerebral Amyloid Angiopathy. His PhD with Prof. Mathias Jucker (Hertie Institute of Clinical Brain Research, University of Tübingen, Tübingen, Germany), led to significant contributions on biomarkers' research using murine models of Alzheimer´s Disease. His research activities involve basic, clinical and translation research, as well as clinical trials in amyloid related pathologies (Alzheimer´s Disease, Cerebral Amyloid Angiopathy and Transthyretin amyloidosis). Most recent and relevant work has been published in Neuron 2016, EMBO Mol Med 2015, JNNP 2015, STM 2013. His current research focuses on new diagnostic and prognostic biomarkers of brain disorders highly prevalent in Portugal using parallel murine and human cohorts of Mixed dementia, Vascular cognitive impairment and Stroke, at i3S and CHUP. His translation research also involves multiple clinical trials, with a PI role in trials with disease modifying treatments for Alzheimer´s Disease.

Dr. Luís Maia, MD-PhD graduated from Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP) in 1998, is a Clinical Neurologist at *Centro Hospitalar e Universitário do Porto* (CHUP) since 2006, received his PhD in translational neurosciences in 2015 from the University of Tubingen – Germany after completing his thesis at the Hertie Institute for Clinical Brain Research. Since 2016 he is a Professor of Neurology at ICBAS-UP and a Researcher at i3S.

Eduardo Tizzano, MD, PhD

Director,
Dept. of Clinical and Molecular Genetics
and Rare Diseases,
Hospital Valle Hebron,
Barcelona, Spain

Professor Eduardo Tizzano, MD. PhD. was born in La Plata (Argentina) and is specialist in Pediatrics and Medical Genetics. He is actually Director of the Department of Clinical and Molecular Genetics and Rare Diseases of the Hospital Valle Hebron, one of the largest University Hospitals in Spain and Europe.

He graduated and doctorate from the University of La Plata (Argentina) and received medical training and medical practice in the two largest Pediatric Hospitals in South America: Ricardo Gutierrez and Juan P Garrahan (both located in Buenos Aires, Argentina). He was Postdoctoral fellow in the Hospital for Sick Children in Toronto, Canada (1990-1993) and Researcher and Consultant Faculty member in Pediatrics at the Hospital Sant Pau, Barcelona (1994-2013). His main areas of research include the characterization of SMA during human development, genotype-phenotype correlations, identification of modifier genes and validation of biological markers publishing extensively in these areas as well as in other genetic disorders. He is very active in academic and research activities at national and international levels collaborating with different neuromuscular and genetic centers and participates as PI and collaborator in clinical trials. Prof. Tizzano has close liaisons to regional, national and international patient support groups (ASEM, FUNDAME, FEDER, SMA Europe, AFM, UILDM, EURORDIS) as well as scientific groups and societies (CIBERER, AEGH, SEGCD,ESHG). He was recipient of several prizes in the field of rare diseases and neuromuscular disorders including the Queen Sophia Prize for his cumulative clinical, research and social work in SMA. He is also curator of the Spanish SMA Registry (FUNDAME), member of the TGODC (Treat-NMD), and coordinates SMA sample biobank and clinical research collaborations in Spain with the purpose to define therapeutic targets for the disease and support clinical trial readiness.

Hans van Bokhoven, PhD

Full professor of Neurogenetics,
PI and Group Leader,
Radboud University Medical Center and
Donders Institute for Brain, Cognition and Behaviour,
Nijmegen, The Netherlands

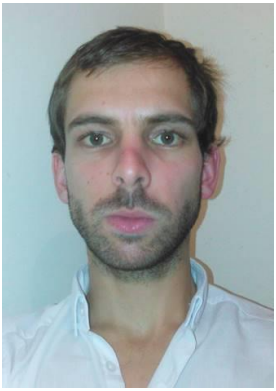
Hans van Bokhoven is heading the Molecular Neurogenetics Unit at the interface between the Departments of Human Genetics and Cognitive Neuroscience. The key goal is to reveal novel neurobiological concepts by resolving the genetic and epigenetic networks that are disrupted in neurodevelopmental disorders, including intellectual disabilities, autism spectrum disorder, and epilepsy. The team uses a multi-level strategy that combines clinical genetics (Dr. T. Kleefstra, Dr. B. De Vries), functional genomics and molecular & cellular neurobiological approaches, such as the generation and characterization of in vitro (primary neurons and hiPSC-derived human neural cells) and in vivo model organisms (Dr. N. Nadif Kasri). We aim to integrate pure fundamental neurobiological research with clinical applications and personalized medicine. In particular, our expertise in the generation of patient-derived neural lineages and their neuro-physiological analysis at single cell and network level (MEA) is setting the stage in this novel research field.

Education:
1987 - MSc in Agricultural Sciences (Engineer), Wageningen University, Netherlands.
1993 - PhD (Molecular Biology), Wageningen University, Netherlands.

Previous scientific or professional activities:

1992-1996:	Postdoc, Dept. of Human Genetics, Radboudumc, Nijmegen.
1996-2003:	Assistant professor Human Genetics.
2003-2010:	Associate professor/Research co-ordinator, Dept. Human Genetics.
2004-present:	PI of the Nijmegen Center for Molecular Life Sciences.
2008-present:	PI Donders Centre for Neuroscience (Nijmegen); PI Radboudumc.
2010-present:	Professor Molecular Neurogenetics.
2012-present:	Central Commission Human-related Research The Hague (CCMO); 10% appointment.

Scientific production:
- H-index (Web of Science): 67
- 280 articles with >13,250 total citations (~50 citations/article)
- Coordinator of two large EU collaborative projects: Euro-MRX and GENCODYS.



André M. Travessa, MD, MSc

Medical Genetics Fellow
Department of Medical Genetics,
Santa Maria Hospital (Centro Hospitalar Lisboa Norte)
Lisbon, Portugal

André Travessa graduated in medicine at Lisbon University Medical School in 2014. He is currently a medical genetics fellow in the Department of Medical Genetics of Santa Maria Hospital (Centro Hospitalar Lisboa Norte) and a teaching assistant of histology and developmental biology at Lisbon University Medical School.

He is member of the Portuguese Society of Human Genetics, the European Society of Human Genetics and the GruPEDGE working group, and member of the scientific committee of the Association for the Blind “Bengala Mágica”.

He published five papers in international peer-reviewed journals and presented several communications in national and international conferences and meetings. His main areas of interest are genetic skeletal disorders (including osteogenesis imperfect) and intellectual disability. He is involved in two research projects in the field of osteogenesis imperfecta.

He collaborates with other departments and research groups, including the Instituto de Genética Médica y Molecular (Hospital Universitario La Paz, Madrid, Spain).

Heloísa G. Santos, MD, PhD

President, SPGH Bioethics Committee
Department of Medical Genetics,
Santa Maria Hospital (CHLN) /
Faculty of Medicine, University of Lisbon
Lisbon, Portugal

Consultant in Clinical Genetics and Paediatrics. President of SPGH Bioethics Committee. Head of Genetics Unit of Paediatric Service, Hospital Santa Maria (1974–1999). Training with CO Carter in medical genetics and cytogenetics in Clinical Genetics Unit, Institute of Child Health, London (1978). First Director of Medical Genetics Service of Hospital Santa Maria, Lisbon (1999–2004). GENOMED—Consultant in Medical Genetics of Institute Molecular Medicine of Faculty Medicine. University Lisbon (2004-2007). Permanent Consultant in Medical Genetics of the Portuguese Directorate-General of Health since 1996. University Assistant of Medical Genetics in Faculty of Medical Sciences of University of Lisbon (1977-1982). University Assistant of Medical Genetics of Faculty of Medicine University of Lisbon (FMUL) (1983-1991). PhD in Genetics (1991), FMUL. Invited Professor of Medical Genetics in FMUL (1991–2004). Lecture in Bioethics in the Bioethics Centre of FMUL (from 2000). Member of UNESCO International Bioethics Committee (2002-2006). President of Bioethics Council of Portuguese National Health Institute, INSA (2012–2015); President of Bioethics Committee of Portuguese Paediatrics Society until 2016. National Genetics Award -1991; Tuberous Sclerosis Association Award—1994; INSA AWARD—2017. Member of ESHG (from 1978), BSMG (from 1984), Portuguese Society of Paediatrics (from 1970), SPGH (Honorary), Tuberous Sclerosis Association (Honorary). European Society Human Genetics (ESHG) —member from 1978. Host and local President of the ESHG 30th Annual Meeting (Lisbon, 1997). Scientific Programme Committee Member (1997–1999). Portuguese Society Human Genetics—Founder member (1996), President in 1997 (first) and 2004. Honorary Member (2011), President of SPGH Bioethics Committee.

Over 120 publications in Medical Genetics and Bioethics, most in international journals and books.

Research leader in several scientific projects.

André Dias Pereira, PhD

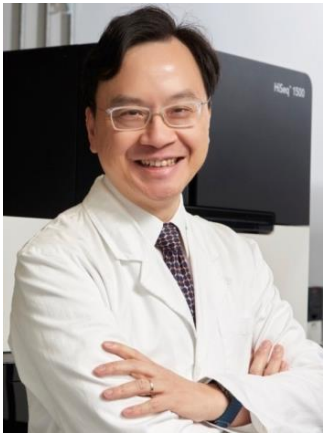
Professor of Law
President of *Centro de Direito Biomédico*,
Faculty of Law, University of Coimbra
Coimbra, Portugal

André Pereira is a Professor of Civil Law at the University of Coimbra (Portugal), Director of the Centre for Biomedical Law and a Member of the National Council of Ethics for Life Sciences.

Pereira's academic career started with his graduation in law at the University of Coimbra (1998) and since then he has obtained a post-graduate degree in both Medical Law (1999) and Civil Law (2002) and in 2003 he defended his Master's thesis. He obtained his PhD in law (summa cum laude) in 2014, with the thesis "Medical Liability and Patients' Rights".

Moreover, Pereira is Researcher at the University of Coimbra Institute for Legal Research. He is member of several Ethics committees (Portuguese Society of Human Genetics, National Institute of Legal Medicine and Forensic Sciences, AIBILI Ethics Committee) and Member of the Committee for Animal Well-being of IMBC - Institute of Molecular and Cellular Biology (Porto).

At the international level, Pereira is a Fellow of ECTIL (European Centre of Tort and Insurance Law, Vienna, Austria), has been a Governor of the World Association for Medical Law (2012-2018) and a Member of the International Academy of Comparative Law.



Y. M. Dennis Lo,

SBS MA, DM, DPhil, FRCP, FRCPath, FRS JP

Associate Dean (Research)
Director, Li Ka Shing Institute of Health Sciences
Chairman, Department of Chemical Pathology
Li Ka Shing Professor of Medicine
Professor of Chemical Pathology
Faculty of Medicine,
The Chinese University of Hong Kong,
Hong Kong SAR, China

Dennis Lo is the Associate Dean (Research) of the Faculty of Medicine, the Director of the Li Ka Shing Institute of Health Sciences and Chairman of the Department of Chemical Pathology of The Chinese University of Hong Kong.

He received his undergraduate education from the University of Cambridge, and his Doctor of Medicine and Doctor of Philosophy degrees from the University of Oxford.

He discovered the presence of cell-free fetal DNA in maternal plasma in 1997 and is a key driver of non-invasive prenatal diagnosis. He has also pioneered many non-invasive approaches for detecting cancer-associated molecular aberrations in blood.

He is a Fellow of the Royal Society (UK) and a Foreign Associate of the US National Academy of Sciences, and has been awarded the King Faisal International Prize in Medicine in 2014 and the Future Science Prize in 2016.

ORADORES_SIMPÓSIOS | SPEAKERS_SYMPOSIA

Alba Mota, PhD

Field Application Scientist, Iberia
Agilent Technologies
Madrid, Spain

Alba Mota studied Biochemistry and Biomedicine at the Autonoma University of Madrid (Spain). She obtained her PhD in Molecular Bioscience working at MD Anderson Cancer Center (Madrid) and performing a short-term research stay in the Memorial Sloan Kettering Cancer Center (New York) in order to decipher intra-tumor molecular heterogeneity through Next-Generation Sequencing (NGS). After that, she carried out a post-doctoral research project in the Spanish National Center for Cardiovascular Research. She is currently a Field Application Scientist in Agilent Technologies, covering the Genomics solutions for Iberia area.

Hannes Arnold, PhD

Technical Sales Specialist for Central EMEA
10x Genomics
Munich, Germany

Hannes (Hans Peter) Arnold studied Biology, Chemistry, and Practical and Theoretical Education at the LMU in Munich (Germany). He received his PhD on Molecular Biology at the Max-Planck-Institute for Biochemistry in Martinsried (Germany). He continued to work in several positions in industry at the interface between sales and science. He worked in the last seven years as Sales Specialist and Business Development Manager with different companies in the field of Next Generation Sequencing (among them Illumina). He joined 10x Genomics in December 2017 as Technical Sales Specialist for Central EMEA.

PALESTRAS | LECTURES

The genetic landscape of Intellectual disability

Anita Rauch
University of Zurich, Institute of Medical Genetics, Switzerland

Intellectual disability (ID) is characterized by significant impairment of intellectual and adaptive functions with onset during childhood. The level of such deficits is commonly measured by standardized tests and a value below 69-75 of the resulting, normally distributed intelligence quotient (IQ) is defined as ID. Boosted by broad applications of whole-exome sequencing (WES), more than 1000 genes affecting a variety of pathways with a large spectrum of associated phenotypes have been identified as underlying monogenic causes in about 50% of patients with otherwise undiagnosed neurodevelopmental disorders. As an explanation of variability in ID severity within the same disorders, there is growing evidence that next to mutation specific effects, familial genetic background and polygenic risk variants known to be associated with normal IQ distribution may influence expressivity of monogenic defects or may even mimic the latter in a minority of cases. While in offspring of consanguineous couples, autosomal recessive genes are commonly found as disease causes, a major distribution of de novo pathogenic variants in outbred populations was shown. Nevertheless, recent data indicate, that compound heterozygosity for autosomal recessive disorders may also significantly contribute to ID, but is difficult to diagnose due to the plethora of inherited variants of unknown functional significance. Further explanations for monogenic pathogenic variants escaping WES diagnoses are parental mosaicism or de novo variants in recessive alleles leading to unwarranted neglecting of pathogenic variants by trio-approaches, as well as imprinting and repeat expansion disorders, and larger indels not reliably detectable by WES. A further unresolved question is the contribution of non-coding variants for which causality is difficult to proof.

Main references:

Rauch-A et al. “Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study” Lancet 2012

Gilissen-C. et al. “Genome sequencing identifies major causes of severe intellectual disability” Nature 2014

Finucane-B et al. “Shift happens: family background influences clinical variability in genetic neurodevelopmental disorders” Genetics in Medicine 2016

Kochinke-K et al. “Systematic Phenomics Analysis Deconvolutes Genes Mutated in Intellectual Disability into Biologically Coherent Modules” Am J Hum Genet 2016

Deciphering Developmental Disorders Study “Prevalence and architecture of de novo mutations in developmental disorders” Nature 2017

Niemi-MEK et al. “Common genetic variants contribute to risk of rare severe neurodevelopmental disorders” Nature 2018

Hu-H et al. “Genetics of intellectual disability in consanguineous families” Mol Psychiatry 2018

Short P. et al. “De novo mutations in regulatory elements in neurodevelopmental disorders” Nature 2018

Papuc-SM et al. “The role of recessive inheritance in early-onset epileptic encephalopathies: a combined whole-exome sequencing and copy number study” Eur J Hum Genet, in press

The role of Imprinting in Cancers

David Monk

Cancer Epigenetics and Biology Program, Bellvitge Institute for Biomedical Research (IDIBELL), Barcelona, Spain

---

Genomic imprinting is the parent-of-origin specific monoallelic transcription, regulated in part by allelic difference in DNA methylation established in the male and female germline and maintained throughout somatic development. In addition to being indispensable for growth, imprinted genes have been suggested to play a crucial role in driving oncogenic switch or suppressing tumour development. Deregulated expression, which includes the reactivation of the normally silent allele (commonly referred to as loss-of-imprinting, LOI) or the silencing of the transcribed allele, has been implicated in childhood cancer (especially Wilms' tumours) associated with the classical imprinting disorder Beckwith–Wiedemann syndrome (BWS). During this presentation we will cover the basis of BWS-associated cancer aetiology, progressing to the role of imprinted genes in adult tumour types. Finally, I will discuss some of our more recent observations from our laboratory. In brief, we note that methylation profiles at imprinted differentially methylated regions (DMRs) do not often represent genuine epigenetic changes but simply the accumulation of underlying copy-number aberrations (CNAs), and that reoccurring copy-number alterations influence allelic expression. This is exemplified by loci with copy-number neutral loss-of-heterozygosity (cnnLOH) or amplifications that are expressed from the appropriate parental chromosomes, which is indicative of maintained imprinting, but with altered dosage.

Imprinting errors in spermatogenic cells of infertile patients

Joana Marques

(C. Joana Marques[1,2], Filipa Carvalho[1,2], Mário Sousa[3,4], Alberto Barros[1,2,4])

[1]Genetics Unit, Department of Pathology, Faculty of Medicine, University of Porto (FMUP), Portugal; [2]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; [3]Department of Microscopy, Laboratory of Cell Biology, Multidisciplinary Unit for Biomedical Research-UMIB, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto Portugal; [4]Centre for Reproductive Genetics A Barros, Porto, Portugal

---

Genomic imprinting is an epigenetic mechanism regulating gene expression and resulting in monoallelic expression of imprinted genes. Imprinting marks are erased in Primordial Germ Cells (PGCs) and re-established during gametogenesis, according to the sex of the germ line. Parental imprints have to be faithfully maintained during mitotic and meiotic cell divisions, in order for correct imprints to be transmitted to the embryo, resulting in normal embryo development and placental function.

Male infertility is often accompanied by a decrease in sperm count (oligozoospermia) or even absence of sperm (azoospermia) in the semen, suggesting a failure in the spermatogenic process. We have previously shown that methylation errors at imprinted genes, either in the form of DNA hypermethylation or hypomethylation, occur in sperm from oligozoospermic and azoospermic infertile patients. We have also shown that imprinting marks are already established in human adult spermatogonia and are maintained throughout spermatogenesis, possibly through a tight control by DNA methyltransferases (DNMTs). More recently, we have analysed other players in the (de)methylation process, the TET enzymes that convert 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), observing an altered expression in germ cells from infertile patients. These results bring new clues for epigenetic mechanisms involved in the molecular regulation of human spermatogenesis.

Acknowledgements

Our research is partly funded by the Portuguese Foundation for Science and Technology (FCT), through Strategic Funds (Pest-OE/SAU/UI0215/2014; UID/BIM/04293/2013), Project Grants (POCI/SAU-MMO/60555/04; PTDC/BIA-BCM/121276/2010) and FCT Investigator Award to CJM (IF/00047/2012).

Establishing the human epigenome in development and pluripotency

Peter Rugg-Gunn

(Amanda Collier, Peter Chovanec, Stefan Schoenfelder, Csilla Varnai, Peter Fraser, Anne Corcoran and Peter Rugg-Gunn)

Babraham Institute, Cambridge, UK

Human pluripotent stem cells (hPSCs) exist in multiple states that are broadly termed naïve and primed. Both cell states can self-renew, but are functionally and molecularly distinct. Naïve hPSCs largely recapitulate the transcriptome and epigenome of pre-implantation embryos, and primed hPSCs are similar to early post-implantation embryos. This is an important distinction because these two developmental stages differ enormously in gene regulation and in epigenetic hallmarks such as X-inactivation status and DNA methylation. The research in my lab is focused on understanding the mechanisms of epigenetic and gene regulatory changes as hPSCs transition between the two states, with the aim of applying that information to more precisely control cell fate decisions and to better understand human development. Recently, we have mapped the transcriptional and epigenetic dynamics during cell state change, discovering that different reprogramming methods drive different trajectories. We have also examined the 3D genome organisation of naïve and primed hPSCs. By applying new network-scale computational approaches, we have interrogated the organisation at multiple genomic scales, ranging from a global overview to local communities that recapitulate architectural domains, down to individual promoter-enhancer interactions. Investigating chromatin topology and activity in human pluripotent states offers new insights into features of gene regulatory control during human development.

An algorithm for CLN2 Diagnosis

Miguel Leão
Department of Genetics, S. João Univ. Hospital Center, Porto, Portugal

---

Clinical picture of Neuronal Ceroid Lipofuscinosis (NCL).

Current nomenclature of NCL.

Clinical features of CLN2 and differential diagnosis with other progressive myoclonic epilepsies.

MRI and EEG findings of CLN2.

Natural history and management of CLN2.

Biochemical and genetic diagnosis of CL2.

Diagnostic algorithms.

CLN2 disease - from early diagnosis to early intervention

Eva Wibbeler

(Eva Wibbeler, Angela Schulz)

Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

---

CLN2 disease, a rare, inherited, pediatric, neurodegenerative lysosomal storage disorder caused by TPP1 deficiency, is characterized by seizures, language and motor function loss, blindness and early death.

Early diagnosis has become essential as a first approved therapy is available. Early diagnosis can be improved by testing TPP1 enzyme activity in patients with language developmental delay plus onset of seizures.

A first therapy for CLN2 disease has been developed: A phase 1/2 study (NCT01907087) demonstrated that intracerebroventricular (ICV) infusion of 300 mg cerliponase alfa, a recombinant human TPP1 enzyme, every other week for 48 weeks slowed progression in motor and language function. An ongoing extension study (NCT02485899) assesses the long-term safety and efficacy of ICV-administered Cerliponase alfa in children with CLN2 disease for up to 240 weeks.

Cumulative data from both studies were used to evaluate long-term safety (assessed by adverse events (AEs) frequency) and efficacy (assessed by changes in the CLN2 clinical rating scale motor and language (ML) domains). Treated patients were compared to patients from an independent natural history study.

24 subjects were initially treated with Cerliponase alfa in the phase 1/2 study (9 male, 15 female, mean (SD) age 4.3 years (1.24)); 23 subjects are still enrolled in the extension study (96 to 161 weeks total exposure, median 116 weeks). Safety data showed that all subjects had adverse events (AEs); most were Grade 1-2 and part of the underlying neurodegenerative disease. Common drug-related AEs included pyrexia, vomiting, and convulsion. Twenty (83%) subjects had at least one serious AE, which were mostly consistent with neurodegenerative disease in a pediatric population. Efficacy data showed a significant attenuation of the rate of decline in the motor language score under treatment with Cerliponase alfa: Treated patients had a mean rate of decline of 0.27 scoring points/ 48 weeks ($p<0.0001$) compared to a rate of decline of 2.0 points/48 weeks in untreated patients. The responder (<2 point loss per 48 weeks) rate at 96 weeks (87 %, $p <0.0002$) was unchanged compared to that observed at 48 weeks, suggesting a persistent treatment effect.

Conclusion: These data suggest that enzyme replacement therapy with ICV-administered cerliponase alfa has an acceptable safety profile and a sustained effect over time.

Harnessing the immunotherapy revolution for the treatment of colorectal cancer: neo-antigens and beyond

Noel de Miranda

(J. van den Bulk[1], D. Ruano[1], M.E. IJsselsteijn[1], M. Visser[2], R. van der Breggen[1], K. Peeters[3], T. Duhen[4], R. Duhen[5], A.D. Weinberg[4,5], S.H. van der Burg[2], E.M.E. Verdegaal[2], N.F. de Miranda[1])

[1]Department of Pathology, [2]Department of Medical Oncology, [3]Department of Surgery, Leiden University Medical Center, The Netherlands. [4]AgonOx, Inc. Portland, OR, USA. [5]Earle A. Chiles Research Institute, Portland, OR, USA.

Innovative treatment options are required to improve cure rates in advanced colorectal cancer patients. Immune checkpoint blockade therapy (anti-PD-1) was shown to be effective in colorectal cancers with high mutation burden (e.g. mismatch repair-deficient cancers) as anti-tumour reactivity is largely explained by the recognition of somatically mutated antigens (neoantigens). No immunotherapeutic strategies are currently available for patients diagnosed with low mutation burden colorectal cancer. We hypothesized that if neo-antigen-reactive T cells were present in low mutation burden patients, the latter could benefit from immunotherapeutic interventions that stimulate neo-antigen recognition and the triggering of a robust anti-tumour immune response.

In order to detect neo-antigens, whole exome and RNA next-generation sequencing analyses were performed in cancer and healthy tissues from colorectal cancer patients. Corresponding neo-epitopes were synthesized and tested for their ability to induce immune cell activation in T cells isolated from the tumour tissues (TIL) and from peripheral blood. Neo-antigen-specific T cell responses were identified in the majority of patients that presented with tumours carrying 25 to 36 transcribed, non-synonymous variants. Up to six different neo-antigens were recognized per tumour, which resulted in a higher detection rate than anticipated based on published data. Moreover, we discovered the merits of isolating $CD39^+CD103^+CD8^+$ T cells for detection of a broad recognition of HLA class I-restricted neo-antigens. This $CD39^+CD103^+CD8^+$ T cell subset comprises the majority and a broader repertoire of neo-antigen-specific T cells compared to bulk TIL populations or lymphocytes derived from peripheral blood.

In conclusion, we developed a neo-antigen screening pipeline to unlock the immunogenic potential of colorectal cancers with low mutation burden. We have detected a relatively high number of neo-antigens that are recognized by tumour- and/or PBMC-derived T cells in mismatch repair proficient, low mutation burden colorectal cancer patients, and show the importance of the $CD39^+CD103^+CD8^+$ T cell subset for neo-antigen-based immunotherapies. These findings warrant the further exploration of the potential to employ neo-antigen-targeted therapies to improve clinical outcomes of colorectal cancer patients.

The genetic basis of response to checkpoint inhibitors
Why do some patients respond and how can we predict who will?

Vassiliki Kotoula
Dept of Pathology, School of Medicine, Aristotle University of Thessaloniki, Greece

Cancer immunotherapy has revolutionized cancer care. This year, the development of immune checkpoint inhibitors (ICIs) has scooped Nobel prices in Medicine. These drugs block immune checkpoint molecules (CTLA-4, PD-1, PD-L1) and thus release the brakes to allow host immune response that destroys the tumor. ICIs are effective in different types of cancer but, unfortunately, not in all patients, while they may elicit life-threatening adverse events (1-3). ICIs are targeted drugs. A bit ironically though, the presence of ICI molecular targets in tumors does not adequately predict efficiency of treatment with ICIs. DNA mismatch repair deficiency has been approved as the first tumor-type-agnostic biomarker predictive for response to an ICI in 2017 and tumor mutational burden is being assessed with the same rationale (3-6). All these markers are characteristics of the malignant cells within a tumor.

Malignant tumors, however, are tissues, i.e., multicellular structures composed out of malignant cells that are placed in a growth-permissive stroma infiltrated by different amounts and functional types of host immune cells. Tumor microenvironments greatly vary with respect to the characteristics of immune infiltrates (7). These, in turn, vary according to the genomic contexture of the tumor, to the genetic contexture of the host, and to the functional status of the host immune system. The PD1 pathway has a plethora of diverse roles in regulating host immunity (8), which is also influenced by microbiota that may also interfere with response to ICIs (9). The recent comprehensive description of the immune landscape in cancer (10) attempts to integrate the various aspects determining the tumor immune response status, with six subtypes that differ by somatic aberrations in cancer cells (mutations, genomic stability) and by characteristics of the tumor microenvironment (immune infiltrates and stromal cells). Further issues with ICIs are that these drugs are more effective in combination with other anticancer drugs, e.g., cytotoxic chemotherapeutics (8, 11).

The development of robust biomarkers to assist prediction of response, of clinical benefit, of adverse events, and of therapeutic combinations with ICIs is essential to advance the field towards precision immuno-oncology. Apparently, next to the conventional tumor genomics, in order to safely and effectively modulate the PD1 pathway therapeutically, the complex immunological status of the patient should be considered.

References

1. M. J. Overman, M. S. Ernstoff, M. A. Morse, Where We Stand With Immunotherapy in Colorectal Cancer: Deficient Mismatch Repair, Proficient Mismatch Repair, and Toxicity Management. American Society of Clinical Oncology Educational Book, 239-247 (2018).

2. M. A. Postow, R. Sidlow, M. D. Hellmann, Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. New England Journal of Medicine 378, 158-168 (2018).

3. M. Nishino, N. H. Ramaiya, H. Hatabu, F. S. Hodi, Monitoring immune-checkpoint blockade: response evaluation and biomarker development. Nature Reviews Clinical Oncology 14, 655 (2017).

4. C. E. Steuer, S. S. Ramalingam, Tumor Mutation Burden: Leading Immunotherapy to the Era of Precision Medicine? Journal of clinical oncology : official journal of the American Society of Clinical Oncology 36, 631-632 (2018).

5. X. Shen, B. Zhao, Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. BMJ (Clinical research ed.) 362, k3529-k3529 (2018).

6. Y. Goto, Tumor Mutation Burden: Is It Ready for the Clinic? Journal of clinical oncology : official journal of the American Society of Clinical Oncology, JCO2018793398 (2018).

7. D. S. Chen, I. Mellman, Elements of cancer immunity and the cancer-immune set point. Nature 541, 321-330 (2017).

8. A. H. Sharpe, K. E. Pauken, The diverse functions of the PD1 inhibitory pathway. Nature Reviews Immunology 18, 153 (2017).

9. V. Gopalakrishnan, B. A. Helmink, C. N. Spencer, A. Reuben, J. A. Wargo, The Influence of the Gut Microbiome on Cancer, Immunity, and Cancer Immunotherapy. Cancer cell 33, 570-580 (2018).

10. V. Thorsson et al., The Immune Landscape of Cancer. Immunity 48, 812-830.e814 (2018).

11. W. H. Fridman, L. Zitvogel, C. Sautes-Fridman, G. Kroemer, The immune contexture in cancer prognosis and treatment. Nature reviews. Clinical oncology 14, 717-734 (2017).

CAR-T for the treatment of T cell malignancies

John F. DiPersio
Washington University School of Medicine, St. Louis, MO

T-cell malignancies represent a class of devastating hematologic cancers with high rates of relapse and mortality in both children and adults for which there are currently no effective or targeted therapies. Despite intensive multiagent chemotherapy regimens, fewer than 50% of adults and 75% of children with T-ALL survive beyond five years. For those who relapse after initial therapy, salvage chemotherapy regimens induce remissions in 20-30% of cases. Allogeneic stem cell transplant, with its associated risks and toxicities, is the only curative therapy. Targeted therapy against T-cell malignancies represents a significant unmet medical need. Such targeted therapies have shown great potential for inducing both remissions and even long-term relapse-free survival in patients with B-cell leukemia and lymphoma. Engineered T-cells that express a chimeric antigen receptor (CAR) directed against T-cell malignancies are limited by several significant obstacles, but are a promising cancer immunotherapy. First, the shared expression of target antigens between T effector cells and T-cell malignancies results in fratricide, or self-killing, of CAR-T cells. Second, harvesting adequate numbers of autologous T-cells without contamination by malignant cells is, at best, technically challenging and prohibitively expensive. Third, the use of genetically modified CAR-T cells from allogeneic donors may result in life-threatening graft-vs.-host disease (GvHD) when infused into immune-compromised HLA-matched or mismatched recipients. We hypothesized that deletion of CD7 and the T-cell receptor alpha chain (TRAC) using CRISPR/Cas9 in CAR-T targeting CD7 (UCART7) would result in the efficient targeting and killing of malignant T-cells without significant effector T-cell fratricide or induction of GvHD. We chose to target CD7 on malignant T-cells because it is over expressed on the vast majority of T-cell and NK-cell malignancies. Second, germline biallelic deletion of CD7 resulted in mice with normal T-cell numbers, T-cell subsets, and T-cell function. We generated a CD7 CAR using an anti-CD7 single chain variable fragment (scFv) created using commercial gene synthesis and cloned into the backbone of a 3rd-generation CAR with CD28 and 4-1BB internal signaling domains. The construct was modified to express CD34 via a P2A peptide to enable detection of CAR following viral transduction. Human primary T-cells were activated using anti-CD3/CD28 beads for 48 hours prior to bead removal and electroporation with CD7 gRNA, TRAC gRNA, and Cas9 mRNA. On day three, T-cells were transduced with lentivirus particles encoding either CD7 CAR or CAR CD19 control and allowed to expand for a further six days. Transduction efficiency and ablation of CD7 and TRAC was confirmed by flow cytometry. Multiplex CRISPR/Cas9 gene-editing resulted in the simultaneous deletion of both CD7 and TRAC in 72.8%$^{\pm}$1.92 of cells, as

determined by FACS analysis. To prevent alloreactivity, $CD3^+$ CAR-T were removed from the product by magnetic depletion. UCART7 effectively killed T-ALL cell lines (CCRF-CEM, MOLT3, and HSB2) and human primary T-ALL blasts in vitro. Next, we tested the capacity of UCART7 to kill primary T-ALL in vivo without xenogeneic GvHD. Considerable expansion of alloreactive T-cells, severe GvHD (mean clinical GvHD score = 5.66), and a robust graft vs. leukemia effect were observed in recipients of WT T-cells. In contrast, GvHD was completely absent, T-cells were undetectable, and considerable tumor burden was observed in mice receiving $TRAC^{\Delta}$ T cells. Mice receiving UCART7 had no GvHD and unlike UCART19 controls, effectively cleared T-ALL blasts. Fratricide-resistant and allo-tolerant "off-the-shelf" UCART7 signifies a novel strategy for treatment of relapsed and refractory T-ALL and non-Hodgkin's T-cell lymphoma.

Towards modifying disease progression and onset in Huntington Disease (HD) and other genetic neurodegenerative disorders: hopes and challenges

G. Bernhard Landwehrmeyer
Department of Neurology, Ulm University, Ulm, Germany

Conceptually, there is a generic treatment for monogenetic, dominant disorders: dialing down the further production of mutant gene products by targeting mRNA levels. In my presentation, I will describe the effects of an Antisense- Oligonucleotide (ASO), the ASO IONIS-HTT_{Rx} (RG6042) in patients with early Huntington Disease (HD). In this first HTT-lowering drug trial, the effects of multiple doses of intrathecally administered RG6042 by monthly injections over 13 weeks in 46 early stage HD patients were explored. The drug was well tolerated and the levels of the protein product huntingtin in cerebral spinal fluid (CSF) were significantly reduced, suggesting that RG6042 is a promising therapeutic with potential to modify disease progression in HD. A global development program by Roche including a Phase-III study ("Generation-HD1") to explore the long term safety and the benefits of RG6042 applied over two years will likely be launched in 2019. Aside from these non-allele selective approaches using ASOs, efforts are underway to study the safety and efficacy of stereopure ASOs developed by WAVE Life Sciences targeting single nucleotide polymorphism (SNPs) on the mutant HTT allele in two Phase-Ib/IIa clinical trials (PRECISION-HD 1/2). In addition, HTT-lowering gene therapies using miRNA and adeno-associated viral vectors (AAVs) are advancing towards first in man studies. Studies using virally delivered zinc-finger-repressor complexes are in late preclinical development stages. These gene therapeutic approaches using viral vectors as well the intrathecal application of ASOs come with delivery issues: clinical benefits will likely depend on making sure that brain regions relevant for clinical symptoms and signs are reached to a sufficient extent. Lastly, orally administered, CNS penetrant small molecules decreasing the further production of huntingtin gene products are under investigation.

Aside from HD, other monogenetic dominant neurodegenerative disorders are currently under active investigation using similar approaches, including autosomal dominant spino-cerebellar ataxias (SCAs) as well as motor neuron disorders (SOD1, SBMA, C9orf). There is a silver lining on the horizon that gene silencing using the approaches described may allow to modify the so far relentless progressive nature of these neurodegenerative disorders and may allow to postpone symptom onset in carriers of the respective mutations.

LRP10 genetic variants in familial Parkinson's disease and dementia with Lewy bodies: a genome-wide linkage and sequencing study

Wim Mandemakers
Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands

The aim of this study was to identify a novel gene implicated in the development of Parkinson's disease (PD), PD-Dementia (PDD), and dementia with Lewy bodies (DLB).

Background: There are clinical, pathological, and molecular overlaps suggesting that PD, PDD and DLB are parts of a continuum of Lewy Body diseases. Yet, in most patients with familial forms of PD, PDD or DLB, variants in the known disease-causing genes (i.e. *SNCA*, *LRRK2*) are not found, suggesting that other causative or predisposing genes remain to be identified.

Method: We initially performed genome-wide linkage analysis in an Italian family with dominantly inherited PD (10 affected individuals, average onset age 59.8 years). In the second stage, we sequenced the candidate gene in an international multicenter series of 660 unrelated probands, including 430 clinically diagnosed familial PD (n=420) or PDD (n=10), 62 clinically diagnosed DLB, and 168 pathologically confirmed PD (n=49), PDD (n=74) or DLB (n=45). Sequencing data from 645 individuals with aortic aneurysms (who were not neurologically examined) were used as controls. In the third stage, we screened independent series of clinically diagnosed PD patients and controls with no signs nor family history of PD, PDD and DLB from Sardinia (412 PD, 242 controls), Taiwan (831 PD, 431 controls) and Portugal (223 PD, 138 controls) for specific variants.
We also performed mRNA and brain pathology studies in three patients carrying disease-associated variants. Last, we carried out functional protein studies in in vitro models, including neurons from induced pluripotent stem-like cells.

Results: In the initial kindred, we detected significant linkage of PD to chromosome 14, and nominated *LRP10* as disease-causing gene. In stage II, among the international series of 660 probands, we identified 8 patients (4 PD, 2 PDD, 2 DLB), who carried different rare, potentially pathogenic *LRP10* variants, while only one carrier was found among 645 controls (aortic aneurysms). In stage III, two of the eight variants were detected in 3 additional PD probands (2 from Sardinia and 1 from Taiwan), but in none of the controls. Out of the total 11 probands with *LRP10* variants, 10 had a positive family history of disease, and DNA was available from 10 affected relatives (in 7 of these families). The *LRP10* variants were present in 9 of these 10 relatives, providing independent, albeit limited, evidence of co-segregation with disease. Post-mortem studies in 3 patients carrying distinct *LRP10* variants showed severe Lewy-body pathology. Three of the variants severely affect *LRP10* expression and mRNA stability (by cDNA analysis), four other variants affect protein stability (by cycloheximide-chase experiments), and the remaining two variants affect protein localization (by immunocytochemistry), pointing to loss of the LRP10 function as a common pathogenic mechanism.

Conclusion: This work identifies *LRP10* pathogenic variants as a novel genetic cause of synucleinopathies. Elucidating the function of the LRP10 protein can offer novel insights into mechanisms, biomarkers and therapeutic targets.

Fluid Biomarkers in Neurodegenerative Diseases

Luís F. Maia

Neurology Department, Hospital Santo António – CHUP; ICBAS –UP; Molecular Neurobiology, IBMC - Instituto de Biologia Molecular e Celular/i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto

---

Abnormalities in brains of patients with neurodegenerative diseases, like Alzheimer's Disease (AD), start long before the first clinical symptoms. Identifying individuals at such preclinical stages is crucial, since these disease stages are considered the most promising periods for a successful treatment. Such diagnosis relies largely on biochemical and imaging biomarkers. However, there is a lot to be known on the longitudinal biomarker dynamics, particularly in the earliest preclinical stages.

Transgenic mouse models for neurodegenerative diseases like AD and Parkinson`s Disease and related disorders are excellent models for the brain proteopathic lesions but have rarely been used to study clinically relevant body fluid disease biomarkers. Using transgenic mice overexpressing human amyloid precursor protein (APP) we showed that CSF Aβ and t-Tau follow the trends predicted to occur in AD patients. CSF Aβ reflected brain Aβ-pathological changes and CSF t-Tau seems a marker of Aβ pathology progression at later stages. When exploring other markers of neuronal and axonal dysfunction in the plasma of different neurodegenerative mouse models, we found that Neurofilament L (NfL) reflected disease progression and also constituted a marker of treatment response. Such an approach, by allowing the direct comparison of quantifiable brain pathology and by avoiding the diagnostic uncertainty and co-morbidities present in human cohorts, holds great translational value.

In fact, familial and sporadic cases from multicenter human cohorts of AD, like the Dominantly Inherited Alzheimer Network (DIAN) and Alzheimer´s Disease Neuroimaging Initiative (ADNI) have already translated our findings in the clinic, and are on track to establish new diagnostic and patient follow-up strategies.

Novel disease biomarker panels may open new perspectives in identifying and stratifying subjects at risk for AD and other neurodegenerative diseases for preventive disease-oriented treatments.

Advanced therapies in spinal muscular atrophy: beyond the clinical trials

Eduardo F. Tizzano
Area de Genetica Clinica y Molecular, Hospital Vall d´Hebron, Barcelona, Spain

Spinal muscular atrophy (SMA) is a disease with an autosomal recessive inheritance pattern that leads to degeneration and loss of motor neurons in the spinal cord, producing denervation and weakness. SMA is classified into four main types according to age of onset and maximal milestones achieved, representing a continuous spectrum of clinical manifestations ranging from very compromised neonates to adults with minimal manifestations. All patients show homozygous absence or mutations of the SMN1 as the determinant gene of the disease and their phenotype is mainly influenced by the number of copies of a paralogue gene, SMN2, which is present in all of them. However, the genotype-phenotype correlation is not absolute and other modifiers are under investigation to clarify discordant cases. Following regulatory approval of the first tailored treatment for SMA by means of antisense oligonucleotides as a splicing modifier and with other advanced therapies under clinical investigation such as gene therapy, the prospects for care of these patients have changed. Early testing, including pre-symptomatic newborn screening, allows a prompt confirmation of diagnosis that is currently changing proactive measures and opportunities for therapy based in the actual landscape of new treatments. Together with expansion in standard of care, multidisciplinary follow-up and evolving phenotypes, SMA constitutes an example of confluent efforts of different groups that transcends the disease itself. Indeed, it brings new expectations and perspectives to researchers and patients of many other rare genetic diseases awaiting discovery or application of advanced therapies to change the concept of being incurable or intractable.

The genetics of cognitive epigenetics

Hans van Bokhoven

Molecular Neurogenetics Unit, Radboud university medical center, Donders Institute for Brain, Cognition and Behaviour, Nijmegen, The Netherlands

---

Intellectual disabilities (ID) comprise a highly diverse group of cognitive disorders. To date, mutations in some 1000 genes have been associated with ID and this number is rapidly increasing driven by next generation sequencing efforts. The large number of ID-associated gene mutations presents an opportunity to identify common mechanisms of disease as well as molecular processes that are of key importance to human cognition. Given the disproportionately high number of epigenetic genes associated with ID, epigenetic regulation of gene transcription is emerging as a process of major importance in cognition. Epigenetic regulatory complexes involving multiple ID genes provide an excellent tool for fundamental research into the epigenetic mechanisms underlying learning and memory. Our research is focused on the elucidation of the role of an epigenetic module involving euchromatic histone methyltransferase 1 (EHMT1) and other proteins underlying a recognizeable ID syndrome, denoted Kleefstra syndrome. The investigation of this epigenetic module in vitro and in model organisms such as Drosophila melanogaster and mice has revealed an important role in dendritic branching, synaptic morphology and plasticity across species, providing a basis for rationalized intervention strategies.

CRISPR-Cas9 – Method and potential applications

André Travessa

Department of Medical Genetics - Hospital de Santa Maria, Centro Hospitalar de Lisboa Norte, E.P.E., Lisbon, Portugal

Genome editing is the process of precisely modifying the nucleotide sequence of the genome of an organism or cell.

Different techniques to genome editing have been emerged over time, including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and, more recently, clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated-9 (Cas9).

Although many scientists have contributed to the development of genome editing technology, an essential contribution was given in 2012 by Jennifer Doudna and Emmanuelle Charpentier, who discovered how bacteria use the CRISPR/Cas9 system to protect themselves from viruses.

CRISPR/Cas9 technology has been used for many purposes, including regulation of endogenous gene expression, epigenome editing, live-cell imaging of chromosomal loci, generation of animal models, edition of RNA, and high-throughput screening.

CRISPR/Cas9 technology is being explored in research and holds promise for the treatment and prevention of a wide variety of diseases, including not only Mendelian disorders, such as cystic fibrosis, hemophilia, and sickle cell disease, but also more complex diseases, such as cancer, heart disease, and human immunodeficiency virus (HIV) infection.

Genome editing - from benefits to ethical limits

Heloísa Santos
SPGH Bioethics Committee / S. Genética, HSM, FMUL, Lisbon, Portugal

The CRISPR / Cas 9 technology, the new method of genome editing (GE) intervention, is far from having a practical successful implementation and it is difficult to identify all the ethical challenges and hazards related to future practical use. Theoretically, CRISPR / Cas 9 has a very large potential and can alter DNA whether in bacterium, plant, animal or human being. The possible applications have an almost limitless range of areas. Crops (resistance and tolerance to disease and pests), biotechnology (pharmaceutical products and biofuels), biomedicine (cell-based therapies, xenotransplantation, gene therapies, including germinative interventions), military and industrial applications and can also affect wildlife and ecosystems. This frighteningly powerful tool must only be used to improve social and health conditions of human being.

We present international recommendations for governance and further actions (UK and other countries scientific societies) to be discussed and to promote final ethical debate.

GE is a research subject that needs to be strong regulated, with a permanent international monitoring and public debate.

Atual Legislação Portuguesa e Documentos de Bioética

André Gonçalo Dias Pereira

Presidente do Centro de Direito Biomédico – FDUC; Professor da Faculdade de Direito da Universidade de Coimbra; Membro do CNECV

Estamos a assistir à chamada "Quarta Revolução Industrial" (Klaus Schwab)! Neste cenário, têm-se multiplicado, inter alia, os avanços promissores no âmbito da Engenharia Genética, dando-se aso a discussões ético-jurídicas de relevo e que ameaçam redesenhar o próprio tecido social de uma forma nunca antes vista. De modo a evitar-se um desenvolvimento desenfreado da ciência à margem do Direito e da Ética, impõe-se, cada vez mais, um urgente debate acerca da forma como as novas potencialidades podem ser empregues. Atualmente, já existe um amplo rol de normas jurídicas e bioéticas que visam a tutela de uma das componentes mais sagradas do nosso organismo - o genoma - e da identidade genética dos indivíduos, evitando que a pessoa humana seja reduzida a mero instrumento que serve a ciência e os seus interesses específicos. É na análise crítica de algumas dessas normas que nos vamos focar durante a nossa intervenção. Certo é, desde já, que, em tempos em que o vazio ético-legislativo pode significar uma verdadeira ameaça para a nossa "Humanidade", dispor de regulamentação compreensiva e adequada será a nossa única opção – e não temos dúvidas de que a criação de nova regulamentação ou a eventual alteração daquela que já existe deve ser levada a cabo de forma transparente e com ampla participação democrática, especialmente se tivermos em conta que estamos a tomar decisões fracturantes que irão, em certa medida, moldar o futuro da própria Humanidade.

Unlocking the diagnostic potential for circulating DNA

Y. M. Dennis Lo

Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China

---

My group reported the presence of cell-free fetal DNA in maternal plasma in 1997. Since then, this technology has been developed into a platform for noninvasive prenatal testing (NIPT) that can be used for the screening of multiple types of fetal chromosomal aneuploidies, various types of single gene disorders and even for fetal whole genome sequencing. Apart from circulating fetal genetic markers, recent work has also led to the development of fetal epigenomic and transcriptomic analyses from maternal plasma. Excitingly, the global success of NIPT has triggered researchers in other fields to explore the use of circulating nucleic acid technology in multiple areas, notably in the liquid biopsy of cancer. Circulating nucleic acid technology has therefore brought about a paradigm shift in diagnostic medicine.

COMUNICAÇÕES ORAIS | ORAL PRESENTATIONS

OP1 | BASIC RESEARCH

Horizontal transmission of mutation-driven drug resistance in cancer

Susana Junqueira Neto[1,2,3]; Joana Marques[1,2]; Roland Rad[4,5]; Sónia A. Melo[1,2,3]; José Carlos Machado[1,2,3]; José Luís Costa[1,2,3]

1 - i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto; 2 - IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto; 3 - FMUP – Faculdade de Medicina da Universidade do Porto; 4 - Medizinische Klinik, Technische Universität München; 5 - German Cancer Research Center and German Cancer Consortium, Heidelberg

---

Therapy resistance is a major problem in the treatment of cancer. Despite the benefits of targeted therapies, the majority of patients become resistant due to specific genetic mutations. Although mitosis is the obvious mechanism for transmission of resistance mutations, the rate of cell division may not explain the rapid growth of the resistant tumor and the multilocal recurrence of the disease. Thus, we hypothesized that, in addition to mitosis, the specific mutations responsible for resistance can be horizontally transferred between cancer cells (cell communication-based mechanisms).

To address this premise we developed an in vivo experimental strategy to evaluate horizontal transfer of the well-known target therapy resistance mechanism in lung cancer. Immunodeficient mice were inoculated subcutaneously with two different lung cancer cell lines that harbor mutations either involved in sensitivity or resistance mechanisms to target therapy (Erlotinib). In order to test whether growth of the tumor derived from sensitive cells is influenced by the coexistence of tumor resistant cells, sensitive and resistant cells were engrafted in different flanks of the same animal. Tumors' kinetics were monitored and genetic analysis of the tumors performed using NGS and digital PCR.

Our results have shown that, in the presence of resistant cells, tumor sensitive cells acquire resistance twice as fast compared with the control (mice engrafted with sensitive cells alone), suggesting a different resistance mechanism to Erlotinib. The DNA analysis of sensitive tumors revealed the acquisition of the T790M resistant mutation by ~20% of animals when the tumor sensitive cells were in the presence of resistant tumor cells, and the acquisition of an alternative mechanism of resistance in controls. Our data show a preliminary evidence for a role of cell communication-based mechanisms in the transfer of specific mutations between cancer cells.

PhD Scholarship: SFRH/BD/115099/2016
FEDER funds through the COMPETE 2020 - POCI, Portugal 2020, FCT: POCI-01-0145-FEDER-007274 and PTDC/DTP-PIC/2500/2014, and by NORTE 2020, in the framework of the project NORTE- 01-0145-FEDER-000029.

OP2 | BASIC RESEARCH

Genetic variation in Portuguese individuals

Daniel Martins [2]; Hugo Froufe[1]; Diogo Pinho[1]; Cristina Barroso[1]; Maria Simões[1]; Conceição Egas[1,3]

1 - Genoinseq-Next Generation Sequencing Unit, Biocant, BiocantPark, Núcleo 04, Lote 8, 3060-197 Cantanhede, Portugal.; 2 - Universidade do Minho, Largo do Paço, 4704-553 Braga, Portugal.; 3 - Center for Neuroscience and Cell Biology, University of Coimbra, 3004-504 Coimbra, Portugal

---

The in-depth study of the genomics of single populations has contributed significantly to the enlargement of known SNVs in databases. Each single population study has contributed with 18 to 57% of novel SNVs. The new genetic information is particularly relevant as a reference for clinical purposes. Global-scale initiatives as the 1000 Genomes Project (1kG) already include Iberian population; however, no Portuguese individuals were included in this cohort. Furthermore, to our knowledge, gnomAD, the most extensive genomic dataset, does not include Portuguese individuals either. We believe that a Portuguese collection of genomic information would greatly benefit molecular diagnosis in Portuguese patients.

We sequenced seventy exomes of Portuguese individuals with the Ion Proton technology. Reads were mapped using TMAP against the hg19 reference sequence and the variants were called by the Torrent Variant Caller. Variants were inserted in a MongoDB No-SQL Database, and the 1kG and gnomAD information for each variant was uploaded to the same database.

The exomes of the Portuguese individuals contained 275,159 variants, 135,723 (49.3%) of which were exonic. The majority of the variants found in exons were SNVs (118,922, 87.6%). Around 20% (23,245; 19.5%) and 29% (34,277; 28.8%) of the SNVs were novel in gnomAD and 1kG, respectively. The percentage of newly found variants is similar to that reported by other relatively low-scale studies.

The present study is an important contribution to enriching large-scale genomic initiatives and, to stand as a useful auxiliary reference for genetic analyses of Portuguese patients. Attaining to previous national initiatives, a larger project may improve the general knowledge regarding the Portuguese population variant profile.

Funding: In2Genome (CENTRO-01-0247-FEDER-017800), GenomePT (POCI-01-0145-FEDER-022184) and Strategic Project (POCI-01-0145-FEDER-007440).

OP3 | BASIC RESEARCH

Intronic cis-regulatory elements regulate CDH1 gene expression and function

Rita Matos[1,2]; Bárbara Mesquita[1,2]; Patrícia Oliveira[1,2]; Sofia Valente[1,2]; Hugo Pinheiro[1,2]; Joana Carvalho[1,2]; Anabela Ferro[1,2]; David Huntsman[3,4,5]; Carla Oliveira[1,2,6]

1- i3S (Instituto de Investigação e Inovação em Saúde), Universidade do Porto (UP), Portugal; 2- IPATIMUP (nstitute of Molecular Pathology and Immunology), UP, Porto, Portugal; 3- Department of Pathology and Laboratory Medicine, University of British Columbia (UBC), Vancouver, Canada; 4- Centre for Translational and Applied Genomics (CTAG), BC Cancer Agency, Vancouver, Canada; 5- Genetic Pathology Evaluation Centre, UBC and Vancouver General Hospital, Vancouver, Canada; 6 - Faculty of Medicine of UP, Porto, Portugal

---

Cis-regulatory elements (CREs) are non-coding DNA regions, capable of integrating different protein signals important for the expression of specific genes in a controlled temporal and spatial manner. As vital regulators of gene transcription, CREs are important mediators of the relationships between genes and their protein products. Genomic structural variations in CREs may affect gene regulatory networks, explaining certain disease etiologies. In this work, we are studying *CDH1*, a tumor suppressor gene widely disrupted in epithelial cancers. We aim at unveiling the role of potential intronic CREs (iCREs) in *CDH1* expression regulation, since its exonic alterations do not explain all the abnormal *CDH1*-associated phenotypes.

Bioinformatics analyses on *CDH1* locus mining ENCODE data for chromatin accessibility, epigenetic marks, transcription factor binding sites and other regulatory elements, allowed to identify putative *CDH1* iCREs. To ascertain their functional relevance, we edited each iCRE separately in a gastric cancer cell line by using CRISPR-Cas9, as well as *CDH1* exon2 as positive control. All engineered cell clones were purified by single-cell sorting and the CRISPR-Cas9 editing was confirmed by sequencing. *CDH1* expression was assessed by qRT-PCR and a single base primer-extension assay, while E-cadherin expression level and pattern were determined by western blotting and immunocytochemistry, respectively.

We identified two putative iCREs (iCRE1 and iCRE8) of *CDH1* and successfully generated homogeneous clonal cell lines with fine-mapped deletions and inversions in clones' DNA sequences. Our results indicate that intronic rearrangements at iCRE1 region impair *CDH1*/E-cadherin expression, possibly leading to allelic imbalance through the differential binding of transcription factors. Moreover, iCRE1-edited cells can have a similar phenotype to those harboring exon2 deletions. No obvious *CDH1* loss of function was detected for iCRE8-edited clones. This study highlights iCRE1 as a *cis*-regulatory element of *CDH1*/E-cadherin expression, supporting a potential involvement of structural variations at *CDH1* intronic sequences in disease etiology.

Common p53 mutations induce IRES-mediated translation of oncogenic shorter p53 isoforms

Bruna F. Pereira[1,2]; Rafaela Lacerda[1,2]; Maria López-Iniesta[3]; Luísa Romão[1,2]; Marco M. Candeias[1,3]

1 - Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; 2 - Center for Biodiversity, Functional and Integrative Genomics, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; 3 - Molecular and RNA Cancer Unit, Graduate School of Medicine, Kyoto University, Kyoto, Japan

---

At least half of all tumors exhibit mutations in the tumor suppressor *p53* gene. Indeed, the fact that *p53* is frequently mutated in cancer led to its identification as an oncogene, when first described in 1979. Later, it was classified as a tumor suppressor, due to the clarification of its wild-type role in maintaining genome integrity and preventing malignant transformation. The *p53* gene can encode for many p53 isoforms, by alternative splicing, alternative promoters and internal translation initiation mechanisms. While full-length p53 (FL-p53) protein works as a tumor suppressor by regulating many biological processes such as cell cycle, apoptosis, senescence and DNA repair, shorter p53 protein isoforms seem to play different roles in the cell. Recently, we have shown that the most common p53 mutations induce the expression of shorter p53 isoforms. Furthermore, we found that shorter p53 isoforms are implicated in cancer progression as they promote enhanced cell survival, proliferation, adhesion and formation of invasive cell structures. Here, with a bicistronic system containing two reporter genes (*Renilla* luciferase and firefly luciferase), we show that expression of shorter p53 isoforms is mediated by a non-canonical translation initiation mechanism regulated by an Internal Ribosome Entry Site (IRES) in the p53 mRNA. By investigating the effect of common p53 missense mutations on the function of this new IRES, through bioluminescence assays and Western blot analysis, we show that some p53 cancer mutations have a preponderant role in IRES-mediated translation induction of shorter p53 isoforms. With the obtained results we identified a new mechanism by which p53 cancer mutations promote tumorigenesis, which may lead to new understandings of the onset and progression of some types of tumors as well as to the development of new cancer therapies.

Acknowledgments: This work is partially supported by Fundação para a Ciência e a Tecnologia (PTDC/BIMONC/4890/2014), by Grants-in-Aid 16K21111 and 18K07229 from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan and by Takeda Foundation.

OP5 | Basic Research

Liquid biopsy: looking beyond EGFR mutations

Joana Reis[1,2,3]; Gabriela Fernandes[1,2,4]; Venceslau Hespanhol[1,2,4]; José Carlos Machado[1,2,3]; José Luís Costa[1,2,3]

1- i3S (Instituto de Investigação e Inovação em Saúde), Portugal; 2- IPATIMUP (Instituto de Patologia e Imunologia Molecular da U. Porto), Portugal; 3- Faculdade de Medicina da U. Porto, Portugal; 4- Departamento de Pneumologia, Hospital de São João, Portugal

---

Many of the diagnosed lung tumors contain genetic alterations and are eligible for targeted therapy. The genetic analysis is usually performed through tumor biopsy, an invasive procedure that may not be representative of the genomic landscape. To overcome these limitations, liquid biopsy has gained relevance allowing access to tumor DNA in a non-invasive manner. For the patients with genetic mutations, several approaches have been developed, allowing a non-invasive disease monitoring based on the continuous analysis of the driver alteration in cfDNA. For the patients whose tumors harbor gene fusions, the lack of liquid biopsy strategies is related to the difficulty of handling and evaluating cfRNA.

The aim of this work was to develop a comprehensive liquid biopsy strategy possible to be applied to lung cancer patients, including those with gene translocations.

To do so, plasma samples from 200 NSCLC patients were obtained at different stages of disease. In order to assess tumor derived mutations, cfDNA was extracted and evaluated with the Oncomine Lung cfDNA Assay. To evaluate the presence of fusion transcripts in circulation, a methodology for the simultaneous extraction of cfDNA and cfRNA was optimized and evaluated with the Oncomine Lung cfTNA Research Assay.

We showed that tumor derived genetic variants could be identified in plasma with a cfDNA input of 5ng and a sensitivity of 81,5%. The tracking of somatic mutations was used to evaluate response to therapy, including the expansion of resistance mechanisms or disease progression, which could be detected 2 months prior any clinical signs. Moreover, aside from also being able to detect genetic rearrangements, it was possible to identify concomitant mutations.

We developed a methodology that enables the detection of genetic mutations and gene fusions, allowing to expand the clinical application of liquid biopsy to patients that otherwise would not be enrolled in this type of non-invasive monitoring.

FEDER funds through the COMPETE 2020—POCI, Portugal 2020, FCT: POCI-01-0145-FEDER-007274 and PTDC/DTP-PIC/2500/2014, and by NORTE 2020, in the framework of the project NORTE- 01-0145-FEDER-000029.

National study on osteogenesis imperfecta – genotype and phenotype in 150 Portuguese patients

André M. Travessa[1]; Patrícia Dias[1]; Miriam Aza-Carmona[2]; Joana Rosmaninho-Salgado[3]; Teresa Saraiva[4]; Ana Grangeia[5]; Marta Amorim[6]; Miguel Gonçalves-Rocha[7]; Graça Araújo[8]; Heloísa Santos[1]; Ana Medeira[1]; Isabel Cordeiro[1]; Juliette Dupont[1]; Oana Moldovan[1]; Ana Beleza[3]; Joaquim Sá[3]; Jorge M. Saraiva[3]; Lina Ramos[3]; Margarida Venâncio[3]; Sofia Maia[3]; Sofia Fernandes[3]; Gabriela Soares[4]; João P. Freixo[6]; Anabela Bandeira[9]; João Campagnolo[10]; Fátima Godinho[11]; M. Cassiano-Neves[12]; Viviana Tavares[11]; Filomena Teixeira[8]; Teresa Kay[6]; Renata Oliveira[5]; Ana Fortuna[4]; Sérgio B. Sousa[3]; Karen E. Heath[2]; Ana Berta Sousa[1]

1 - Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal.; 2 - Instituto de Genética Médica y Molecular (INGEMM) e Skeletal dysplasia Multidisciplinary Unit, IdiPAZ, Hospital Universitario La Paz, UAM, Madrid, Espanha e CIBERER, ISCIII, Madrid, Espanha.; 3 - Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal.; 4 - Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar Universitário do Porto, Porto, Portugal.; 5 - Serviço de Genética Médica, Centro Hospitalar de São João, Porto, Portugal.; 6 - Unidade de Genética Médica, Hospital Dona Estefânia, Cento Hospitalar Lisboa Central, Lisboa, Portugal.; 7 - Unidade de Genética Médica, Hospital de Braga, Braga, Portugal.; 8 - Serviço de Pediatria, Hospital Dr. Nélio Mendonça, Funchal, Portugal.; 9 - Serviço de Pediatria e Centro de Referência para Doenças Metabólicas, Centro Hospitalar Universitário do Porto, Porto, Portugal.; 10 - Serviço de Ortopedia Infantil, Hospital Dona Estefânia, Cento Hospitalar Lisboa Central, Lisboa, Portugal.; 11 - Serviço de Reumatologia, Hospital Garcia de Orta, Almada, Portugal.; 12 - Serviço de Ortopedia Infantil, Hospital CUF, Lisboa, Portugal.

Context: Osteogenesis imperfecta (OI; prevalence 1/10-20.000) is a group of rare hereditary disorders characterized by increased susceptibility to bone fractures. OI is caused by type I collagen variants in 90% of cases while variants in a growing number of other genes have been identified in the remaining cases. Our aim was to characterize the clinical and mutational spectrum of OI in Portugal and to correlate genotype and phenotype.

Methods: Clinical data of OI patients were collected through analysis of patient medical records and clinical evaluation when indicated. Sanger and/or different next-generation sequencing (NGS) based strategies were used for molecular analysis. Only patients with a definitive clinical/molecular diagnosis of OI were considered.

Results: In total, 150 patients (47 children, 97 adults and 6 fetuses) of 121 families were included. Ninety-three cases were classified as mild OI, 29 as moderate, 19 as severe, and 7 as extremely severe (2 unclassifiable). The male to female ratio was 1:1,3.

In 110 families (90.1%) and 92.7% (139/149) of total patients, 95 distinct pathogenic or likely pathogenic variants were identified in 7 different known OI genes: 65 in *COL1A1*, 19 in *COL1A2*, two each in *SERPINF2*, *TMEM38B* and *BPM1*, and one each in *CRTAP*, *FKBP10*, *IFITM5*, *PPIB* and *WNT1*; 42 are novel.

Of the 123 type 1 collagen-related patients of 94 families, 78 had quantitative mutations and 45 had qualitative mutations.

Autosomal recessive (AR) OI was diagnosed in 11 patients and heterozygous variants in severe AR OI genes were found in 4 patients with mild OI. After extended NGS analysis, a variant in a candidate gene was found in one index patient, and no mutation was identified in six.

Conclusions: This is the first study on OI in a large Portuguese cohort. Our results are in accordance with other populations: predominance of *COL1A1/2* variants with higher frequency of quantitative mutations in patients with mild OI. Patients with *IFITM5* and *FKBP10*-related OI had recognizable phenotypes. Molecular diagnosis in OI, besides genetic counseling, is becoming more important for prognosis, presymptomatic testing and therapeutic recommendations.

Ancestral Origin and diffusion of Val50Met mutation in Transthyretin-Related Familial Amyloid Polyneuropathy (ATTRV50M) in the Portuguese populations

Cátia Leal[1,2]; Teresa Coelho[3]; Diana Santos[1]; Jorge Sequeiros[1,4,5]; Isabel Alonso[1,5]; Alda Sousa[1,4]; Miguel Alves-Ferreira[1,4]; Carolina Lemos[1,4]

1- UnIGENe, IBMC (Instituto de Biologia Molecular e Celular), i3S (Instituto de Investigação e Inovação), U. Porto, Portugal; 2- FMUP (Faculdade de Medicina da U. Porto), Portugal; 3- UCA (Unidade Corino de Andrade), CHUP (Centro Hospitalar Universitário do Porto), Portugal; 4- ICBAS (Instituto Ciências Biomédicas Abel Salazar), U. Porto, Porto, Portugal; 5- CGPP, IBMC (Instituto de Biologia Molecular e Celular), i3S, U. Porto, Portugal

---

Aims/Context: We intend to determine the ancestral origin path of the Portuguese Transthyretin (TTR) Val30Met mutation. TTR Val30Met related familial amyloid polyneuropathy (FAP) is an autosomal dominant systemic amyloidosis, characterized by a great phenotypic variability observed across all major worldwide foci, particularly in age-at-onset (AO). The hypothesis that population differences in AO could be explained by different mutation origins has long been put forward. In Portugal, differences in the AO between the different disease clusters have also been reported, where families from Northern Portugal show a mean earlier AO than families from the inner mountains. Hence, the observed regional differences prompted us to question whether the Portuguese Val30Met mutation has a single ancestral origin or has independently arisen multiple times.

Methods: An extensive family-based haplotype study was conducted in 49 families (a total of 201 Val30Met carriers and relatives) originated from Northern Coast, Inland and Central Coast of Portugal, using two sets of SNPs and STRs markers covering the *TTR* gene. DMLE+ and ESTIAGE software were used to estimate mutation age.

Results: Our results showed a common haplotype of different lengths shared by almost all Val30Met carriers, suggesting a major single-founder effect for Val30Met in the Portuguese population. According to the age estimates analysis, we hypothesize that the mutational event took place 1450–1475 years ago, and its dispersion occurred from Póvoa de Varzim and Vila do Conde to Inland region, corroborating initial hypothesis of Val30Met's migration flow related to fishing/farmer activities. Although AO variability observed among Val30Met Portuguese kindreds seems not attributable to different mutational origins, striking haplotypic differences downstream STR D18S1133 were found between Northern Coast vs. Inland and Central Coast carriers. Future investigations of this genetic surrounding region may provide new insights on AO variability.

Conclusion: Herein, we provide further evidence towards Val30Met ancestral origins and its mutational path within Portugal.

OP8 | CLINICAL RESEARCH

RAS mutational analysis in plasma ctDNA from patients with mCRC

Manuela Pinheiro[1]; Ana Peixoto[1]; Patricia Rocha[1]; Isabel Veiga[1]; Carla Pinto[1]; Catarina Santos[1]; Pedro Pinto[1]; Joana Guerra[1]; João Silva[1]; Manuel R. Teixeira[1,2]

1 - Department of Genetics, Portuguese Oncology Institute of Porto, Porto, Portugal; 2 - Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, University of Porto, Largo Prof. Abel Salazar, 4099-003 Porto, Portugal

---

Aim: Mutations in the RAS genes are negative predictors of response to anti-EGFR monoclonal antibodies in metastatic colorectal cancer (mCRC). The detection of mutations in circulating tumor DNA (ctDNA) has emerged as a noninvasive strategy to assess the molecular profile of advanced cancer patients and to follow the clonal evolution during therapy. This study aimed to perform RAS mutational analysis in plasma ctDNA from mCRC patients using two different techniques, BEAMing Digital PCR technology and the highly-automated real-time PCR platform Idylla, which present limits of detection of 0.01% and 0.2%, respectively.

Methods: This study includes 21 patients with mCRC, previously tested for RAS mutations in tumor tissue by Idylla assays. Blood samples were collected at diagnosis (before surgery and chemotherapy) in 16 patients and after surgery (but before chemotherapy) in two patients. In three patients the blood sample was collected at disease progression (after surgery and chemotherapy), but none of them was performing chemotherapy at the time. DNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit and RAS mutation analysis was conducted using OncoBEAM RAS CRC and Idylla ctRAS assays in 21 and 9 patients, respectively.

Results: Of the 21 cases, 14 presented a mutation in tumor tissue. Using the OncoBEAM test we detected RAS mutations in 12 patients, being the concordance rate with tumor tissue testing 86% (12/14). Of the 9 cases tested by Idylla ctDNA analysis (all positive in tumor tissue), the mutation previously identified in tumor tissue was detected in 55% (5/9) of the cases. Regarding the four negative cases (two also not detected by the OncoBEAM test), two of them had low amount of cell-free DNA and the other two presented a mutant fraction below 0.5% (detected by OncoBEAM test).

Conclusion: The concordance rate between tumor tissue and plasma samples was higher for OncoBEAM RAS CRC test, demonstrating a higher sensitivity than Idylla for detection of RAS mutations in plasma. These results confirm that ctDNA can be an adequate DNA source for tumor genotyping in mCRC patients. These results will be updated.

OP9 | CLINICAL RESEARCH

Molecular characterization of CHUP's MODY patients

Francisco Laranjeira[1]; Isaura Ribeiro[1,2]; Ana Amado[3]; Eugénia Pinto[1]; Joana Vilaverde[3]; Ana Rita Soares[4]; Sónia Rocha[1]; André Carvalho[3]; Ana Marmiesse[5]; Sofia Teixeira[3]; Jorge Dores[2,3]; Cláudia Amaral[3]; Isabel Palma[3]; Cláudia Falcão Reis[4]; Maria Teresa Pereira[3]; Cláudia Freitas[3]; Célia Soares[4]; Maria João Oliveira[3]; Raquel Almeida[3]; Gabriela Soares[4]; Ana Fortuna[2,4]; Dulce Quelhas[1,2]; Helena Cardoso[2,3]

1- Unidade de Bioquímica Genética, Centro de Genética Médica Jacinto de Magalhães (CGMJM), Centro Hospitalar Universitário do Porto (CHUP); 2- Unidade Multidisciplinar de Investigação Biomédica (UMIB), Instituto de Ciências Biomédicas Abel Salazar, U. Porto; 3– S. de Endocrinologia (SEndoc), CHUP; 4– S. de Genética Médica, CGMJM, CHUP; 5- Unidade de Diagnostico y Tratamiento de errors congénitos do Metabolismo, Complexo Hospitalar Universitario de Santiago de Compostela

---

Introduction: Maturity-onset diabetes of the young (MODY) is a group of monogenic disorders of autosomal dominant transmission resulting from a primary defect in insulin secretion, associated with pancreatic β-cell dysfunction, with fourteen different genes been implicated to date. Approximately 2% of diabetic patients has a monogenic cause but it is frequently misdiagnosed as type 1 or type 2 diabetes. Mutations in the glucokinase gene, *GCK*, and genes coding for hepatocyte nuclear factor 1α, 1β and 4α, *HNF1A*, *HNF1B* and *HNF4A*, are the most common causes of MODY, accounting for up to 70% of all patients.

Methodology: A cohort of 98 patients with clinical suspicion of MODY type diabetes, followed either at SEndoc or SGM, underwent molecular genetics testing at UBG. Molecular genetics studies in all 98 patients were initiated by Sanger sequencing of one or more of the following genes, according to clinical suspicion: *HNF1A*, *GCK*, *HNF4A* and *HNF1B*. As second tier approach in 4 patients next-generation sequencing (NGS) panel was used, performed at UDTECM.

Results: We present the molecular characterization of 26 patients where a definite or probable causing mutation was identified. Sanger sequencing accomplished that in 23 families, thus 23% - 65% are HNF1A-MODY, 26% are GCK-MODY and 9% are HNF1B-MODY. NGS panel identified the putative cause of the disease in 3 out of 4 families (75%). Four novel mutations were found: c.1146_1156del and c.1422_1424delGCCinsCAG in *HNF1A*, c.863T>C in *GCK* and c.2474G>T in *ABCC8*.

Conclusions: The prevalence of HNF1A-MODY and GCK-MODY in our cohort is much below the values described. NGS approach is a valuable tool for such a genetically heterogeneous condition and hopefully it will allow for a better characterization of our MODY patients after the second tier study is completed in the remaining 72 families. Accurate genetic diagnosis is important to help assistant physicians on treatment management reducing the risk of diabetic complications.

OP10 | CLINICAL RESEARCH

Identification of molecular alterations in neurotransmission and synaptic genes in Autism Spectrum Disorder

Joana Vilela[1]; Hugo Martiniano[1]; Sonija Luzi[1]; Joana Marques[1]; João Santos[1]; Muhammad Asif[1]; Célia Rasga[1]; Guiomar Oliveira[2]; Astrid Vicente[1]

1 - INSA - Instituto Nacional de Saúde Dr. Ricardo Jorge; 2 - Centro Hospitalar e Universitário de Coimbra

---

Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder, which affects brain function. It is characterized by the presence of social communication deficits and restricted, repetitive patterns of behavior. ASD has a strong genetic component and there is evidence in support of many putative risk genes. However, the genetics and the biological processes underlying the disease are still incompletely understood and will require the integration of intermediate phenotypic analysis provided by direct observation tools such as brain imaging.

Objective: The objective of this work was to identify molecular alterations in neurotransmission and synaptic (NS) genes that play a role in ASD etiology and can be related to brain imaging phenotypes.

Method: We selected candidate NS genes through literature review and the KEGG Pathways and Gene Ontology (GO) databases. Genes were analysed for the presence of rare CNVs (Copy Number Variants) and SNVs (Single Nucleotide Variants) in large experimental ASD and control datasets available respectively through the Autism Genome Project (2446 cases and 9649 controls) and the Autism Sequencing Consortium (1829 ASD cases and 997 controls). Rare SNVs with high or damaging impact in proteins, splice site and UTR variants were also analysed in a subset (N=1539) of the SNV cohort applying a gene-based test (SKAT-O test).

Results: We identified 41 NS genes exclusively targeted by rare CNVs in ASD subjects and 13 NS genes more frequently targeted by rare CNVs in ASD subjects, when compared to controls. We also identified 328 NS genes targeted by rare predicted pathogenic SNVs in ASD subjects. Additionally, we also found that 14 NS genes are strongly associated with ASD with the SKAT-O test. Some of the genes detected are strong ASD candidates such as *CACNA1A, CACNA1C, CACNA1D, GRIN2B, PIK3R2, SCN2A, SHANK2, SHANK3* and *SLC6A1*.

Conclusions: The results show an excess of structural alterations and several variants with predicted pathogenic impact in proteins, reinforcing the putative role of the NS pathways in ASD. We will integrate this results with clinical and brain imaging data to identify biomarkers for the disease.

OP11 | CLINICAL CASE REPORTS

A Case of Coffin-Siris syndrome due to a novel mutation in ARID2

André M. Travessa[1]; Patrícia Dias[1]; Carla Pereira[2]; Ana Berta Sousa[1]

1 - Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal.; 2 - Unidade de Endocrinologia Pediátrica, Serviço de Pediatria Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal.

Background: *De novo* variants in the *ARID2* gene, a subunit of the SWI/SNF complex, have been recently linked to intellectual disability (ID) with features resembling Coffin-Siris syndrome (CSS) in 14 patients. This new syndrome was named CSS type 6. We report the 15th patient with CSS type 6. We aim to compare his clinical findings with those of other patients with CSS type 6 and other molecular etiologies of CSS.

Case description: We present a 17-year-old boy who was referred for genetic evaluation at 14 year of age because of short stature and ID. Family history was unremarkable. Gestation, delivery and neonatal period were uneventful. Growth evolved with short stature and relative macrocephaly, and psychomotor development evolved with moderate to severe ID, autism spectrum disorder and attention-deficit/hyperactivity disorder. Other medical problems included recurrent pneumonia and bronchiectasis, intermittent strabismus, hydronephrosis, umbilical hernia, premature teeth loss, and diabetes mellitus type 1. Metabolic screening, Fragile X syndrome mutation analysis and array-CGH have been previously performed, and were normal. Physical examination at 14 years of age showed proportionate short stature, coarse facial appearance, cubitus valgus, and cervical kyphosis. No limb or ectodermal changes were observed. Skeletal X-ray showed mild and nonspecific vertebral changes. The clinical features were not suggestive of a particular diagnosis. Whole exome sequencing identified a novel heterozygous likely pathogenic variant, c.3511C>T, p.(Gln1171*), in the *ARID2* gene, establishing the diagnosis of CSS type 6.

Conclusions: We present the case of a patient with CSS type 6. As previously reported in patients with mutations in *ARID2*, our patient has coarse facial features, short stature and ID. He doesn't have fifth-digit nail hypoplasia, a typical feature of CCS that is absent in the majority of CSS type 6 patients. He also presented diabetes mellitus type 1, which was never reported in patients with this novel entity. The *ARID2* gene should be included in the analysis of exome or gene panel data in patients with short stature and coarse facial features.

OP12 | CLINICAL CASE REPORTS

The clinical value of DNA and RNA Liquid Biopsies in a case of prostate cancer

Joana Cardoso[1,2,3]; Ana Pereira[2]; Teresa Lourenço[2]; Maria José Rego De Sousa[2]; José Germano Desousa[2]; José Germano Rego De Sousa[2]

1 - 1. Ophiomics - Precision Medicine, Lisboa, Portugal; 2 - 2. Centro de Medicina Laboratorial Germano de Sousa, Lisboa, Portugal; 3 - 3. iNOVA4Health-Advancing Precision Medicine, NOVA University of Lisbon, Lisboa, Portugal

---

A 69 year old man diagnosed with a prostate cancer, currently in progression and a family history of neoplasic disease (multiple prostate cancers, lymphatic cancer), was referred to genetic counselling. Germline genetic testing could reveal the origin of familial cancer and a somatic genetic testing could reveal new potential therapy targets.

We investigated this patient using three distinct approaches: 1) germline DNA testing by NGS using a gene panel comprising 3 genes (BRCA1, BRCA2 and HOXB13) involved in familial prostate cancer; 2) somatic testing of tumor DNA by NGS using a panel of mutational hotspots located in 50 genes and 3) somatic evaluation of a Androgen Receptor (AR) splicing variant involved in resistance to castration therapy on tumor RNA by quantitative real-time PCR (qPCR). Since no archived material from the original tumor was available, we have used a liquid biopsy approach to assess the tumor though its cell-free circulating tumor DNA (cfDNA) and RNA (cfRNA).

The results obtained with the prostate cancer panel revealed that the germline DNA of the patient was negative for mutations in the 3 analyzed genes. In addition, the analysis of the AR splicing variant (AR-V7) with cfRNA revealed that the expression levels of AR-V7 variant were similar to the levels of healthy controls and consequently it is likely that the patient will likely benefit from the anti-AR therapy currently implemented. Finally the results of the cfDNA analysis revealed a mutation in the ATM gene (p.Trp1933LeufsTer32) and a somatic mutation in the RET gene (p.Thr608Ser) with allelic frequencies of 50,5% and 2,6%, respectively. The ATM variant is a germline mutation validated with Sanger sequencing in the patient's germline DNA.

In conclusion, both liquid biopsy approaches allowed us to analyze the prostate tumor even in the absence of archived material. We could discard the presence of potential resistance to anti-AR therapy through the splicing variant AR-V7 in the cfRNA, to identify a RET variant potentially targetable with Tyrosine Kinases inhibitors and an ATM germline variant in the patients' cfDNA, currently under evaluation in other family members.

Wiedemann-Steiner Syndrome – clinical and molecular characterization of 9 patients from four national hospital centers

Sofia Fernandes[1]; Mariana Soeiro E Sá[2]; Inês Carvalho[3]; Márcia Rodrigues[4]; Renata Oliveira[5]; Pedro Louro[6]; Joaquim Sá[1]; Patricia Dias[2]; Jorge M Saraiva[1,7]; Sergio B Sousa[1,8]; Célia Soares[9,10]; Gabriela Soares[9]

1 - Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; 2 - Medical Genetics Unit, Department of Pediatrics, Centro Hospitalar Lisboa Norte, Hospital de Santa Maria, EPE, Centro Académico de Medicina de Lisboa, Lisboa, Portugal; 3 - Medical Genetics Unit, Hospital Dona Estefânia, Centro Hospitalar Lisboa Central, Lisboa, Portugal; 4 - Medical Genetics Unit, Department of Pediatrics, Centro Hospitalar Lisboa Norte, Hospital de Santa Maria, EPE, Centro Académico de Medicina de Lisboa, Lisboa, Portugal (former worker at Medical Genetics Unit, Hospital Dona Estefânia, Centro Hospitalar Lisboa Central, Lisboa, Portugal; 5 - Medical Genetics Unit, Centro Hospitalar de São João, Portugal (former worker at Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal); 6 - Portuguese Oncology Institute Francisco Gentil, Lisboa (former worker at Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal); 7 - University Clinic of Pediatrics, Faculty of Medicine, Universidade de Coimbra, Coimbra, Portugal; 8 - University Clinic of Genetics, Faculty of Medicine, Universidade de Coimbra, Coimbra, Portugal; 9 - Medical Genetics Department, Centro de Genética Médica Jacinto Magalhães (CGMJM), Centro Hospitalar Universitário do Porto, EPE, Porto, Portugal; 10 - Unit foi Multidisciplinary Research in Biomedicine, ICBAS-UP, Porto, Portugal

---

Context: Wiedemann-Steiner Syndrome (WSS) is a rare autosomal dominant condition characterized by intellectual disability, short stature, typical facial features (narrow palpebral fissures, long eyelashes, thick eyebrows, wide nasal bridge) and hypertrichosis (mainly "hairy elbows"). WSS is caused by loss-of-function variants in *KMT2A* gene, which is involved in histone modification, leading to chromatin remodelling defects.

Methods: Clinical and molecular characterization of all cases with WSS diagnosis observed at 4 Portuguese hospital centres based on retrospective analysis of patient medical records. Clinical exome (7/9) or *KMT2A* targeted NGS (2/9) was or is being performed in all cases.

Results: We describe 9 unrelated patients, 5 males and 4 females. The age of clinical and/or molecular diagnosis ranged between 4 and 29 years (median 10 years). The main reasons for referral were intellectual disability (6/9) and dysmorphic features (4/9). All individuals had mild to moderate intellectual disability (9/9), behavioural problems (9/9), craniofacial dysmorphisms: narrow palpebral fissures (8/8) and downslanted (5/6), long eyelashes (7/8), thick eyebrows (5/6) and wide nasal bridge (7/8). Hypertrichosis *cubiti* was present in 8/8 and often became less evident with

age. Short stature was present in 2/8 individuals, recurrent infections in 5/6 and sleep apnoea in 2/4 cases, one of which required non-invasive ventilation. We identified 5 novel *KMT2A* heterozygous variants (1 pathogenic, 4 likely pathogenic), 1 previously described pathogenic variant, no variant was detected in 1 case and in 2 we are waiting for results.

Discussion: In general, our data are in accordance with the literature. Short stature is the only feature that seems to have lower prevalence than expected. The variety of clinical features is wide and clinical suspicion is often challenging. In 6/9 cases, the diagnosis was not initially considered and only achieved after clinical exome sequencing and reverse phenotyping. Detailed description of populational cohorts of WSS patients are important for families and health professionals, leading to a better-informed genetic counselling and surveillance.

OP14 | CLINICAL CASE REPORTS

A new case of Bain type X-linked syndromic intellectual disability

Mariana Soeiro E Sá[1]; Oana Moldovan[1]; Ana Berta Sousa[1]

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

Variants in *HNRNPH2* have recently been associated with Bain type X-linked syndromic intellectual disability (IDXSB) in female patients who share a common neurological phenotype of global developmental delay/intellectual disability (DD/ID), hypotonia, behaviour abnormalities (including autism spectrum disorder), movement disorders, seizures, as well as post-natal microcephaly and dysmorphic features. Growth delay, gastrointestinal problems and musculoskeletal anomalies are also common.

Herein we describe a 17-year-old girl with moderate ID, stereotypic behaviour, wide gait, post-natal microcephaly, growth delay, scoliosis, gastroesophageal reflux, joint hyperlaxity, and dysmorphic features, namely posteriorly rotated external ears, upslanting palpebral fissures, blepharophimosis, prominent nasal bridge and columella, short wide philtrum, wide mouth, micrognathia, transverse left palmar crease, long fingers, 5th finger clinodactyly, pes planus, and long wide halluxes. Since her first referral at 3 years and 6 months of age, she underwent etiological investigation with peripheral blood karyotype, subtelomeric FISH analysis and array-CGH, which were all normal. Lastly, trio-based whole exome sequencing, including the patient and her unaffected mother and brother, identified a heterozygous pathogenic variant in *HNRNPH2*, c.617G>A, p.(Arg206Gln), previously described in association with IDXSB.

HNRNPH2 belongs to a large group of RNA-binding proteins that control pre-mRNA alternative splicing by affecting spliceosome assembly at splice sites, thus regulating gene expression. So far, only 6 cases have been reported in the medical literature, although there are reports of up to 30 girls with *HNRNPH2* variants that can be found through patient's associations. Most mutations described so far are missense mutations affecting conserved residues in the nuclear localization sequence. The mechanism of pathogenicity is not clear, but a gain-of-function effect has been suggested based on studies of other RNA binding proteins. The lack of identified male patients, in addition to the gene's location in the X chromosome, suggests possible male lethality.

OP15 | CLINICAL CASE REPORTS

Genotype-phenotype correlations in five patients with large NF1 deletions

João Rodrigues Alves[1]; Catarina Machado[1]; Juliette Dupont[1]; Rosário Silveira-Santos[1]; Ana Sousa[1]; Oana Moldovan[1]; Ana Berta Sousa[1]

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

---

Introduction: A minority of neurofibromatosis type 1 (NF1) patients have large deletions encompassing the *NF1* gene and its flanking regions, and frequently have a distinctive phenotype that includes overgrowth, intellectual disability (ID) and high risk of malignancy. The contribution of specific genes located within the deletion interval to the phenotype has recently emerged. We describe five such patients, aiming to correlate genotype to phenotype.

Clinical report: Four female patients, aged 3 to 12-years old, and a 21-year old male harbouring large *NF1* deletions were observed in our Department. All fulfilled clinical criteria for NF1. Two had cutaneous neurofibromas and one a plexiform neurofibroma. Three presented birth weight >4000g and all were tall for their age in infancy. Mild ID and minor dysmorphisms were observed in all patients, and a distinctive Weaver-like phenotype in two of them. Whole-gene *NF1* deletions were identified by MLPA. ArrayCGH was subsequently performed, revealing three recurrent 1.4 Mb deletions (Type-2) and two atypical deletions.

Discussion: Besides typical NF1 stigmata, the main common manifestations in our patients were overgrowth and mild ID. Codeletion of *RNF135* has been linked both to overgrowth and ID, and the former was encompassed in all cases. Additionally, codeletion of *SUZ12* has been associated with increased risk of malignant peripheral nerve sheath tumours (MPNST), and point mutations in this gene were recently reported in two Weaver-like patients. In this group, two out of four patients with *SUZ12* deletions presented Weaver-like features. The young age of these four patients may explain the absence of precursor lesions or MPNST, and close follow-up is warranted. Our data is consistent with the association between *RNF135* and overgrowth, and might add evidence regarding the link between *SUZ12* and a distinctive Weaver-like phenotype. Patients with *NF1* deletions require special clinical care and surveillance, and further investigation is needed to elucidate the role of other genes in the phenotype and tumorigenesis process in these patients.

OP16 | CLINICAL CASE REPORTS

A new mutation in RPL10 associated with X-linked syndromic intellectual disability in two families and literature review

Mário Laço[1,2]; Margarida Venâncio[1]; Lauren Grote[3]; Mike Friez[4]; Estelle Chanudet[5]; Chiara Bachelli[5]; Hywel Williams[5]; Sérgio Sousa[1,6]

1 - Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; 2 - Faculty of Health Sciences, Universidade da Beira Interior, Covilhã, Portugal; 3 - Division of Clinical Genetics, Children's Mercy, Kansas City, Kansas, U.S.A.; 4 - Greenwood Genetic Center, Greenwood, SC, USA; 5 - Centre for Translational Omics, Genetics and Genomic Medicine UCL Great Ormond Street Institute of Child Health, London, UK; 6 - University Clinic of Genetics, Faculty of Medicine, Universidade de Coimbra, Coimbra, Portugal

---

Context: Putative pathogenic variations in more than 700 genes have been associated with intellectual disability (ID), 100 of them located in the X chromosome and implicated in X-linked ID (XLID). *RPL10* variants have been associated with a spectrum of phenotypes ranging from isolated autism spectrum disorder to a dysmorphic syndrome with microcephaly and growth retardation.

Case reports: We report four cases from two unrelated families, two brothers (17 and 10 years old) both born prematurely from a non-consanguineous healthy Portuguese parents (family 1, F1) and two siblings from European American descendant, an 18 year old male and a his maternal half brother who died at the age of 18 months (family 2, F2). All cases presented severe developmental delay/ID, absent speech, short stature, post-natal microcephaly, non-independent walking with ataxia, feeding difficulties and strikingly similar phenotypic features.

In addition, in F1 the older brother developed early-onset epilepsy while the younger brother had congenital heart defect, undescended testicles, micropenis, vesico-ureteral reflux. In F2, the adult male had generalized epilepsy, dystonia, acquired hypothyroidism and delayed puberty, while the younger brother had cleft palate.

Whole exome sequencing in F1 and a XLID NGS panel in F2 identified a common genetic variation - an hemizygosity for a novel missense variant, c.218A>G (p.Asn73Ser) in exon 5 of the *RPL10* gene. This variant was not reported in the literature nor in ExAC or other databases and in silico analysis points out to likely pathogenicity. In F1, the cognitively normal mother was heterozygous for this variant, which was found to be *de novo* on her, and had skewed X-inactivation.

Conclusion: To our knowledge, these are the 7th and 8th families reported with syndromic XLID caused by *RPL10* missense variants and p.Asn73Ser the 6th variant described. The present report expands and helps to delineate the *RLP10*-related mutational and clinical spectrum. Our four cases have a highly consistent and distinct phenotype and comparing with all cases described in the literature, at least a second *RPL10*-related (endo) phenotype seems to exist.

OP17 | CLINICAL CASE REPORTS

Copy Number Variation: susceptibility loci associated with variable expressivity/incomplete penetrance – A Retrospective Analysis

Raquel Gouveia Silva[1]; Sónia Custódio[1]; Rosário Silveira-Santos[1]; Raquel Rodrigues[1]; Eva Rolo[1]; Oana Moldovan[1]; Juliette Dupont[1]; Patrícia Dias[1]; Catarina Machado[1]; Mariana Soeiro E Sá[1]; André Travessa)[1]; Ana Berta Sousa[1]; Ana Sousa[1]

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

---

Introduction: Copy Number Variants (CNVs) in susceptibility *loci* are recurrent findings in laboratories performing array Comparative Genomic Hybridization (aCGH) analysis. Incomplete penetrance and variable expressivity pose serious problems to adequate clinical significance ascertainment, which is particularly problematic in the prenatal setting. In this study we aim to address CNVs associated with susceptibility *loci* identified in patients with neurodevelopmental disorders.

Methods: We collected all CNVs identified between 2012 and 2017 in a cohort of 2031 cases analyzed by aCGH (CGX-HD180k, PerkinElmer). Of 467 CNVs, 383 belonged to index cases. We selected 63 associated with incomplete penetrance in the literature, and analyzed them considering chromosome region, frequency, classification, inheritance and clinical features.

Results/Discussion: Selected CNVs were located on 1q21.1, 15q11.2, 15q13, 16p11.2, 16p12.2, 16p13.11 and 17q12. 37 were duplications and 26 were deletions. The most frequently duplicated regions were 15q11.2 (11/37) and 1q21 (9/37). Duplications of 1q21, 16p11.2 and 17q12 were classified as either pathogenic or of uncertain clinical significance, depending on available data and correlation with the patient's phenotype. Duplications of the remaining regions above were classified as likely benign. Segregation analysis was performed in 19 cases, all were inherited except for a 17q12 *de novo* duplication. The most commonly deleted regions were 15q11.2 (8/26) and 16p11.2 (7/26). All deletions were classified as pathogenic, except for deletions of 15q11.2 which were considered likely benign. Parental studies were performed in 14 cases, but only four were *de novo* (one each 1q21 and 17q12, and two 16p11.2). Classification of CNVs correlated with reported penetrance levels for each *locus*. Four CNVs were identified prenatally: a 16p12.2 dup and a 15q11.2 del were not reported; a 1q21 del *de novo* with additional cytogenetic findings was classified as pathogenic; and a 1q21 dup of paternal origin was classified as of uncertain significance.

Conclusions: This study highlights the need for multidisciplinary analysis and data sharing among laboratories.

OP18 | CLINICAL CASE REPORTS

A familial case with a ZMYND11 mutation: syndromic intellectual disability with a recognizable phenotype

Pedro M Almeida[1,2]; Fabiana Ramos[1]; Jorge Saraiva[1,3,4]

1 - Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; 2 - Faculty of Health Sciences, University of Beira Interior, Covilhã; 3 - University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra, Portugal; 4 - Clinical Academic Center of Coimbra, Coimbra, Portugal

Introduction

Mutations in the zync finger MYND-type containing 11 (*ZMYND11*) gene were identified in patients with a syndromic intellectual disability/developmental delay and other signs and symptoms, namely behavioural disorders, hypotonia, mild craniofacial dysmorphism, brain anomalies and seizures. The *ZMYND11* protein functions as a transcriptional repressor and plays an inhibitory role in the muscle and neuronal differentiation process. To date only eight cases are describe in the literature, all with loss-of-function mutations. The *ZMYND11* gene is thought to be a critical gene in 10p15.3 microdeletion syndrome.

Clinical Report

A six-year-old girl, born at 37 weeks to non-consanguineous parents, was referred to our unit due to psychomotor delay, west syndrome, laryngomalacia, a major aortopulmonary collateral artery and dysmorphic features. Her mother has intellectual disability, epilepsy and short stature. She took 2g of valproate and 1g of levetiracetam during the first 7 months of pregnancy. There is also a history of ID in the grandmother and other maternal relatives.

We first performed array-CGH to the girl that did not reveal CNVs clearly pathogenic. After, we decided to perform a broad gene panel analysis in the mother that found a probable pathogenic frameshift variant in the *ZMYND11* gene. That variant is also present in the girl and her grandmother.

Discussion

The observed symptoms of ID/global development delay, disorder of speech development, behavioural problems and specific facial phenotype can be considered as phenotypic core characteristics of a clinically recognisable syndrome with invariable neurodevelopmental disorder that is caused by loss-of-function *ZMYND11* mutations. However there are very few patients described yet. So, the use of a broad NGS panel can be reasonable.

Our patients have a frameshift mutation that is in accordance with this mechanism of haploinsufficiency, and its cosegregation with the disease in three affected family members and their specific phenotype confirms the pathogenic status of this variant.

The girl has a more severe phenotype probably due to her exposure to valproate during pregnancy.

COMUNICAÇÕES EM PAINEL | POSTER PRESENTATIONS

HIGHLIGHTS

Cell-free DNA methylation of selected genes allows for early detection of the major cancers in women

Sandra P. Nunes[1,2]; Catarina Moreira-Barbosa[1]; Sofia Salta[1]; Susana Palma De Sousa[3]; Inês Pousa[4]; Júlio Oliveira[4]; Marta Soares[4]; Licínio Rego[5]; Teresa Dias[5]; Jéssica Rodrigues[6]; Luís Antunes[6]; Rui Henrique[1,7,8]; Carmen Jerónimo[1,8]

1- Cancer Biology & Epigenetics Group—Research Center, Portuguese Oncology Institute of Porto (CI-IPOP), Porto, Portugal; 2- Master in Oncology, Institute of Biomedical Sciences Abel Salazar, University of Porto (ICBAS-UP), Porto, Portugal; 3- Breast Cancer Clinic and Dept. of Medical Oncology, IPOP, Porto, Portugal; 4- Lung Cancer Clinic and Dept. of Medical Oncology, IPOP, Porto, Portugal; 5- Digestive Tract Pathology Clinic and Surgical Oncology, IPOP, Porto, Portugal; 6- Dept. of Epidemiology, IPOP, Porto, Portugal; 7- Dept. of Pathology, IPOP, Porto, Portugal; 8- Dept. of Pathology and Molecular Immunology, ICBAS-UP, Porto, Portugal

---

Context/Aims: Breast (BrC), colorectal (CRC) and lung (LC) cancers are the three most common and deadly cancers in women. Cancer screening entails an increase in early stage disease detection but is hampered by high false-positive rates and overdiagnosis/overtreatment. Aberrant DNA methylation occurs early in cancer and may be detected in circulating cell-free DNA (ccfDNA), constituting a valuable biomarker and enabling non-invasive testing for cancer detection. We aimed to develop a ccfDNA methylation-based test for simultaneous detection of BrC, CRC and LC.

Methods: CcfDNA from BrC, CRC and LC patients and asymptomatic controls were extracted from plasma, sodium-bisulfite modified and whole-genome amplified. *APC, FOXA1, MGMT, RARβ2, RASSF1A, SCGB3A1, SEPT9, SHOX2* and *SOX17* promoter methylation levels were determined by multiplex quantitative methylation-specific PCR. Associations between methylation and standard clinicopathological parameters were assessed. Biomarkers' diagnostic performance was also evaluated.

Results: A "PanCancer" panel (*APC, FOXA1, RASSF1A*) detected the three major cancers with 72% sensitivity and 74% specificity, whereas a "CancerType" panel (*SCGB3A1, SEPT9* and *SOX17*) indicated the most likely cancer topography, with over 80% specificity, although with limited sensitivity.

Conclusions: CcfDNA's methylation assessment allows for simultaneous screening of BrC, CRC and LC, complementing current modalities, perfecting cancer suspects' triage, increasing compliance and cost-effectiveness.

Funding: This work was supported by a grant from Research Center of Portuguese Oncology Institute of Porto (PI 74-CI-IPOP-19-2016). C.M.-B. was supported by Núcleo Regional da Madeira da Liga Portuguesa Contra o Cancro & Diário de Notícias. S.S. is supported by a PhD fellowship IPO/ESTIMA-1 NORTE-01-0145-FEDER-000027. J.R. is supported by a fellowship PI 110-CI-IPOP89 2018-CES 329-017.

Induction of apoptosis increases the sensitivity of detection of relevant mutations in circulation

Joana Marques[1,2]; Susana Junqueira Neto[1,2,3]; Jorge Pinheiro[4]; José Carlos Machado[1,2,3]; José Luís Costa[1,2,3]

1- i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto; 2- IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto; 3- FMUP – Faculdade Medicina da Universidade do Porto; 4- Serviço Anatomia Patológica, Hospital São João, Portugal

---

The study of tumor-derived DNA, as a liquid biopsy strategy, has revealed its clinical relevance as a biomarker for cancer management. However, the intrinsic low abundance of ctDNA and the complexity of the methodologies available difficult the detection of tumor mutations in plasma. Thus, we hypothesize that induction of apoptosis may increase the levels of ctDNA in circulation and enable the use of routine approaches for the detection of relevant mutations in plasma of cancer patients.

In vitro, H1975 lung cancer cell line was treated with docetaxel for different periods of time to evaluate the time dependent effect of single dose treatment on the levels of apoptosis. The levels of ctDNA release were also assessed by quantification of DNA extracted from culture medium. *In vivo* studies were then performed in immunodeficient C57BL/6 xenografted mice. The impact of docetaxel treatment on the levels of apoptosis in the tumor tissue was analyzed by IHC, 24h and 48h after treatment. In parallel, cfDNA was extracted from the plasma of xenografted mice to determine the effect of treatment on DNA release levels. The fraction of ctDNA within total cfDNA was determined by qPCR.

The *in vitro* studies have shown an increase on the levels of apoptosis and ctDNA release upon docetaxel treatment, in a time dependent manner with greater impact at 48h. Similarly, the *in vivo* results have shown that a single dose treatment had an impact on tumor apoptosis, mainly at 48h, which correlated with increased levels of cfDNA detected in plasma. The specific detection of increased levels of tumor-derived DNA, by targeting a mutation of the xenografted cell line, confirmed the influence of a single dose treatment on ctDNA release. This study provides new insights regarding a better timing for blood collection, approximately 24h to 48h after treatment. This new approach may overcome the low abundance of ctDNA and accelerate the standardization of liquid biopsy on the clinical routine.

FEDER funds through the COMPETE 2020—POCI, Portugal 2020, FCT: POCI-01-0145-FEDER-007274 and PTDC/DTP-PIC/2500/2014, and by NORTE 2020, in the framework of the project NORTE- 01-0145-FEDER-000029.

Evidence for a role of nonsense-mediated mRNA decay pathway genes in Autism Spectrum Disorder

Ana Rita Marques[1,2]; Hugo Fmc Martiniano[1,2,3]; João Pedro Santos[1,2]; Joana Vilela[1,2]; Muhammad Asif[1,2]; Guiomar Oliveira[4,5]; Luísa Romão[2,6]; Astrid Moura Vicente[1,2,7]

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

---

Introduction: Autism Spectrum Disorder (ASD) is a highly heterogeneous neurodevelopmental disorder with an unclear etiology. Genetic factors are estimated to account for 50 to 80% of the familial ASD risk, but most of the genetic determinants are still not known and a role for other regulatory mechanisms is likely. The nonsense-mediated decay (NMD) pathway controls mRNA quality and plays an important role in the regulation of the transcriptome. Mutations in genes involved in the NMD pathway have been linked to neurodevelopmental disorders, with intriguing evidence for an involvement of mutations in the *UPF3B* gene, a core component of the NMD pathway, in ASD.

Methods: In this study we explored the potential role of NMD factors in ASD. For this purpose, a list of 153 genes involved in the NMD pathway was generated using AmiGO, Reactome and a systematic literature review. The frequency of Copy Number Variants (CNVs) targeting NMD genes in ASD patients (n=3570) was compared with control subjects (n=9649), using the Fisher's exact test corrected for multiple testing. We also screened for Single Nucleotide Variants (SNVs) in NMD genes in whole exome sequencing data (WES) from 1338 ASD subjects, to identify loss of function mutations.

Results: We found five NMD genes targeted by CNVs exclusively present in at least two ASD subjects, and two NMD genes more frequently targeted by CNVs in ASD subjects, when compared to controls. In the ASD WES dataset we identified 43 high impact variants in 28 NMD genes, including the *UPF3B* and *ACE*, two genes previously implicated with ASD.

Discussion: The discovery of 33 NMD genes that are intriguing candidates for ASD in large patient genomic datasets provides evidence supporting the involvement of the NMD pathway in ASD pathophysiology.

Acknowledgements
Support for this work was provided by Fundação para a Ciência e a Tecnologia (grant PD/BD/113773/2015 to Ana Rita Marques).

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

Vânia Gonçalves[1,2]; Paulo Matos[2]; Joana Pereira[1,2]; Andreia Henriques[1,2]; Peter Jordan[1,2]

1 - Instituto Nacional de Saúde Doutor Ricardo Jorge; 2 - Biosystems & Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa

Distinct genetic subtypes have been described in colon cancer, one of which involves overexpression of RAC1b, a variant generated by alternative splicing. Aberrant splicing is known to occur in cancer and can be caused by mutation in a gene or splicing factor but also represents a dynamic response to oncogene- induced cellular signaling and in this case it may be pharmacologically targeted. Here we explore how signaling pathways are involved in the deregulation of alternative RAC1b splicing in colorectal tumor cells.

HT29 cells represent serrated colorectal tumors with BRAF gene mutation V600E in one allele and RAC1b overexpression. Cells were transfected with shRNA vectors directed against target candidate protein kinase transcripts and their effects on RAC1b levels analyzed 24h later by Western Blot and qRT-PCR. Treatment with kinase inhibitors or anti-inflammatory drugs was performed 24h and 48h prior to cell lysis.

Two kinases, SRPK1 and GSK3β, were found required to sustain RAC1b levels and both were shown to act upon the phosphorylation of splicing factor SRSF1, which binds to and promotes the inclusion of the alternative exon in RAC1b. SRPK1 knockdown or pharmacological inhibition reduced SRSF1 phosphorylation decreasing its nuclear translocation and concomitantly RAC1b splicing. The same regulatory pathway was also found to be controlled by GSK3β. Interestingly, GSK3β phosphorylation was identified to serve as target for the anti-inflammatory drug ibuprofen, which inhibits RAC1b overexpression.

Together, our results demonstrate that alternative splicing is deregulated by oncogenic signal transduction pathways and it may be drug revertable.

Acknowledgements: This work was supported by the Fundação para a Ciência e Tecnologia, Portugal and Association Maratona da Saúde.

National study on TRPV4-related skeletal dysplasias – clinical and molecular characterization of eleven Portuguese patients

João Parente Freixo[1]; Oana Moldovan[2]; Sílvia Sequeira[3]; Ana Beleza[4]; Ana Carvalho[5]; Patrícia Dias[2]; Rui Gonçalves[1]; Teresa Kay[1]; Jorge Saraiva[5,6]; Miriam Aza-Carmona[7]; Karen E. Heath[7]; Sérgio B. Sousa[5,8]

1- S. de Genética Médica, Hospital Dona Estefânia, CHLC, EPE, Lisboa, Portugal; 2- S. de Genética Médica, Dept. de Pediatria, Hospital de Santa Maria, CHLN, EPE, Centro Académico de Medicina de Lisboa, Lisboa, Portugal; 3- Unidade de Doenças Metabólicas, Hospital Dona Estefânia, CHLC, EPE, Lisboa Portugal; 4- Clinical Genetics Service, Guy's and St Thomas' NHS Foundation Trust, London, UK; 5- S. de Genética Médica, Hospital Pediátrico, CHUC, Coimbra, Portugal; 6- Dept. de Pediatria, Faculdade de Medicina, Universidade de Coimbra, Coimbra, Portugal; 7- Institute of Medical and Molecular Genetics (INGEMM), IdiPAZ, UAM and Skeletal dysplasia multidisciplinary Unit (UMDE), Hospital Universitário La Paz, Madrid, Spain and CIBERER, ISCIII, Madrid, Spain; 8- Dept. de Genética Clínica, Faculdade de Medicina, Universidade de Coimbra, Coimbra, Portugal

AIMS: Heterozygous dominant (likely) gain-of-function variants in the *TRPV4* gene are responsible for a continuous phenotypic spectrum of skeletal dysplasias. Interestingly, some *TRPV4* variants also cause neuromuscular disorders or both skeletal and neuropathic. We studied genotype-phenotype correlations and natural history on this group of disorders in the Portuguese population.

METHODS: Clinical and molecular characterization of all cases with *TRPV4*-related skeletal dysplasias observed at 3 Portuguese hospital centres based on retrospective analysis of medical records and clinical re-evaluation when indicated.

RESULTS: We describe 11 patients from 8 different families, 5 males and 6 females, last evaluated between 2 and 37 and clinically diagnosed between 1 and 23 years.

One variant of unknown significance (VOUS) and four different pathogenic heterozygous *TRPV4* missense variants were identified. Two of them were recurrent and three were novel. Five patients had metatropic dysplasia (MD), four of which harbored the recurrent variant p.Pro799Leu and one had a novel variant affecting the same residue p.Pro799Ala. The p.Arg594His recurrent mutation was identified in two unrelated patients presenting with spondylo-metaphyseal dysplasia Kozlowski type (SMDK). A novel variant, p.Gly595Glu, was identified in three patients from the same family who showed an intermediate phenotype between autosomal dominant brachyolmia and spondylo-epiphyseal dysplasia Maroteaux type. In addition, in one patient presenting with isolated bilateral Perthes disease, a novel variant, p.Ala293Asp, classified as a VOUS, was identified. This variant is absent from gnomAD and in silico analyses predict that it is deleterious.

CONCLUSION: Our results reinforce phenotypic homogeneity associated with specific *TRPV4* missense variants such as p.Pro779Leu or Ala in MD and p.Arg594His in SMDK. This is the second report on a *TRPV4* variant possibly involved in susceptibility to bilateral Perthes disease. Detailed description of patient cohorts with such rare disorders are crucial for families and health professionals, leading to a better-informed management and surveillance of possible complications.

C9orf72 expansion is associated with accelerated decline of respiratory function and decreased survival in amyotrophic lateral sclerosis

Gabriel Miltenberger-Miltenyi[1]; Vasco A Conceição[2]; Marta Gromicho[2]; Ana Catarina Pronto-Laborinho[2]; Susana Pinto[2]; Peter M Andersen[3]; Mamede De Carvalho[2,4]

1 - Physiology Institute, Faculty of Medicine, Instituto de Medicina Molecular, University of Lisbon, Lisbon, Portugal; 2 - (1) Physiology Institute, Faculty of Medicine, Instituto de Medicina Molecular, University of Lisbon, Lisbon, Portugal; 3 - (2) Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden; 4 - (3) Department of Neurosciences and Mental Health, Hospital de Santa Maria-CHLN, Lisbon, Portugal

Introduction: Respiratory insufficiency is the main cause of death in amyotrophic lateral sclerosis (ALS). As the *C9orf72* repeat expansion represents the most common genetic risk factor for this disease, we studied whether *C9orf72* modulates respiratory function and survival.

Methods: Demographic and clinical data, and *C9orf72* status were collected from 372 ALS patients followed in our center. Multiple regressions controlling for the *C9orf72* expansion, diagnosis delay, region of onset, age, gender, and comorbid frontotemporal dementia were performed to evaluate the functional and respiratory status of the patients at baseline and during disease progression — assessed using the global ALSFRS-R score and its respiratory subscore, and the predicted forced vital capacity (%FVC). A Cox regression controlling for the same variables was carried out to analyse survival.

Results: At baseline, 32/372 (8.60%) patients carried the *C9orf72* repeat expansion. During disease progression, the ALSFRS-R_{global} and ALSFRS-$R_{respiratory}$ scores were not significantly influenced by the mutation ($p>0.8$ and $p>0.3$, resp.). On the contrary, we found that the *C9orf72* mutation is an independent risk factor for a faster %FVC decline ($p=0.001$) and shorter survival ($p=0.002$).

Conclusions: In ALS patients the *C9orf72* expansion is independently associated with accelerated respiratory dysfunction and thus with a shorter survival. This finding indicates a new pathogenic mechanism of *C9orf72* in ALS.

Success evaluation of Assisted Reproductive Technology in couples with chromosomal abnormalities

Ana Rita Jesus[1]; Sandra Soares[2]; Joaquina Silva[3]; Alberto Barros[3,4,5]; Sofia Dória[4]

1 - MMED- Faculdade de Medicina do Porto; 2 - Serviço de Medicina da Reprodução - HSJ Porto; 3 - Centro de Genética da Reprodução Prof Doutor Alberto Barros; 4 - Serviço de Genética, Departamento de Patologia, Faculdade de Medicina do Porto; 5 - Instituto de Ciência e Inovação em Saúde I3S, Universidade do Porto

---

Introduction: Infertility is estimated to affect 15% of couples, and chromosome abnormalities play an important role in its etiology. An increasing amount of couples has been using Assisted Reproductive Technology (ART). The main objective of this work is to access the reproductive success of ART techniques in infertile couples with chromosomal abnormalities comparing to a control group also submitted to ART with normal karyotype.

Methods: A retrospective analysis of all karyotypes performed, from 2010 to 2017, in the Genetics Service of FMUP, with the clinical indication of infertility, were considered for the study. Only couples were included, and two groups were established attending the presence or absence of chromosomal abnormalities (cases and controls). Data regarding type of infertility, couples' ages, ART techniques performed and their reproductive success were obtained from medical records. An independent t-test and chi-square tests were performed for comparative analysis.

Results: We found a prevalence of 6,62% (162/2446) of chromosome abnormalities in our population. Chromosomal anomalies were found in 83 men (35,02%) and 154 women (64,98%). Low grade mosaicism was the most prevalent anomaly, affecting 49,79% of individuals, followed by translocations (23,31%) and sex chromosomes abnormalities (13,93%). There was no statistical difference between groups regarding mean age, type of infertility and number of procedures necessary to the reproductive success. On average, 2,21 fertility treatments were necessary to achieve a successful outcome. There was a slightly higher rate of success in the control group (49,80% vs. 46,29%; p=0,402), with fewer losses of pregnancy (19,35% vs. 12,76%). However, this was not statistically significant.

Conclusion Although the differences regarding success rate between groups were not found statistically significant, we still advocate that cytogenetic analysis should be performed routinely in all infertile couples due to the fact that it might help deciding the best treatment options and minimize the risk of transmission of anomalies to the offspring.

Extending genetic analysis of hereditary myopathies beyond next-generation targeted and exome sequencing

Ana Gonçalves[1,2]; Manuela Santos[3]; Ana Fortuna[2,4]; Teresa Coelho[5]; Ricardo Taipa[6]; Manuel Melo-Pires[6]; Johan T Den Dunnen[7]; Mário Sousa[2,8,9]; Rosário Santos[1,2,*]; Jorge Oliveira[1,2,*]

1- Unidade de Genética Molecular, Centro de Genética Médica Jacinto Magalhães, (CGMJM), Centro Hospitalar e Universitário do Porto (CHUP), Porto; 2- Unidade Multidisciplinar de Investigação Biomédica (UMIB), Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (ICBAS-UP), Porto; 3- Consulta de Doenças Neuromusculares e S. de Neuropediatria, CHUP, Porto; 4- S. de Genética Médica, CGMJM, CHUP, Porto; 5- S. de Neurofisiologia, Dept. de Neurociências, CHUP, Porto; 6- Unidade de Neuropatologia, CHUP, Porto; 7- Dept. of Human Genetics and Clinical Genetics, Leiden University Medical Center, Leiden; 8- Dept. de Microscopia, Laboratório de Biologia Celular, ICBAS-UP, Porto; 9- Centro de Genética da Reprodução Prof. Alberto Barros, Porto; *-Equally contributing authors

Background: Next-generation sequencing applications have significantly improved the research and diagnostic yield of genetically heterogeneous conditions. A subset of patients remains unsolved even upon WES analysis. We describe 3 complex cases of hereditary myopathies.

Methods: P1: new-born; severe nemaline myopathy; brother with similar phenotype. A combination of WES, exon-level microarray (XON), RNA and genomic breakpoint sequencing was done. P2 and P3: suspected Becker Muscular Dystrophy. No pathogenic variants in *DMD* were identified by conventional testing. In P2, bioinformatic, transcriptomic and genomic approaches (SB, long-range PCR and single-molecule real-time sequencing) were applied. In P3, RT-PCR, cDNA-MLPA, low-coverage whole genome sequencing (LC-WGS) and breakpoint sequencing approaches were used.

Results: P1: WES failed to explain an autosomal-recessive condition. XON array showed a large heterozygous deletion in *KLHL41*, where WES had identified a single heterozygous missense variant. This novel deletion was confirmed by RNA and genomic breakpoint sequencing. Family studies confirmed compound heterozygosity for the two variants and co-segregation with the disease. P2: An aberrant transcript was identified, containing a 103nt insertion between *DMD* exons 51 and 52, with no similarity to the gene. This corresponded to the partial exonization of a LINE-1 sequence. Further characterization identified the deep-intronic insertion of a complete LINE-1. P3: *DMD* cDNA studies disclosed the absence of exons 75-79. Automated structural variant calling from LC-WGS was inconclusive, but BAM file inspection showed a putative breakpoint within intron 74, as some reads had homology with a region upstream of *PRDX4* (Xp22.11). Breakpoint sequencing showed a ~8Mb inversion comprising part of *DMD* and upstream of *PRDX4*.

Conclusions: This work revealed unexpected and hitherto unknown mutational events underlying some myopathies. Together with solid bioinformatics, WGS complemented by transcriptome analysis has the potential to detect the majority of mutation types.

Funding: CHUP research grant 336-13(196-DEFI/285-CES);UMIB funded by FCT(Pest-OE/SAU/UI0215/2014).

MEF2C haploinsufficiency syndrome: a clinical report of two deletions and one mutation

Raquel Gouveia Silva[1]; Catarina Machado[1]; Juliette Dupont[1]; Oana Moldovan[1]; Ana Berta Sousa[1]

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

Introduction

MEF2C haploinsufficiency syndrome was recently recognized as a neurodevelopmental disorder, leading to excitatory/inhibitory imbalance and neurobehavioral dysfunction, which plays a role in the pathogenesis of autism spectrum disorder (ASD). It is characterized by moderate to severe psychomotor delay and intellectual disability, epilepsy or febrile seizures, brain abnormalities, stereotypic movements and minor dysmorphism, caused by either microdeletion at 5q14.3 (44 cases reported until now) or point mutations (eight patients, according to the literature) in the *MEF2C* gene. No significant phenotypic differences between point mutations and microdeletions have been observed so far.

Clinical Report

Two young women, aged 18 and 22, and a 12 year-old girl presented with axial hypotonia, autistic traits with midline stereotypies, intellectual disability, affecting language more severely, epilepsy, that was difficult to control initially with anti-epileptics, and sleep disorder. The youngest also had ataxic gait, strabismus and delayed myelination on cerebral magnetic resonance imaging. All three cases presented some minor dysmorphic features. In the other patients, array CGH identified a *de novo* copy loss at 5q14.3. Whole exome sequencing was performed after extensive investigation in the youngest patient, revealing a *de novo* heterozygous pathogenic point mutation in the *MEF2C* gene.

Discussion

MEF2C haploinsufficiency syndrome should be considered in the differential diagnosis of patients with severe intellectual disability and Rett-like features. Up to now, not many cases have been reported, making it difficult to establish significant phenotypic differences between point mutation and microdeletion patients. Very recently, treatment with NitroSynapsin, a new dual-action compound related to the FDA approved drug memantine showed benefit in a *MEF2C* haploinsufficiency mouse model.

Detection of copy number variations in rare Mendelian disorders using whole exome sequencing

Susana Sousa[1]; Paulo Silva[1]; Susana Barbosa[1]; Ana Lopes[1]; Ana Filipa Brandão[1]; Patrícia Arinto[1]; Sara Morais[1]; Isabel Alonso[1]; Jorge Sequeiros[1,2]

1- CGPP – Centro de Genética Preditiva e Preventiva, IBMC – Instituto de Biologia Molecular e Celular, i3S – Instituto de Investigação e Inovação em Saúde; 2- Universidade do Porto, Portugal

---

Introduction: Copy number variations (CNVs) are important elements of human genetic diversity which are commonly observed in the human population and have been increasingly recognized as an important etiology of many human diseases. Whole exome sequencing (WES) has been widely accepted as a robust and cost-effective approach for clinical genetic testing of small sequence variants. Detection of CNVs within WES data has become possible through the development of various algorithms, software programs and statistical methods that use the comparison of coverage between the affected patient and controls. Incorporation of these algorithms into WES bioinformatics pipelines increases the diagnostic yield. In this study, we demonstrate the challenges and feasibility of analyzing CNVs using WES data.

Methods: We performed WES on an Illumina HiSeq 4000, on patients with rare genetic diseases and analyzed CNVs using Golden Helix's VarSeq software. CNVs detected as likely causative are confirmed by qPCR or MLPA. When a causative SNV in a recessive disorder is detected we fine-tune our CNV analysis for this gene with more lenient parameters on a case-by-case basis.

Results: Last year we detected 12 CNVs as probably causative, but just one deletion could be confirmed by qPCR, leading to 91,7% of false positives (FPs). Using this know-how, optimizing the regions of interest flanks (to 100bp), and using healthy CNV controls from 1kG Project, ClinGen, ClinVar, DECIPHER, DGV, ExAC and our internal CNV database, we were able to filter many of these FPs and common CNVs. Therefore, during the current year, we analyzed 701 patients where we detected 79 CNVs as probably causative, of which 33 cases were confirmed by qPCR or MLPA (58,2% of FPs).

Conclusion: Although we had a high rate of FPs, we were able to identify potential disease-causing CNVs using WES data in 4,7% of the 701 patients studied. The confirmation by orthogonal methodologies validated the detected CNVs and software analysis. Therefore, it is possible to increase the diagnostic yield by combining SNV and CNV analysis by WES in order to improve the molecular diagnosis of patients with rare Mendelian disorders.

Prognostic and Mutational Genomic Signatures of Breast Cancers in the era of Precision Medicine

Joana Cardoso[1,2,3]; Ana Pereira[1]; Maria Jose Rego De Sousa[1]; José Germano De Sousa[1]; José Germano Rego De Sousa[1]

1 - 2. Centro de Medicina Laboratorial Germano de Sousa, Lisboa, Portugal; 2 - 1. Ophiomics - Precision Medicine, Lisboa, Portugal; 3 - 3. iNOVA4Health-Advancing Precision Medicine, NOVA University of Lisbon, Lisboa, Portugal

---

In the era of precision medicine the clinical management of breast cancer patients with *ER*-positive, HER2-negative disease is still challenging. A wide spectrum of different risk profiles fits within this group of patients which include women who derive little benefit from adding chemotherapy to adjuvant endocrine therapy to women with a high-risk of relapse and thus to whom treating with chemotherapy is very appropriate. In addition to prognosis, little is currently done to assess the heterogeneous DNA mutations harboured in the primary breast tumor that can dictate drug response and also prognosis.

In order to assist the clinicians in making treatment decisions (addition of chemotherapy) after consideration of conventional markers new molecular tests predicting the outcome of breast cancer patients have been developed and its prognostic value recognized by main current international guidelines. Furthermore the new massive sequencing technologies (NGS) can also aid clinicians to uncover the most relevant mutations.

We have recently implemented in our lab the usage of EndoPredict® genomic signature (Myriad Genetics), a new RNA-based 12-genes molecular score (EP-Score) predicting the 10-year likelihood of distant recurrence in patients with early-stage, ER-positive, and HER2-negative breast cancer treated with adjuvant endocrine therapy only. This 2nd generation genomic signature, estimates an individualized risk score (EPclin) through the integration of the EP-Score with two clinical variables ("tumor size" and "number of positive lymph nodes"). In addition, to better tailor therapy decisions we have implemented laboratory and bioinformatics NGS workflows to assess the mutational landscapes of archived breast tumors material prior to therapy.

We have already applied EndoPredict® to a patient cohort that included ductal and lobular, T1 to T2 and N0 to N1 breast tumors, all resulting in molecular-assisted therapy decisions. We will describe a particular case where the combined usage of EndoPredict® and mutational landscape provided valuable information to drastically reduce the risk of recurrence.

COMUNICAÇÕES EM PAINEL | POSTER PRESENTATIONS

P12 | BASIC RESEARCH

GenoinVar: an end-to-end solution for exome sequencing and variant analysis

Maria José Simões[1]; Bernabé Diéguez[1]; Cristina Barroso[1,2]; Hugo Froufe[1]; Conceição Egas[1,2]

1 - Genoinseq, Next-Generation Sequencing Unit, Biocant; 2 - Center for Neuroscience and Cell Biology, University of Coimbra

---

Genetic diagnosis is a determinant information for clinical practice in many cases. The increasing use of untargeted solutions based on next-generation sequencing has proved to be cost-effective in the diagnosis of a wide range of conditions. Furthermore, implementation of multidisciplinary approaches has also led to a significative reduction in time to diagnosis.

We developed an end-to-end solution from blood sample to exome sequencing and variant analysis to accelerate the discovery of causal genetic variants. This solution was developed in the framework of the In2Genome project, a multidisciplinary project to integrate whole exome sequencing in clinical practice routine involving experts of Genoinseq, Coimbra Genomics and the Genetics Unit of the Coimbra Pediatric Hospital (CHUC).

The solution involves whole exome sequencing of extracted DNA on the Illumina NextSeq platform. Exome capture uses a combined solution from IDT and Illumina that reduces non-specific hybridizations and increases performance, namely the percentage of on-target reads. Obtained sequencing metrics are uniform and above specifications, more than 94% of the reads mapped on-target and 98% of the target bases had coverage higher than 20X.

Candidate variant selection is then performed in our ExomeLoupe platform, an intuitive and user-friendly Windows software for variant prioritization and interpretation. The user can select the variants of interest by gene, HPO term or disease; genomic position; clinical significance based on ClinVar; population frequency; and/or predictive effect according to different tools. ExomeLoupe works on encrypted data, also enabling file storage and sharing in a protected manner according to the new European General Data Protection Regulation.

Under this end-to-end solution we tested molecular diagnosed patients selected by CHUC within the In2Genome project and successfully identified the previously reported variants. GenoinVar represents a true end solution for identifying causal variants in clinical or research contexts.

Funding: In2Genome (CENTRO-01-0247-FEDER-017800), GenomPT (POCI-01-0145-FEDER-022184) and Strategic Project (POCI-01-0145-FEDER-007440).

How DIS3L2 meets NMD-targets: I'm really into "U"!

Paulo J Da Costa[1,2]; Margarida Saramago[3]; Sandra C Viegas[3]; Cecília Arraiano[3]; Luísa Romão[1,2]

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

The nonsense-mediated mRNA decay (NMD) pathway selectively degrades mRNAs carrying a premature translation-termination codon but also regulates the abundance of a large number of physiological RNAs that encode full-length proteins. Also, NMD regulates the levels of many physiological PTC-free mRNAs that encode full-length proteins. In human cells, NMD-targeted mRNAs are degraded by endonucleolytic cleavage and exonucleolytic degradation from both 5' and 3' ends. This is achieved by a process not yet completely understood that promotes the decay of the mRNAs in 5'-to-3' and 3'-to-5' by the XRN1 and exosome, respectively. In yeast, Dis3/Rrp44 protein is the catalytic subunit of the exosome, but in humans, there are three known paralogues of this enzyme: DIS3, DIS3L1, and DIS3L2. Conversely to its counterparts, DIS3L2 exoribonuclease activity is independent of the exosome. In order to unveil the role of DIS3L2 in NMD, we performed its knockdown in HeLa cells and measured the mRNA levels of various natural NMD targets. Our results show that DIS3L2 is involved in NMD-targets decay. Besides that, DIS3L2 acts directly on NMD-targets and interacts with the key NMD factor UPF1. We also show that DIS3L2-mediated decay depends on the activity of the terminal uridylyl transferases (TUTases) 4 and 7, which adds non-templated uridines to the mRNAs 3' end, marking these mRNAs for DIS3L2 degradation. Together, our findings establish a direct role of DIS3L2 in NMD in an uridylation-dependent manner.

P14 | BASIC RESEARCH

Identification of Copy Number Variation by array-CGH in Portuguese Children and Adolescents Diagnosed with Autism Spectrum Disorders

Sidonie Monteiro[1]; Joel Pinto[2,3]; Miguel Leão[2,4]; Sofia Dória[2,3]

1 - Faculdade de Medicina da Universidade do Porto; 2 - Serviço de Genética, Departamento de Patologia da Faculdade de Medicina da Universidade do Porto; 3 - Instituto de Ciência e Inovação em Saúde I3S, Universidade do Porto; 4 - Consulta de Genética Médica - HSJ

Background: Autistic spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental diseases. ASD affect several children and can be manifested in earlier stages of life. Array CGH offers superior sensitivity for identification of submicroscopic chromosomal abnormalities and it is advocated to be the first tier genetic testing for patients with ASD. Copy Number Variants (CNVs), that is known to predispose to these neurodevelopmental disorders, may play a role in the etiology of ASD. The main objective of this work was to establish a clinical association between array-CGH results and ASD.

Methods: 253 patients admitted to a neurogenetic consultation and diagnosed with ASD were selected for array-CGH (Agilent 4x180K microarrays). CNVs were classified as benign, pathogenic, likely pathogenic, VOUS and pathogenic in recessive forms.

Results: 3,557% (9/253) of CNVs were classified as pathogenic CNVs. Considering also the likely pathogenic CNVs the rate increases to 11,462% (29/253). This is similar to the frequencies found in the literature (around 10%). Some unexpected CNVs not always correlated to the ASD pathophysiology were also found. Taking into account a phenotype-genotype correlation the patients were divided in two groups. In the group with this correlation, we found 22 pathogenic or likely pathogenic CNVs (10 deletions and 12 duplications). Within this group we were able to perform parents studies in 6 cases, 5 inherited and 1 *de novo*.

Conclusion: The identification of copy number variations in children and adolescents with autistic disorders highlight the relevance of array comparative genomic hybridization as the first-tier genetic test.

Next sequencing generation (NGS) namely using specific ASD panels for the most common mutations has been applied in many patients, nevertheless we underline the need for better data in NGS for reducing the uncertain significance of the results.

Epigenetic modifications could contribute for second trimester pregnancy spontaneous losses

Sara Vasconcelos[1]; Carla Ramalho[2,3]; Joana Marques[1,2]; Sofia Dória[1,2]

1 - Genetics Unit, Department of Patology, Faculty of Medicine, University of Porto, Portugal; 2 - I3S – Instituto de Investigaão e Inovação em Saúde, University of Porto, Portugal; 3 - Department of Obstrectics and Gynecology, Centro Hospitalar São João and Faculty of Medicine, Porto, Portugal

---

Introduction: Miscarriage is considered the most common complication in pregnancy. Despite its heterogenic causes, the reasons behind 40-50% of miscarriages are still not well understood. Epigenetics has a central role in the regulation of fetal growth and development, so methylation patterns abnormalities or aberrant imprinting gene have been considered to be possible candidates.

Material and Methods: A total of 70 placenta and fetal tissues samples from 12-24 weeks loss of pregnancies were studied. Cases were divided in idiopathic spontaneous abortions (ISA) with or without fetal growth restriction (FGR). Controls were selected from pregnancy losses due to infections causes. RNA expression levels of genes involved in methylation (*DNMTs*) and hydroxymethylation (*TET*s), imprinted genes (*IGF2*, *CDKN1C*, *KCNQ1*, *PHLDA2*, *MEST*, *PEG10*) and a non-imprinted gene (*LEP*) were analyzed by RT-qPCR. MS-MLPA and bisulfite cloning sequencing were performed for *MEST* promotor and two imprinted control regions. The global levels of 5-hmC in both tissues were analyzed using ELISA technique.

Results: Upregulation of *TETs*, *DNMT3A*, *IGF2* and *CDKN1C* genes and downregulation of *MEST* gene were observed in placentas without FGR; *DNMT3B* was downregulated in placentas with RCF; In the fetal tissue with FGR, the *TET3* gene was shown to be downregulated; In the fetal tissue with or without FGR, *IGF2* and *LEP* were upregulated; The global levels of 5-hmC were higher in the placenta comparing to the fetal tissue.

Discussion: Our study shows epigenetic deregulations in ISA. We observed changes in the expression of three imprinted genes, highlighting the importance of *IGF2* during the second trimester of pregnancy. Changes in the *LEP* gene expression was also observed, stressing the molecular complexity underlying human miscarriage. For the first time, global levels of 5-hmC were evaluated in both placenta and fetal tissue of ISA.

This work arises the question whether a possible epigenetic change present in the ISA could be the cause or a consequence of the abnormal development of pregnancy.

P16 | BASIC RESEARCH

Ultra-low coverage nanopore sequencing identifies thousands of structural variants in a tumour genome

Catarina Silva[1,2]; Bárbara Marques[1]; Sónia Pedro[1]; Hildeberto Correia[1]; Luís Vieira[1,2]

1 - Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa; 2 - ToxOmics - Centro de Toxicogenómica e Saúde Humana, Nova Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa

---

Objectives/Context: Nanopore sequencing is a recent technology which allows direct sequencing of DNA. It uses an ionic current to move DNA strands through nanopores embedded in an electrically-resistant membrane. The changes in nanopore signals are recorded by an ASIC chip placed below the membrane and translated into a specific base sequence. Nanopore sequencing provides a better alternative than short-read technologies to identify structural variants (SV) because of the ability to sequence entire DNA molecules. In this work we performed nanopore sequencing of a tumour genome to detect several types of SV.

Methods: We used the MinION device (Oxford Nanopore Technologies) to sequence the genome of an anonimized solid tumour sample. The rapid sequencing kit was used to prepare three 1D genomic DNA libraries which were sequenced consecutively using a single flow cell. Current measurements were converted to reads using the MinKNOW software and these were basecalled using Albacore. The resulting fastq files were mapped to the human genome using LAST. Reads harboring SV were identified using Picky. Copy number alterations were compared to those obtained using a CytoScan HD Array (ThermoFisher Scientific).

Results: The 3 MinION runs produced a total of 2,34 gigabases of DNA. The mean read length was 4508 bases and the longest read had 74369 bases. The genome was covered at a depth of 0.68X. A total of 447420 reads were aligned to the genome of which 65382 (14,4%) corresponded to breakpoint-containing reads. A total of 3470 SV with a minimum of 2 read-support were called including 2996 deletions, 196 duplications, 56 translocations, 216 insertions and 6 inversions. The vast majority of SV detected by Picky had a length below the array resolution. However, only a few of the larger copy number alterations were called because of coverage gaps.

Conclusion: Nanopore sequencing is a fast and sensitive approach to detect SV in the human genome. A 1-2X genome coverage will provide an almost complete picture of SV for investigating rearrangements in tumour cells.

This work was supported by Toxomics and GenomePT project (POCI-01-0145-FEDER-022184).

P17 | BASIC RESEARCH

Identification and characterization of two BRCA intragenic duplications

Manuela Pinheiro[1]; Ana Peixoto[1]; Catarina Santos[1]; Carla Escudeiro[1]; Rui Santos[1]; Carla Pinto[1]; Pedro Pinto[1]; Joana Guerra[1]; Susana Bizarro[1]; João Silva[1]; Manuel R. Teixeira[1,2]

1 - Department of Genetics, Portuguese Oncology Institute of Porto, Porto, Portugal.; 2 - Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, University of Porto, Largo Prof. Abel Salazar, 4099-003 Porto, Portugal

---

Aim: Deletions or insertions of large genomic sequences within coding regions are usually pathogenic because they can disrupt the reading frame and lead to loss of function. On the other hand, it is difficult to infer the effect of duplications without identifying the breakpoints and determining the orientation and location of the duplicated sequence. We aimed to characterize the genomic breakpoints of two intragenic duplications detected in *BRCA1* and *BRCA2* genes.

Methods: We studied two index patients from two HBOC families, one presenting a *BRCA1* (NM_007294.3) exons 4 to 6 duplication and another presenting a *BRCA2* (NM_000059.3) exons 17 to 18 duplication, detected by MLPA. To characterize the genomic breakpoints of these two rearrangements, we performed long range PCR using duplication specific primers.

Results: The *BRCA1* rearrangement consisted of an in tandem direct 12035-bp duplication, comprising the *BRCA1* region between 440-bp downstream of exon 3 and 870-bp downstream of exon 6 (c.134+440_441+870dup). The *BRCA2* rearrangement consisted of an 8451-bp in tandem direct duplication, comprising the *BRCA2* region between 2083-bp upstream of exon 17 and 1512-bp upstream of exon 19 (c.7806-2083_8332-1512dup). These duplications are predicted to cause frameshifts that create a premature stop codon.

Conclusion: Regarding the duplication of *BRCA1* exons 4 to 6, the breakpoint junction presented a complete homology sequence of 25-bp between *BRCA1* intron 3 and 6 and two highly homologous Alu elements were found in the genomic sequences flanking the breakpoint. Concerning the *BRCA2* exons 17 to 18 duplication, the breakpoint junction presented a complete homology sequence of 16-bp between *BRCA2* intron 16 and 18 and two highly homologous Alu elements were also found in the genomic sequences flanking the breakpoint. Concluding, we identified and characterized the genomic breakpoints of two duplications that occurred in tandem and in direct direction in the *BRCA1* and *BRCA2* genes, which likely occurred by homologous recombination mediated by Alu elements. We also consider that these intragenic duplications are the genetic defect underlying HBOC syndrome in these families.

Fabry disease associated with the GLA p.Phe113Leu variant: Evidence for a common ancestor between Portuguese and Italians

Diana Martins[1]; Susana Ferreira[1]; Luísa Pereira[2]; Nicoletta Chiesa (Italy)[3]; João Oliveira[4]

1 - Genetics Unit, Department of Pathology, Faculty of Medicine, University of Porto, Porto, Portugal; 2 - i3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal; 3 - Department of Pediatrics, University of Torino, Torino, Italy; 4 - Medical Genetics Outpatient Clinic, São João Hospital Centre, Alameda Hernâni Monteiro, 4200-319 Porto

---

Fabry disease (FD) is an X-linked lysosomal storage disorder with a heterogeneous spectrum of clinical manifestations that are caused by mutations in the α-galactosidase A gene (*GLA*). More than 900 *GLA* variants are currently reported most of them being family-specific. The most common causative mutation in Portuguese subjects diagnosed with FD is the p.Phe113Leu, which is associated with a late-onset cardiac variant of FD, and also appears to be prevalent in the Italian population. Notably, the Portuguese and Italian mutation carrier families share an identical five microsatellite haplotype that encompasses approximately 3Mb around *GLA* gene, suggesting an inheritance derived from a common ancestor. In order to estimate the most likely date for the occurrence of p.Phe113Leu mutation in Europeans we genotyped 100 healthy males from each the Portuguese and the Italian populations and expanded our haplotype analysis to a total of eleven gene-flanking microsatellite markers spanning a physical distance of approximately 23Mb in 95 individuals (21 Leu113 allele carriers and 74 controls). For each Portuguese and Italian control cohort we identified 91 different haplotypes none of them shared by the FD individuals carrying the *GLA* mutation. We identified a common haplotype in 10 out of 17 Portuguese mutation carriers and inferred it as the associated to the founder. Based on the pattern of linkage disequilibrium at closely linked marker loci the age of the mutation was estimated using the Decay of Haplotype Sharing MAPping software which allowed calculating the time to the most recent common ancestor in 16.7 generations. The mutation was therefore estimated to have arisen at the end of the 15th century which may be associated to the escape of the Sephardim Jews from the Iberian Peninsula in the period of the Inquisition. Exiled Jews spread throughout the world, including northern Italy, in pursuit of refuge.

P19 | BASIC RESEARCH

Analysis of NAPRT genetic variants and gene expression in tumors

Sara Duarte-Pereira[1,2]; Sérgio Matos[2]; José Luís Oliveira[2]; Raquel M. Silva[1,2]

1 - Department of Medical Sciences & iBiMED, University of Aveiro, Portugal; 2 - IEETA/DETI, University of Aveiro, Portugal

Background: Nicotinate phosphorybosyltransferase (Naprt) is an emerging target in cancer therapy, as Naprt loss is associated with EMT phenotype. Whether this is due to mutations in the gene is largely unknown.

Goal: To study the role of *NAPRT* variants in the regulation of gene expression, we analyzed CNV (copy number variation) and eQTLs (expression quantitative trait loci) that alter the expression of *NAPRT* in tumor samples from the PanCancer project of The Cancer Genome Atlas (TCGA).

Methods: We obtained *NAPRT* gene expression and CNV data from the cBioPortal and single-tissue cis-eQTL data from the PancanQTL database. In 16 types of cancer, we analyzed the correlation between CNV and expression, and the number and effect of eQTLs in *NAPRT* and surrounding genes.

Results: Most of the studied cancer types through the cBioPortal had no *NAPRT* alteration in more than 80% of the samples. Amplification of the locus was the most common alteration, though it did not fully overlap with gene upregulation. Ovarian cancer was the exception with *NAPRT* amplified in 33% and upregulated in 58% of those cases.

In the PancanQTL database, we found several *NAPRT* eQTLs that were not reported in GTEx, which comprises non-pathological samples, meaning that these might be cancer specific variants. LGG (low grade glioma) had 38 specific variants and 18 more were present in several cancer types. For example, rs34979030 is common to 11 different types of cancer. On the other hand, rs10099003 appears to influence *NAPRT* gene exclusively in prostate cancer.

Conclusion: Though *NAPRT* gene has a high number of genetic variants, their frequency in cancer is low. However, some have important effects on gene expression and might be cancer specific, stressing the need to characterize *NAPRT* gene expression to improve patient stratification for tailored therapeutics.

Funding: COMPETE/FEDER UID/BIM/04501/2013 to iBiMED, PEst-OE/EEI/UI0127/2014 to IEETA and SFRH/BD/108890/2015 to SDP.

P20 | BASIC RESEARCH

Altered expression of epigenetic regulators in azoospermic patients

Diogo Rodrigues[1]; Mário Sousa[2]; Filipa Carvalho[1,3]; Alberto Barros[1,3,4]; Joana Marques[1,3]

1 - Serviço de Genética, Faculdade de Medicina, Universidade do Porto, Portugal; 2 - Departamento de Microscopia, Laboratório Biologia Celular, Unidade Multidisciplinar de Investigação Biomédica – UMIB, Instituto de Ciências Biomédicas Abel Salazar (ICBAS) Universidade do Porto, Portugal; 3 - I3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; 4 - Centro de Genética da Reprodução A Barros, Porto, Portugal

---

Introduction/Objectives: Major epigenetic events take place during spermatogenesis. DNMT and TET enzymes play a pivotal role in this process, converting cytosine into 5-methylcytosine (5mC), and 5mC into 5-hydroxymethylcytosine (5hmC), respectively. 5mC is crucial for gene silencing and genomic imprinting, and 5hmC appears to have a role in the demethylation process. The importance of a correct epigenetic reprogramming during spermatogenesis is highlighted by methylation defects in germ cells of men facing fertility problems. Here, we measured *DNMT* and *TET* expression in germ cells of patients presenting infertility due to Oligozoospermia, Obstructive Azoospermia, and Secretory Azoospermia. We also analysed *TET* promoter methylation, and global DNA hydroxymethylation content.

Methods: A total of 58 testicular biopsies and 23 sperm samples were analysed. Nucleic acids extraction was performed using TRIzol method. Gene expression study was performed by Real-Time PCR, and DNA methylation profiles were evaluated by standard Bisulfite Sequencing. Global DNA hydroxymethylation content was measured by ELISA.

Results: We observed increased expression of TET1, TET2 and DNMT3A in cases of Secretory Azoospermia (SAZ), especially in Sertoli-cell only Syndrome, whereas DNMT1 decreased. In sperm from oligozoospermic patients, the results were similar to SAZ. *TET1* and *TET2* promoter methylation was very low in both controls and cases, with no significant differences, as was the case for 5hmC global levels.

Conclusion: Increased expression of *TET* enzymes and decreased expression of *DNMT1* indicate that normal dosage of these epigenetic regulators might be crucial for correct spermatogenesis to occur. Our results highlight the need for a better understanding of epigenetic regulation of spermatogenesis in order to contribute to successful IVF treatments.

P21 | BASIC RESEARCH

The role of Neurexin (NRXN2) genetic network in migraine susceptibility

Miguel Alves-Ferreira[1,2,3]; João Luís Neto[1,2]; José Pereira-Monteiro[1,2]; Jorge Sequeiros[1,2,3]; Isabel Alonso[1,2]; Alda Sousa[1,2,3]; Carolina Lemos[1,2,3]

1 - UnIGENe, IBMC - Institute for Molecular and Cell Biology, University Porto, Porto, Portugal; 2 - i3S - Instituto de Investigação e Inovação em Saúde, University Porto, Porto, Portugal; 3 - ICBAS - Instituto Ciências Biomédicas Abel Salazar, University. Porto, Porto, Portugal

---

Aims/Contex: Our aim is to explore the role of Neurexin (*NRXN2*) and other components of the synaptic vesicle machinery, involved in the regulatory mechanisms of neurotransmitter release, in migraine susceptibility.

Migraine is a common neurological disorder, reducing the quality of life of patients and their families. This complex disorder affects about 15% of the general population, being two to four times more common in women than in men.

In the last years, we have centered our attention to the synaptic vesicles' molecular machinery and life cycle, with a central role in neurotransmitter release and its regulation. One example is neurexin (*NRXN2*), which establish connections between the fusion proteins of intracellular and synaptic vesicles, interacting with other important components of this mechanism as synaptotagmin, $GABA_A$*-R* or *CASK*.

Methods: Four tagging single nucleotide polymorphisms (SNPs) of *NRXN2* were analyzed in 183 cases and 265 controls. To evaluate association between *NRXN2* SNPs and migraine, a multivariable-logistic regression was performed. Allelic and haplotypic frequencies were estimated. Interaction between *NRXN2-SYT*, *NRXN2-GABRE* and *NRXN2-CASK* was assessed by a multivariable-logistic regression and confirmed by a multifactor dimensionality reduction analysis.

Results: We found two strong and significant synergistic interactions between migraine liability and the following gene pairs: *NRXN2-GABRE* and *NRXN2-CASK* that remained significant after 1000-fold permutation-based correction.

Conclusions: For the first time a genetic interaction was found among *NRXN2*, one of $GABA_A$-receptors and *CASK genes* showing a synergetic effect of interaction between these genes in migraine susceptibility.

These genes interactions may be a small part of a higher network of genes, allowing us to better understand migraine etiology and leading to the development of new therapeutic approaches.

P22 | BASIC RESEARCH

The role of alternative splicing cis-regulation for breast cancer risk

Juliana Machado[1]; Ramiro Magno[1]; Joana M. Xavier[1]; Ana-T. Maia[1]

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

Aims/Context: Recent genome-wide association studies (GWAS) have revealed the association of hundreds of single nucleotide polymorphisms (SNPs) with breast cancer (BC) risk. However, they fail to pinpoint the underlying biological mechanism for this risk. Interestingly, most risk-associated SNP loci are located in non-coding regions, suggesting possible regulatory roles, such as altering the binding of transcription or splicing factors, as well as miRNAs.

There has been a bias in the functional characterisation of GWAS loci towards the effect of regulatory SNPs on transcription factor binding. Our aim is to determine the extent of the contribution of rSNPs influencing splicing among known breast cancer susceptibility loci.

Methods: We screened genome-wide significant ($P \leq 5x10\text{-}8$) breast cancer risk associated SNPs from published GWASes for association with alternative splicing isoforms. To this end, we optimised existing bioinformatics tools and used RNA-seq expression data from normal breast samples, available from GTEx project, to identify splicing quantitative trait loci (sQTL).

Results and Conclusions: We found that rs6456883 is a significant cis-sQTL for the expression of ZNF311 gene isoforms, and that three more SNPs, rs6682326, rs3008282 and rs2906324, are also significant cis-sQTLs for the expression of RPL23AP53 gene isoforms. We are currently performing their functional characterisation, to reveal the mechanism by which these variants regulated alternative splicing. Our work is starting to reveal the extent by which alternative splicing plays a role in known breast cancer susceptibility, and also paves the way to further testing of other candidate loci.

P23 | BASIC RESEARCH

The role of eIF3 subunits in the mechanism of nonsense-mediated mRNA decay

Patrícia Martins Dias[1]; Claudia Onofre[1]; Juliane Menezes[1]; Luísa Romão[1]

1 - Instituto Nacional de Saúde Doutor Ricardo Jorge

Premature translation-termination codons (PTCs or nonsense codons) can arise from mutations in germ or somatic cells. The introduction of a PTC into an mRNA can trigger nonsense-mediated decay (NMD), an important mRNA surveillance mechanism that typically recognizes and degrades mRNAs containing PTCs to prevent the synthesis of C-terminally truncated proteins potentially toxic for the cell. The physiological importance of NMD is manifested by the fact that about one third of genetic disease-associated mutations generate PTCs.

The mammalian translation initiation factor 3 represents the most complex eukaryotic initiation factor (eIF) in mammalian cells. This factor comprises 13 subunits (eIF3a to eIF3m), each one playing an important role in translational control. Disruption of eIF3 initiation factor activity can lead not only to cancer but also neural physiological alterations, and to act as a mediator of infection cascade. Although some eIF3 subunits (for example, e and g) have been implicated in NMD, others were not studied yet. With the aim to identify other eIF3 subunits involved in NMD, we have depleted each one of the eIF3 subunits in HeLa cells and tested its effect in the expression of PTC-free or PTC-containing reporter human β-globin genes. Our data show that eIF3l and eIF3j subunits have an important role in targeting mRNAs for NMD. We will describe the molecular mechanisms underlying these observations.

Acknowledgements

This work was partially supported by Fundacão para a Ciência e a Tecnologia (PTDC/BIM-MEC/3749/2014 and UID/MULTI/04046/2013 to BioISI from FCT/MCTES/PIDDAC).

P24 | BASIC RESEARCH

Modulation of protein translation mediated by upstream open reading frames (uORFs) in PERK mRNA

Rafael Fernandes[1,2]; Luísa Romão[1,2]

1 - University of Lisboa, Faculty of Sciences, BioISI - Biosystems & Integrative Sciences Institute, Campo Grande, C8 bdg, 1749-016 Lisboa, Portugal; 2 - National Institute of Health Dr. Ricardo Jorge, Department of Human Genetics, Lisboa, Portugal

Genome-wide studies pointed out translation as a major regulator of gene expression, being a key post-transcriptional mechanism by which cells rapidly change their gene expression pattern in response to diverse stimuli. Upstream open reading frames (uORFs) are examples of *cis*-acting elements that can regulate translation initiation. A uORF is defined as a coding sequence located within the 5'untranslated region (5'UTR) of an mRNA, and is typically considered a repressor of main ORF (mORF) translation. This can be due to the recognition of the uORF start codon by the preinitiation complex. In this case, when the translating ribosome encounters the uORF stop codon, the translation machinery disassembles, avoiding mORF translation if the ribosome cannot reinitiate at the main start codon. ATF4, CHOP and GADD34 are stress-response proteins encoded by uORF-harboring transcripts with translation repression activity, which is responsible for maintaining a low expression of these proteins in normal conditions. However, when ER stress occurs, the unfolded protein response (UPR) is activated and eIF2α is phosphorylated by PERK. In these cases, the availability of the preinitiation complex is reduced, favoring translation of the mORFs. The stress-response proteins are therefore up regulated, triggering a cascade of events aiming stress resolution and cell survival.

In this work we intended to determine if PERK is regulated at the translational level in normal and ER stress conditions. We have validated the annotated sequence of *PERK* 5'UTR using 5'RACE, and we have selected uORFs based on ribosome profiling data already available. Then, we have cloned the 5'UTR into a reporter plasmid, in frame with firefly luciferase ORF. Using site-directed mutagenesis, we have made constructs with mutated uORFs to evaluate their impact in translation efficiency. Our data suggest that the uORFs have a repressive effect in mORF translation, and we are now dissecting the mechanisms that drive this regulation.

Acknowledgments

Partially supported by UID/MULTI/04046/2013 center grant from FCT to BioISI. RF is recipient of a fellowship from BioSys PhD programme (SFRH/BD/114392/2016) from FCT.

P25 | BASIC RESEARCH

The role of GSTT1 and GSTM1 gene polymorphisms in bronchial asthma

Margarida Cortez[1,2]; Joana Ferreira[2,3]; David Sarmento[2,3]; Carla Carvalho[2,3]; Andreia Matos[2,3]; Manuel Bicho[2,3]

1 - CHLN_HSM, ImmunoAllergy, Lisbon, Portugal; 2 - Genetics Laboratory, ISAMB, Lisbon Medical School, Portugal; 3 - Institute of Scientific Research Bento Rocha Cabral, Lisbon, Portugal

Aims: *GSTM1* and *GSTT1* null polymorphisms could be associated with the inability of glutathione S-transferases (GSTs) variants of the enzymes to detoxify the reactive oxygen species (ROS).

Methods:For GSTT1 and GSTM1 we analyzed asthmatics (n= 96) compared with control group (n=160); the polymorphisms were analyzed by Multiplex-PCR.Control of asthma assessed by ACQ7 and PAQLQ. Statistical analysis was performed with PASW-24 establishing a significance level of $p< 0.05$.

Results: In asthmatics there are 61 females and 35 males; in controls: 93 females and 67 males (p=0.468). The mean age ±SD of the asthmatics was 38.69 ± 20.013 years .The mean age ±SD in the control group was 44.15 ± 12.63 years (p=0.019). In asthmatics genotype frequencies of GSTT1*0 were : 50 (52.1%) and GSTT1+ were : 46 (47.9%); in control group genotype frequencies of GSTT1*0 were : 49 (30.6%) and GSTT1+ were : 111(69.4%). The GSTT1*0 is more frequent among asthmatics (p=0.001). In asthmatics genotype frequencies of GSTM1*0 were : 51(53.1%) and GSTM1+ were : 45(46.9%); in control group genotype frequencies of GSTM1*0 were : 72 (45.0%) and GSTM1+ were : 88(55.0%). There is no statistical differences (p=0.258). The genotype GSTT1*0 confers a risk of being asthmatic of 2.747 times when compared with GSTT1+ genotype and adjusted for age: OR^b: 2.747 [1.602-4.713]; p<0.001. The genotype GSTT1*0 confers a risk of being allergic asthmatic of 4.863 times when compared with GSTT1+ genotype and adjusted for gender: OR^b: 4.863 [1.137-20.788]; p=0.033.

Conclusion: According to our results GSTT1*0 polymorphisms could lead to different genotype specific response to therapy and different endotypes/phenotypes among asthmatic patients.

Effects of cryopreservation on spermatic parameters, DNA integrity and mitochondrial activity: a preliminary study

Patrícia Pinho[1,2]; Regina Arantes-Rodrigues[1,3]; Zélia Gomes[4]; Miguel Brito[4]; Osvaldo Moutinho[4]; Isabel Gaivão[2,5]; Francisco Peixoto[6]; Bruno Colaço[3]; Rosário P. Leite[1]

1-Genetics/Andrology Laboratory, Hospital Centre of Trás-os-Montes and Alto Douro (CHTMAD), Vila Real; 2-Dept. of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real; 3-Centre for the Research and Technology of Agro-environmental and Biological Sciences (CITAB), UTAD, Vila Real; 4-Dept. of Obstetrics and Gynecology, CHTMAD, Vila Real; 5-Animal and Veterinary Research Centre (CECAV), UTAD, Vila Real; 6- CQVR, UTAD, Vila Real, Portugal

---

Cryopreservation is a routine technique used in assisted reproductive technology (ART).

The aim of the study was to compare the impact of cryopreservation on spermatic parameters, DNA integrity and mitochondrial activity between normozoospermic men (group N) and men with altered spermatic parameters (group A).

This study used 26 sperm samples from men who attended the infertility consultation. Motility, vitality, morphology, sperm concentration (according to WHO, 2010), DNA damage (Comet Assay) and mitochondrial activity (MitoTracker^{TM} Red FM) were assessed before and after cryopreservation. DNA fragmentation (TUNEL) was assessed before, and SOD and GR enzymes activity after cryopreservation.

In fresh samples, the motility and normal morphology were significantly higher in samples of group N ($p<0,05$). The DNA integrity, measured by Comet Assay (N= 94,8 AU vs A= 79,6 AU; p=0,247) and TUNEL (N=4,6% vs A=6,5%; p=0,258), was similar between the two groups, but the percentage of spermatozoa with active mitochondria was significantly lower in group A (N=71,4% vs A=59,1%; p=0,035). The results showed that the motility, vitality, midpiece abnormalities, DNA damage and active mitochondria tend to be more affected by cryopreservation on group A. The DNA damage (N=163,6 AU vs A=180,7 AU; p=0,471) increased 126,8% in group A and 72,6% in group N (p=0,057), and spermatozoa with active mitochondria (N=25,8% vs A=16,1%; p=0,021) decreased 72,9% in group A and 63,9% in group N (p=0,165). SOD and GR enzymes activity were slightly higher on group A. Spearman correlation coefficients reinforced that the better the quality of fresh samples is, the less the quality becomes affected by cryopreservation ($p<0,05$).

As expected and despite the small number of samples, sperm samples with altered spermatic parameters tend to have higher DNA damages/fragmentation, less active mitochondria and a higher oxidative stress, demonstrating a lower resistance to cryopreservation. Concerning the implications of our results, it´s also urgent to enhance efficiency of freezing systems.

P27 | BASIC RESEARCH

Study of genetic variations associated with channelopathies in cases of sudden death

Laura Caine[1]; Benedita Silva[2]; Agostinho Santos[2]; Beatriz Abreu[1]

1 - FMUP; 2 - INMLCF

CONTEXT - Sudden Death (SD) is a public health problem that has taken on a growing concern in the general population and has raised awareness of the need for a precise autopsy diagnosis with genetic information that may be useful in preventing other family members' deaths. In young people (≤ 40 years), victims of SD, studies report that a complete medical-legal autopsy does not reveal a cause of death in 30% of cases and these events are designated as cases of Sudden Unexplained Death (SUD). For these unexplained cases, an important diagnostic contribution may be provided by genetic analysis, which should be conducted during the investigation about the cause of death. It is estimated that 1/3 of the SUD can be explained by channelopathies which are hereditary pathologies caused by mutations in genes that lead to dysfunctions in the cardiac ion channels. The main phenotypes seen in patients with these dysfunctions are: Long QT Syndrome, Short QT Syndrome, Brugada Syndrome, and Catecholaminergic Polymorphic Ventricular Tachycardia.

METHODS - In this study will be used peripheral blood samples from SUD cases. Genetic analysis will be performed by Next Generation Sequencing in KCNQ1, KCNH2, SCN5A, CACNA1C, CACNB2b, SCN10A, KCNJ2, RyR2 and CASQ2 genes, that according to the literature are associated with channelopathies.In cases of SUD in which variations are detected in the genes analyzed, a DNA sample will be requested from the direct relatives of these victims in order to assess the risk of having a genetic condition that makes them susceptible to SD.

CONCLUSION - With the results of this scientific investigation we hope to demonstrate the viability and the feasibility of the conducted genetic studies, in the medical-legal investigation of SUD cases in young people in Portugal, in order to reduce considerably the inconclusive diagnoses about the etiology of death. In addition, with the genetic evaluation of relatives of SUD victims, we intend to contribute to an effective prevention of new cases of SD.

P28 | BASIC RESEARCH

Human Epidermal Growth Factor Receptor 2 at one Portuguese district hospital

Joana Liz Pimenta (Portugal)1; Mariana Rocha (Portugal)1; Marta Souto (Portugal)1; Pedro Botelho (Portugal)1; António Teira (Portugal)1; Miguel Barbosa (Portugal)1; Rosário Leite (Portugal)1

1 - Centro Hospitalar de Trás-os-Montes e Alto Douro, Vila Real, Portugal

Context: The human epidermal growth factor receptor 2 (HER2) plays an important role in the development of some types of cancer and is considered a prognostic biomarker. The accuracy in determining HER2 status is essential because there are anti-HER2 targeted therapies that are able to reduce recurrence and improve survival. This new era for cancer patients is only possible thanks to Human Genetics.

Methods: Observational prospective study conducted between August 18th of 2016 to September 19th of 2018. Sample of the patients tested for HER2 status in the Genetic Laboratory of the Centro Hospitalar de Trás-os-Montes e Alto Douro. The technique applied to determine HER2 was Fluorescence in situ Hybridization (FISH) and was performed using Zytovision dual-probe according to the manufacturer's protocol. The technique applied to manage the tissue was the fixation with formalin embedded in paraffin. The statistical analysis was performed through the Microsoft Office Excell 2016 and IBM SPSS Statistics version 23.

Results: It was analysed a sample of 108 patients, with median age of 60±12 years (minimum and maximum ages was 30 years and 85 years, respectively), 95.3% of whom were female (103 patients) and 4.6% were male (5 patients). HER2 was positive in 20.4% (22 patients), equivocal for 10.2% and negative for 69.4% (75 patients). The medium time since the sample was received in the laboratory till the results were 6±4days.

Conclusion: The rate of HER2 positive in this study is accordingly to the literature. It may be too early to evaluate the impact of the HER2 status determination in the district population of the hospital. Accurate testing is extremely important since anti-HER2 target therapy has shown a great impact in the overall and disease free survival. Therefore the population in need, regardless where they are from, should have access to Genetic Laboratories.

P29 | CLINICAL RESEARCH

A Portuguese Tool for Quality Assessment of Genetic Counselling by Genetics Healthcare Professionals

Catarina Costa[1]; Marina S. Lemos[2]; Carolina Lemos[1,3]; Miguel Alves-Ferreira[1,3]; Jorge Sequeiros[1,3]; Milena Paneque[1,3]

1 - CGPP – Centro de Genética Preditiva e Preventiva, IBMC – Instituto de Biologia Molecular e Celular, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; 2 - FPCEUP - Faculdade de Psicologia e de Ciências da Educação, Universidade do Porto, Portugal; 3 - ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

Background: Recent studies on patients' and professionals' views in Portugal highlighted a need for instruments and quality indicators of genetic counselling practice. In response, a novel tool was developed using the Reciprocal-Engagement Model (REM) as a theoretical-practical foundation as well as evidence-based insights from national research

Methods: After pre-test validation, the scale was submitted to psychometric validation, we used a sample of 30 participants that were mainly medical geneticists, who evaluated 81 counseling sessions, carried out at main national services between January and April 2017.

Results: Based on empirical and statistical criteria the best items were selected. The final 50 items-version comprises five dimensions: education, the counselees' characteristics as part of the process, relationship between counselor and counselee, potential effects of the process on the counselee, and services provision. Results also showed consistent psychometric properties of the scale, which was supported on theoretical and practice concepts of genetic counselling.

The professionals involved in the validation process, highlighted as very relevant for assessment of their practice the association of each genetic counselling principle with specific goals, strategies and behaviors, in the REM model and, accordingly, underlying the structure of the new instrument.

Conclusions: The constructed scale is a pioneer tool in Portugal and perhaps the first practical application of the REM in the context of genetic services in Europe. Research on quality assessment of genetic counseling practice, using the Portuguese new scale as the measure instrument, will in turn inform the applicability of the REM to our national context and others.

With this study, we would like to raise the discussion on how relevant this new tool can be for further investigation on genetic counselling field and its potential impact in the improvement of genetic services in our country.

An algorithm for the detection of common copy number alterations in cancer

Luísa Esteves[1]; Francisco Caramelo[2]; Ilda P. Ribeiro[1,3]; Isabel M. Carreira[1,3,4]; Joana B. Melo[1,3,4]

1 - Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 2 - Laboratory of Biostatistics and Medical Informatics, IBILI - Faculty of Medicine, University of Coimbra, 3000-354, Coimbra, Portugal; 3 - CIMAGO - Center of Investigation on Environment, Genetics and Oncobiology - Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 4 - CNC, IBILI, Group of Aging and Brain Diseases: Advanced Diagnosis and Biomarkers, Coimbra, Portugal

---

Aims/Context: Copy number alterations (CNAs) are critical for cancer origination and progression. Recurrent CNAs - regions that have a high enough probability to be altered in at least some subjects of the cohort - are determinant to the detection of driver alterations, since those will be present in a considerable amount of the analyzed genomes and are disease-specific as opposed to random subject-specific alterations – passenger alterations. The disease-specific genetic signature, derived from the common CNAs, can then be defined as a function of the probability of alteration for a given region.

Materials and Methods: A method for the determination of recurrent CNAs and the underlying probability distribution of the cancer in study, was developed**.** The algorithm partitions a dataset of array CGH or SNP array segmented profiles, by chromosome, recovering the overlapping regions, their breakpoints and probability of alteration. In order to test this algorithm, simulated datasets with known properties were generated. CNA data from three cancer types, obtained by array CGH, was downloaded from The Cancer Genome Atlas (TCGA) and subjected to the algorithm. All analyses were performed using R and Matlab.

Results: The algorithm performed well for 1000 tested simulated datasets, retrieving correctly both the regions' breakpoints and their probability of alteration. The error for that probability was found to decrease as the number of subjects in a cohort increased. The algorithm performed well in real datasets, retrieving correctly the most frequently altered regions and was successfully used to compare between groups established within the cohorts.

Conclusions: The study of copy number alterations is crucial to understanding the development and progression of several conditions, the most prominent of those being cancer. Reducing considerably the number of regions to analyze as well as generating more structured data is essential to reduce noise on the complex datasets generated by genome-wide technologies.

P31 | CLINICAL RESEARCH

Prevalence of X-aneuploidies and X-structural abnormalities in a Portuguese population with primary amenorrhea or premature ovarian insufficiency

Alexandra Estevinho[1]; Ana R Neves[2]; Jorge M Saraiva[1]; Luís M Pires[3]; Joana B Melo[3,4,5]; Isabel M Carreira[3,4,5]; Eunice Matoso[1,3,4]

1- Cytogenetics Laboratory, Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal; 2- Dept. of Obstetrics B, CHUC, Coimbra, Portugal; 3- Laboratory of Cytogenetics and Genomics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 4- CIMAGO-Centro de Investigação em Meio Ambiente, Genética e Oncologia, Coimbra, Portugal; 5- CNC-IBILI Consortium, Universidade de Coimbra, Portugal

---

Amenorrhea affects 1-3%of women of the reproductive age. Primary amenorrhea(PA) is defined as the absence of menarche in 14-year-old girls, without the development of secondary sexual characteristics, or absence in 16-year-old girls with normal development of secondary sexual characteristics. Premature ovarian insufficiency (POI) is defined as primary ovarian defect characterized by secondary amenorrhea for at least 4-6 month before 40 years old. Chromosome abnormalities, namely the ones involving the X chromosome are strongly associated with both PA and POI.

The objective of this work was to identify the prevalence of chromosome abnormalities in a group of female patients affected by PA or POI.

We studied 87 patients, 24 with PA and 63 with POI. Chromosome analysis was performed on blood lymphocytes, using GTG high resolution banding technique, according to standard procedures. Complementary molecular studies were performed when needed.

The study revealed that chomossomal abnormalities were present in 20 of the 87 studied patients. Nine presented aneuploidies of the X chomosome: 45,X, 47,XXX, and mosaics with variable expression of the 45,X/47,XXX/48,XXXX/46,XX cell lines. Three carried a mos 46,X,i(Xq)/45,X and only one a mos 46,XY/46,XX. Five exhibited structural aberrations: Xp, Xq deletions and a X-autosome translocation. Two showed sporadic alterations: mos 47,XX,+21/46,XX and 45,XX,der(13;14).

Our study revealed a 23%prevalence of chromosomal abnormalities in 87 women studied. Aneuploidies of the X-chromosome, 45,X and 47,XX, are associated with ovarian failure, but in mosaic state it depends on the type of cell-lines present and its distribution among different tissues. Structural X abnormalities like i(Xq) and X-autosome translocation are associated with chromosome pairing failure and gonadal dysfunction. On the other hand, the Xp22.33p22.31, Xq13q24 and Xq22.1deletions observed are overlapping imbalances with POI critical regions, already described. The results support the importance of chromosomal studies to provide an etiologic explanation, disclosing candidate critical regions for ovarian failure and providing a more ascertained genetic counseling.

Autosomal recessive spinocerebellar ataxia 20 – From genotyping to phenotyping, back and forth

Nuno Maia[1,2]; Gabriela Soares[3]; Teresa Temudo[4]; Isabel Marques[1,2]; Bárbara Rodrigues[1,2]; Ana Maria Fortuna[2,3]; Rosário Santos[1,2]; Arjan De Brouwer (Netherlands)[5]; Paula Jorge[1,2]

1 - Unidade de Genética Molecular, Centro de Genética Médica Jacinto de Magalhães (CGMJM), Centro Hospitalar Universitário do Porto; 2 - Unidade Multidisciplinar de Investigação Biomédica (UMIB), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto; 3 - Unidade de Genética Médica, Centro de Genética Médica Jacinto de Magalhães (CGMJM), Centro Hospitalar Universitário do Porto; 4 - Serviço de Neurologia Pediátrica, Centro Hospitalar Universitário do Porto; 5 - 5Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen

Background Extensive genetic heterogeneity of Intellectual-Disability (ID) syndromes and overlapping clinical features require a "genotype-first" approach to determine the underlying molecular defect. Although diagnostic yield in ID disorders has improved, mostly due to next-generation sequencing, still around 50% of cases remain unsolved. **Methods** As part of an ID research project, exome-sequencing was performed in a 25-yr old female with severe developmental delay, hypotonia, macrocephaly, cerebellar atrophy, stereotypies and facial dysmorphisms. Karyotype, aCGH and extensive metabolic studies were normal. A similar phenotype was observed in her 10-yr younger brother. Parents are non-consanguineous and there is no family history. **Results** Assuming autosomal recessive (AR) inheritance, genes carrying two rare non-synonymous coding variants, splicing variants, or indels were prioritized, but segregation studies of those variants were inconclusive. Review of clinical phenotype pointed towards intellectual disability-coarse facies-macrocephaly-cerebellar hypotrophy syndrome, but a single heterozygous nonsense variant was identified in *SNX14* gene: c.1195C>T (shared by the two sibs and the mother). Based on the clinical phenotype, *SNX14* exome data read-depth was screened for CNVs. Low coverage exons were sequenced by Sanger sequencing and Low Cycle Number (LCN) PCR was carried out to exclude exon deletions/duplications. LCN PCR results were confirmed by qPCR amplification which revealed a low copy number of exons 10 to 13: c.(854+1_855-1)_(1171+1_1172-1)del (shared by the two sibs and the father). Characterization of deletion breakpoints by Sanger sequencing and expression studies are ongoing. **Conclusions** We describe the first non-consanguineous family with two siblings affected by AR spinocerebellar ataxia 20 (MIM 616354), carrying *SNX14* compound heterozygous variants. This study underscores detailed phenotyping and laboratory-clinician crosstalk as key elements to guide the genetic investigation and, therefore, to increase ID diagnostic yield.

Funding UMIB is supported by National Funds through the FCT in the frameworks of UID/Multi/0215/2016 project UMIB/ICBAS/UP.

Genetic susceptibility for hypertension development in the Portuguese population

Ildegário Semente[1,2]; Joana Ferreira[1,3]; Laura Aguiar[1,3]; Andreia Matos[1,3]; Manuel Bicho[1,3,4]; Paula Faustino[2,4]; Ângela Inácio[1,3,4]

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

---

Hypertension is a multifactorial disease involving both environmental and genetic factors. It is characterized by the presence of elevated arterial tension levels and is frequently found in association with metabolic and hormonal disturbances, cardiac hypertrophy and high vascular toning.

This study aimed to investigate the potential implication of common polymorphisms located in *NOS3*, *HBA* and *G6PD* genes in hypertension development in the Portuguese population.

We performed a case-control study among a sample of 243 Portuguese chronic hypertensive patients and 134 healthy subjects (matched control group). DNA was obtained from peripheral blood samples. The number of repetitions in *tandem* (VNTR) in intron 4 of *NOS3* gene was characterized by PCR, the SNP rs1050829 in *G6PD* gene was analysed by PCR-RFLP and the -3.7kb alpha-thalassemia deletion in *HBA* gene was searched by Gap-PCR. The statistical analyses were made using the statistics tool SPSS 25.0, with the statistical significance level set to *p-value* <0.05.

Results show that the presence of allele 4a in VNTR of *NOS3* gene is associated to a higher risk of having hypertension [OR = 2.297; CI (95%) = 1.206-4.376; p = 0.011]. Furthermore, there is a significant difference in relation to the genotypic frequency of that polymorphism between genders, being the female gender with allele 4a associated to a higher risk of being hypertensive (p = 0.004). No significant differences were verified for the G6PD variant nor for the presence of the alpha-thalassemic between the hypertensive and the normotensive groups.

In conclusion, this study indicates that the presence of allele 4a in *NOS3* provides genetic susceptibility for hypertension development in the Portuguese population. This result emphasizes the importance of the endothelial-derived nitric oxide metabolism in this pathology due to its contribution to the vasodilatation process. The identification of contributing genetic variants for the susceptibility to hypertension may allow recognizing the vulnerable individuals and classifying patients in subgroups with distinct genetic characteristics in order to delineate the better prevention and therapeutic strategies.

Genetic predisposition to breast/ovarian cancer due to pathogenic variants in other genes than BRCA1/BRCA2. Experience from Synlab Genetic center

Natália Salgueiro[4]; Ariana Conceição[4]; Juliette Dupont[1]; Nataliya Tkachenko[2]; Gabriela Soares[2]; Miguel Gonçalves-Rocha[3]; Cláudia Martins[4]; Diana Pinto[4]; João Gonçalves-Rocha[1]; Patricia Dias[1]; Oana Moldovan[1]; Catarina Machado[1]; Renata Oliveira[3]; Joana Goncalves[4]; Ana Berta Sousa[1]; Ana Fortuna[2]; Margarida Reis-Lima[4]

1 – S. de Genética, Centro Hospitalar de Lisboa Norte; 2 – S. de genética, Centro Hospitalar do Porto; 3 - Unidade de Genética Médica, Hospital de Braga; 4 - Unidade de Genética Molecular, Synlab-GDPN, Porto

Introduction: Multiple recent studies show that a percentage of high-risk individuals have germline pathogenic variants in cancer risk genes others than BRCA1 and BRCA2, such as ATM, BRIP1, CDH1, CHEK2, NBN, PALB2, RAD51C, RAD51D, PTEN, TP53, EPCAM, STK11.

In the last few years, Next-Generation-Sequencing (NGS) has enabled the analysis of a greater number of genes in patients with suspected Hereditary Breast and Ovarian Cancer (HBOC). Our aim is to confirm the prevalence of cancer predisposing pathogenic/likely pathogenic (P/LP) variants in genes other than the BRCA1 and BRCA2 in our sample.

Methods: We studied 211 patients who meet the National Comprehensive Cancer Network (NCCN 2. 2017) guidelines for HBOC genetic testing. This study included NGS analysis of 18 actionable genes: BRCA1, BRCA2, ATM, BRIP1, CDH1, CHEK2, NBN, PALB2, RAD51C, RAD51D, PTEN, TP53, EPCAM, STK11, MLH1, MSH2, MSH6 e PMS2. The NGS was performed on Ilumina Platform, using the Trusight Cancer Kit (Ilumina).

Results: Our study revealed 26 P/LP variants in the 18 genes analysed (26/211= 12,3%). Of these, 13 (6,2%) were detected in BRCA1/2 and 13 (6,2%) in the other genes**.** The non-BRCA1/2 genes variants represent 50% of total P/LP variants detected and included ATM, BRIP1, CHEK2, MLH1, MSH6, PALB2, PMS2 and RAD51C genes. One patient had co-occurrence of pathogenic variants in BRCA1 and RAD51C genes.

Discussion: The discovery of new genes involved in Genetic Predisposition to HBOC and the advent of NGS have enabled the use of multigene panel as an accurate, faster and economic test. In our study, the rate of P/LP variants in non-BRCA1/BRCA2 genes was 6,2% which is consistent to those reported in literature. The use of a multigene panel for testing HBOC high-risk patients may identify 50% more individuals with hereditary cancer gene P/LP variants than testing BRCA1/BRCA2 alone. Finally, we conclude that the use of a multigene panel (only with clinical actionable genes) for patients with suspected HBOC risk should be the standard approach. This approach will increase clinical management for substantially more individuals and their families.

Identification in laryngeal cancer of a genomic and epigenetic signature associated with recurrence

Ilda P. Ribeiro[1,2]; Francisco Caramelo[3]; Margarida Ribeiro[1]; Jorge Miguéis[4]; Joana B. Melo[1,2]; Isabel M. Carreira[1,2]

1 - Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 2 - iCBR_CIMAGO—Center of Investigation on Environment Genetics and Oncobiology—Faculty of Medicine University of Coimbra, Coimbra, Portugal; 3 - Laboratory of Biostatistics and Medical Informatics, IBILI - Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 4 - Departments of Otolaryngology, Coimbra University Hospitals, Coimbra, Portugal

---

Background: Laryngeal cancers represent one-third of all head and neck cancers, being associated with a high morbidity and mortality. These tumors are often diagnosed in patients with significant smoking history and can involve different subsites of the larynx, with different implications in symptomatic presentation, patterns of spread, and treatment strategies. The low 5 year-survival rate due to the frequent late diagnosis and recurrences development make vital to identify biomarkers and molecular signatures to predict the recurrences development and early tumors detection. The aim of this study was to perform the genomic and epigenetic characterization of laryngeal cancer samples in order to identify a molecular signature to predict the development of relapses and metastases. **Methods:** Tumor and non-tumor laryngeal tissue samples from twenty-one patients diagnosed with laryngeal cancer were analyzed using array Comparative Genomic Hybridization (aCGH) and Methylation Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA). The follow-up periods ranged from 7 to 46 months. **Results:** aCGH revealed gains and losses in the great majority of chromosomes, being gains frequently observed in 3q, 7p, 8, 9q, 11q, 12p, 17q and 18p and losses in 3p, 9p, 11p and Y. Copy number alterations observed at *THBS1* gene in tumor samples and *VHL* gene in non-tumor samples were correlated with development of relapses and metastases in our cohort (odds-ratio 5.5 and 6.0, respectively). Likewise, *WT1* gene promoter methylation in tumor and non-tumor samples was associated with the development of relapse and metastasis (odds-ratio 3.3 and 3.5, respectively). **Conclusion:** Specific molecular signatures can be useful to guide the early diagnosis of this neoplasm and their recurrences, through the detection of genetic and epigenetic altered patterns in tumor lesions and even in morphologically non-tumor or potentially malignant lesions. Further studies in large cohorts are needed to validate the clinical application of these potential biomarkers in the prediction of relapses and metastases development.

Clinical and genetic characterization of a cohort of patients with Hypertrophic Cardiomyopathy

Teresa Saraiva[1]; Patrícia Rodrigues[2]; Maria Abreu[1]; Ana Soares[1]; Claudia Reis[1]; Gabriela Soares[1]; Ana Fortuna[1,3]

1- Medical Genetics Department, Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar Universitário do Porto, EPE, Porto, Portugal; 2 - Cardiology Department, Centro Hospitalar Universitário do Porto, EPE, Porto, Portugal; 3 - Unit for Multidisciplinary Research in Biomedicine, ICBAS-UP Porto, Portugal

Context: Hypertrophic Cardiomyopathy (HCM) is the most common genetic cardiovascular condition with an estimated prevalence of 0.6% in the general population. In up to 60% of cases, the disease is an autosomal dominant trait caused by variants in cardiac sarcomere protein genes.

Methods: We present a retrospective review of HCM adult patients from Centro Hospitalar do Porto, followed in the cardiology outpatient clinic. Clinical and molecular characterizations were performed.

R**esults:** The study included 54 patients, with a male to female ratio of 1.65 and a mean age of 54.5 years at diagnosis. The major reason for referral was the presence of cardiovascular symptoms (72%). Family history of sudden cardiac death and of HCM was present in 16 (30%) and 6 (11%) patients, respectively. During a median follow-up of 3 years, 9 patients had de novo atrial fibrillation (17%), 3 experienced heart failure episode requiring hospitalization (6%), stroke events occurred in 2 patients (4%), 1 had ventricular tachycardia (2%) and 1 died (2%). Cardioverter defibrillator was implanted in 3 patients (6%) and septal alcoholization was performed in 1 (2%). Regarding ECG assessment, ST-T abnormalities were the most common findings (41%) and echocardiographic examinations revealed an average interventricular septum thickness of 16.6 mm and ejection fraction of 68.7%. Obstructive HCM was present in 13 patients (24%), while 41 (76%) had nonobstructive HCM. Molecular genetic testing was performed in 45 patients (83%) and included NGS for *MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2* and *TPM1* (n=35), DNA sequence analysis for *MYBPC3, MYH7* and *TNNT2* (n=6) and familial study (n=4). A total of 16 pathogenic/likely pathogenic variants were identified (35%). *MYBPC3* variants were the most common. Patients with pathogenic variants exhibited an earlier onset of disease and poorer clinical outcomes than those without molecular confirmation.

Conclusion: Our cohort is characterized by a relatively benign clinical course and majority of patients were managed primarily through medication. Major adverse cardiac events were more frequent in positive genotype HCM patients.

P37 | CLINICAL RESEARCH

Our experience with BRCA1/2 families: medical, social and psychological needs

Mariana Soeiro E Sá[1]; Juliette Dupont[1]; Alexandra Leonardo[1]; Ana Berta Sousa[1]

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

---

Aims: To identify the number of patients with cancer predisposing syndromes (CPS) seen at our Medical Genetics division; describe their medical history; assess motivations for genetic testing; evaluate the degree of knowledge about CPS and determine the number of at-risk individuals tested per family.

Methods: We analyzed a sample of patients with mutations in *BRCA1/2* through medical files, a clinical interview and a two-part questionnaire filled by the physician and anonymously by the patient. Statistical analysis was done after codification.

Results: We collected data from 33 patients (27 women/6 men) from 22 families with mutations in *BRCA1* (n=15) and *BRCA2* (n=18). Mean age of first cancer was 42 years. Mean age at genetic testing was 42.3 years for non-index cases. 15/22 eligible women chose prophylactic oophorosalpingectomy. All 13 women with prior history of unilateral breast cancer (BC) or conservative surgery opted for prophylactic mastectomy vs. 3/10 women without prior history of BC. Most eligible women undergo annual mammogram and MRI. All eligible women undergo transvaginal US for ovarian cancer (OC) screening, but only 4 at the recommended interval. An average of 22.8% at-risk individuals was studied in each family. All patients discussed their diagnosis within the family, mainly due to recognizing the importance of knowing one's cancer risk (n=27) and wanting to motivate family members to get tested (n=23). Patient's main motivation for genetic testing was doctor's counsel (n=21). Most patients (n=29) consider genetic testing "Very Important". Patients overestimate their cancer risks and 51.5% are not clear regarding heredity. Most patients report normal levels of anxiety and depression.

Conclusions: Genetic testing for CPS is motivated by desire to know personal risk of cancer, but doctor's counsel has the most impact. Even though genetic testing is considered very important and discussed within the family, the number of at-risk individuals studied is relatively low and they are tested later than recommended. More investment is needed in educating patients regarding CPS.

Clinical, histological and molecular characterization of Duchenne/Becker Muscular Dystrophy patients with a pathogenic variant involving exon 44 in DMD gene – a study from a multidisciplinary Unit in a tertiary Hospital

Ana R. Soares[1]; Ana Gonçalves[2,6]; Ricardo Taipa[3]; Cristina Garrido[4]; Melo Pires[3]; Ana M. Fortuna[1]; Teresa Coelho[5]; Rosário Santos[2,6]; Manuela Santos[4]

1- S. de Genética Médica, Centro de Genética Médica Dr. Jacinto Magalhães (CGMJM), Centro Hospitalar Universitário do Porto (CHUP); 2- Unidade de Genética Molecular, CGMJM, CHUP; 3- Unidade de Neuropatologia, S. de Anatomia Patológica, CHUP; 4- Unidade de Neuropediatria, S. de Pediatria, CHUP; 5- Unidade de Neurofisiologia, CHUP; 6- Unidade Multidisciplinar de Investigação Biomédica - Instituto de Ciências Biomédicas Abel Salazar (UMIB/ICBAS), Porto

Context: Dystrophinopathies include a spectrum of X-linked muscle diseases caused by pathogenic variant in *DMD* gene, ranging from mild form of Becker muscular dystrophy, with dystrophin qualitatively and quantitatively reduced, to severe and progressive form of Duchenne dystrophy, with absent dystrophin, muscle weakness with loss of ambulation (LOA) and cardiomyopathy. Steroids and better care have changed the disease course. Genotype-phenotype correlation has been attempted, as with the "reading frame hypothesis", but there are exceptions, such as frameshift or non-sense mutations involving exons 8 or 44 that seem to cause a phenotype milder than predicted. With the advent of new treatments, the best patient characterization is essential.
The aim of this work was to characterize all cases diagnosed at our center who had a pathogenic variant involving exon 44 of *DMD* gene.

Methods: From 1992 to 2017, all patients were characterized according to age of onset, clinical presentation [at diagnosis, at 10yrs and at last observation], histological findings on biopsy, genetic variant and established treatments.

Results: Over the last 25yrs, we found 6 patients with a pathogenic variant involving exon 44, with mean age at diagnosis of 7.7yrs. Clinically, LOA was at <10yrs in 1case, >10yrs in 4 cases and 1 case is still ambulant at age 14. At diagnosis, 2 patients had cardiac manifestations and 3 had developmental delay; at age 10, all had global weakness, 2 had LOA and 1 had scoliosis; at last consultation (ages of 10-27yrs), 3 cases had scoliosis, all had cardiac disease and 4 had respiratory manifestations. Four patients started steroids <10yrs, 1case at 12yrs and the only 2 steroid naïve patients died before 3rd decade of life. Muscle biopsy revealed absent dystrophin in 2 cases and irregular dystrophin in 3. Molecular study found 5 deletions and 1 missense variant.

Conclusion: These results can reflect variability in the care and treatment of patients over time. We were able to show that there is no clear correlation between genetic alteration, biopsy result and clinical presentation in this small group of patients, making genetic treatment more challenging.

P39 | CLINICAL RESEARCH

Interstitial triplication 20p11.22p11.21, in a girl with development delay and vertebral anomalies, disclosed by array-CGH

Eunice Matoso[1,2,4]; Lina Ramos[1]; Jorge Saraiva[1]; Alexandra Estevinho[1]; Susana I Ferreira[2]; Joana B Melo[2,3,4]; Isabel M Carreira[2,3,4]

1 - Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra; 2 - Cytogenetics and Genomics Laboratory, Faculdade de Medicina da Universidade de Coimbra; 3 - CNC.IBILI Consortium, Universidade de Coimbra.; 4 - CIMAGO, Faculdade de Medicina da Universidade de Coimbra

---

Interstitial triplications leading to partial tetrasomies are rare chromosomal aberrations, the majority involve chromosome 15q11q13 region, showing a middle inverted repeat. Sporadic intrachromosomal triplications have also been described for other chromosomes and regions, but involving the region 20p11.2 has never been reported. Nonetheless, tetrasomy of the full 20p was reported in two pre-natal cases carrying an isochromosome and associated with multiple congenital abnormalities; and in one post-natal patient due to inherited translocation and secondary non-disjunction, presenting moderate development delay. Partial duplications encompassing the region 20p11.2 are not so rare and display an overlapping phenotype although less severe than the present report.

We describe a patient with a *de novo* interstitial triplication of 20p11.2 detected using oligonucleotide array comparative genomic hybridization (array-CGH) and confirmed by multiplex ligation-dependent probe amplification (MLPA). The imbalance encompasses 19 RefSeq genes, two of which are *PAX1* and *SSTR4*, whose deregulation may play a critical role in the development of the vertebral anomalies and the intellectual disability. The patient share consistent features with the phenotype of the trisomy 20p syndrome, including: psychomotor retardation and vertebral anomalies, but additionally exhibits microcephaly and severe language impairment.

In conclusion, we report the first tetrasomy 20p11.22q11.21 due to an interstitial triplication, the patient exhibits the main clinical features of the trisomy 20p syndrome. This imbalance could delineate a minimal critical region, disclosing candidate genes, that we propose to be *PAX1* and *SSTR4*, whose over-expression could be responsible for the vertebral anomalies and the intellectual disability respectively.

P40 | CLINICAL RESEARCH

The role of medical geneticists in the integrated care offered to patients with hereditary cancer syndromes

Maria Lopes-De-Almeida[1]; Luzia Garrido[2]; João Paulo Oliveira[3,4]; Sérgio Castedo[2,4,5]; Carla Oliveira[4,5]

1 - Medical Genetics Unit, Centro Hospitalar e Universitário de Coimbra; 2 - Oncogenetic consultation, Breast Center, Centro Hospitalar Universitário de São João; 3 - Medical Genetics Unit, Centro Hospitalar Universitário de São João; 4 - Medical Faculty of the University of Porto; 5 - i3S – Instituto de Investigação e Inovação em Saúde & Ipatimup – Institute of Molecular Pathology and Immunology of the University of Porto

Hereditary cancer syndromes (HCS) represent about 5-10% of all cancers, and patients and their family members who are carriers of cancer-predisposing mutations, should be followed by specialized teams, undergo pre-symptomatic testing and be offered specific oncologic surveillance and/or preventive interventions.

However, it is estimated that only 20-30% of people with HCS are currently identified.

The European Reference Networks (ERN) on genetic tumour risk syndromes (GENTURIS), is a virtual interconnected system, involving healthcare providers across Europe, that aims to improve the identification of these syndromes, minimize variation in clinical outcomes, design and implement guidelines, develop registries and biobanks, support research, and empower patients, by giving them access to a more accurate diagnosis, surveillance and treatment, according to the specific pathogenic variant identified.

The aim of this work is the presentation of an algorithm for the diagnosis and follow-up of patients and families with HCS in Portugal, where medical geneticists play a central role. This algorithm predicts the integration of a medical geneticist as part of every multidisciplinary team, and interaction with the ERN GENTURIS. This would allow collecting informed consents based on the guidelines of ERNs, collect data in a centralized manner, discuss rare and difficult cases, optimize individual care and offer the most adequate options to these patients. We started by identifying the number and location of medical geneticists in Portugal, as well as hospitals requesting genetic tests without involving geneticists, and concluded that the offer is limited. This reinforces the need to keep the number of National Reference Centres for HCS low, thereby allowing the concentration of highly qualified professionals, and the creation of a system enabling less equipped hospitals to access virtual multidisciplinary consultations.

In the short range, the proposed algorithm would allow more families with an HCS to be identified, diagnosed and treated, by granting access of healthcare providers to expertise in the field of HCS through the ERN.

P41 | CLINICAL RESEARCH

Recurrent chromosomal breaks in a cohort of head and neck cancer

Ilda P.Ribeiro[1,2]; Luísa Esteves[1]; Francisco Caramelo[3]; Thomas Liehr (Germany)[4]; Joana B. Melo[1,2,5]; Isabel M. Carreira[1,2,5]

1 - Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 2 - CIMAGO - Center of Investigation on Environment, Genetics and Oncobiology - Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 3 - Laboratory of Biostatistics and Medical Informatics, IBILI - Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 4 - Institute of Human Genetics, Jena University Hospital, Friedrich Schiller University, Jena, Germany; 5 - CNC, IBILI, Group of Aging and Brain Diseases: Advanced Diagnosis and Biomarkers, Coimbra, Portugal

---

Aims/Context: Head and neck squamous cell carcinoma (HNSCC) presents complex chromosomal rearrangements and the molecular mechanisms behind its development remain elusive. The identification of recurrent chromosomal breakpoints could help understand these molecular mechanisms and consequently lead to the discovery of potential diagnostic biomarkers.

Materials and Methods: Array comparative genomic hybridization was performed in 104 HNSCC patients and the chromosomal breakpoints involved in gene amplification or loss were analysed. The data analysis was performed using the R programming language.

Results: Frequent breakpoints were found in chromosomes 12p, 8p, 3q, 14q, 6p, 4q, Xq and 8q. Chromosomes 6, 14, 3, 8 and X were more prone to present breaks than other chromosomes. We observed that low copy repeat DNA sequences are located at or in close proximity to breakpoint sites, ranging from 0 to 200 bp flanking the breakpoint site. LINES, SINES and Simple Repeats were the most frequent repeat elements identified in these regions.

Conclusions: Overall these results demonstrate selective, frequent genomic breaks involving several chromosomic regions. The presence of DNA low copy repeats elements in or close to the breakpoint site may contribute to the genomic instability but may not be the only explanation for the common rearrangement events observed in specific chromosome regions of HNSCC patients.

Genomic classification-based model for the distinction between intra- and extrahepatic cholangiocarcinoma

Inês Tavares[1]; Ilda P. Ribeiro[1,2]; Ricardo Martins[2,3,4]; Luísa Esteves[1]; Margarida Abrantes[2,3]; Filomena Botelho[2]; José G. Tralhão[2,3,4]; Joana B. Melo[1,2,5]; Isabel M. Carreira[1,2,5]

1 - Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 2 - CIMAGO - Center of Investigation on Environment Genetics and Oncobiology - Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 3 - Biophysics Unit, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 4 - Surgery A, Surgery Department of Coimbra University Hospital, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 5 - iCBR - Coimbra Institute for Clinical and Biomedical Research, Coimbra, Portugal

Introduction: Cholangiocarcinoma (CCA) is a rare malignant tumor originating from the epithelial cells of the biliary tree. 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%). This tumor is commonly classified as intrahepatic (ICC) and extrahepatic (ECC), based on anatomical location. The aim of this study was to analyze the copy number variation of tumor samples from patients with ICC and ECC, through array-CGH, and therefore to develop a genomic model to differentiate between these two anatomical tumor types.

Methods: The minimum common regions of alteration were determined using the array-CGH results from 10 ECC and 13 ICC patients. The most important regions for the distinction between ICC and ECC were selected by Gini's coefficient given by a Variable Importance Plot in a bootstrapping scheme applied to a balanced set regarding the number of cases in each class. A two-class support vector machine (SVM) algorithm for statistical classification was applied to these data to test the ability of the selected regions to distinguish between the two classes.

Results: The array-CGH results obtained revealed some common alterations between the patients. Gain of 2q37.3 and Xp and loss of 3p, 11q11, 14q, 16q, Yp and Yq were the most common alterations observed ICC patients. Regarding the ECC patients, gain of 2q37.3 and 16p25.3 and loss of 3q26.1, 6p25.3-22.3, 12p13.31, 17p, 18q and Yp were the most common alterations shown within this group. The developed genomic model identified further chromosomal regions that seem to enabled the distinction between ICC and ECC, namely 3q26.1, 6p25.3, 14q32.33 and Xq26 (Figure 3), with an accuracy of 71.43%, 95% CI [43, 100]%.

Conclusions: This genomic model is very important whereas previous genetic studies have shown that there are differences between these two anatomical types and may be helpful in the clinical management of the patients. Our findings support the idea that ICC and ECC may be two closely related but different biologic entities.

Project developed under the research grant from NRC-LPCC/CIMAGO

P43 | CLINICAL RESEARCH

Phenotypic description of patients with chromosome 3q29 deletion or duplication

Célia Azevedo Soares[1,2]; Ana Rita Azevedo Soares[1]; Teresa Saraiva[1]; Gabriela Soares[1]; Maria João Nabais Sá[2,3]; Ana Maria Fortuna[1,2]

1 - Medical Genetics Department, Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar Universitário do Porto, EPE, Porto, Portugal; 2 - Unit for Multidisciplinary Research in Biomedicine, ICBAS-UP Porto, Portugal; 3 - Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

Context: While some cytogenetic variants have an established genotype-phenotype correlation, deletions or duplications on chromosome 3q29 are still being cytogenetically and phenotypically characterized. A recurrent 1.6Mb heterozygous deletion established the cytogenetic diagnosis of the 3q29 deletion syndrome, associated with global developmental delay, intellectual disability, dysmorphisms and psychiatric disorders. Duplications on the 3q29 region are associated with intellectual disability, obesity, and minor dysmorphisms. The correlation between the duplicated regions on 3q29 and clinical phenotype remains elusive. Our aim is to contribute to the effort of delineating the phenotype of copy number variants of chromosome 3q29 region.

Methods: The clinical features of eight patients were evaluated, including four index cases with 3q29 deletions identified by multiplex ligation-dependent probe amplification (MLPA); two 3q29 duplications index cases and one familial case identified by MLPA, and one index case with a duplication identified by array comparative genomic hybridization (array-CGH).

Results: All four patients with deletions presented delayed global development. The only common feature in patients with duplications was a bulbous nose. One patient with a deletion on the 3q29 region, identified by MLPA, was also diagnosed with a pyruvate dehydrogenase deficiency that contributed to his complex clinical features. One patient with a *de novo* 3q29 duplication identified by array-CGH had a personal and familial history of microcephaly and visual defects that were not attributed to the duplication.

Conclusions: This case series shows that patients with 3q29 deletions or duplications have some overlapping clinical features. Nevertheless, in some patients the phenotype could be related to a multiloci genotype, or a phenotypic expansion of the known 3q29 deletions/duplications. A thoughtful interpretation of cytogenetic and clinical features is essential to diagnose and follow-up patients with an atypical presentation.

Female FMR1 full-mutation carriers: clinical and molecular characterization

Nataliya Tkachenko[1]; Gabriela Soares[2]; Ana Rita Soares[2]; Célia Azevedo Soares[2,4]; Ana Rita Gonçalves[3,4]; Nuno Maia[3,4]; Isabel Marques[3,4]; Paula Jorge[3,4]; Rosário Santos[3,4]; Ana Maria Fortuna[2,4]

1- Medical Genetics Dept., Centro de Genética Médica Doutor Jacinto de Magalhães (CGMJM), Centro Hospitalar Universitário do Porto (CHUP), Porto, Portugal; 2- Medical Genetics Dept., CGMJM, CHUP, Porto, Portugal; 3- Molecular Genetics Unit, CGMJM, CHUP, Porto, Portugal; 4- Unidade Multidisciplinar de Investigação Biomédica (UMIB), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal

---

Introduction: Fragile X Syndrome (FXS) is one of the main causes of intellectual disability (ID), affecting all males carrying a full-mutation (FM) in the *FMR1* gene. About 50% of females carrying a FM present with some degree of ID, usually less severe. As there are few studies on female FM carriers, the aim of this study was to describe the clinical and molecular characteristics of these females and to outline the genotype-phenotype correlation.

Methods: Data from all female cases observed at our genetics consultation that had a FM in the *FMR1* gene diagnosed at our lab was collected. A checklist was designed based on medical experience and literature reports with the most frequent signs and symptoms easily detected through clinical exam. Whenever necessary, molecular study was re-evaluated and completed with different techniques. In all cases, the pattern of X-chromosome inactivation was performed using HUMARA assay.

Results: From 12 cases identified, 10 were included in the study, belonging to 7 families. The mean age at FXS diagnosis was 18,3 years (ranging from 6 to 47). In 6 cases the female patient was the index case, under investigation for neurodevelopmental disorder, and in 3 cases the index case was an affected male relative. All cases had learning difficulties, 2 of them with moderate ID; 3 cases had ADHD and none had ASD. Six cases presented with nonspecific dysmorphic features. The main manifestation was behaviour disturbance.

Molecular test revealed a FM in *FMR1* gene, ranging from 250 to 600 CGG repeats. In 7 cases the mutation was maternally inherited and in 3, heredity was unknown. Eight cases showed random X-chromosome inactivation pattern, and remaining cases are in progress.

Conclusion: All findings were consistent with previous literature reports. Mild dysmorphic features and absence of systemic manifestations make the diagnosis more difficult, so it should be suspected in females with learning difficulties/ID, with or without behaviour alterations. The authors wish to stress that this molecular diagnosis requires specific laboratory techniques and that FM is not detectable by *array*-CGH or NGS tests used in ID diagnosis workout nowadays.

P45 | CLINICAL RESEARCH

Correlation between peripheral cytopenias and cytogenetic changes in the bone marrow in a paediatric population. Experience of 22 years

Maria Do Céu Silva[1]; Ana Paula Ambrosio[1]; Neuza Bacalhau[1]; Mónica Viegas[1]; Hildeberto Correia[1]

1 - Instituto Nacional de Saúde Doutor Ricardo Jorge, IP

The haemogram is the most frequent request and an essential tool in the diagnosis of different pathologies in paediatric age, especially in haematological diseases. Peripheral cytopenias is the first laboratory finding suggestive of haematological disease, such as myelodysplastic syndrome, idiopathic thrombocytopenic purpura, among others. Confirmation of these pathologies should include the study of bone marrow, with analysis by different methodologies, including conventional cytogenetic karyotype analysis.

In this work, we intend to present and establish a correlation between the results obtained by conventional cytogenetics in bone marrow samples and observation of peripheral cytopenias in a paediatric population over 22 years.

A retrospective 22-year series (1995-2017) of 154 bone marrow samples from a paediatric population was analysed, which at the initial diagnosis presented peripheral cytopenia. The samples were process according to the established protocol for chromosome analysis in bone marrow, including cell culture, for each biological product, followed by a cytogenetic study to identify the karyotype.

In the 154 samples analysed with peripheral cytopenias, 31 were bicytopenias, 33 pancytopenias, 21 neutropenias, 11 anaemias and 58 thrombocytopenias, of which 22 were of idiopathic origin. We identify 15 samples with abnormal karyotype, some of which presented a complex karyotype. Samples with abnormal karyotypes had pancytopenia or bicytopenia at the same time.

Peripheral cytopenias are extremely important for suspicion of paediatric haematological diseases, especially in myelodysplastic syndrome. Conventional cytogenetic analysis of the bone marrow plays a fundamental role in the confirmation of these pathologies, their clinical evolution and the choice of proper therapy, However, micro-arrays should be performed with the aim of identifying micro-deletions / duplications or loss of heterozygosity that are characteristic in this group of pathologies.

P46 | CLINICAL RESEARCH

Fanconi anemia: still an underdiagnosed disease? Retrospective analysis based on 25 years of cytogenetic evaluation

Beatriz Porto[1]; Cláudia Oliveira[1]; José Barbot[2]

1 - ICBAS - Instituto Ciências Biomédicas Abel Salazar; 2 - PFARN - Associação Portuguesa para a Anemia de Fanconi

Fanconi anemia (FA) is a recessive disorder clinically characterized by progressive bone marrow failure (BMF) and congenital abnormalities with variable presentation. The rarity of the disease, its phenotypic variability and the late onset of BMF generate a consensus that considers FA as a disease susceptible to underdiagnosis/late diagnosis. This collides with the benefits inherent to a timely diagnosis, which is decisive both for patient's outcome and appropriate genetic counseling. An effort is being carried out, among the clinical specialties involved with the morbility of FA, to promote a timely diagnostic accuracy.

Despite the recent research advances about genetic diversity and pathophysiology of FA, the evaluation of DEB-induced chromosome instability still remains the first line diagnostic test. The ICBAS Cytogenetics Laboratory has been a reference laboratory in the application of this test since 1992. A total of 667 DEB-tests requested for suspicion/exclusion of FA were performed, with 66 patients diagnosed with FA. In the present work we evaluated the evolution of the diagnostic accuracy over 25 years on the basis of the following parameters: clinical specialties that requested the test; number of tests/year; number of diagnosis/year; age at the time of diagnosis.

The main specialties that requested DEB-tests were Hematology and Pediatrics, and the relation between them evolved in favor of the second over the time. The number of tests/year increased over the years. The number of diagnoses/year, whose overall mean (2.4) was higher than the expected (according to FARF estimative), remained stable over the years, as opposed to the age at diagnosis, which decreased during the same period.

In conclusion, the results point to an improvement in diagnostic accuracy. The number of tests/year increased, revealing greater clinical sensibility to FA diagnosis, with progressive intervention of other specialties than Hematology. As a result, we observed a significant decrease of the age at diagnosis. The high overall mean of diagnoses/year may be due to the presence of a gypsy population where there is a founding effect associated with consanguinity.

P47 | CLINICAL RESEARCH

Genome-wide association study in chronic obstructive pulmonary disease (COPD): associations between genetics and clinical measures

Tatiana Silva[1]; Filipa Machado[1]; Gabriela Moura[2]; Alda Marques[1]

1 - Lab3R – Respiratory Research and Rehabilitation Laboratory, School of Health Sciences (ESSUA), University of Aveiro (UA), Aveiro, Portugal and Institute for Biomedicine - iBiMed, UA, Aveiro, Portugal; 2 - Institute for Biomedicine - iBiMed, UA, Aveiro, Portugal and Department of Medical Sciences, UA, Aveiro, Portugal

Chronic Obstructive Pulmonary Disease (COPD) is common and progressive condition with increasing relevance in Western countries. Patient's genetic background is bound to play a role in this disease since clinical variables are not sufficient to explain its onset and/or progression. Our goal was to study the genetic profile of these patients and explore association with clinical measures commonly associated with a deterioration of symptoms (e.g., number of exacerbations) in order to pinpoint genetic variants that could help clinicians predict COPD prognosis.

Methods: A pilot study of 40 patients with stable COPD (68 ± 9 years old) was conducted. Patients were recruited from routine pulmonology appointments and primary health care centres. Sociodemographic, anthropometric and general clinical data were collected. DNA was extracted from saliva samples and genotyped with the Illumina Beadchip array. Individual variants were tested for association with clinical variables (Airway obstruction levels (FEV1pp) and number of exacerbations) and interactions with smoking behavior was tested through a Genome-wide association study (GWAS).

Results: FEV1pp was associated with 2 SNPs (mapped to 2 genes) and the number of exacerbations was affiliated with 195 SNPs (mapped to 29 genes). Gene enrichment analysis using the GO term revealed an enrichment of genes associated with the inflammatory response. In addition, a greater number of relevant SNPs / genes resulted from inclusion in the GWAS of the patients' smoking status, which suggests a better stratification of the subjects based on this additional information.

Conclusion: This pilot study showed significant genetic variations in patients with COPD, related to several clinical variables considered relevant for this disease. Further studies will be conducted with a larger set of patients and matched healthy controls, as a way to further characterize this population and to explore the interplay between genetics and COPD progression. Our ultimate goal shall be to identify potential new biomarkers for personalized interventions in COPD.

P48 | CLINICAL RESEARCH

Cancer challenges: common pathogenic ATM variants

Pedro Louro[1]; Patrícia Machado[1]; Ana Clara[1]; Ana Luís[1]; Isália Miguel[1]; Sandra Bento[1]; Sidónia Santos[1]; Sofia Fragoso[1]; Fátima Rodrigues[1]; Irina Coelho[1]; Joana Parreira[1]; Paula Rodrigues[1]; Fátima Vaz[1]

1 - Instituto Português de Oncologia de Lisboa Francisco Gentil

---

Context: Ataxia-telangiectasia (AT) is caused by biallelic pathogenic variants in ATM gene, which encodes a protein kinase that plays an important role in cellular responses to DNA damage. Classic AT is characterized by early childhood-onset cerebellar ataxia, telangiectasias of the conjunctivae, immunodeficiency, radiosensitivity, and a predisposition to malignancy. Non-classic forms of AT include adult-onset AT and AT with early-onset dystonia. The study of AT patients' families have shown a 2 to 5-fold increased risk of breast cancer for females who are monoallelic carriers. Men who are monoallelic carriers have an increased risk of prostate cancer. Monoallelic carriers also have an increased chance to develop pancreatic cancer and possibly other cancers, like gastric, colorectal and ovarian cancer.

Methods: Clinical and molecular characterization of all cases with monoallelic ATM variants observed at the Familial Cancer Risk Clinic of Instituto Português de Oncologia de Lisboa Francisco Gentil, based on retrospective analysis of patient medical records. Next-generation sequencing was performed in all cases.

Results: We report 6 patients, 5 females and 1 male. Four of the female patients had breast cancer and the male had Hodgkin's lymphoma. The other female does not have history of cancer and was a relative of an affected female patient. In all families, there were several female relatives with breast cancer, not tested yet. To our knowledge, no family has an AT case.

Discussion: At this moment, we cannot rule out that Hodgkin' lymphoma is from the spectrum of cancers caused by pathogenic ATM variants. ATM gene investigation results are being released every month, with fresh insights, making it difficult to update patients' management in a fair, coherent and cost-effective process. As far as we know, carrier frequency for pathogenic ATM variants is about 1%. As such, this can pose a serious healthcare challenge, further aggravated by the massification of genetic testing. Genetic counselling should be offered to these patients and genetic testing of their partners should be discussed, especially if they also have a suggestive family history of cancer.

P49 | CLINICAL RESEARCH

High performing AmplideX® PCR/CE for Myotonic Dystrophy type I is concordant with a combination of PCR and Southern Blot analysis~

Isabel Marques[1,2]; Nuno Maia[1,2]; M Rosário Santos[1,2]

1 - Unidade de Genética Molecular, Centro de Genética Médica Dr Jacinto de Magalhães (CGMJM), Centro Hospitalar Universitário do Porto; 2 - Unidade Multidisciplinar de Investigação Biomédica (UMIB), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto

Context: Myotonic dystrophy type 1 (DM1) is among the most common adult-onset forms of inherited neuromuscular disorders, with a prevalence of ~1/8000 individuals worldwide. It is a triplet repeat disorder caused by an expansion of >50 CTGs in the 3'UTR of the *DMPK* gene.

Somatic mosaicism is common and the number of repeats can be different within and between tissues in a single individual, and this number can change over time. The age of onset and severity of the condition are roughly correlated with the size of the expansion.

Methods: The conventional diagnostic strategy is based on a Fluorescent-PCR (FP-PCR) and/or a Triplet-Primed-PCR (TP-PCR), both of which rarely amplify above 100 repeats. This is followed by Southern Blot (SB) analysis to resolve apparent homozygous genotypes and to quantify the size of the expansion.

We evaluated the AmplideX® PCR/CE *DMPK* kit as part of an early access program. This technology combines PCR and capillary electrophoresis (CE) to size of alleles with up to 200 repeats, with an optional agarose gel electrophoresis (AGE) protocol to size larger alleles. The kits were tested on gDNA samples extracted from peripheral blood, amniotic fluid, chorionic villi and cell cultures, from a total of 101 patients and 5 controls that had been previously genotyped by conventional methods.

Results: Genotyping with the AmplideX® PCR/CE assay was 100% concordant with previous results, showing 100% sensitivity and specificity, including zygosity resolution. CE profiles enabled clear differentiation between normal and expanded alleles while simultaneously identifying repeat mosaics. The high sensitivity enabled molecular analysis of samples with limited amounts of gDNA.

Conclusion: The AmplideX® *DMPK* technology resolves zygosity and demonstrates high accuracy in both sizing of up to 200 repeats and detecting expansions of >200 repeats, as well as low abundance mosaics. Sizing of alleles with over 200 repeats can be achieved using an AmplideX® PCR/AGE *DMPK* assay protocol. This technology is a simpler and faster approach than the combination of conventional techniques previously required for the molecular diagnosis of DM1.

Genotypic differences between southwestern Europe and Africa: a comparative study in eNOS, G6PD, GSTM1 and GSTT1 genes

Laura Aguiar[1,2]; Ildegário Semente[1,2,3]; Andreia Carvalho[1,2,4]; Cristina Caroça[5,6,7]; Helena Caria[8,9]; Paula Faustino[3,10]; Manuel Bicho[1,2,10]; Ângela Inácio[1,2,10]

1 - Instituto de Investigação Científica Bento da Rocha Cabral; 2 - Laboratório de Genética, Faculdade de Medicina da Universidade de Lisboa; 3 - Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge; 4 - Faculdade de Ciências da Universidade de Lisboa; 5 - Otolaryngology Department, NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa; 6 - Hospital CUF Infante Santo; 7 - Centre for Toxicogenomics and Human Health (ToxOmics), Genetics, Oncology and Human Toxicology, Nova Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa; 8 - BioISI-Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa; 9 - Departamento de Ciências Bioemédicas, Escola Superior de Saúde, Instituto Politécnico de Setúbal; 10 - Instituto de Saúde Ambiental, Faculdade de Medicina da Universidade de Lisboa

Aims/Context: The aim of the present work was to compare the genotypic frequency in genes involved in oxidative stress, in southwestern Europe and Africa. The genes under analysis were: endothelial nitric oxide synthase (*eNOS*), glucose-6-phosphate dehydrogenase (*G6PD*) glutathione S-transferases mu (*GSTM1*) and theta (*GSTT1*).

Methods: We analyzed 273 DNA samples from Portugal and 202 samples from Africa (24 DNA samples from Mozambique and 178 DNA samples from São Tomé and Príncipe). For the *eNOS* gene, polymorphic analysis of variable number of tandem repeats (VNTR) in intron 4 (27 bp tandem repeat) was performed by polymerase chain reaction (PCR). Characterization of the rs1050829 in *G6PD*, was obtained by PCR followed by restriction fragment length analysis. A multiplex PCR assay was used for simultaneous detection of *GSTM1* and *GSTT1* polymorphisms. All statistical tests were performed with SPSS 24.0 software.

Results: Results show that concerning *eNOS*, genotypes presenting the 4a allele have a lower frequency in Portugal than in Africa ($p<0.001$). Interestingly, only in Africa we found the rare alleles 4c, 4d and 4y. For the *G6PD* gene, there is a lower frequency of genotypes with the G allele in Portugal once compared with the African populations ($p<0.001$). Regarding *GSTM1* and *GSTT1,* the presence of the null genotypes was more common in Africa, than in Portugal ($p=0.001$ and $p<0.001$, respectively).

Conclusions: Our results show differences in the geographical distribution of four polymorphisms in *eNOS*, *G6PD*, *GSTM1* and *GSTT1* genes. These differences may be related with different selective pressures provided by the different climatic environments in southwestern Europe and the equatorial and sub-equatorial Africa. Indeed, conditions such as drought and high light intensity have been documented to be able to promote ROS production and oxidative stress.

P51 | CLINICAL RESEARCH

Interpreting sequence variants: a daily challenge in a clinical molecular genetics laboratory

Rita Bastos Ferreira[1]; Ana Filipa Brandão[1]; Ana Margarida Lopes[1]; Susana Sousa[1]; Patrícia Arinto[1]; Sara Morais[1,2]; Paulo Silva[1]; Susana Barbosa[1]; Diana Santos[2]; Isabel Alonso[1,2]; Jorge Sequeiros[1,2,3]

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

---

High-throughput next-generation sequencing has been a crucial tool for Medical Genetics, as it has allowed the identification of new underlying genetic causes for many Mendelian diseases. With the large amount of data generated, this approach brought a major challenge - filtering, classification and clinical interpretation of variants present. Despite the increasingly ability of variant classification and genotype-phenotype correlation, there are still many variants with uncertain clinical significance.

To describe variants identified in genes causing Mendelian disorders, the CGPP pipeline was based on the ACMG guidelines that recommend the classification of variants into 5 categories (pathogenic, likely pathogenic, uncertain significance, likely benign, and benign), based on criteria such as population frequencies, *in silico* predictions, functional analysis and segregation data. Our aim was to present some challenging cases detected at our laboratory.

Using virtual gene panels, based on Whole Exome Sequencing (WES), on an 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.

Here we present some cases of variants identified at our lab that highlight some of the caveats in variant classification, as variants that *a priori* would be reported as "likely pathogenic" and probably are not responsible for the patient phenotype, and other variants that could be classified as "uncertain significance" or "likely benign" that are probably causing the disease in that patient and family.

The use of high-throughput sequencing technologies in diagnostic settings is becoming more prominent, although these data needs to be interpreted with great caution, given the complexities outlined previously. It is critical to reach consensus between healthcare providers and clinical laboratories, so that both have a common understanding of how variants are classified and, thus, are able to provide a better service of patient counselling and management.

P52 | CLINICAL RESEARCH

Proximal 1p36 deletions: Report of 3 patients and review of the literature

Maria Abreu[2]; Cláudia F. Reis[2]; Ana Maria Fortuna[2,3]; Gabriela Soares[2]

2 - Medical Genetics Department, Centro de Genética Médica Jacinto de Magalhães (CGMJM), Centro Hospitalar Universitário do Porto, E.P.E., Porto, Portugal; 3 - Unit for Multidisciplinary Research in Biomedicine (UMIB), ICBAS-UP Porto, Portugal

Introduction: Terminal 1p36 deletion may be the most common terminal microdeletion in humans, with a prevalence of ~1/5000 live-births and a phenotype encompassing developmental delay, characteristic facial features, growth retardation, microcephaly and variable other congenital anomalies. The breakpoints of 1p36 deletion are variable among patients. Despite being less common, proximal 1p36 deletions have also been described in the literature as disease-causing, with an associated critical region. These deletions have been described with a variable phenotype and genotype, and some attempts at genotype-phenotype correlation have been made.

Methods: We report three patients from our center with a proximal 1p36 deletion detected by array-CGH. We reviewed the literature for other case reports of proximal 1p36 deletions and discussed the genotype-phenotype correlations.

Results: The deletions in all three patients encompassed the proposed proximal critical region, and one of the patients also presented a 10q duplication of unknown significance. All patients shared a few phenotypical features: global developmental delay, wide nasal bridge and narrow chin. Less consistent features included intrauterine growth restriction, microcephaly, short stature, hypotelorism, underdevelopment of the midface, arched eyebrows, palpebral upslanting, tubular nose, and predominantly motor delay (2/3). Other malformations and dysmorphic features were present in only one of the three patients.

Discussion: Some features present on proximal 1p36 deletions – such as small stature, microcephaly, hypotelorism, and seizures – occured only when genes outside the critical region are involved. This was not the case for global developmental delay, among other features, which was present regardless of the placement of the deletion. Other cases need to be evaluated to draw conclusions on the genotype-phenotype correlation; however, current data suggest that proximal 1p36 deletion is a spectrum of syndromes with some degree of overlap rather than a specific syndrome with a single critical region.

P53 | CLINICAL RESEARCH

Patients' Voices in Portugal - when rare becomes common

Catarina Costa[1]; Isabel Alonso[1]; Jorge Sequeiros[1,2]; Milena Paneque[1,2]

1 - CGPP – Centro de Genética Preditiva e Preventiva, IBMC – Instituto de Biologia Molecular e Celular, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; 2 - ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

Rare disease is a health condition that affects 1 in 2000 people in the european population. Patients' associations have an important role in the identification of new patients and families and bridging resources among them. Nevertheless, little is known regarding our national associations, and their main roles and needs. The present exploratory study aimed to characterize a group of Portuguese associations, mostly on rare diseases, using a qualitative methodology.

Semi-structured interviews were conducted with a sample of 38 representatives, from 23 national patients' associations. Recordings were transcribed and analyzed using the thematic analysis method. Three conceptual categories emerged from their discourses relating to: (a) mission of associations, (b) the current context of associative work in our country and (c) present strategies and opportunities for patients' associations in Portugal. Most common limitations to their work, as mentioned by the patients' association, were: its voluntary character, low literacy and poor involvement of the population and professionals, lack of funding and non-recognition of the empowering role of associations. On other hand, those with a close contact with medical specialists were the ones with best results on their educational activities and community awareness. Involvement with European homologous associations was an indicator of success in the associative work, as it allowed for the exchange of experiences and is a mean to obtain new knowledge.

The results showed that much remains to be done for all families and associations to facilitate the process of "living with the disease." This was the first national study reflecting on the work of patients' associations of rare diseases in Portugal and aimed to contribute to new strategies in favor of the rare patients and their relatives.

Associations play a key role in patient advocacy. It is possible to reinforce their relevant work of articulation with existing resources, facilitating the necessary integration in healthcare services and maximizing their visibility at the national level.

P54 | CLINICAL RESEARCH

Fanconi's anemia - Retrospective study over a period of 37 years

Ana Paula Ambrósio[1]; Maria Do Céu Silva[1]; Neuza Bacalhau[1]; Mónica Viegas[1]; José Furtado[1]; Hildeberto Correia[1]

1 - Instituto Nacional de Saúde Doutor Ricardo Jorge, IP

Fanconi anemia (FA) is a rare disease, with an estimated frequency of 1 to 5 per 1,000,000 births, which may increase in some ethnic group (like Ashkenazi Jewish and Gypsy). It's an autosomal recessive disease that may have an X-linked transmission. Patients with FA may have congenital malformations, bone marrow failure, hypersensitivity to clastogenic agents, chromosomal fragility, and increased susceptibility to oncological diseases. Due to the great complexity of this pathology, the first approach to diagnosis consists of the detection of chromosomal aberrations (breaks, structural rearrangements, rings) in peripheral blood cells in culture with clastogenic agent such as diepoxybutane (DEB) or mitomycin C (MMC).

We intend to present the results of chromosome instability studies induced by DEB and MMC performed in our institution.

A retrospective 37 years series (1980-2017) of 274 samples sent to the cytogenetic laboratory with suspicion of FA and 28 samples of relatives of patients with FA were perform. The samples were process according with the protocol established by the International Fanconi Anemia Registry (IFAR).

In the 274 analysed samples, 39 cases with AF were identify. In the cytogenetic studies of relatives with AF, 2 positive cases were identified for FA. Abnormal karyotypes were also observed in 8 samples suspected of AF.

In this study, 41 new cases of AF were identify, mainly from the Lisbon and Tagus Valley regions and some specific cases from Azores, the central region and from the Portuguese speaking African countries (PALOP). This study evidences that the majority of the presented cases are underdiagnosed. These results do not allow to estimate a frequency of patients with AF in Portugal, since it does not include individuals from all Portuguese regions, and are include two individuals of PALOP origin. It would be interesting to carry out Next generation sequencing on the Fanconi positive samples in order to obtain in a single assay the analysis of the various genes involved in the pathology thus identifying the genetic change causing the disease.

P55 | CLINICAL RESEARCH

Genomic characterization of Monoclonal Gammopathies patient

Alexandra C. Oliveira[1]; Luís M. Pires[1]; Ana C. Gonçalves[2,3]; Artur Paiva[2,4]; Catarina Geraldes[2,5]; Adriana Roque[5]; Joana B. Melo[1,2]; Ana B. Sarmento-Ribeiro[2,3,5]; Isabel M. Carreira[1,2]

1 - Cytogenetics and Genomics Laboratory, Faculty of Medicine of the University of Coimbra (FMUC), Coimbra, Portugal; 2 - iCBR-CIMAGO - Centre of Investigation on Environment Genetics and Oncobiology - FMUC, Coimbra, Portugal; 3 - Laboratory of Oncobiology and Haematology and University Clinic of Haematology, FMUC, Coimbra, Portugal; 4 - Cytometry Operational Management Unit, Clinical Pathology Service of Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal; 5 - Clinical Haematology Department of CHUC, Coimbra, Portugal

Introduction: Monoclonal Gammopathies (MG) are a set of disorders of the haematopoietic system, resulted from the clonal proliferation of malignant plasma cells in the bone marrow, due to genetic/epigenetic alterations and environmental factors. Even though not all MG are considered malignant neoplasms, which is the case of Monoclonal Gammopathy of Undetermined Significance (MGUS), they can evolve to Multiple Myeloma (MM), a malignant plasma cells neoplasm, that still remains with no cure. A good establishment of MM risk stage is mandatory and genetic analysis has been proposed as a good strategy for a more accurate prognosis. Interphase Fluorescence *in Situ* Hybridization (i-FISH) is the only technique applied in the clinical practice for MG patients' genetic analysis, even though it seems not to be enough.

Therefore, the aims of this project were the genomic and molecular characterization of MG patients and the validation of Array Comparative Genomic Hybridization (aCGH) and Multiplex Ligation-dependent Probe Amplification (MLPA) technologies in the MG patients' prognosis.

Methods: A total of 13 patients were studied (3 patients with MGUS, 5 patients with MM and 5 patients with relapsed MM). Plasma cells ($CD138^{+}$) from bone marrow samples were isolated through immunomagnetic separation, being then selected to perform aCGH and/or MLPA.

Results: From the 10 studied samples by aCGH, 7 presented 13q deletions, 5 presented 1q gain, trisomy 9 was detected in 4 samples and 14q deletions were detected in 4 samples. MLPA technology only partially confirmed the genetic alterations detected by aCGH. Furthermore, it allowed the study of other 3 samples which were not possible to be analysed by aCGH.

Conclusions: This pilot study contributed to the unveiling of the genomic alterations behind MG in the studied cohort, thus allowing the first steps in the possible validation of aCGH and/or MLPA as complementary techniques to i-FISH in the clinical practice. The study of more cases is needed to confirm the usefulness of these approaches.

Patients' views: a new tool for quality assessment of genetic counselling

Márcia Carvalho[1,2]; Milena Paneque[2]; Fidjy Rodrigues[3]; Jorge Saraiva[3,4,5]; Alexandra Leonardo[6]; Ana B. Sousa[6]; Vânia Machado[7]; Renata Oliveira[8]; Miguel Rocha[9]; Jorge Sequeiros[2,10]; Marina S. Lemos[1]

1- Faculty of Psychology and Education Science, University of Porto, Portugal; 2- UnIGENe and CGPP - Center for Predictive and Preventive Genetics, IBMC - Institute for Molecular and Cell Biology, i3S - Institute for Research and Innovation in Health Sciences, University of Porto, Portugal; 3- Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra (CHUC), Portugal; 4- University Clinic of Pediatrics, Faculty of Medicine of University of Coimbra (FMUC), Portugal; 5- Clinical Academic Center of Coimbra, Portugal; 6- S. de Genética Médica, Centro Hospitalar Lisboa Norte – Hospital de Santa Maria, Centro Académico de Lisboa, Portugal; 7- Hospital de Santo Espírito da Ilha Terceira, Portugal; 8– S. de Genética Médica, Centro Hospitalar de São João, Portugal; 9- Unidade de Genética Médica, Hospital de Braga, Portugal; 10– Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal.

Background: It is consensual that appropriate genetic counselling is essential when a genetic test is offered or where there is a risk for a genetic condition. Assessing quality of genetic counselling, however, is a challenge for genetic services worldwide, given the scarcity of effective tools available to this effect. Recent studies in Portugal reinforced precisely this lack of tools, as well as the need to define quality indicators that support them. A pioneer instrument for quality assessment of genetic counselling practice by professionals has been developed. Currently, we started the construction of an analogous tool designed for quality assessment from the consultand's perspective. Here, we present the methodological design and preliminary results of the development and the validation process of this new tool.

Method: The proposed scale was submitted to pre-test validation with 7 consultands at CGPP, between June and August of the current year. Cognitive interviews with 5 of these participants were performed to explore in-depth the adequacy of the items, instructions and scale options. All main national genetic services were invited to collaborate in the recruitment of a minimum sample of 120 patients, for the validation process. This study was approved by the FPCEUP Ethics Committee.

Results: Based on the above mentioned procedures, the present version of the scale has 52 items, organized in 5 dimensions focusing on: (1) relevance of the genetic information; (2) the way consultand's emotional issues and personal characteristics were addressed; (3) the relationship and communication issues; (4) genetic counselling outcomes; and (5) services provision.

Conclusions: The process of psychometric validation started last September and should be completed next May. In the short-term, it is expected that this tool will allow an evaluation of genetic consultations and services from the users standpoint, as well as a comprehensive understanding of the genetic counselling process, where both the professionals' and the consultands' views can be compared.

P57 | CLINICAL CASE REPORTS

TBL1XR1 single gene CNV: copy loss in mother and daughter with ID and ADHD

Sónia Custódio[1]; Rosário Silveira-Santos[1]; Oana Moldovan[1]; Ana Sousa[1]; Ana Berta Sousa[1]

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

Introduction: The genetic diagnosis of intellectual disability (ID) has witnessed a major breakthrough in the past decade with the routine use of microarray technology. While many ID related genes are now uncovered, others are still in need of more data to support a stronger clinical delineation.

Case Report: We report a six year old girl referred to our genetics department for ID, behavioral problems and epilepsy. She also presented attention deficit hyperactivity disorder (ADHD), and clinical observation revealed unspecific facial dysmorphic features. Array-CGH analysis detected a 1.22 Mb deletion, on 3q26.32, encompassing *TBL1XR1* gene (PerkinElmer® GGX-HD 180k, Genoglyphix v3.1). Subsequent FISH studies established that the variant was inherited from the affected mother and was absent in her maternal grandmother and two maternal uncles, who have no phenotype.

Discussion and Conclusion: *TBL1XR1* gene encodes a protein that is part of both nuclear receptor corepressor (N-CoR) and histone deacetylase 3 (HDAC 3) complexes, and plays an essential role in transcriptional activation mediated by nuclear receptors. Point mutations in this gene have been previously related to autism and Pierpont Syndrome while microdeletions were shown to cause syndromic ID. However, there are only a few reported patients. In our case, this single gene CNV segregating with the ID phenotype in the family, provides further evidence of *TBL1XR1* haploinsufficiency and

Interstitial 6q22.1-q22.31 microdeletion: narrowing the critical region for ID/DD and movement disorders

Eva A. Rolo[1]; Rosário Silveira-Santos[1]; Patrícia Dias[1]; André M. Travessa[1]; Ana Sousa[1]; Ana Berta Sousa[1]

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

Introduction: Interstitial 6q22 deletions are a group of rare cytogenomic disorders clinically characterized by a variable phenotype including intellectual disability (ID)/developmental delay (DD), hypotonia, movement disorders, growth retardation, cardiac defects, upper limb malformations, facial dysmorphism, and Prader-Willi-like features. Considering the rarity and phenotypic heterogeneity of 6q22 microdeletions, genotype-phenotype correlations can be improved by overlapping and characterizing small microdeletions within the region.

Methods: We describe a 15-year-old girl with unremarkable family history referred to our genetics department due to ID and movement disorder, including ataxic gait, difficulties with gross and fine motor skills, poor motor coordination and tremor. Physical examination showed nonspecific dysmorphism precluding any definitive diagnosis. *Array*-CGH analysis was performed (PerkinElmer® CGX-HD 180K, Genoglyphix v3.1), and the results were further evaluated by FISH.

Results and Discussion: *Array*-CGH analysis detected a *de novo* interstitial deletion on 6q22.1q22.31 [arr[GRCh37] 6q22.1q22.31(117092352_119430150)x1dn], encompassing nine OMIM genes (*GPRC6A, RFX6, VGLL2, ROS1, GOPC, NUS1, PLN, MCM9,* and *ASF1A*). A retrospective evaluation, combining our case with the few reported 6q22 microdeletions, allowed us to find an overlapping region of 1.2 Mb encompassing six genes (*GPRC6A, RFX6, VGLL2, ROS1, GOPC,* and *NUS1*). Among these*, VGLL2, GOPC,* and *NUS1* seem to play a major contribution to the phenotype of 6q22 microdeletion. Particularly, the *GOPC* gene, which encodes a trans-Golgi-associated protein with a role in synapse development and function, has been proposed as a candidate gene for ataxia and other movement disorders. The motor features observed in our patient reinforce this hypothesis, contributing to narrow down the 6q22.1 critical genomic region associated with ID and movement disorders.

P59 | CLINICAL CASE REPORTS

Chromosome 16p13.11 Copy Number Variation in prenatal and postnatal detected by array-CGH – an update

Mariana Val[1]; Isabel M. Carreira[1,2,3]; Luís M. Pires[1]; Joana Ribeiro[1,2,3]; Nuno Lavoura[1]; Susana I. Ferreira[1,2]; Margarida Venancio[4]; Fabiana Ramos[4]; Joana B. Melo[1,2,3]

1 - Laboratório de Citogenética e Genómica, Faculdade de Medicina da Universidade Coimbra, Coimbra, Portugal; 2 - iCBR-CIMAGO - Centro de Investigação em Meio Ambiente, Genética e Oncobiologia, Faculdade de Medicina da Universidade Coimbra, Coimbra, Portugal; 3 - CNC-IBILI, Universidade de Coimbra, Coimbra, Portugal; 4 - Serviço de Genética Médica, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

---

Context: Chromosomal region 16p13.11 is structurally complex, subdivided into three single-copy sequence blocks called intervals I, II and III. Each block is flanked by low-copy repeats (LCRs) with highly homologous DNA sequences making it prone to non-allelic homologous recombination (NAHR) being a major source of *de novo* genomic rearrangements. 16p13.11 copy number variants (CNVs) have variable sizes (0,8 to 3,3Mb), encompassing one or more of the three intervals. Interval II (chr16:15.48-16.32Mb, GRCh37/hg19) CNVs, have the higher number of patients reported so far, involving a set of eight genes, including *NDE1*, referred as a strong candidate gene for neurodevelopmental disorders. Clinical features of patients with microdeletions or microduplications at chromosome 16p13.11, have been associated with a range of neurodevelopmental disorders including autism spectrum disorders (ASD), attention-deficit hyperactivity disorder (ADHD), intellectual disability (ID) and schizophrenia.

Methods: A cohort of 1700 patients, including prenatal and postnatal samples, showing ID, ASD and congenital anomalies was studied by Agilent 180K oligonucleotide array-CGH and the inheritance was established whenever possible.

Results and conclusions: We have identified 20 patients with 16p13.11 CNVs (10 deletions and 10 duplications), 3 detected in prenatal and 17 in postnatal. The majority of the patients showed high clinical variability with a wide range of phenotypic manifestations: developmental delay, autism, speech delay, learning difficulties, behavioural problems, epilepsy, microcephaly and physical dysmorphisms. In our data, an higher male:female 16p13.11 CNV ratio has not been detected. In ~70% of the cases, the alteration was inherited from unaffected parents confirming that duplications and deletions at 16p13.11 represent incomplete penetrance but that predispose to a range of neurodevelopmental disorders.

P60 | CLINICAL CASE REPORTS

A case of germ-line mosaicism in Tuberous Sclerosis

Ana Grangeia[1,2]; C. Joana Marques[2,3]; Susana Fernandes[2,3]; Miguel Leão[4]

1 - Serviço Genética Médica, Centro Hospitalar Universitário de S. João, Portugal; 2 - i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; 3 - Serviço de Genética, Faculdade de Medicina, Universidade do Porto, Portugal; 4 - Unidade de Neurogenética, Serviço Genética Médica, Centro Hospitalar Universitário de S. João, Portugal

Introduction: The diagnosis of Tuberous Sclerosis Complex (TSC) is established on clinical and/or molecular criteria. TSC is an autosomal dominant disorder caused by pathogenic variants in the *TSC1* or *TSC2* genes. Here we reported a family case with two affected siblings with TSC and two clinically unaffected parents. Both children have the same apparently *de novo* pathogenic variant in the *TSC2* gene. **Methods**: DNA was isolated from whole blood cells from all family members and from the skin cells and spermatozoa in the father. The screening of the pathogenic variant was performed by Sanger sequencing of the exon 37 of the *TSC2* gene. **Results**: The two children, aged 6 and 10 years old have the clinical diagnosis of TSC with molecular confirmation by the identification of a novel pathogenic variant, c.4783G>C (p.Gly1595Arg), in the *TSC2* gene exon 37. The genetic screening of the *TSC2* variant in the parent's blood cells DNA did not identify the pathogenic variant in any of them. Since the father presents a skin with inflammatory signs in the face previously diagnosed as a rosacea, the possibility of a somatic mosaicism was proposed. However, the familial variant was not detected in the skin cells DNA. In order to evaluate the possibility of germ-line mosaicism in the father, the *TSC2* gene was analyzed in DNA isolated from the spermatozoa. Two alleles were identified, confirming the existence of germ-line mosaicism. **Conclusions**: If the pathogenic variant found in the proband cannot be detected in leukocyte DNA of either parent, two possible explanations are *a de novo* pathogenic variant in the proband or germline mosaicism in a parent. When neither parent has the pathogenic variant or clinical evidence of the disorder, the *TSC1* or *TSC2* pathogenic variant is likely *de novo*. In these cases a low (1%) recurrence risk is usually given. In our family the demonstration of germ-line mosaicism increases the estimated risk of recurrence and has implications for genetic counseling. The identification of the causal variant and the evidence of the germ-line mosaicism allow the possibility of prenatal diagnosis, including preimplantation genetic diagnosis for future pregnancies.

P61 | CLINICAL CASE REPORTS

Epidermolysis bullosa: mandatory molecular diagnosis can change the future

Catarina S. Rosas[1]; Lina Ramos[1,2]; Fábia Mota[3]; Margarida Fonseca[4]; Leonor Ramos[5]; Marciana Dias[4]; Jorge M. Saraiva[1,6]; Gabriela Mimoso[4]

1 - Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra; 2 - University of Beira Interior, Health Sciences Department; 3 - Paediatrics Unit, Centro Hospitalar Tondela-Viseu; 4 - Neonatal Unit, Maternidade Bissaya Barreto, Centro Hospitalar e Universitário de Coimbra; 5 - Dermatology Department, Centro Hospitalar e Universitário de Coimbra; 6 - University Clinic of Paediatrics, Faculty of Medicine, University of Coimbra

Introduction: Dystrophic Epidermolysis Bullosa (DEB) is a genetic skin disorder which affects the connective tissue. It is characterized by severe mucocutaneous fragility, leading to the appearance of skin and mucosa blistering and erosions with minimal trauma. The different types of EB, which lead to different prognosis, are extremely difficult to distinguish in early infancy and the molecular study is mandatory. In this particular type of EB, a precocious eviction of minimal trauma prevents even the development of skin cancer or digital amputation. The only gene known to cause DEB is COL7A1 and the diagnosis is established with the identification by molecular genetic testing of biallelic pathogenic variants, in the case of recessive DEB (RDEB). We report 2 cases of RDEB who share a common genetic variant between them.

Clinical report: Case 1 – Newborn, from consanguineous parents, and a paternal aunt with DEB, who declined molecular genetic test (MGT). Extensive ulcerated lesions in the ankles and feet and scattered vesiculobullous lesions were evident at birth. Sequence analysis of the COL7A1 gene identified 2 variants in compound heterozygosity (c.4118C>A and c.7249C>T) endorsing the diagnosis of DEB. Case 2 – Newborn, from nonconsanguineous parents, with no relevant background. He presented, at birth, similar feet ulcerated lesions and exuberant lesions of the orolabial mucosa. A homozygous variant was identified in COL7A1 gene (c.7249C>T).

Discussion: Inheritance from the parents was confirmed in both cases, interestingly, with the consanguineous couple presenting with 2 different variants and the other couple, distant in origin, sharing 1 of these variants, in both members. The clinical observation of an affected adult allowed a single gene test in the first case, contrarily to the other case, in which a NGS panel with 21 genes was required. To our knowledge, the variant c.4118C>A has never been described in the literature. It introduces a STOP codon, leading to a truncated protein and was classified as likely pathogenic. The specific diagnosis in these two cases allowed the establishment of the correct prognosis and the maintenance of adequate care.

P62 | CLINICAL CASE REPORTS

Cockayne Syndrome: a new phenotype related to a already described variant

Pedro M Almeida[1,2]; Sara M. Ribeiro[1]; Isabel Monteiro[3]; Fabiana Ramos[1]; Jorge Saraiva[1,4,5]; Lina Ramos[1,2]

1- Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; 2- Faculty of Health Sciences, University of Beira Interior, Covilhã; 3- Hospital do Divino Espírito Santo, Ponta Delgada, Açores; 4- University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra, Portugal; 5- Clinical Academic Center of Coimbra, Coimbra, Portugal

---

Introduction: Cockayne syndrome (CS), first described in 1936, is a rare autosomal recessive multisystem disorder, with an incidence estimated 1:200000 births, mainly characterised by prenatal or postnatal growth disorders, intellectual deficit, neuromotor difficulties, impaired vision and hearing, skeletal abnormalities and premature ageing. Disease severity and the age of onset are variable. Mutations in *ERCC6* gene (responsible for production of CSB protein) and in *ERCC8* gene (responsible for production of CSA protein) have been found to cause OFCD syndrome. These proteins play a vital role in the repair and "decoding" of DNA, which are necessary for the proper functioning of cells. Studies performed thus far have failed to delineate clear genotype-phenotype relationships.

Clinical Report: A one-year-old girl, born at 40 weeks from consanguineous parents, was referred due to congenital cataracts, microcephaly, short stature and arthrogryposis. She had a history of diminution in movements and intrauterine growth restriction in prenatal development. She was re-evaluated in the first 6 months of life with severe microcephaly, growth failure and severe mental retardation. Brain MRI showed diffuse hypomyelination of the cerebral white matter, hypoplastic corpus callosum and small lens. We performed an array-CGH that was normal and then we did a broad gene panel analysis that found a homozygous c.201C>T (p.Thr204Lys) variant in the *ERCC8* gene. Study of the parents confirmed that they are heterozygous for this variant.

Discussion: CS belongs to the family of NER(nucleotide excision repair)-related disorders. There is a large variation in severity of this disorder, which led to the categorisation of three types. Our patient has type 2, which is a neonatal severe form, typically letal in the first decade of life. The biallelic probable pathogenic missense variant in the *ERCC8* gene identified in our case was already described, in compound heterozygous. However this patient hasn´t congenital cataracts, small lens, severe arthrogryposis and intrauterine growth restriction.

The homozygous state can be the cause of the more severe phenotype, that wasn´t observed before.

P63 | CLINICAL CASE REPORTS

Small supernumerary ring chromosome (1) mosaicism associated with a Kabuki-like phenotype

João Rodrigues Alves[1]; Rosário Silveira-Santos[1]; Juliette Dupont[1]; Ana Sousa[1]; Ana Berta Sousa[1]

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

---

Introduction: Small supernumerary marker chromosomes (sSMC) are rare and are associated with a variable phenotype in relation with the origin of the duplicated genetic material. Kabuki Syndrome (KS) is a genetic disorder caused by pathogenic heterozygous variants in *KMT2D* or *KDM6A*. We report a patient with a sSMC derived from chromosome 1 who shares some similarities with KS patients. Our aim is to contribute to the phenotypic characterization of this uncommon chromosomal abnormality.

Case report: A 3-year-old girl with irrelevant family history presented with global developmental delay and dysmorphisms. Gestation and delivery were uneventful, but floppiness and developmental delay were noticed soon after birth. Non-verbal cognition was significantly impaired and aggressive behaviour was present. Dysmorphic features included hypertelorism, long palpebral fissures, eyebrows with sparseness of the lateral third, and short columella, resembling KS gestalt. ArrayCGH identified a mosaic 40.76 Mb pericentromeric duplication at 1p13.3q21.2, together with a mosaic 4.69 Mb duplication at 10q22.2q22.3. Karyotyping subsequently revealed a supernumerary ring marker chromosome.

Discussion: To date only eighty-eight sSMC derived from chromosome 1 have been reported. Although the phenotype is very variable, ranging from normality to severe intellectual disability (ID), some correlations have been drawn: the region 1p12 to 1q12 appears to be non-dosage dependant, and the size of the sSMC seems to correlate with severity. This case reinforces the finding that near-centromere partial trisomy 1 results frequently in dysmorphisms, ID and hypotonia. The resemblance to KS was interestingly reported 20 years ago in a patient presenting an interstitial duplication of the short arm of chromosome 1, with significant overlap to ours. More patients are needed to allow possible candidate genes to be suggested. No clinical significance could be attributed to dup 10q22.2q22.3. In conclusion, we illustrate a distinctive phenotype of a rare chromosome abnormality, while emphasizing the importance of resorting to complementary classical cytogenetic studies.

P64 | CLINICAL CASE REPORTS

Renpenning syndrome: a rare syndrome in two Portuguese patients

Marta Marques[2]; Fabiana Ramos[2]; Sofia Maia[2,3]; Jorge M. Saraiva[2,4]

2 - Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; 3 - University Clinic of Genetics, Faculty of Medicine, University of Coimbra, Portugal; 4 - University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra, Portugal

Introduction: Renpenning syndrome (MIM #309500) is a rare X-linked recessive disorder caused by *PQBP1* mutations. This gene encodes a protein predominantly expressed in the central nervous system during development, playing an important role in neurodevelopment and neuronal functions. The syndrome is characterized by intellectual disability, leanness, microcephaly, short stature (relative to familial target measurements) and dysmorphisms.

Clinical description: We report two boys, who are the first and only children of two non-consanguineous couples and single cases in the families. Both have developmental delay, prenatal microcephaly, leanness, congenital heart defect and non-specific dysmorphisms.

Results and discussion: A next-generation sequencing panel of 6110 genes was performed and the pathogenic *PQBP1* (NM_005710.2) c.459_462del (p.Arg153Serfs*41) variant was identified in both patients in hemizygosity and was proven to be inherited in both. This variant is one of three recurrent variants that occur in an AG hexamer in *PQBP1* exon 4. It has been proposed a gain-of-function mechanism for this 4bp deletion, namely that the mutant protein binds preferentially to non-phosphorylated FMRP through a new C-terminal epitope and promotes its ubiquitin-mediated degradation, causing synaptic dysfunction.

The phenotypes of both patients were in concordance with the literature, even though one of the cases did not have short stature relative to familial target measurements. However, the dysmorphic features were not considered to be recognizable. Furthermore, both boys were single cases in the families, which made the diagnosis of an X-linked intellectual disability more challenging. The availability of new technologies in genetics allowed an accurate genetic counseling of the families, namely the identification of healthy female carriers, who have a 25% risk of having an affected child.

P65 | CLINICAL CASE REPORTS

Psychosocial experiences of young adults at risk for transthyretin familial amyloid polyneuropathy: early versus late-onset Portuguese case report

José D. Pereira[1,2,3,4]; Marina S. Lemos[5,6]; Milena Paneque[1,2,3,4]

1- i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal.; 2- IBMC – Institute for Molecular and Cell Biology, Universidade do Porto, Porto, Portugal.; 3- Centre for Predictive and Preventive Genetics (CGPP), Universidade do Porto, Porto, Portugal.; 4- ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.; 5- FPCEUP – Faculdade de Psicologia e de Ciências da Educação, Universidade do Porto, Porto, Portugal.; 6- CPUP – Centro de Psicologia, Universidade do Porto, Porto, Portugal.

Transthyretin familial amyloid polyneuropathy (FAP) has been characterized by its early-onset (before 40 years) in Portugal and a few studies have discussed the psychosocial impact of the disease in our population. Recently, more late-onset (after 50 years) Portuguese cases have been described. Patients with this particular later onset are frequently probands of their families, pointing out an unexpected family attribute and making more complex its management. Family members may experience greater difficulties in adapting to this new and severe condition and its genetic risk. Research on individual and family psychosocial experience of this particular form of FAP is not yet available. We sought to identify psychosocial effects of life paths related to the disease pattern.

The present case report explored psychosocial experiences according to the familial pattern of disease onset. We describe two clinical cases of Portuguese young adults with genetic risk for early- and late-onset FAP, respectively. After written consent, semi-structured interviews were conducted, recorded and analysed using thematic analysis.

The first case was a 23 years old female who had an extended period and a close experience with the disease in her relatives. The participant was aware of the consequences of FAP for her future life plan, if she was a carrier. The second case was a 23 years old male who did not know much about the disease. After learning about two months ago that his mother has late-onset FAP (without history of symptoms), he had no expectations about the consequences of the disease for his life.

This case report is the commencement of a large research project on this topic. First insights suggest that some specific issues related with the familial pattern of disease onset may have a role in the psychological experience of at-risk subjects that perform presymptomatic testing. Personal experience with FAP seems to influence the psychosocial impact of presymptomatic testing, making the management of the disease more challenging by the families with late-onset FAP. This preliminary report may have some implications for practice in the context of genetic counselling.

Recombinant chromosome derived from two independent translocations of the same maternal homologues: Incidental finding in a fetus

Cláudia Alves[1]; Mafalda Lopes[1]; Isabel Cerveira[2]; Carolina Ferreira[2]; Fernanda Baltar[1]; Rita Monteiro[1]; Cecília Correia[1]; Margarida Reis-Lma[1]

1 - GDPN SYNLAB Portugal; 2 - Unidade de Medicina Fetal, Serviço de Ginecologia e Obstetrícia, CHTV-Hospital de S. Teotónio

Context: Complex chromosome rearrangements (CCR) account for a very small number of cases described in the literature. It is very rare that both homologues of the same chromosome pair are involved each in a different rearrangement. We report a case where a reconstructed derivative chromosome was observed in a fetus of a female carrier of two different translocations involving both chromosomes 4.

Methods and Results: A 22 years-old primigesta was referred for an invasive procedure due to cystic hygroma, fetal hydrops, omphalocele, malformation of the left forearm/hand and of the right foot. CVS was performed on the 13th week of gestation.

QF-PCR revealed trisomy 18 in a male fetus. Karyotype analysis showed trisomy 18 resulting from a CCR involving chromosomes 4, 10 and 18.

Parental cytogenetic studies were carried out. Two reciprocal translocations involving both homologues of chromosome 4 were observed in the mother: one between one chromosome 4 and a 10; and the other between the chromosome 4 homologue and an 18. Her karyotype was described as 46,XX,t(4;10)(q21.1;p13),t(4;18)(q33;q21.1).

This unveiled the presence of a recombinant chromosome 10 in the fetus, resulting from (at least one) recombination event between the derivative 10 of t(4;10) and the derivative 4 of t(4;18).

Fetal autopsy confirmed the ultrasound findings and revealed also microcephaly, alterations of the cerebral cortication and cardiopathy.

Discussion: Depending on the type and size of the chromosomal segments involved in a CCR, it is acknowledged that the chance of viable conceptuses is low when such events are observed. Trisomy 18 was the net imbalance of this particular case. If only QF-PCR was requested, the CCR could have been missed before a new pregnancy, and genetic counselling not offered. Such a set of translocations involving both homologues of a particular chromosome pair and the observation of a newly formed chromosome as a result of a recombination event between two derivatives of different translocations, makes this a unique case.

Cytogenetic Findings Associated With Increased Nuchal Translucency: A 20-years Retrospective Study

Bianca Silva[1]; Sílvia Pires[1]; Cristina Candeias[1]; Elisa Lopes[1]; Manuela Mota Freitas[1]; Maria Do Céu Ribeiro[1]; Paula Oliveira[1]; Natália Oliva-Teles[1]

1 - Unidade de Citogenética, Centro de Genética Médica Doutor Jacinto Magalhães/Centro Hospitalar Universitário do Porto - EPE, Porto, Portugal

Context: Nuchal translucency (NT) is the normal fluid-filled subcutaneous space identified at the posterior triangle of the fetal neck during the late first trimester and early second trimester of pregnancy (11.3-13.6 weeks). When increased over 95th percentile (P95) NT is considered the most sensitive ultrasound marker in the screening of chromosomal abnormalities, being associated with a number of cytogenetic findings such as Trisomy 21,18 and 13, Turner syndrome and triploidy.

Aim: To determine the prevalence of cytogenetic abnormalities in fetuses with NT higher than P95 in ultrasound screening, referred for cytogenetic studies at Centro de Genética Médica Doutor Jacinto Magalhães in a 20-year period, and compare the results with the previously publications.

Methods: A retrospective study of 927 fetal samples with NT above P95 referred for cytogenetic studies was performed between January 1997 and December 2016. The absolute and relative frequencies of the variables under study were determined and a comparison of proportions to assess the relationship between the increase in NT and the incidence of cytogenetic abnormalities were calculated.

Results: Of the 927 fetal samples with increased NT, 11.4% presented chromosomal abnormalities, slightly lower than the reported in the literature. The proportion of abnormal karyotypes in fetuses with NT above P99 (0.048) is significantly higher than in fetuses with NT between P95 and P99 (0.014). Trisomy 21 was the most prevalent anomaly (63.2%), followed by Trisomy 18 (10.4%) and Trisomy 13 (5.7%). The average maternal age of fetuses with chromosomal abnormality (34.7 ± 5.7 years) was significantly higher when compared with those without anomaly (30.9 ± 5.6 years).

Conclusion: Our study corroborates the association between the increase of NT and abnormal karyotypes and consolidates NT measurement as a sensitive marker in the screening of chromosomal alterations. We can also postulate that increased fetal NT thickness combined with maternal age provides an effective screening method.

P68 | CLINICAL CASE REPORTS

Cytogenetic characterization and follow-up of Chronic Myeloid Leukaemia

Inês Lobo[1]; Susana Lisboa[2]; Lurdes Torres[2]; Joana Vieira[2]; Susana Bizarro[2]; Nuno Cerveira[2]; Cecília Correia[2]

1 - Faculty of Sciences, University of Porto; 2 - Department of Genetics, Portuguese Oncology Institute of Porto (IPO-Porto), Porto, Portugal

---

Introduction: Chronic Myeloid Leukaemia (CML) is a clonal disease of the bone marrow responsible for the abnormal growth of granulocytic cells.In the majority of the cases the Philadelphia (Ph) chromosome is detected, as a result of the reciprocal translocation t(9;22)(q34;q11.2), leading to the formation of the *BCR-ABL1* gene fusion. The discovery of this molecular abnormality allowed the development of targeted molecular therapy with selective tyrosine kinase inhibitors (TKI),resulting in excellent cytogenetic and molecular responses.

Material and Methods: Conventional cytogenetic studies were performed in 22 patients, 7 at diagnosis and 15 in follow-up (5 of these were previously submitted to hematopoietic stem cell transplant,HSCT).

Results and discussion: All 7 diagnostic patients showed the t(9;22)(q34;q11.2) and started TKI treatment. Of these, 3 presented additional chromosomal abnormalities: 1 showed loss of chromosome Y, 1 had an additional Ph chromosome and one, 3 months after TKI treatment, showed an additional sub-clone with multiple chromosomic abnormalities that disappeared 2 months later.

Of the 15 follow-up patients, 6 were in complete cytogenetic remission (CCgR) (no Ph+ metaphases). Five female patients had undergone HSCT: 4 exhibited a normal male karyotype, reflecting the presence of donor cells in the transplanted bone marrow and 1, transplanted with a donor of the same sex, showed a normal female karyotype. Of the remaining 4 patients, 1 showed the t(9;22)(q34;q11.2) translocation in 97% of the metaphases 3 months after TKI treatment. Three months later, he had persistence of the disease and showed the presence of two additional cytogenetically independent clones in Ph negative metaphases (trisomy 8 and monosomy 7).Two patients had a partial cytogenetic remission (PCgR) (7% and 15% positive metaphases, respectively).Finally, one patient had a minimal cytogenetic remission (minCgR) (70% positive metaphases) and 2 months later a PCgR (33% positive metaphases).

Conclusion: We studied CML patients at different stages treated with distinct approaches and observed distinct cytogenetic responses associated with different phases of this disease.

P69 | CLINICAL CASE REPORTS

Kaufman Oculocerebrofacial Syndrome: A new variant in UBE3B and literature review

Sara M Ribeiro[1]; Nuno Oliveira[2]; Gabriela Mimoso[3]; Margarida Fonseca[3]; Luís Matos[4]; Joana Mesquita[3]; Margarida Venâncio[1,5]; Jorge M. Saraiva[1,6]; Lina Ramos[1,7]

1- Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal; 2- Pediatric Unit, Centro Hospitalar do Baixo Vouga, EPE, Aveiro, Portugal; 3- Neonatal Intensive Care Unit, Maternidade Bissaya Barreto, CHUC, Coimbra; 4- Obstetric Unit, Maternidade Bissaya Barreto, CHUC, Coimbra; 5- University Clinic of Genetics, Faculty of Medicine, University of Coimbra (FMUC), Portugal; 6- University Clinic of Pediatrics, FMUC, Coimbra, Portugal; 7- Faculty of Health Sciences, University of Beira Interior, Covilhã

Introduction: Kaufman Oculocerebrofacial syndrome (KOS) is a rare autossomal recessive disorder characterized by severe development delay with ocular and craniofacial abnormalities. UBE3B is a calmodulin-regulated E3 ubiquitin ligase and its modulation implicates a role for calcium signaling in mitochondrial protein ubiquitylation, protein turnover, and disease. To date, only 21 cases of *UBE3B* biallelic pathogenic variants are described in literature linked to Kaufman Oculocerebrofacial syndrome.

Case Report: We report a newborn with the diagnosis of KOS based on clinical and molecular findings.

The prenatal ultrasound examination at 24 weeks of gestation revealed a hypoplastic nasal bone, micrognathia and a cardiac septal hypertrophy. He was born at 30 weeks. He was hypotonic and his respiratory effort was absent and was stabilized with non-invasive ventilation. He had a triangular face, micrognathia, blepharophimosis, bilateral postaxial polydactyly and dysplastic ears. He had inspiratory stridor and did laryngoscopy that identified structural defects of the epiglottis. Maxillofacial tomography showed multiple structural malformations that could condition hearing loss; transfontanellar ultrasound revealed a permanent diffuse hyperechogenicity. He evolved into obstructive apnea, restrictive miocardiopathy, failure to thrive and inevitably died.

The genetic investigation revealed a homozygous mutation c.2481dup p.(Lys828*) in *UBE3B* gene that confirmed the diagnosis.

Discussion: The clinical and molecular findings in our case are in accordance to the literature. *UBE3B* pathogenic alleles identified in individuals with typical KOS are missense substitutions in highly conserved amino acid residues of the HECT domain or frameshift and nonsense variants that are predicted to lead to protein truncation. These variants presumably abolish the E3 ligase enzyme activity and alter the mitochondrial physiology.

In conclusion, the new NGS approaches in newborns with congenital anomalies are an effective and fast way to establish the diagnosis that could lead to a better management in pediatric intensive care unit*s.*

P70 | CLINICAL CASE REPORTS

Intrachromossomal triplication of 10p13-p12.2 in a 3-year-old girl

Carolina Almeida[2]; Joel Pinto[2]; Maria Pinho[2]; Maria Moreira[2]; Miguel Leão[1,3]; Sofia Dória[1,2]

1 - Genetics Service, Department of Pathology, Faculty of Medicine, University of Porto, Portugal; 2 - Institute of Health Research and Innovation (I3S), University of Porto, Porto, Portugal; 3 - São João Hospital Center, Porto, Portugal.

Context: A 3-year-old girl with hypotonia, corpus callosum agenesis, intellectual disability and developmental delay was referred for genetic studies.

Methods: Array comparative genomic hybridization (aCGH) analysis, quantitative polymerase chain reaction (qPCR) and karyotype were performed.

Results: Microarray analysis of DNA extracted from the patient's peripheral blood showed an apparent homozygous 7.9 Mb duplication (Mean Log Ratio = 0.942) in the region of 10p13-p12.2, spanning nucleotides 14,813,839-22,759,158 (hg19). To determine the location of the additional material, karyotype was performed and an intrachromossomal triplication was observed. Additionally, q-PCR on parent's peripheral blood showed a normal result.

Conclusion: Intrachromossomal triplications are rare complex chromosomal rearrangements and only few patients have been described in the literature. In the Decipher database we found only one patient with similar size duplication (6,55Mb) although not in triplication. This patient had congenital anomalies and intellectual delay. The likely mechanism to explain triplication is non-allelic homologous recombination (NAHR) mediated by segmental duplications (Dhaibani et al, 2017). These segmental duplications are estimated to comprise approximately 5% of the human genome and could facilitate an unequal recombination between homologous regions. The present triplication was found to be *de novo* and includes potential dosage-sensitive genes, but has not been previously reported.

P71 | CLINICAL CASE REPORTS

When rarity clashes with frequency: genetic analysis of a Portuguese patient with thrombocytopenia-absent radius syndrome

Catarina Monteiro[1,2]; Jorge Oliveira[2,3]; Ana Gonçalves[2,3]; Eugénia Cruz[1]; Catarina Lau[1,3]; Margarida Lima[1,3]; Sara Morais[1,3]; Rosário Santos[2,3]

1 - Serviço de Hematologia Clínica, Centro Hospitalar Universitário do Porto; 2 - Unidade de Genética Molecular, Centro de Genética Médica Dr. Jacinto Magalhães, Centro Hospitalar Universitário do Porto; 3 - Unidade Multidisciplinar de Investigação Biomédica - Instituto de Ciências Biomédicas Abel Salazar (UMIB/ICBAS)

Background Thrombocytopenia-absent radius (TAR) is an ultrarare syndrome (~1/240000 new-borns) mainly presenting with bilateral absence of the radius and thrombocytopenia that potentiates bleeding episodes during infancy. The skeletal anomalies in TAR are variable, ranging from amelia to the absence of radii or other defects in the lower limbs. TAR is of autosomal recessive inheritance, usually with compound heterozygosity for variants in *RBM8A*: often a rare null allele (caused by a 200-kb deletion) or, less frequently, variants originating premature stop codons, combined with a non-coding SNP on the other allele.

Methods A female patient was diagnosed at birth with TAR, based on the physical examination and clinical evaluation. X-ray showed radial aplasia with bilateral cubital hypoplasia. Thrombocytopenia present during the neonatal period improved after 1.5 yrs of age. At 24 yrs of age, she presents mild thrombocytopenia (last platelet count of $66x10^9$/L). Genetic studies resorted to NGS analysis with a haematology gene panel and the P297 MLPA kit. Blood RNA was subjected to RT-PCR followed by Sanger sequencing. **Results** No microdeletion was identified by MLPA. The binary alignment map file generated by NGS was manually inspected at the *RBM8A* locus, enabling the identification of two heterozygous variants: c.-21G>A and c.342-2A>G. The former, reported to be pathogenic, has a frequency of ~2.8% in population databases. The latter, as yet unreported, alters a canonical splice-site sequence; *RBM8A* transcript analysis confirmed skipping of exon 5.

Conclusions Aside from the novel splicing variant, this case is didactic considering the rarity of TAR syndrome and its unique genetic characteristics. As NGS usually generates a large number of variants, especially in more comprehensive approaches, variant filtering is used to reduce the analytical burden. Typically, variants with population frequencies above 1% are excluded, which in the present case would result in failure to detect the pathogenic c.-21G>A SNP in *RBM8A*.

Funding Work funded by *Fórum Hematológico do Norte*. CM holds scholarship Ref. BI.03/2018/HEMATCLINICA/CHP from *Centro Hospitalar Universitário do Porto*

Microdeletions/microduplications in 22q11.2: 2015-2018 study using SALSA® MLPA® Probemix P250 DiGeorge

Cristina Candeias[1,2*]; Manuela Mota Freitas[1,2*]; Natália Oliva Teles[1,2]

1 - Unidade de Citogenética, Centro Genética Médica Doutor Jacinto Magalhães/Centro Hospitalar Universitário do Porto - EPE, Porto, Portugal; 2 - Universidade do Porto, Unidade Multidisciplinar de Investigação Biomédica (UMIB/ICBAS,UP), Porto, Portugal.
*equal contributors

Context: Microdeletions/microduplications in chromosome 22q11.2 region cause a variety of disorders, including DiGeorge syndrome (DGS; OMIM #188400), Velocardiofacial syndrome (VCFS; OMIM #192430), 22q11.2 Distal deletion syndrome (OMIM **#** 611867) and chromosome 22q11.2 Microduplication syndrome. DGS and VCFS syndromes have a large clinical overlap and are both caused by deletions of a specific 1-3 Mb region on chromosome 22q11.2. The overall birth prevalence of 22q11.2 deletions appears to be approximately 1 in 4,000, with 75% of these patients having cardiac abnormalities.

Material and methods: Between January 2015 and September 2018, 145 individuals were studied in the Cytogenetic Unit of CGMJM/CHUP,EPE using SALSA® MLPA® Probemix P250 (MRC-Holland™).

Results: From the 145 individuals studied, 32 (22%) showed alterations: 19 microdeletions and 13 microduplications.

Conclusions: Although both deletions and duplications are expected to occur in equal proportions as reciprocal events caused by LCR-mediated rearrangements, very few 22q11.2 microduplications have been identified. This may probably be due to the less severe phenotype associated with the microduplication and therefore fewer patients are referred for cytogenetics studies.

The great phenotypic variability associated with these syndromes makes it difficult to establish either the diagnosis or the prognosis of these patients. Thus it is very important to work in a multidisciplinary team with geneticists, cardiologists, immunologists, psychiatrics and psychologists for a better follow up and genetic counselling of these patients and families.

P73 | CLINICAL CASE REPORTS

Importance of cytogenetic analysis in B-cell chronic lymphocytic leukemia: a case report

Marta Souto[1]; Bruno Mesquita[2]; Catarina Valença[3]; Pedro Botelho[1]; Margarida Costa[2]; Ana Carvalho[2]; Marisol Guerra[2]; Manuel Cunha[2]; Osvaldo Moutinho[4]; Rosário Pinto Leite[1]

1 - Laboratório de Genética, Centro Hospitalar Trás-os-Montes e Alto Douro (CHTMAD); 2 - Serviço de Hematologia, CHTMAD; 3 - Universidade de Trás-Os-Montes e Alto Douro; 4 - Serviço de Ginecologia / Obstetrícia, CHTMAD

Introduction: Chronic lymphocytic leukemia (CLL) is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent lymphocytes. In 95% of the cases, the cells of origin are clonal B cells (B-CLL) and this is the most prevalent form of leukemia in adult individuals in the Western World. The cytogenetics anomalies associated are trisomy of chromosome 12 and deletions on chromosomes 6q21, 13q14, 11q22-q23 and 17p13. However, detection of these abnormalities through classical cytogenetics is difficult and that´s why Fluorescent *in situ* Hybridization (FISH) is usually performed to study these patients.

Material and methods: The authors present a case of an 80-year old women diagnosed with B-CLL. She presented in 2017 with an history of axillar and retro auricular adenopathies with a cyclic growth of 5 months, stable lymphocytosis of 9080U/L without anemia or thrombocytopenia, elevated LDH 368U/L and multiple large adenopathies at physical exam. After one month she presented progressive enlargement of submandibular adenopathy, night sweets and increase of LDH. The cervical FNA cytology revealed a prolymphocytic transformation of CLL. She started chemotherapy and at the moment she has fulfilled 5 cycles with good response and normalization of LDH.

Classical and molecular cytogenetic were performed before treatment. Cultures with B-mitogens and FISH panel for CLL were applied.

Results: FISH analysis were normal. Classical cytogenetic analysis revealed a complex karyotype with two abnormal cell lines: one with a translocation between the long arms of chromosomes 6 and 10 and a deletion of the short arm of chromosome 9 (10 metaphases) and the other cell line had also a derivative of chromosome 1 with extra material on the long arm and a translocation involving chromosomes 7, 12 and 22 (2 metaphases).

Conclusion: FISH did not detect any of the cytogenetic alterations present and these could justify the acceleration of prolymphocytic leukemia. The detection of a complex karyotype is important to the prognostic and have therapeutic implications for the patient.

P74 | CLINICAL CASE REPORTS

Early onset PTEN germline mutation detected by whole-exome-sequencing

Ana Pereira[1]; Teresa Lourenço[1]; Maria José Sousa[1]; Fernando Ferreira[2]; Joana Monteiro[2]; José Germano Sousa[1]; Germano De Sousa[1]

1 - Centro de Medicina Laboratorial Germano de Sousa; 2 - Unidade Local de Saúde do Baixo Alentejo –Hospital José Joaquim Fernandes

---

INTRODUTION: *PTEN* hamartoma tumor syndrome (PHTS) is used to describe all individuals, irrespective of the clinical diagnosis or syndrome, with an identified heterozygous germline *PTEN* mutation due to it phenotypic heterogeneity. The PHTS includes Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), *PTEN*-related Proteus syndrome (PS), and Proteus-like syndrome. Pediatric criteria for consideration of PTEN hamartoma tumor syndrome includes macrocephaly as a required criteria

CLINICAL REPORT: We present a 2 years old girl with macrocephaly, *aplasia cutis*, large forehead, laryngomalacia, sleep apnea and left external jejunal vein aneurysm and normal psychomotor development. Whole-exome-sequencing performed after normal karyotype and arrayCGH analysis revealed a missense variant in *PTEN*: c.517C>T (p.Arg173Cys) classified as pathogenic by *Human Genome Database Mutation (HGMD) and ClinVar.*

DISCUSSION: The most serious consequences of PHTS relate to the increased risk of cancers, including thyroid, breast, endometrial, and, to a lesser extent, renal cancers. In this regard, the most important aspect of management of any individual with a *PTEN* mutation is increased cancer surveillance to detect any tumors at the earliest, most treatable stages. Identifying a pathogenic *PTEN* mutation allows for targeted genetic testing in family members and initiation of cancer screening and risk reduction in relatives who test positive and are therefore at increased risk.

P75 | CLINICAL CASE REPORTS

1q21.1 recurrent microdeletion: report of two cases

Ana Pereira[1]; Ana Sousa[1]; Teresa Lourenço[1]; Maria José Sousa[1]; Joana Monteiro[2]; Fernando Ferreira[2]; José Germano Sousa[1]; Germano De Sousa[1]

1 - Centro de Medicina Laboratorial Germano de Sousa; 2 - Unidade Local de Saúde do Baixo Alentejo –Hospital José Joaquim Fernandes

INTRODUTION: 1q21.1 recurrent microdeletion (OMIM# 612474) is not a clinically recognizable syndrome as some individuals have no clinical phenotype and others have variable findings that include developmental delay and facial dysmorphisms. This region is there for classified as susceptibility *loci* with a penetrance of 23-55% (95% CI).It is inherited in an autosomal dominant manner with some deletions occurring *de novo* (18%-50%). The frequency of 1q21.1 microdeletion is approximately 0.2% of individuals referred to chromosomal microarray analysis.

CLINICAL REPORT: We present clinical and molecular findings in two unrelated patients with overlapping 1q21.1 microdeletions, identified by arrayCGH (Agilent 180K). Case 1 is a *de novo* 2.68 Mb deletion encompassing 16 OMIM genes (arr[GRCh37] 1q21.1q21.2(146514772_149202620)x1dn) detected in a 4-month-old girl with severe facial dysmorphisms, *aplasia cutis,* laryngomalaciaand syndactilia. Case 2 is a 1.3 Mb deletion encompassing 10 OMIM genes (arr[GRCh37] 1q21.1q21.2 (146514772_147824207x1) detected in a 19-month-old girl with psychomotor development delay, facial dysmorphisms, elbow arthrogryposis, and lower limbs hyper laxity. Parental studies still ongoing on the case 2.

DISCUSSION: The diagnosis of this recurrent microdeletion is established by detection of ~1.35-Mb heterozygous deletion at 1q21.1. Both patients shares facial dysmorphisms as the only clinical feature described in this copy number variant. Those two cases highlights the useful to perform chromosomal molecular analysis instead of karyotype as a first-tier clinical diagnostic test for individuals with developmental disabilities, and/or congenital anomalies. Achieving an earlier diagnosis allows routine pediatric care; routine developmental and learning assessments as well as identification of at risk family members.

12q13.3 microduplication: new case report and literature comparison

Catarina S. Rosas[1]; Lina Ramos[1,2]; Jorge M. Saraiva[1,3]; Susana Ferreira[4]; Ana B. Roseiro[5]; Francisco Vale[5]; Joana B. Melo[4]; Isabel M. Carreira[4,6,7]; Sérgio B. Sousa[1,8]

1- Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal; 2- Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal; 3- University Clinic of Paediatrics, Faculty of Medicine, University of Coimbra (FMUC), Coimbra, Portugal; 4- Cytogenetics and Genomics Laboratory, FMUC, Coimbra, Portugal; 5- Department of Orthodontics, Dentistry Area, FMUC, Coimbra, Portugal; 6- CIMAGO-Center of Investigation on Environment Genetics and Oncobiology - FMUC, Coimbra, Portugal; 7- CNC-IBILI-Group of Aging and Brain Diseases: Advanced Diagnosis and Biomarkers, Coimbra, Portugal; 8- University Clinic of Genetics, FMUC, Coimbra, Portugal

Introduction: Interstitial duplication of the long arm of chromosome 12 is a very rare cytogenetic condition. To our knowledge, few cases have been described, only two presenting with homogenous 12q13 duplication [Dallapiccola et al., 2009; Bertoli et al., 2013]. These cases presented with duplications spanning, respectively, around 4,8Mb and 1,2Mb. The first was considered as a phenocopy of Wolf-Hirschhorn syndrome (WHS). The last had a similar presentation, in early infancy, later evolving into a phenotype no longer suggestive of that same syndrome.

Clinical Report: We report a 15–year-old girl, born from non-consanguineous parents, who presented with prenatal short stature. She had an uncomplicated birth and neonatal period. She maintained the short stature postnatally and developed a complex clinical picture characterized by severe global developmental delay, craniofacial dysmorphisms (craniofacial asymmetry, hypertelorism, downslanting palpebral fissures, thin upper lip, broad nose with wide bridge and bulbous tip) and remarkable oral and teething anomalies. Noonan Syndrome was initially suspected and excluded. Array CGH (Agilent 180k) at the age of 13 identified a de novo homogenous 12q13 microduplication, spanning around 577kb (chr12:53,508,848-54,085,982), involving 18 OMIM genes, 3 of them described in the Morbid Map (AAAS, SP7, AMHR2), located within the above-mentioned duplication regions and which was classified as likely pathogenic. A detailed comparison with these previously described cases was performed.

Discussion: Despite in our case WHS had never been suspected, there are clearly overlapping features with the patient reported by Dallapiccola et al. [2009], such as short stature, severe development delay and craniofacial resemblance. The same happened for the patient described by Bertoli et al. [2013], with comparable features as the intellectual disability, although milder, but with the absence in our case of features such as ocular, cardiac or skin anomalies. This can be explained by the smaller size of the duplication identified in our patient, helping to delineate the critical region responsible for the common features.

P77 | CLINICAL CASE REPORTS

Congenital myopathies: going back to the old microscope in the age of futuristic gene panels

Mário Laço[1]; Sara Ribeiro[1]; Fátima Negrão[2]; Olinda Rebelo[3]; Jorge Oliveira[4]; Joaquim Sá[1]; Jorge Saraiva[1]; Lina Ramos[1]

1 - Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; 2 - Maternidade Bissaya Barreto, Centro Hospitalar e Universitário de Coimbra; 3 - Laboratório de Neuropatologia, Centro Hospitalar e Universitário de Coimbra; 4 - Unidade de Genética Molecular, Centro de Fenética Médica Doutor Jacinto de Magalhães, Centro Hospitalar do Porto

---

Congenital myopathies are a group of genetic muscle disorders (1:50,000 live births) characterized by hypotonia and weakness, which start usually at birth. These disorders are genetically and histologically heterogeneous, and until recently, the diagnosis was done only on the basis of major morphological features observed on muscle biopsy. Different genes have been identified in the extensive phenotypic and histological expression of these disorders and next-generation sequencing (NGS) has uncovered new genes and new pathogenic mechanisms. We present a prospective evaluation of a newborn from Uzbek descendant with a congenital myopathy.

Case report:

A male child was born at 32 weeks of gestation from healthy parents with a previous healthy son. Pregnancy was complicated by polyhydramnios. The child was delivered by caesarean section due to pelvic presentation. At birth, the newborn had non-autonomous breathing movements, a generalized hypotonia and practically no spontaneous reflexes. After a detailed review of pregnancy and family history, the mother described the fetal movements as decreased in comparison to her previous gestation, and mentioned several neonatal deaths of newborns sons from sisters and aunts. Prader-Will and Steinert's myotonic dystrophy were excluded. Death occurred on the tenth of life and post-mortem muscle and nerve biopsy suggested a probable myotubular congenital myopathy. A congenital myopathies NGS-panel (23 genes) identified the variant c.1467 + 1G> A (r.spl?) in intron 13 of the *MTM1* gene.

Although this variant has been previouly described to be associated with X-linked myotubular myopathy (Herman et al. 2002), our aim is to remember that a careful diagnostic strategy supported by muscle biopsy is essential to guide the molecular study to a single gene or a select group of genes, which is more cost-effective than large NGS studies and produce less “variants with uncertain significance”. Even our strategy could have been improved, should we have valued the family history of male neonatal deaths and the signs of a myotubular myopathy, clearly indicating an X-linked disease associated to *MTM1* gene, and therefore, a single gene test!

P78 | CLINICAL CASE REPORTS

A rare case of 48,XXXY syndrome with multiple cell lines

José Seixo[1,2]; Mónica Mónica[1,3]; Cristina Candeias[1,3]; Manuela Mota[1,3]; Joana Damásio[4]; Natália Oliva-Teles[1,3]

1 - Unidade de Citogenética, Centro Genética Médica Doutor Jacinto Magalhães/Centro Hospitalar Universitário do Porto - EPE, Porto; 2 - Faculdade de Ciências da Universidade do Porto (FCUP); 3 - Universidade do Porto, Unidade Multidisciplinar de Investigação Biomédica (UMIB/ICBAS,UP); 4 - Serviço de Neurologia, Centro Hospitalar Universitário do Porto - EPE

Sexual chromosomal abnormalities affect at least 1/400 live births and include karyotypes such as 47,XXX; 47,XXY; 47,XYY and 45,X. The addition of a copy of an X or a Y chromosome is a rare occurrence. 48,XXXY Syndrome is a rare sexual aneuploidy, characterized by the presence of two extra X chromosomes in men and has an estimated frequency of 1/50.000 male live births. The phenotype of individuals with this karyotype may include hypogonadotropic hypogonadism, gynecomastia, high stature and facial dysmorphisms such as hypertelorism, epicanthal folds, prominent mandible, protruding lips and clinodactyly of the fifth finger.

The authors present a case of a 48,XXXY syndrome mosaic with five cell lines. A man aged 65 years was referred for chromosomal studies, presenting with clinical features of Klinefelter's syndrome, namely high stature, long hands, broad hips and female characteristics. The karyotype was established as: **mos 48,XXXY[70]/47,XXY[20]/49,XXXXY[3]/51,XXXXXXY[2]/46,XY[5]**

Centromere X FISH was performed and 234 metaphases and interphase nucleous were analyzed. It confirmed the cell line proportion and also allowed to see a cell with 50,XXXXY constitution.

Karyotypes such as 48,XXXY or 49,XXXXY are usually considered variants of the Klinefelter syndrome since they share phenotypic characteristics. However increased risks of congenital malformations and additional medical complications in individuals with these karyotypes make it imperative to distinguish these from patients with 47,XXY.

The phenotypic effects in mosaic patients, often with a 46,XY cell line, are variable and sometimes not very and therefore the majority of these patients remains undiagnosed. Thus, the diagnosis of these patients and their characterization is essential for better differentiation and understanding of this type of anomalies.An early diagnosis may prevent future physiological or psychological complications. In addition, because patients with 48,XXXY syndrome are often infertile, their early diagnosis may play an important role in counseling and in infertility context, increasing the possibility of the generation of offspring by medically assisted reproductive techniques.

P79 | CLINICAL CASE REPORTS

Molecular characterization of a rare case of fetal mosaic 10p tetrasomy in routine prenatal diagnosis surveillance

Joana Trindade[1]; Vânia Ventura[1]; Paula Lindo[1]; Alexandra Cadilhe[2]; Diogo Cunha[2]; Maria João Oliveira[1]; Alina Queirós[1]; Cláudia Alves[1]; Cecília Correia[1]; Margarida Reis-Lima[1]

1 - GDPN SYNLAB Portugal; 2 - Unidade de Medicina Fetal DPN, Serviço de Ginecologia/Obstetrícia, Hospital de Braga

---

Introduction: Tetrasomy 10p is an extremely rare chromosomal disorder. So far there are only two reports in the literature of partial tetrasomy 10p, in prenatal period.

In partial trisomy 10p clinical manifestations may vary greatly in range and severity, depending on the size and content of the duplicated segment. The most frequent findings on ultrasound scan include craniofacial and skeletal anomalies.

Methods: A 35-year-old healthy pregnant woman was referred for amniocentesis at 22 weeks of gestation due to multiple severe fetal anomalies: hypoplastic nasal bone, nuchal edema, lemon-shaped head, abnormally shaped cerebellum, bilateral cysts of plexus choroideus, left pyelocalycial dilation and club feet.

Aneuploidy screening (Multiplex-PCR) and karyotype analysis were performed. Anomalies in karyotype lead to 60K CGH-array analysis.

Results: QF-PCR revealed absence of aneuploidies in a male fetus.

Fetal karyotype showed mosaicism for an isochromosome 10p: 47,XY,+idic(10)(q11.2)[11]/46,XY[27].

CGH-array analysis confirmed a duplication (47 Mb in size) of the segment 10p15.3-q11.22 of chromosome 10, classified as pathogenic.

So far only the maternal blood sample was received for karyotype analysis, which revealed a normal female constitution (46,XX).

Pregnancy was terminated and fetal autopsy is in progress.

Discussion: In the present case, the marker chromosome detected in the fetal karyotype has been characterized as a dicentric isochromosome 10 with breakpoint at 10q11.22.

The array CGH results, together with cytogenetic analysis are consistent with a tetrasomy in mosaic of the whole short arm and partial long arm of chromosome 10, including the centromere.

The results can explain the fetal sonographic findings observed in this case.

We emphasize the importance to use complementary techniques for a more accurate and better characterization of the abnormalities/diagnosis, which allow medical decisions to be held more safely and genetic counselling be offered.

P80 | CLINICAL CASE REPORTS

Stressing the benefit of a multidisciplinarity approach in Pre-natal Diagnosis: a Beckwith-Wiedemann Syndrome case report

Elsa Carvalho[1]; Marta Moreira[1]; Isabel Cerveira[2]; Natália Salgueiro[1]; Ariana Conceição[1]; Margarida Reis-Lima[1]; Carolina Ferreira[2]

1 - GDPN-SYNLAB; 2 - Unidade de Medicina Fetal, CHTV-Hospital de S. Teotónio, Viseu

Introduction: Beckwith-Wiedemann Syndrome (BWS; OMIM#130650) is an overgrowth disorder characterized by macrosomia, macroglossia, omphalocele, organomegaly and developmental abnormalities. Its incidence is estimated to be 1 per 13,700 live births. BWS patients are prone to the development of embryonal tumors (most commonly Wilms' tumor or nephroblastoma).

BWS is a genetic heterogeneous disorder caused by dysregulation of gene expression in the imprinted 11p15 chromosomal region. Various 11p15 region defects have been implicated and epigenetic defects account for about two thirds of cases.

Methods and Results: We present a case in which the only fetal ultrasound finding detected at 21 weeks of gestation of a 37 years old healthy woman, was omphalocele. Echocardiogram was normal. Aneuploidy screennig and Array-CGH analysis were performed and revealed a normal result. The clinical suspicion of BWS led to further molecular investigation.

MS-MLPA analysis using the kit ME030-C3 from MCR-Holland was performed in order to evaluate the methylation status of 11p15 region. The result revealed hypomethylation of the IC2 (imprinting center 2). This result is described in the literature (present in 50% of BWS patients) and is consistent with the clinical suspicion diagnosis of the fetus.

Discussion: Prenatal diagnosis of fetuses with BWS can help obstetricians and pediatricians in the decision-making process for prenatal, perinatal and postnatal care.

Most of the affected cases are diagnosed after birth and it is often difficult to diagnose it prenatally. Currently, ultrasound is a fundamental tool for the prenatal detection of affected cases, chiefly if several signs are present.

In general population, ultrasound prenatal screening to identify clinical findings suggestive of a diagnosis of BWS, may lead to the consideration of chromosome analysis, microarray, and/or molecular genetic testing. Once again, we emphasize that a multidisciplinarity, that includes dialogue between laboratory and obstetricians, clinical geneticists among others, is crucial for an accurate and successful Prenatal Diagnosis, prevention and genetic counselling.

NOTES

DESIGN

Miguel Neves

IMAGEM

OportoDrawings