19ª Reunião Anual da Sociedade Portuguesa de Genética Humana

19ª REUNIÃO ANUAL da SOCIEDADE PORTUGUESA de GENÉTICA HUMANA

SPGH

5 - 7 NOVEMBRO 2015

Ordem dos Médicos

PORTO

2015

19ª REUNIÃO ANUAL

SOCIEDADE PORTUGUESA DE GENÉTICA HUMANA

5 a 7 de Novembro de 2015
Ordem dos Médicos
PORTO

SPGH Sociedade Portuguesa de Genética Humana

Caros Colegas

Desde a última reunião da SPGH no Porto, em 2012, muito mudou na nossa cidade, no nosso país e Europa e na Genética Humana.

A SPGH tem conseguido afirmar-se com estabilidade crescente ao longo destes 19 anos, o que podemos considerar uma proeza de monta, tendo em conta alguns tempos mais conturbados que tivemos que ultrapassar.

Agora, em 2015, o nosso Porto cresceu e está na moda com grande visibilidade a nível internacional, e a Genética Humana afirmou-se transversalmente em todos os níveis na Medicina e Saúde em geral, ao longo da revolução genómica.

A nossa Reunião Anual é um momento especial de convívio, de reencontros e partilha de conhecimento, mas é também uma oportunidade para parcerias e descobertas. Por vezes não fazemos ideia do que já se faz em Portugal ou por Portugueses e com que nível de excelência.

O programa deste ano tenta lançar-nos numa atitude de actualização da visão dos caminhos futuros da Genética Humana em várias áreas, mas também nos mantém com os pés na terra em relação à utilidade das recentes descobertas da investigação básica e clínica que se podem aplicar ao tratamento e melhoria da qualidade de vida de quem é afectado de doenças genéticas. Haverá sempre o habitual destaque para os melhores trabalhos apresentados deinvestigação básica e clínica.

Temos o privilégio de ter connosco o actual Presidente da *European Society of Human Genetics*, Prof. Doutor Feliciano Ramos-Fuentes, que nos falará do treino da Genética Humana na Europa, numa perspectiva de formação de qualidade e exigência, mas também de reconhecimento transversal a nível dos vários países europeus.

Agradecemos aos nossos Colegas das Comissões da SPGH, que contribuíram de forma fulcral para a realização desta reunião e seu programa, e também para a gestão da SPGH ao longo do ano. Agradecemos também aos Oradores e Moderadores que aceitaram o nosso convite para fazerem esta reunião acontecer.

A organização esforçou-se para proporcionar uma boa reunião científica e agradável estadia no Porto, contamos com a vossa participação activa para que esta reunião seja produtiva.

A Comissão Organizadora

Margarida Reis Lima | Susana Fernandes | Cecília Correia

Comissão Organizadora | Organizing Committee

Margarida Reis Lima | Susana Fernandes | Cecília Correia

Comissão Científica | Scientific Committee

Ana Berta Sousa | Carla Oliveira | Filipa Carvalho
Joana Melo | Jorge Saraiva | Lina Ramos
Luísa Romão | Manuel Teixeira | Paula Faustino
Sofia Dória | Susana Fernandes

Apoio ao Secretariado Local | Secretariat

Ana Paula Neto
Beatriz Queiroz
Joana Vieira
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CGC genetics
www.cgcgenetics.com

FDNA
Accessible Genetics

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

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LABCO
Quality Diagnostics

Pfizer

Prata

ALEXION

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genzyme
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INSTITUTE FOR MOLECULAR AND CELL BIOLOGY

ipatimup

mercatura

SRQ SOQUÍMICA

STAB vida
Your easy genetics laboratory

Parceiros Científicos & Institucionais
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A SPGH agradece o apoio Científico/Institucional das seguintes organizações
The PSHG thanks the Scientific/Institutional support of the following organizations

Porto.

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FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

IPOPORTO
INSTITUTO PORTUGUÊS DE ONCOLOGIA DO PORTO FG, EPE

ORDEM DOS MÉDICOS

FUNDAÇÃO LUSO-AMERICANA

FCT
Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA

anbioq
ASSOCIAÇÃO NACIONAL DE BIOQUÍMICOS

ORDEM DOS BIÓLOGOS

ORDEM DOS FARMACÊUTICOS 1835

spdm
Sociedade Portuguesa de Doenças Metabólicas

Sociedade Portuguesa de Neurologia

SOCIEDADE PORTUGUESA DE NEUROPEDIATRIA

SOCIEDADE PORTUGUESA DE ONCOLOGIA

Raríssimas
INVESTIGAÇÃO INOVAÇÃO INTERNACIONALIZAÇÃO

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PROGRAMA CIENTÍFICO

SCIENTIFIC PROGRAMME

PROGRAMA | PROGRAMME

Day 1 Thursday, November 5

Clubs (Parallel Sessions)

14:00 **Registration Opening**

14:30 **Human Variome Project Meeting (UNESCO)**
Chairs: Maria Luís Cardoso, Rosário Santos (CGMJM)

16:30 **Cytogenetic and Molecular Genetics Club**
Chairs: Sofia Dória (FMUP), Rosário Santos (CGMJM)

16:30 **Medical Genetics and Clinical Dysmorphology Club**
Chairs: Ana Fortuna (CGMJM), Gabriela Soares (CGMJM)

Day 2 Friday, November 6

08:30 **Registration Opening**

09:00 **Opening & Welcome Session**
Margarida Reis Lima, Susana Fernandes, Cecília Correia

09:15 **Neuromuscular Disorders**
Chairs: Ana Fortuna, Rosário Santos

Genetic variant databases: their present and future roles in Human Genetics
Jorge Oliveira (Porto, CGMJM/CHP)

Advances in diagnosis and treatment of Duchenne muscular dystrophy
Kevin Flanigan (Columbus, USA, Nationwide Children's Hospital)

10:30 **Personal genomics, horoscopes and forensic genetics**
Chair: Sérgio Castedo

António Amorim (Porto, IPATIMUP)

11:00 Coffee Break / Poster Viewing

11:30 **Pre-clinical studies for the development of pharmacological treatment in hereditary amyloidosis**
Chairs: Hildeberto Correia, Juliette Dupont

Maria João Saraiva (Porto, IBMC)

12:00 **Oral Communications I**
Chairs: Lina Ramos, Sofia Dória

13:00 Lunch / Poster Viewing

14:30 **Prenatal Diagnosis**
Chairs: Filipa Carvalho, Isabel Carreira

aCGH in PND: data, decisions and dilemmas
Paula Rendeiro (Porto, CGC Genetics)

NIPT workflow implementation and technology considerations
Marina Baldi (Roma, IT, GenomaLaboratory)

15:30 **Face2Gene: facial dysmorphology automated analysis in clinical genetics**
Chair: Miguel Rocha

Nicole Fleischer (NYC, USA, FDNA)

16:00 Coffee Break / Poster Viewing

16:30 **From genes to genomes in Medical Genetics: new possibilities and new challenges**
Chairs: Jorge Pinto-Basto, João Silva

Joris Veltman (Nijmegen, NL, RadboudUMC)

17:30 **SPGH Assembly**

20:30 Conference Dinner

Day 3 Saturday, November 7

08:45 **Oral Communications II**
Chairs: Ana Berta Sousa, Luisa Romão

10:00 **News in diagnosis and treatment of Lysosomal Storage Disorders (LSD)**
Chairs: João Paulo Oliveira, Carolino Monteiro

Paula Garcia (Coimbra, CHUC)

10:30 **The future of Human Genetics training in Europe**
Chairs: Margarida Reis Lima, Joana Melo

Feliciano Ramos-Fuentes - President, ESHG (Zaragoza, ES, H.Clínico Universitario)

11:00 Coffee Break / Poster Viewing

11:30 **Oncogenetics**
Chairs: Manuel Teixeira, Carla Oliveira

Cancer Immunotherapy and Tumor Escape
Haaken Norell (Lisboa, IMM)

The changing landscape in BRCA1/2 testing for ovarian cancer predisposition and treatment: is tumor testing first the new standard practice?
Nicoline Hoogerbrugge (Nijmegen, NL, RadboudUMC)

13:00 Lunch / Poster Viewing

14:00 ***De novo* mutations in Intellectual Disability**
Chairs: Patrícia Maciel,Sérgio B. Sousa

Joris Veltman (Nijmegen, NL, RadboudUMC)

14:45 **GenEthics: Bioethics Committee Session**

Bioethical evaluation of the new reproductive techniques for mitochondrial disorders prevention
Chairs: Jorge Sequeiros, Heloísa G.Santos

Impact and clinical spectrum of mitochondrial disorders
Isabel Santana (Coimbra, CHUC)

Mitochondrial disorders prevention - the new reproductive medicine techniques and its dilemmas
Célia Ventura (Lisboa, INSA)

Ethical considerations and recommendations
Heloísa G.Santos (Lisboa, President of SPGH Ethics Committee)

15:45 **SPGH 2015 Award Conference**

16:15 **Basic and Clinical Research Awards Ceremony**

16:30 Closing Session

ORADORES CONVIDADOS

INVITED SPEAKERS

JORGE OLIVEIRA

Current position

"Técnico Superior Saúde, ramo genética" (since 2003)
Clinical Laboratory Geneticist, certified by the European Board of Medical Genetics (2015)
Unidade de Genética Molecular, Centro de Genética Médica Dr. Jacinto Magalhães,
Centro Hospitalar do Porto, E.P.E., Porto, Portugal
E-mail: jorge.oliveira@chporto.min-saude.pt

Main Professional and Research Areas

Genetic basis and mutational spectrum of hereditary myopathies.
I am currently applying massively parallel sequencing technology (gene panels and whole exome sequencing) to identify new genetic causes for these myopathies.

Academic Education

2013 – PhD student in Biomedical Sciences, ICBAS, University of Porto, Portugal
2000 – 2002 MSc in Molecular Genetics, University of Minho, Portugal
1995 – 1999 Lic. Degree in Biology, University of Aveiro, Portugal

Scientific Work

Number of invited lectures and oral presentations (Aug. 2015): 14.
Number of publications in peer-reviewed journals (Aug. 2015): 20 (11 as first author).
Number of posters in scientific meetings (Aug. 2015): 48 (14 as first author).
Scientific awards: 4 (2 as first author).
Developed and maintains four internationally recognized locus-specific Databases.

KEVIN FLANIGAN

Current Position

Principal Investigator at the Nationwide Children's Hospital Center for Gene Therapy and co-Director of the NCH Muscular Dystrophy Association Clinic

Program Director
Center for Gene Therapy
The Research Institute, WA3014
Nationwide Children's Hospital
700 N. Children's Drive
Columbus, Ohio 43205
Kevin.Flanigan@NationwideChildrens.org

Main Research areas and Scientific Work

Dr Flanigan has been a member of the Executive Board of the World Muscle Society since 2001, and is a member of the Executive Committee of TREAT-NMD, the international alliance directed toward establishing the infrastructure to ensure that promising new therapies reach patients as quickly as possible. His laboratory work is directed toward the molecular characterization and therapy of neuromuscular diseases, and the identification of genetic modifiers of disease. He is also conducted multiple clinical trials in Duchenne muscular dystrophy, including trials of gene modifying therapies such as nonsense suppression and exon skipping.

Academic Education and Professional Career

Dr. Flanigan trained in Neurology and Neuromuscular Disease at Johns Hopkins University, and pursued a post-doctoral fellowship in Human Molecular Biology and Genetics at the University of Utah. After 14 years on the faculty in Utah, he moved to Ohio State University as Professor of Pediatrics and Neurology

ANTÓNIO AMORIM

Current Position

Coordinator of the GABBA (Graduate Program in Areas of Basic and Applied Biology), University of Porto.

Member of the Editorial Board of: The Scientific World Journal, Frontiers in Genetics, Open Forensic Sciences Journal

National Contact Point of EUROFORGEN-NoE - European Forensic Genetics Network of Excellence

Leader of the Population Genetics Research Group at IPATIMUP

Coordinator of the MSc Program in Forensic Genetics (FCUP).

Main Professional and Research Areas

Formal and population genetics and evolution. Pure and applied genetics, forensics (human and non-human paternity and kinship expertise) and diagnosis of genetic diseases.

Academic Career and Scientific Work

Degree in Biology by the Faculty of Sciences, University of Porto, in 1974; PhD in Anthropology in 1983. Full Professor since 1993.

Awards: Seeds of Science Special, Ciência Hoje, 2012;Estímulo à Excelência, FCT (Fundação para a Ciência e a Tecnologia), 2005

Publication Record: 374 articles, 4903 citations, h-index: 36 (Thomson Reuters update, 02/22/2015)

MARIA JOÃO SARAIVA

Maria João Mascarenhas Saraiva received a BSc in Biology from the University of Porto,

Portugal, in 1976, and an MSc in Biochemistry from the University of London, in 1978. Between 1980 and 1984, she did a PhD in Biochemistry at the University of Porto, and qualified as Professor of Biochemistry in the University of Porto in 1991.

She worked for different periods as a Visiting Scientist at the College of Physicians and Surgeons at Columbia University, New York.

She is Director of the Molecular Neurobiology Group at IBMC, Porto.

Maria João Saraiva was awarded the Seiva Prize for Services to Science by the City of Porto, in 1996, and the Gulbenkian Prize in Science, in 2009.

She has published over 220 articles in peer reviewed journals, several reviews on the subject of molecular biology of misfolding diseases of the central and peripheral nervous system.

PAULA RENDEIRO

Current Position

Chief Technical Officer (CTO) at CGC Genetics, since 2007
Director Laboratory Cytogenetics, CGC Genetics (since 2004)
Director Laboratory Cytogenetics, CGC Centro de Genética Clínica (since 2004)
Quality and Innovation Manager (since 2004)
Member of the Board of the College of Human Biology and Health of the Portuguese Biologist Association (2013-)

Academic Education

Specialist in Human Genetics by the Portuguese Biologist Association (2008)
(1989-1993): Faculdade de Ciências da Universidade do Porto, FCUP (Sciences College of Porto University). Degree in Biology, with technical-scientific specialization in Ecology and Zoological Resources (4 years).

Main Research Areas and Scientific Work

Projects:
- Mechanism of Resistance to Imatinib in Chronic Myeloid Leukemia.
- Genetic study on association between Acute Myeloyd Leukemia and the presence of a polymorphism on Toll-like receptor 9 promotor region.
- Genetic Susceptibility testing for dental caries on Iberic population.
- Genetic Susceptibility testing for obesity on Iberic population.

Responsible for trainees from Masters Degrees (3)
Responsible for trainees from different bachelor's degrees (17)
Has presented 22 talks on scientific meetings (both National and International);
Has been present in 80 National and International scientific meetings;
Has collaborated in the organization of 10 scientific meetings;
Has produced and co-produced more than 73 scientific communications presented in scientific meetings;
Has produced and co-produced more than 47 papers and published abstracts;
Is member of 6 National and International Scientific Societies.

MARINA BALDI

Current Position

Biologist, Genetic Counselor, Forensic Geneticist
Medical Genetics Counselor - Genoma Group
Responsabile Genetista
Laboratorio Genoma
Via di Castel Giubileo 11
00138 - Roma
Mail: baldi@laboratoriogenoma.it

Biosketch

Graduation in Biology University of Rome
PhD in Medical Genetics University of Rome
PhD Forensic Genetics and Criminology
Post doctoral fellow - Dept Medical Genetics University of Roma
Post doctoral fellow Division of Medical Genetics,Cytogenetic Laboratory. Jefferson Medical College, Philadelphia, USA
Training in “Cytogenetic Laboratory" Royal Hospital for Sick Children, Edinburg, UK

NICOLE FLEISCHER

Biosketch

A Biologist by training, Nicole Fleischer has worked for the past 10 years in Clinical Marketing of cutting edge medical technologies.

At FDNA Nicole coordinates research initiatives and as Director of Product Marketing, is daily in contact with Face2Gene users worldwide to make the Facial Dysmorphology Novel Analysis technology better, for the benefit of the whole genetics expert community.

Mother of four, Nicole finished her Biology and MBA studies at the Hebrew University in Jerusalem.

JORIS VELTMAN

Current Position

Prof. Joris Veltman, PhD has been fascinated by the possibilities of genomics technologies to explain the causes of human disease ever since these technologies became available. For this purpose he has built a multidisciplinary research group with expertise in genome technology, molecular biology, computational science and clinical genetics. His group pioneered the use of genomic microarrays as well as next generation sequencing for Mendelian disease gene identification and for clinical diagnostics.

Curriculum Vitae

In the last ten years his group has been using intellectual disability as a model disease to learn the basic concept of genotype-phenotype correlations. The research of his group focuses on genomic architecture and genomic disease, with a particular interest in the role of rare de novo mutations and structural variations in severe neurodevelopmental and prenatally lethal disease.

His work using whole exome sequencing in patients with sporadic forms of intellectual disability and their parents recently provided strong experimental evidence for a de novo paradigm in this disorder.

Joris Veltman is also actively involved in the implementation next generation sequencing approaches in routine clinical diagnosis. His ultimate goal is to advance medical sciences by integrating our knowledge on the impact of genome variation in routine clinical decision-making. In 2011 he obtained an ERC consolidator grant from the European Union to continue his research on studying de novo mutations and their role in intellectual disability.

In 2013 he was appointed full professor Translational Genomics at the Radboud University Medical Center and as of January 2014 he has a joint appointment in Nijmegen at the department of Human Genetics and in Maastricht at the department of Clinical Genetics.

PAULA GARCIA

Biosketch

- desde 1999: Assistente Hospitalar de Pediatria (desde 2006 Assistente Hospitalar Graduada), no Hospital Pediátrico de Coimbra, na Consulta de Doenças Hereditárias do Metabolismo (DHM), Centro de Desenvolvimento Luís Borges, Centro Hospitalar e Universitário de Coimbra

- desde 2005 Membro do Grupo de Trabalho do Centro Nacional Coordenador do Diagnóstico e Tratamento das Doenças Lisossomais de Sobrecarga e Comissão Coordenadora para o Tratamento das Doenças Lisossomais de Sobrecarga (presidente para o triénio 2013-016)

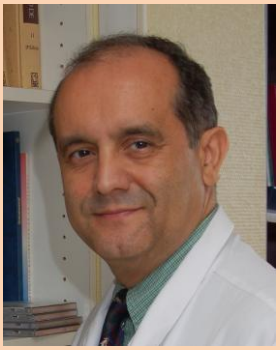
- desde 2011: Consultora em Pediatria

- desde 2012: responsável pela área das DHM da Unidade Coordenadora Funcional Inter Hospitalar Pediátrica da ARS Centro

- 1989: Licenciatura em Medicina, Universidade do Porto

Foi Investigadora Principal em 4 projectos multinacionais na área das DHM. Colaborou em mais de uma dezena de projectos de investigação nacionais e internacionais na área das DHM, incluindo registos internacionais de doentes.
Foi convidada para múltiplas conferências e moderação de mesas em reuniões nacionais e internacionais sob tema das DHM.

38 Publicações de artigos completos em revista indexada na área das DHM, publicados como autora ou co-autora, em revistas nacionais ou internacionais. Apresentou dezenas de trabalhos em reuniões científicas nacionais e internacionais sob a forma de comunicações orais ou posters



FELICIANO RAMOS-FUENTES

Biosketch

1983	M.D. Degree at the University of Extremadura Medical School (Spain)
1988	Ph.D. in Genetics at the University of Zaragoza Medical School, Zaragoza, Spain.
1990 - 92	Fulbright Scholarship. Postdoctoral Fellow. The Children´s Hospital of Philadelphia, Philadelphia, USA.
1993	Board Certified in Clinical Genetics (ABMG, USA)
2005 - 13	President of the Spanish Society of Human Genetics (AEGH).
2006	Chair Professor of Pediatrics, University of Zaragoza Medical School, Zaragoza, Spain.
2008	Member of the Experts Committee of the Spain´s National Strategy for Rare Diseases.
2010	Coordinator of the Spain´s National Reference Center for Cornelia de Lange Syndrome. Scientific advisor of the Spanish Association of families with Cornelia de Lange Syndrome.
2013	Coordinator of the Clinical Group associated to CIBERER at the Hospital Clínico Universitario “Lozano Blesa”.
2013	President of the Genetics Committee of the Hospital Clínico Universitario “Lozano Blesa”
2014	President of the National Comission of Clinical Genetics (Ministry of Health, Spain)
2015 - 16	President of the European Society of Human Genetics (**ESHG**)

- Author of more than 100 scientific papers, many in SCI journals such as *Nature Genetics*, *American Journal of Human Genetics*, *Human Molecular Genetics, Journal of Medical Genetics*, *European Journal of Human Genetics*, *Sci Transl*, *American Journal of Medical Genetics, etc.*

- Author of 48 chapters in Pediatrics and Genetics books.

- Principal Investigator of more than 20 research projects funded by the Spain’s Ministry of Health (ISCIII-FIS) and by the Government of Aragón (regional).

HAAKEN NORELL

Biosketch

2015 - Present:	Invited scientist scholar – Fundação para a Ciência e a Tecologia, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Portugal
2012 - 2014:	Senior postdoctoral researcher – Welcome II Marie Curie fellow, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Portugal
2006 - 2011:	Postdoctoral research fellow, Department of Surgery at the Medical University of South Carolina, USA
2001 - 2006:	Ph.D. student, Department of Oncology and Pathology at Karolinska Institute, Sweden
2006	Ph.D. in Immunobiology including tumor immunology. Department of Oncology and Pathology, Karolinska Institute, Stockholm, Sweden
2001	M.Sc. in Molecular Biotechnology Engineering. Uppsala University, Uppsala, Sweden

Research interests

My research is driven by my long-standing interest in understanding tumor-host interactions, particularly the mechanisms that can be deployed by the immune system to target cancer cells, and how immune pressures are neutralized through tumor immune evasion mechanisms/ selection of tumor cell escape variants.
Since a few years, I lead the research on leukemia and cancer immunotherapy (as a means to target it) within Prof. Silva-Santos's laboratory at Instituto de Medicina Molecular. I currently focus on dissecting the intra-sub-clonal dynamics (through barcode-based lineage tracing technology) of acute myeloid leukemia and its impact on response to treatments (ranging from conventional chemotherapy to cancer immunotherapies) and disease relapse. In this context, I have as Principal Investigator obtained an Exploratory Grant from Fundação para a Ciência e a Tecnologia (FCT) and another two year grant from Associação Portuguesa Contra a Leucemia - SEMAPA, which collectively have allowed me to start elucidating the molecular mechanisms that drive the intra-sub-clonal dynamics and evolution of AML and thereby underlie disease recurrence.

NICOLINE HOOGERBRUGGE

Current Position

Full clinical professor of hereditary cancer and head of the expert centre in hereditary cancer, Radboud University Medical Center, Nijmegen, the Netherlands.

Education/Training

- Medical Studies (Master and M.D. both *cum laude*), Erasmus University Rotterdam, 1983
- PhD, Erasmus University Rotterdam, 1992
- Medical specialization Internal medicine, Erasmus University Rotterdam, 1988

Prof Hoogerbrugge has the ambition of improving detection, diagnosis and treatment of hereditary cancer and preventing cancer in relatives.

Prof Hoogerbrugge now works on improving the effectiveness and efficiency of the diagnostic setting for hereditary ovarian cancer.

She changed the diagnostic setting of hereditary colorectal cancer, by introducing Mismatch repair deficiency analysis (MSI/IHC) analysis for all newly diagnosed CRC patients diagnosed below age 70. This has led to an important increase in the recognition of Lynch syndrome (hereditary colorectal cancer) in the Netherlands.

She was among the very first who made and studied the effects of Apps for successfully improving the recognition of hereditary cancer by doctors and patients themselves in current practice.

She has more than 200 peer-reviewed publications.

Her Research together with others for the past 5 years resulted in:

1. Detection of various new genetic risk factors for colorectal cancer (CRC): *NTHL1, EPCAM, BUB1, BUB3 and FOCAD*.
2. Improved recognition of hereditary cancer (breast, ovarian, childhood and CRC)
3. Knowledge on efficient and effective implementation of guidelines on hereditary cancer.
4. Knowledge on the psychosocial impact of hereditary cancer.
5. Development of vaccination for CRC prevention in Lynch Syndrome

ISABEL SANTANA

Current Position

- Neurologista, Coordenadora da Consulta de Demência e da Unidade de Neuropsicologia do Serviço de Neurologia, Centro Hospitalar e Universitário de Coimbra

- Professora Associada de Neurologia e investigadora do CNC.IBILI, Faculdade de Medicina, Universidade de Coimbra

CÉLIA VENTURA

Formação Académica

Licenciatura em Análises Clínicas e Saúde Pública, 17 valores (ESTeS, 1993)
Frequência da licenciatura em Biologia (Faculdade de Ciências, Univ. de Lisboa)
Mestrado em Bioética, 19 valores, *summa cum laudae* (Universidade Católica Portuguesa)
Doutoramento em Saúde Pública em curso (Escola Nacional de Saúde Pública, Universidade Nova de Lisboa)

Cargos Actuais

- Técnica de Análises Clínicas e Saúde Pública Principal; Investigadora na Unidade de Investigação e Desenvolvimento, Departamento de Genética Humana, INSA
- Auditora interna do INSA, NP EN ISO/IEC 17025 e NP EN ISO 15189 Gestora da Qualidade Substituta do Departamento de Genética Humana do INSA

Actividade Científica e Docência

- Formadora no módulo “systems medicine, genomics and personalized medicine” do programa doutoral BIOSYS da Faculdade de Ciências da Universidade de Lisboa, com o tema “Introduction to Bioethics – Applications in Genetic and Genomic Research”, 11 de Março de 2014.
- Formadora na unidade curricular “Genética Humana” do programa doutoral “Genética Humana e Doenças Infecciosas” do IHMT e da Faculdade de Ciências Médicas, Universidade Nova de Lisboa, com o tema “Biobancos”, 18 de Dezembro de 2014.
- Formadora com a comunicação “Biobancos- Questões Éticas” no CEIC - Comissão de Ética para a Investigação Clínica - 30 de Janeiro de 2105.
- Young Investigator Award com a apresentação “Molecular pathology of factor XI deficiency on the portuguese population” no XVIth Congress of the International Society on Thrombosis and Haemostasis, Florença, 1997.
- Ventura C. (2011). “A bioética em biobancos destinados a investigação genética”. Em Ana Sofia Carvalho e Walter Osswald (Ed.) Ensaios de Bioética 2., Porto: Instituto de Bioética da Universidade Católica Portuguesa.
- Ventura C. (2011). Biobancos e Investigação genética: Orientações Éticas. Lisboa: Instituto Nacional de Saúde Doutor Ricardo Jorge.

Participação em Grupos de Trabalho

Membro da Comissão de Ética da Sociedade Portuguesa de Genética Humana.
Membro do grupo de trabalho para o projecto de Portaria de Gestão de Documentos do INSA.
Membro do grupo de trabalho para a criação de um biobanco central INSA.

HELOÍSA G. SANTOS

Biosketch

- Geneticista Médica e Pediatra
- Directora do Serviço de Genética Médica do Hospital S. Maria,Lisboa (1999 - 2004)
- Doutoramento em Genética em 1991 (Classificação: Muito Bom com Distinção e Louvor)
- Professora Auxiliar Convidada da disciplina de Genética da Faculdade de Medicina de Lisboa
- Prémio Nacional de Genética em 1991
- Consultora Permanente de Genética da Direcção Geral da Saúde. Atualmente é também membro da Comissão de Genética.
- Sócia fundadora e primeira Presidente da SPGH. Presidente da Comissão de Bioética da SPGH. Eleita em 2011 Sócia Honorária desta Sociedade.

- Membro do International Bioethics Committee da UNESCO (IBC) (2002-2006)
- Além de Presidente da Comissão de Bioética da Sociedade Portuguesa de Genética Humana, é igualmente Presidente da Comissão de Bioética da Sociedade Portuguesa de Pediatria (SPP) e da CES do INSA. É membro do Conselho de Ética e Deontologia Médica da Ordem dos Médicos.

- Cento e dezanove trabalhos publicados, 51 em revistas internacionais.
- Cerca de 400 participações activas em reuniões portuguesas e internacionais
- Participou em múltiplas acções de formação Pós-Graduada dirigidas a médicos e outros técnicos de saúde (enfermagem, áreas laboratoriais, etc.)

- Investigadora do Projecto “Estratégias para Implementação de Programas de Prevenção de Doenças Genéticas e Malformativas: um Estudo Conjunto Brasil/Portugal” (JNICT).
- Investigadora Principal do Projecto “Estudo Clínico e Genético das Osteocondrodisplasias. Registo Português” (FLAD).
- Investigadora Principal do Projecto “Características clínicas, citogenéticas e psicológicas do Síndrome de Turner”, que foi realizado no S. Genética do HSM (Ministério da Saúde).
- Investigadora Principal do Projecto “Estudo molecular das condrodisplasias associadas a mutações do gene FGFR3 na população portuguesa” (FLAD).

PALESTRAS

LECTURES

Genetic variant databases: their present and future roles in Human Genetics

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The human genome reference sequence has been completed more than a decade ago and now we are witnessing the transition to the age of individual genomes. This shift is being driven by the exponentially growing sequencing capacities - the so-called massive parallel sequencing. The potential clinical benefits from the application of this technology are evident and thoroughly discussed in the literature.

However, together with these great expectations came huge challenges. As genetic studies become further comprehensive, the amount of variants generated is enormous, and therefore strategies are required to reduce the analytical burden to identify disease-related variant(s). For a significant number of variants we are still unable to ascertain their true nature/impact, and they are consequently catalogued as unclassified variants. Additionally, for rare pathogenic variants it is often difficult to determine the specific, corresponding phenotype. Genetic variant databases (GVDs) may give a contribution to solve these difficulties. This presentation will review the roles of GVDs in clinical genetics, illustrated with studies in hereditary neuromuscular diseases.

The first example of GVDs, are publically accessible population sequence databases such as dbSNP, EVS, 1000GP and EXAC, having data extremely useful for variant filtering steps in whole-exome or -genome analysis, and for pathogenicity assessment of sequence variants. Locus-specific databases (LSDBs) are another class of GVDs, which collect variants for a specific gene together with clinical items. As detailed in this presentation, LSDBs have contributed to improve the mutational spectrum and genotype-phenotype correlations in Duchenne/Becker muscular dystrophy (D/BMD) and in myotubular myopathy. The Leiden DMD database in particular, was extremely useful to delineate an integrated therapeutic approach for D/BMD by antisense oligonucleotide-mediated exon skipping. Finally, disease databases (such as Clinvar, HGMD and OMIM), although presenting limiting aspects especially in terms of mutational coverage and/or representativeness, may contribute to accelerate genetic diagnostics.

Based on these examples, it is shown that GVDs are now indispensable resources in clinical genetics both for research and diagnostic purposes, and are key players in the global effort to establish a reference dataset of human sequence variants.

Advances in the diagnosis and treatment of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) occurs due to mutations in the X-linked DMD gene, which encodes the protein dystrophin. Absence of dystrophin at the sarcolemma results in myofiber injury, leading to degeneration, necrosis, and inflammation, followed by fibrosis and fatty replacement of muscle. In DMD, diagnosis is typically made by age 5; historically, loss of ambulation occurs by age 12, and death by age 20. The allelic Becker muscular dystrophy (BMD) is milder, and clinically heterogeneous, with loss of ambulation occurring after 15 years of age and even into late adulthood in some individuals.

Genetically, DMD mutations that result in DMD typically disrupt the reading frame of DMD, whereas mutations associated with BMD result in an open reading frame, allowing translation of a partially functional protein.

Several therapeutic approaches are currently in clinical trial for DMD, some of which seek to restore expression of either a full-length dystrophin or an internally-truncated "BMD-like" dystrophin. These include exon skipping therapies, and adeno-associated virus (AAV) gene transfer with microdystrophin transgenes. Other approaches include nonsense mutation suppression, using compounds directed toward readthrough of the mutation, and AAV gene transfer of surrogate genes encoding proteins that can compensate for the missing dystrophin.

In all cases, increasing evidence suggests that dystrophin restoration treatment will be more effective in younger than in older boys, highlighting the need for early diagnosis. Technologic advances have made early molecular diagnosis increasingly common, and the demonstration of successful mutation detection from newborn screening blood spots shows that widespread newborn screening is technically feasible.

Personal genomics, horoscopes, and forensic genetics

Antonio Amorim
IPATIMUP, i3S, and Faculty of Sciences, University of Porto

A lot of hype - even catching US Presidency attention - has been paid to personal genomics. This attention drives at least in part from the promise that the knowledge of an individual's genome would serve as a basis for a personalized medicine, including the selection of the most appropriate therapy as well as the evaluation of risks of developing a disease and preemptively counteract it.

These promises face however skepticism when it comes to common, complex diseases. We will try to evaluate if these promises are fulfillable and to investigate the reasons for the current deceptive status and the possible ways to overcome it.

We will show that (at least in part) the background for the (relative) failure stems from the weakness of the current theoretical and methodological approaches which were based upon the assumption that big data would inherently bring along big science and huge practical results.

If so, what is needed, far from an intensification of the current research path is to readdress complex disease genetics in a solid theoretical framework and to invest more on deep phenotyping rather than on deep sequencing.

Pré-clinical studies for the development of pharmacological treatment in hereditary amyloidosis.

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Familial amyloidotic polyneuropathy (FAP) is a neurodegenerative disease affecting specially the peripheral nervous system. The cause of this life threatening pathology is the extracellular deposition of mutant transthyretin as early non-fibrillar deposits and amyloid in sites that do not synthesize TTR.

We have generated a new mouse model expressing the human transthyretin V30M in a heat shock transcription factor 1 null background which presents extensive and earlier non-fibrillar transthyretin deposition in distinct organs including the peripheral and autonomic nervous system; The novel mouse model is of the utmost importance in testing new therapeutic strategies and to explore pathogenic mechanisms associated with FAP and effects on the autonomic nervous system, namely innervation and neuron cell death.

Based on the knowledge gathered from the various “in vitro” and “in vivo” approaches to TTR aggregation and toxicity, different multicenter clinical trials are underway; we might need more than a single approach to effectively treat FAP.

In particular we have been involved with different approaches based on siRNA to silence liver TTR expression and on removal/reduction of TTR deposits by the synergistic effect of doxycycline and TUDCA, which resulted in significative reduction of TTR deposition and associated tissue markers in the TTR Val30Met transgenic mouse model.

The pre-clinical studies on an animal model which were in the basis for these trials will be discussed as well as approachs to counteract inflammatory cascades.

Array in PND: data, decisions and discussion points

Paula Rendeiro
CGCgenetics, Porto

Chromosomal microarray analysis using comparative genomic hybridization (array CGH) has proven to be a reliable and highly efficient diagnostic tool in the diagnosis of intellectual disability, development delay and congenital malformations, and has already replaced conventional cytogenetics in several countries. Despite this, its introduction in prenatal setting is still not consensual, raising several questions in the way the test should be offered, analysed and interpreted; and also the need for specific pre-test counselling.

Published data clearly highlight the benefits of array CGH in prenatal detection rate of clinically significant alterations, with an increase of 3-13% (depending on the data series) over conventional testing. Also the fact that karyotype alone does not provide an answer to the vast majority of the prenatal tests, clearly justifies the need for additional methods with higher sensitivity, as array CGH. Nevertheless, the implementation of CGH testing in routine prenatal care has not been straightforward.

There are now many centres and hospitals offering the test, but the terms and conditions vary largely among them, and basically each one implementing their own protocol. There are indeed reasons for this variability and, eventually, for the lack of consensus, which lies in the different approaches used. (a) In which pregnancies should array CGH be offered? All pregnancies? High risk pregnancies? Abnormal US pregnancies? (b) What to look for? Large rearrangements? Known syndromes? All variants? (c) What to report? Everything? Only high/full penetrance clinically significant variants? Report or not variants of unknown significance? (d) How to handle incidental findings? (e) Perform karyotype simultaneously? Replace karyotype? It has not been easy to reach one single answer for each one of these questions, making it difficult to reach one single protocol of guidelines. Pre-test counselling could play an important role to overcome the questions related with incidental or unexpected findings.

These difficulties have not prevented the continuous growth in the number of CGH tests done in prenatal context, contributing for more and larger data series. These will, undoubtedly, bring more insight about the type and frequency of this genomic events and their clinical value. This knowledge will be helpful for the construction of a consensus for array CGH use in prenatal diagnosis.

NIPT workflow implementation and technology considerations

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Non-invasive prenatal testing (NIPT), based on the analysis of circulating fetal cell-free DNA (cfDNA) in maternal plasma, has revolutionized the field of prenatal care and methods using massively parallel sequencing (MPS) are now being implemented almost worldwide.

Substantial progress has been made from initially testing for aneuploidies of chromosomes 13, 18 and 21, to testing for sex chromosome aneuploidies, additional autosomal aneuploidies as well as partial deletions and duplications genome-wide.

Although NIPT is associated with significantly reduced risks for the fetus in comparison to existing invasive prenatal diagnostic methods, it presents several implementation challenges.

Here, we review key issues potentially influencing NIPT and illustrate them using both data from literature and in-house data.

We will also discuss on the importance of determining the limit of detection (LOD) of NIPT methods, in order to define the actual lower fetal fraction required to detect common fetal autosomal trisomies, as well as on the advantages to using NIPT approaches with the capability of reliably testing samples with low FF.

Recognition of Syndrome Phenotypes with Facial Dysmorphology Novel Analysis

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Facial analysis systems are becoming more available to healthcare providers to aid in the recognition of dysmorphic phenotypes associated with a multitude of genetic syndromes. These technologies automatically detect facial points and extract various measurements from facial images to recognize dysmorphic features and evaluate similarities to known facial patterns (gestalts).

Face2Gene is a neurogenetic search and reference tool powered by Facial Dysmorphology Novel Analysis technology. This technology leverages advanced algorithms and the cumulative experience of hundreds of genetics professionals and facilitates detection of facial dysmorphic features and recognizable patterns of human malformations to present comprehensive and up-to-date phenotype / genotype databases in real-time.

As more geneticist's use this technology, the algorithms learns and becomes better for the benefit of the entire genetics expert community.

The results of several studies conducted on specific syndromes show that the technology's detection rate is comparable with dysmorphology experts, further suggesting that a clinical application utilizing such technology may be a useful tool for healthcare professionals in clinical settings.

From genes to genomes in medical genetics: new possibilities and new challenges

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Rapid developments in genomics technologies now allow us to sequence all genes (the exome) or even the entire genome of thousands of patients in research and diagnostics. This is completely changing the way genetics studies are done, taking away the major bottleneck of genomic variation detection.

In this presentation I will discuss the technological changes that have occurred in genomics and their impact on medical genetics and its changing role in medicine. Also, I will discuss how to interpret the enormous amount of variation present in individual genomes in the context of a clinically heterogeneous phenotype.

Solving this will require a concerted clinical, biological and bioinformatics approach, resulting amongst others in international agreement on phenotype ontologies, sharing of clinical and genomic data, optimization of variant interpretation tools and the validation of these using relevant biological models.

A major focus of my presentation will be on the use of different next generation sequencing technology and bioinformatics tools and IT infrastructure for large-scale variant detection and data interpretation.

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News in diagnosis and treatment of Lysosomal Storage Disorders

Paula Garcia
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National Coordinator Committee of LSD Treatment

Lysosomal storage disorders (LSDs) are a large and heterogeneous group of rare inherited metabolic diseases. The spectrum of clinical manifestations is extremely variable, ranging from prenatal dead to attenuated late adulthood presentations. There are more than 50 LSDs describe, with a combined incidence of 1/7.700.

News in diagnosis is virtually in the area of genetic testing, like next-generation sequencing techniques (specific gene panels), although, metabolomics is back in importance, in order to counterpart the genetic not described findings.

For the majority of LSDs the current standard of care is mainly supportive and symptomatic. To a few, enzyme replacement therapy (ERT) became the gold standard and is nowadays available to patients with Gaucher type I disease, Fabry disease, MPS types I, II, IV and VI, Lysosomal Acid Lipase deficiency and Pompe disease. The efficacy of this therapeutic approach is limited. This is caused not only by the irreversibility of already established lesions and the inability to treat CNS pathology. Furthermore, the development of antibodies against the exogenously supplied enzymes may have a negative impact on efficacy.

Bone marrow transplant (BMT) and small molecules are also available for some selected cases of MPS, metachromatic leukodystrophy (MLD) and Gaucher and Niemann-Pick type C (NPC) respectively.

Regulatory and commercial incentives provided by political authorities, protecting the orphan and rare diseases, has became a huge encouragement for the interest in developing specific drugs. Certain unmet medical goals are recognized in most of the LSDs and the development of new therapies based on the increasing knowledge of the pathophysiological mechanisms involved, have make conceivable the emergence of novel therapeutic concepts and therapies, including localized (articular, intrathecal / intraventricular) and combined therapies.

New advances on the control of these disorders trespass areas like gene therapy, small molecules (chaperons, substrate deprivation, substrate optimization and codon stop), second-generation enzyme replacement and cellular transplant.

Also, reflecting the complex pathology of the LSDs and the various biological processes involved, a number of investigational strategies, some including branded compounds, are being researched for use as disease modifiers. These approaches include calcium modulation, enhancing exocytosis, regulation of proteostasis, modulation of autophagy and the use of non-steroidal anti- inflammatory drugs. Several clinical trials with these diverse hypotheses are now ongoing on cells, animal models and humans. In the future tailored therapies will be delivered to patients considering their specific clinical and genetic characteristics.

(continued)

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The future of Human Genetics training in Europe

Feliciano Ramos-Fuentes
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Cancer Immunotherapy and Tumor Escape

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Adoptive transfer of T cells that are genetically engineered to express chimeric antigen receptors (CARs) constitutes a very promising immunotherapy against some types of leukemia. This notwithstanding, such mono-specific targeting of a self-antigen (e.g. CD19) often lead to toxicity, due to elimination of also healthy leukocytes, and/or emergence of tumor escape variants that drive relapse. We have thus developed an alternative cellular immunotherapy product composed of γδ T cells that selectively recognizes malignant (but not normal) leukocytes through their Vδ1 TCRs as well as a panel of natural killer cell receptors, including the natural cytotoxicity receptors (NCRs) NKp30 and NKp44. The NCR expression is uniquely induced on Vδ1 TCR expressing T cells *ex vivo,* by a cocktail of cytokines and TCR agonists, endowing these "Delta One T (DOT-) cells®" with enhanced cytotoxicity against leukemia cells *in vitro* and *in vivo*. Stimulated DOT-cells® further produce TNF-α and IFN-γ, but no IL-17 or IL-10. In xenograft models of human chronic lymphocytic leukemia (MEC-1 cells), these effectors significantly inhibited the growth of pre-established primary tumors (in Balb/c $Rag^{-/-}$ $\gamma_c^{-/-}$ mice) and infiltrated and persisted in tumors and multiple tissues, thus preventing tumor dissemination to e.g. the bone marrow and the liver (in NOD-SCID $\gamma_c^{-/-}$ mice). No evidence of treatment-associated toxicity was found in biochemical or histological analyses. These data provide the proof-of-concept for both the safety and efficacy of DOT-cells®, which now swiftly move towards clinical trials.

To dissect why certain cancer cells within primary human leukemia tumors escape and gain resistance to therapies, we have also developed a novel experimental system to quantitatively measure the dynamics of all the single-tumor-cell-lineages that establish in mice xenografted with acute myeloid leukemia (AML). AML remains the deadliest hematological malignancy, in spite of frequent complete remissions after initial treatment. Treatment failure is associated with selection/expansion of specific pre-existing genetic cancer clones, which further gain additional mutations to fuel intra-tumor heterogeneity and relapses. What governs (sub)clonal interference and evolution, as well as the functional impact of these processes, however remains unknown. We use cellular barcoding, where each engrafting cell carry a unique traceable non-coding DNA sequence, to track the *in vivo* fate of the descendants of all leukemia initiating cells over time and space. We will thus dissect the (sub)clonal tumor population dynamics that underpin the impact of each alternative therapy and the following relapses.

The changing landscape in BRCA1/2 testing for ovarian cancer predisposition and treatment: is tumor testing first the new standard of practice?

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Recently, PARP-inhibitors were approved for treatment of *BRCA1* and *BRCA2* mutation-positive epithelial ovarian cancer (OC). Approximately 15% of patients with epithelial ovarian cancer have an inactivating germline *BRCA*-mutation. Additionally, according to the literature, 4-8% of ovarian cancer patients have a tumor specific somatic mutation in *BRCA1* or *BRCA2*, also making them eligible for treatment with PARP-inhibitors.

Due to the high prevalence of germline mutations in patients with epithelial ovarian cancer germline, *BRCA* mutation detection could be recommended to all these OC patients.

For reasons of efficiency tumor-DNA *BRCA*-test in all patients with newly diagnosed OC may be useful as a pre-screen for germline-DNA *BRCA*-testing.

Most patients with OC (80%) are expected to have a negative tumor-DNA *BRCA*-test, requiring no germline testing. The remaining 20% will have a germline or tumor-specific *BRCA*-mutation in tumor-DNA and may benefit from PARP-inhibitors. Only those patients should be referred to clinical genetics for germline *BRCA*-testing. 75% of these are expected to carry a germline *BRCA*-mutation.

This approach may lead to improved detection of patients who may benefit from specific therapy (PARP-inhibitors) and enhanced detection of germline *BRCA*-mutation carriers.

The detection of germline BRCA-mutations may open the way for ovarian cancer and breast cancer prevention for both OC patient and their relatives.

De novo mutations in intellectual disability

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Severe intellectual disability (ID) occurs in 0.5% of newborns and is thought to be largely genetic in origin. The extensive genetic heterogeneity of this disorder requires a genome-wide detection of all types of genetic variation. Microarray studies and, more recently, exome sequencing have demonstrated the importance of *de novo* copy number variations (CNVs) and single-nucleotide variations (SNVs) in ID, but the majority of cases remain undiagnosed.

Recently, we applied whole-genome sequencing to 50 patients with severe ID and their unaffected parents. All patients included had not received a molecular diagnosis after extensive genetic prescreening, including microarray-based CNV studies and exome sequencing. Notwithstanding this prescreening, 84 *de novo* SNVs affecting the coding region were identified, which showed a statistically significant enrichment of loss-of-function mutations as well as an enrichment for genes previously implicated in ID-related disorders. In addition, we identified eight de novo CNVs, including single-exon and intra-exonic deletions, as well as interchromosomal duplications. These CNVs affected known ID genes more frequently than expected.

On the basis of diagnostic interpretation of all *de novo* variants, a conclusive genetic diagnosis was reached in 20 patients. Together with one compound heterozygous CNV causing disease in a recessive mode, this results in a diagnostic yield of 42% in this extensively studied cohort, and 62% as a cumulative estimate in an unselected cohort.

These results suggest that *de novo* SNVs and CNVs affecting the coding region are a major cause of severe ID. I will also discuss more recent studies investigating the role of somatic *de novo* mutations as well as an investigation of risk factors increasing the *de novo* mutation frequency in offspring.

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Mitochondrial diseases - Clinical Spectrum
(Principais características clínicas das Doenças Mitocondriais)

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Mitchondrial diseases (MD)are a clinically heterogeneous group of disorders that arise as a result of dysfunction of the mitochondrial respiratory chain which is crucial for cell surviving and function. In some cases only one a single organ is affected, but most commonly it involves multiple organ systems, like the eye, brain, muscle, kidney, heart, Diabetes, etc. Considering neurological manifestations, common CNS findings include seizures, migraine, stroke-like episodes, chorea, ataxia, spasticity, visual and hearing deficits, but also more global deficits like fluctuating encephalopathy and dementia. Myopathic features are also very common with a great spectrum of severity from exercise intolerance, to a localized external ophtalmoplegia or severe proximal myopathy. This major variability of symptoms may cluster in syndromic categories (like MERRF – Myoclonic Epilepsy with Ragged-Red Fibers), but overlapping phenotypes are being increasingly described and secondary mitochondrial dysfunction is also recognized as an intermediary mechanism of cell-death in neurodegenerative diseases, like Alzheimer's disease, Parkinsonian syndromes and Fronto-temporal Dementia.

Besides the referred clinical heterogeneity, MD brings about major challenges in terms of diagnosis and counseling. Though the diagnosis may be straightforward by a single molecular genetic testing (with the identification of a mtDNA or nuclear gene mutation), many other cases require blood and CSF analysis, brain imaging, muscle biopsy and also multi-gene panel testing or even genomic approaches. Genetic counseling is also complex because MD may be caused by nuclear gene mutations (with both recessive and dominant inheritance) or by mtDNA defects, (classically transmitted by maternal inheritance), but also occurring the novo (mtDNA deletions) and at the same time prenatal genetic testing may be further complicated by mtDNA heteroplasmy and variable penetrance. Management of MD is actually largely supportive and with modest efficacy highlighting prevention strategies like preimplantation genetic diagnosis or nuclear transfer approaches.

Mitochondrial disorders prevention - the new reproductive medicine techniques and its dilemmas

Célia Ventura, Heloísa Santos
SPGH Bioethics Committee

Mitochondria are small endosymbiotic organelles that exist in almost all eukaryotic cells. Originally thought to simply produce energy, they are now known to play an important role in many cellular functions including pyrimidine biosynthesis, ion homeostasis, β-oxidation and cell signalling. Each mitochondrion contains several copies of its own 16,560 kb circular genome (mtDNA) that encodes for 37 genes: 13 components of the oxidative phosphorylation pathway, 2 rRNAs and 22 unique tRNAs. Some mitochondrial protein subunits are coded by nuclear DNA, therefore requiring coordination between the nucleus and mitochondrial DNA. In patients with mitochondrial disease, either all mtDNA copies are mutated (homoplasmy) or there is a mixture of wild-type and mutated mtDNA (heteroplasmy). The clinical phenotype depends on the ratio of mutated to wild-type mtDNA in affected cells, with a threshold effect that varies according to the specific mutation and the affected tissue, but usually ranges between 60-90%. Two possible therapeutic approaches for mitochondrial diseases have been recently described based on the spontaneous segregation of heteroplasmic mtDNA or somatic cell nuclear transfer from patient's proliferating pluripotent cells.

Mitochondria are exclusively inherited through the maternal lineage, with paternal mitochondria targeted for destruction primarily by ubiquitin-dependent proteolysis. The maternal transmission of heteroplasmic mtDNA is complicated by replicative segregation and mitochondrial genetic bottleneck, resulting in marked variation in the levels of mutated mtDNA among the offspring of heteroplasmic mothers. Genetic counselling combined with prenatal or preimplantation genetic diagnosis is offered to carriers of pathogenic mtDNA mutations, but these techniques will only be of value to women who have low levels of heteroplasmy, and decision-making can be difficult due to predictive uncertainties. For women with homoplasmic or high levels of heteroplasmic mtDNA mutations, currently the only option to ensure an unaffected child is whole oocyte donation that has the limitation of not maintaining the genetic link to the mother. These difficulties have led to a search for alternative approaches. The first proposal was cytoplasmic transfer used to enable infertile women to carry a pregnancy. In cytoplasmic transfer, cytoplasm is extracted from a healthy donated oocyte and injected into the oocyte of the recipient-intending mother.

The resulting oocyte will contain some mtDNA from the donor added to the original mother's mitochondria and nuclear DNA. This technique has a limited efficiency to prevent mitochondrial disease, and two emerging cell reconstruction techniques based on in vitro fertilization, spindle transfer and pronuclear transfer, have been applied and are nowadays legal in the UK. Spindle transfer involves transfer of the metaphase II spindle from the unfertilized oocyte of an affected woman to an enucleated donor oocyte. In pronuclear transfer, the nuclear genome from the pronuclear stage zygote of an affected woman is transferred to an enucleated donor zygote. Mitochondrial germline gene replacement therapy raises a number of ethical issues, mainly due to safety and efficacy concerns and to germline modification, transmitted to subsequent generations with effects that may not manifest for many years. Other ethical considerations are the triple parenthood of the child and the slippery slope to rectify problems caused by genes in the nucleus, as mutations in either genome can be the cause of mitochondrial disorders. All these issues will be discussed.

COMUNICAÇÕES ORAIS I

ORAL COMMUNICATIONS I

CO 1 | Molecular Genetics

THE HUMAN TRANSCRIPTOME ACROSS TISSUES AND INDIVIDUALS

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Introduction Gene expression is the key determinant of cellular phenotype, and genome-wide expression analysis has been a mainstay of genomics and biomedical research, providing insights into the molecular events underlying human biology and disease. While expression datasets from tissues/primary cell and individuals has accumulated over recent years, only limited expression datasets have allowed analysis across tissues and individuals simultaneously. The Genotype Tissue Expression Project (GTEx) is developing such a resource, collecting multiple 'non-diseased' tissues sampled from recently deceased human donors. We analyzed the GTEx pilot data freeze which comprised RNA-seq from 1641 samples from 175 individuals representing 43 body sites: 29 solid organ tissues, 11 brain sub-regions, whole blood, and two cell lines, EBV-transformed lymphocytes (LCL), and cultured Fibroblasts from skin.

Results Tissues exhibit characteristic transcriptional signatures that show stability in post-mortem samples. These signatures are dominated by a relatively small number of genes, though few are exclusive to a particular tissue, and vary more across tissues than individuals. Genes exhibiting high inter-individual expression variation include disease candidates associated with sex, ethnicity and age. Primary transcription is the major driver of cellular specificity, with splicing playing a secondary role; except for the brain, which exhibits a characteristic splicing program. Variation in splicing, in contrast, despite its stochasticity, may play a comparatively greater role in defining individual phenotypes.

Discussion Overall, these results underscore the extraordinary value of the GTEx project as a resource to understand tissue-specific transcript regulation as well as transcriptional variation between individuals. This value will be enhanced as the project scales up to an anticipated goal of ~20K RNA samples in 900 individuals.

CO 2 | Molecular Genetics

EXONIZATION OF AN INTRONIC INTERSPERSED NUCLEAR ELEMENT 1 (LINE1; L1) IN THE *DMD* GENE: AN UNPRECEDENTED CAUSE OF BECKER MUSCULAR DYSTROPHY

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Introduction A broad spectrum of mutations in the dystrophin gene (*DMD*), from large deletions/duplications to point mutations, may cause Duchenne/Becker Muscular Dystrophy (D/BMD). This heterogeneity calls for a variety of molecular techniques in the diagnostic workflow. Even considering novel technical approaches such as NGS, some deep intronic mutations may fail to be detected.

Methods We report the case of a patient with disease onset at age 13, high CK levels and reduced dystrophin in muscle biopsy, having an apparent X-linked family history of neuromuscular disease. Routine techniques (multiplex PCR, MLPA and gDNA sequencing) failed to detect any mutations. Muscle *DMD* transcript analysis was subsequently conducted by RT-PCR. Bioinformatic analysis, targeted PCR and Southern blot experiments were used to characterize the mutational event.

Results cDNA analysis revealed an out-of-frame 103 bp insertion between exons 51 and 52, with no homology to the *DMD* genomic sequence; instead, it was found to belong to a long interspersed nuclear element (LINE1 or L1) of ~6 kb, inserted in the middle of intron 51. Partial exonization of this L1 had occurred due to the recognition of cryptic splice sites in intron 51 and in L1 itself. Besides the insertion junctions, characteristic L1 hallmarks were present: 5´UTR, poly A tail, target site duplication flanking the insertion and insertion at a near-consensus L1 endonuclease site. Haplotype and segregation analysis in several family members suggests a de novo mutational event.

Discussion L1 are mobile elements contributing to genome dynamics, but can also act as mutagens giving rise to genetic diseases. Although L1 insertions have been previously described in the *DMD* gene (disrupting exons or promoters) this is the first report of a pathogenic deep intronic L1 insertion. Its detection was only possible due the application of mRNA studies, thus underscoring the importance of these studies in undiagnosed B/DMD cases.

CO 3 | Molecular Genetics

LGMD - GENOTYPIC SPECTRUM AMONG PORTUGUESE PATIENTS OF ROMA GYPSY ETHNICITY

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Introduction The limb girdle muscular dystrophies (LGMD) are a heterogeneous group of progressive disorders affecting predominantly the pelvic and shoulder girdle musculature. The clinical course ranges from severe forms with onset in the first decade and rapid progression, to milder adulthood forms.
Over 29 *loci* have been identified; 8 autosomal dominant (LGMD1A-1H) and 21 autosomal recessive subtypes (LGMD2A-2U). Their prevalence and relative distribution vary widely between populations.
Like other isolated populations, Gypsies are known to have a high rate of consanguinity, leading to the occurrence of founder effects, often with "private" mutations. In particular, the private mutation Cys283Tyr in the gamma-sarcoglycan gene (*SGCG*; LGMD2C) has been reported in these communities by several groups of researchers.

Methods We present the results of a large cohort of 223 individuals of Gypsy ethnicity, pertaining to 78 families, comprised by 67 affected individuals and 156 relatives (34 referred because of either a family history of a muscular disorder or of a consanguineous marriage).
First tier testing was for the common *SGCG* founder mutation (Cys283Tyr). Upon request, negative cases were screened for other LGMD genes and the dystrophin gene (*DMD*).
Newly-detected mutations in the *CAPN3* and *SGCB* genes were subsequently screened for in 100 individuals (undiagnosed patients and relatives).

Results Seventy families (90%) shared the Cys283Tyr mutation. Three further patients presented a novel large deletion in the beta-sarcoglycan gene (*SGCB*; LGMD2E), a novel splice mutation in *CAPN3* and a deletion in the *DMD* gene. The same *CAPN3* mutation was subsequently detected in yet another patient.

Conclusions We show that, besides the known *SGCG* founder mutation, other muscular dystrophy mutations are segregating among Roma Gypsies. Given the endogamous characteristics of this community, it might be justifiable to include these two newly-identified mutations in our first-tier screen.

CO 4 | Cytogenetics and Genomics

NEXT-GEN CYTOGENETICS AND THE HIDDEN COMPLEXITY OF GENOMIC OR CHROMOSOMAL REARRANGEMENTS

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Human developmental abnormalities are devastating conditions that account for almost half of all full-term neonatal deaths in developed countries. For individuals who survive, congenital anomalies often confer lifelong disability and their impact on public health is profound. However, the genetic etiology and genomic architecture contributing to the vast majority of these conditions remain unknown. Separately, and in addition, the genetic etiologies of recurrent infertility remain to be elucidated.

The current low resolution diagnostic techniques are insensitive to the full mutational spectrum contributing to human developmental abnormalities and infertility, the poor understanding of the molecular alterations introduced by genomic rearrangements, and the lack of a fully annotated human genome hinders predictive diagnostics.

This study results from collaboration between a Portuguese Consortium including clinical geneticists and the Developmental Genome Anatomy Project (DGAP) from Harvard Medical School.

First, a group of cases were comparatively analyzed using genomic array and Next-Generation Sequencing (NGS). Subsequently, NGS of whole-genome large-insert libraries was applied for the identification of genomic or chromosomal rearrangements at nucleotide resolution in a series of cases, including two prenatal samples. Presently, this high-throughput technology is the only approach able to identify the full spectrum of structural variants, in a time frame that allows its application even for prenatal samples. The introduction of NGS into clinical cytogenetics surely will create a high-throughput, sequence-based Next-Gen Cytogenetics that will catalyze a dramatic advancement in clinical diagnostics. Therefore the understanding of the molecular pathology of these chromosome rearrangement-associated developmental disorders and infertilities will contribute to an improved prediction of the phenotypic consequences of these rearrangements.

CO 5 | Cytogenetics and Genomics

ANALYSIS OF 24 CHROMOSOMES BY PREIMPLANTATION GENETIC SCREENING USING ARRAY-CGH

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Introduction Preimplantation genetic screening (PGS) for the study of aneuploidies has been used to improve pregnancy rates in patients with advanced maternal age, recurrent miscarriages and repeated implantation failure.
Fluorescence in situ hybridization (FISH) technique has some limitations allowing only the study of a limited number of chromosomes. The introduction of array comparative genomic hybridization (aCGH) allows the analysis all chromosomes in a single cell level. Here we report the results of aCGH in 4 cycles of PGS.

Methods In the 4 cycles, cells for aCGH diagnosis were biopsied at 5th/6th day from trophectoderm with subsequent embryos slow freezing. From the 4 cycles, 41 normally fertilized embryos were obtained (2PN2GP) but only 20 (8, 4, 7 and 1 embryos, respectively) (48.8%) were biopsied. Cells were lysed and DNA was amplified using the Sureplex DNA Amplification System. aCGH technique was performed using the 24sure Microarray Pack (v3.0) according to manufacturers protocol. Slides were scanned with a SureScan Microarray Scanner and the SureScan Microarray software (Agilent Technologies). BlueFuse Multi software was used for analysis.

Results and Discussion From the 20 embryos analysed, 8 were euploid (5, 2, 1, 0 embryos, respectively) (40%) and 12 (60%) were aneuploid with single or several trisomies and/or monosomies. Embryo transfer was already performed in 2 cycles, resulting in one biochemical pregnancy with a subsequent miscarriage.
Array-based technology applied to single cells analysis is a very powerful technique that allows not only the analysis of all the chromosomes but also enables 5th day biopsy of trophectoderm cells minimizing considerably the mosaic findings detected at 3rd day embryo biopsy.

CO 6 | Molecular Genetics

HUMAN MTOR TRANSCRIPT CONTAINS AN IRES ELEMENT THAT GUARANTEES ITS EXPRESSION AND FUNCTION UNDER GLOBAL TRANSLATION IMPAIRING CONDITIONS

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Mammalian target of rapamycin (mTOR) is a conserved serine/threonine kinase that integrates signals from the cellular nutrient- and energy-status, acting namely on the protein synthesis machinery. Deregulation of mTOR signaling is implicated in major diseases, such as cancer, mainly due to its role in regulating protein synthesis. The main mTOR targets are proteins responsible for ribosome recruitment to the mRNA, thus, a specific inhibitor of mTOR, for example rapamycin, leads to global inhibition of translation. Major advances are emerging regarding the regulators and effects of mTOR signaling pathway; however, regulation of *mTOR* gene expression is not well known. Knowing that in stress conditions such as hypoxia, overall protein synthesis is reduced, but synthesis of mTOR protein is not inhibited, we hypothesized that mTOR 5' untranslated region harbors an internal ribosome entry site (IRES) allowing cap-independent synthesis of mTOR protein in stress conditions. By using dicistronic reporter plasmids and luciferase assays we have tested and confirmed this hypothesis. Here, we show that human *mTOR* transcript harbors an IRES element that is formed by a highly folded RNA scaffold capable of binding directly to the 40S ribosomal subunit. We further demonstrate that mTOR IRES is active both in normal and stress conditions, and that its activation status in response to translational adverse conditions parallels mTOR protein levels. Moreover, our data reveal that the IRES-dependent translation of mTOR is necessary for its ability to induce cell cycle progression into S-phase. These results suggest a novel regulatory mechanism of *mTOR* gene expression that integrates the protein profile rearrangement triggered by global translational inhibitory conditions.

COMUNICAÇÕES ORAIS II

ORAL COMMUNICATIONS II

CO 7 | Molecular Genetics

ONCOGENIC MECHANISMS OF *HOXB13* MISSENSE MUTATIONS IN PROSTATE CARCINOGENESIS

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Introduction The recurrent germline mutation *HOXB13* p.(Gly84Glu) (G84E) has recently been identified as a risk factor for prostate cancer (PrCa). We have performed full sequencing of the *HOXB13* gene in 462 Portuguese PrCa patients with early-onset and/or familial/hereditary disease, and identified two novel missense mutations, p.(Ala128Asp) (A128D) and p.(Phe240Leu) (F240L), which were predicted to be damaging to protein function *in silico*. In the present work we aimed to investigate the potential oncogenic role of these mutations *in vitro*, comparing to that of the recurrent G84E mutation and wild-type *HOXB13*.

Methods We induced site-directed mutagenesis in a *HOXB13* expression vector and established *in vitro* cell models of prostate carcinogenesis with stable overexpression of either the wild-type or the mutated *HOXB13* variants. *In vitro* assays were then performed in the established cell populations to evaluate proliferation, apoptosis, anchorage independent growth and invasion.

Results We observed that, while the wild-type *HOXB13* promotes proliferation, also observed with the F240L variant along with a decrease in apoptosis, the A128D mutation decreases apoptosis and promotes anchorage independent growth. No phenotypic impact was observed for the G84E mutation in the cell line model used.

Discussion Our data show that specific *HOXB13* mutations are involved in the acquisition of different cancer-associated capabilities, showing oncogenic mechanisms more consistent with gain of function mutations. Although the evidence is less clear for the G84E variant, the phenotypic impact of *HOXB13* overexpression, the partially different oncogenic properties of the different mutations, the lack of truncating mutations, and the absence of loss of heterozygosity whenever tumors were tested, all concur with an oncogenic role of *HOXB13* in prostate carcinogenesis.

CO 8 | Cancer Genetics

NCOA2 IS A CANDIDATE TARGET GENE OF 8Q GAIN ASSOCIATED WITH CLINICALLY AGGRESSIVE PROSTATE CANCER

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Introduction Prostate cancer (PCa) is the second most frequently diagnosed non-skin cancer in men worldwide and the fifth cause of cancer-related deaths. Tumors harboring 8q gains are associated with poor clinical outcome, but the target genes of this genomic alteration remain to be unveiled. In this study, we aimed to identify potential 8q target genes associated with clinically aggressive PCa.

Methods We first characterized using FISH the relative copy number of 8q (assessed with MYC flanking probes) of a series of 50 radical prostatectomy specimens, with available global gene-expression data and typed for ETS rearrangements, and then compared the gene expression profile of PCa subsets with and without 8q24 gain using Significance Analysis of Microarrays. FISH was subsequently performed with NCOA2 flanking probes.

Results In the subset of tumors with ERG fusion genes (ERG+), five genes were identified as significantly overexpressed (FDR≤5%) in tumors with relative 8q24 gain, namely VN1R1, ZNF417, CDON, IKZF2 , and NCOA2. Of these, only NCOA2 is located in 8q (8q13.3), showing a statistically higher mRNA expression in the subgroup with relative 8q gain, both in the ERG+ subgroup and in the whole series (P=0.000152 and P=0.008, respectively). Combining all the cases with NCOA2 overexpression, either at the mRNA and protein level, we identified a group of tumors with NCOA2 copy number increase, independently of ETS status and relative 8q24 gain. Furthermore, we detected for the first time a structural rearrangement involving NCOA2 in PCa.

Discussion These findings show that NCOA2 is a candidate target gene of 8q gain associated with clinically aggressive prostate cancer and that further studies with larger series are warranted to evaluate if NCOA2 relative copy number gain presents prognostic value independently of the well-established poor prognosis associated with MYC relative copy number gain.

CO 9 | Cancer Genetics

(EPI)GENOMIC DRIVERS OF INTRATUMORAL HETEROGENEITY IN CLEAR CELL RENAL CELL CARCINOMA

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Introduction Genetic alterations and disruption of epigenetic regulatory mechanisms are hallmarks of cancer. Nevertheless, while the genetic contribution for cancer development is easily illustrated by mutations in tumor suppressors or oncogenes, the epigenetic involvement and its functional relevance is far more complex and has only recently become a major focus of cancer research. In this study we aimed at investigating the impact of epigenetic deregulation on important cancer processes in a panel of 28 distinct cancers.

Methods Genomic alterations were retrieved after computational analyses of whole-exome sequencing (WES) data from 8920 tumor–normal matched pairs representing 28 different carcinomas (including 451 patients with ccRCC) from The Cancer Genome Atlas (TCGA). To score intratumor heterogeneity (IH) we calculated the mutant-allele tumor heterogeneity (MATH) using WES data. Linear model analyses were performed to determine the contribution of the different gene groups to genomic instability and IH in ccRCC.

Results Our pan-cancer analysis of all genomic alterations in 28 different cancers revealed that ccRCC ranks amongst those with the lowest frequency of genomic alterations. Notably, ccRCC has the highest frequency of alterations in epigenetic modifier genes. This finding suggests that epigenetic deregulation plays a major role in ccRCC development and progression. In fact, we found that mutations in epigenetic modifier genes are the stronger determinants of genomic instabilities and high IH in ccRCC. Our retrospective analyses further revealed that high IH leads to decreased overall survival of ccRCC patients.

Discussion Our study discloses a novel link between epigenetic deregulation and genomic instability, a cancer hallmark that fuels ccRCC progression. Moreover, the impact of epigenetic deregulation on IH and patient survival paves the way for novel therapeutic interventions in ccRCC.

CO 10 | Clinical Genetics

ETIOLOGICAL INVESTIGATION OF SENSORINEURAL HEARING LOSS: HIGH DIAGNOSTIC YIELD AND INVALUABLE BENEFIT TO PATIENTS AND FAMILIES

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Introduction Hearing loss (HL) is the most prevalent sensory disorder in developed countries, affecting 2-3/1,000 children. Sensorineural HL (SNHL) is mainly caused by environmental or genetic factors, the latter found in 60% of patients. Several syndromes can include HL and are accountable for 30% of genetic SNHL. We describe the results of our approach to SNHL in the setting of a medical genetics clinic.

Methods Medical records of patients with SNHL of unknown cause (isolated or not) referred to the genetics clinic at our hospital from October 2011 to April 2015 were reviewed. Demographic, clinical, audiometric and work-up data were collected and analyzed. Etiological investigation had followed a sequential approach designed in-house and tailored for the assessment of SNHL, with a first tier of comprehensive anamnesis and examination followed by a set of laboratory tests, imaging studies, ophthalmology screening, molecular testing, or other investigations according to phenotype and family history.

Results Data of 167 index patients were analyzed. HL was prelingual in 84%, non-progressive in 79%, profound in 58% and familial in 39% of patients, with pedigree suggestive of autosomal recessive inheritance in 55% of the latter. A genetic cause was determined in 103 patients (61.7%), of which 65% were confirmed by molecular testing. DFNB1 non-syndromic HL due to connexin 26 impairment was the overall leading single etiology (19.3%). Syndromic HL was diagnosed in 1/3 of patients with genetic HL, Waardenburg and Usher syndromes being the most frequent. We propose to discuss bias issues within this cohort and highlight the most relevant SNHL diagnosis.

Conclusions Achieving an etiological diagnosis soon after SNHL identification is paramount for providing adequate management, prognostic information and timely treatment options; it also precludes unnecessary procedures, allows the diagnosis of other family members and refines genetic counseling of patients.

CO 11 | Clinical Genetics

DISEASE EXOME, A POWERFUL DIAGNOSTIC TOOL: POSTMORTEM DIAGNOSIS OF DYSKERATOSIS CONGENITA

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Exome sequencing has become a powerful diagnostic tool to identify the molecular etiology of genetic diseases. We report on a deceased 60yo male with an undiagnosed systemic disease that presented with liver cirrhosis, pulmonary fibrosis, sick sinus syndrome and thrombocytopenia. Multiple tests had been previously performed with no diagnosis. DNA from post-mortem tissue was obtained and disease exome performed.

Disease exome was performed by capture of target regions using oligonucleotide probes (TruSightOne, Illumina) and subsequent NGS (MiSeq, Illumina) of a panel composed by 4813 clinically-relevant genes. Alignment and variant calling was performed using the BWA and GATK, respectively. Variants with MAF<1% were filtered and processed with bioinformatic analysis tools to assess its pathogenicity and potential to explain the clinical phenotype. Pathogenic and likely pathogenic variants were confirmed by Sanger sequencing.

Analysis revealed a novel heterozygous variant NM_198253.2:c.1492G>A (p.Gly498Arg) in the TERT gene, not described in the literature, that affects a highly conserved residue. Bioinformatic analysis suggests that the variant is very likely pathogenic (PolyPhen-2: probably damaging; SIFT: deleterious; dbSNP, ExAC, 1KGenomes, ESP: not present). Mutations in TERT gene cause autosomal dominant dyskeratosis congenita (DKC; type2; MIM 613989) and autosomal recessive DKC (type4; MIM 613989). In the context of the clinical phenotype of this patient, this result supports the diagnosis of DKC (type2).

We report a post-mortem diagnosis of DKC (type2) with a novel variant in the TERT gene, expanding the mutational spectrum of TERT related DKC. Additionally, this result highlights the importance of molecular diagnosis, even post-mortem, as establishing the molecular etiology allows proper genetic counseling to at-risk relatives. Finally, this case illustrates the power of clinical exome sequencing in solving complex diagnostic cases in a clinical setting.

CO 12 | Clinical Genetics

NEXT GENERATION SEQUENCING DATA: FROM NUCLEOTIDE CHANGES TO INTERPRETATION

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The dissemination of Next Generation Sequencing (NGS) technologies created great expectations for faster and more accurate genetic diagnosis, however, increased insight into patients' genomes has still limited impact in genetic diagnosis. One of the hurdles is bioinformatics data analysis as often different commercial software lead to selection of different sets of putative disease-causing variants for the same patient. In addition, a fair number of selected variants are either technical artefacts, or natural human variants without disease association. We aimed at developing bioinformatics NGS data analysis pipelines, custom designed to answer specific biological and/or clinical problems. Two main features underlie our pipelines: 1) customization and data mining to link the clinical question with the NGS data; 2) Open-source software which has rapid turnover and benefits from worldwide bioinformatics expertise, ever improving gold-standard algorithms for read mapping and alignment. Our pipelines incorporate large datasets of information produced by clinicians and selected according to the biological/clinical question. By selecting, trimming and incorporating relevant knowledge, variant calling/selection becomes a more confident and robust process. Along with our pipelines, we are also dedicated to the bioinformatics education of the medical and scientific community, who are key players in the translation of bioinformatics data into biological/clinical meaning. The close relationships with users of our pipelines, allows us to understand the questions that our pipelines raise and fine-tune data analysis and selection strategies. In summary, we are working towards the establishment of bioinformatics data analysis pipelines that are permeable to novel analysis algorithms, and that incorporate interpretation towards solving a clinical problem. With this adaptability, bioinformatics analysis of NGS data can move towards better and more reliable genetic diagnosis.

POSTERS

P 1 | Clinical Genetics

CHROMOSOMAL DISORDERS AND MALE INFERTILITY

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Male factor infertility is considered a complex disorder with a largely unknown etiology that affects about 7% of men. In general, genetic abnormalities account for 15%-30% of condition and Y chromosome microdeletions are also frequent.
The study, based on our casuistic, aimed at contributing to a better understanding of the genetic causes of infertility.

A group of 410 idiopathic infertile men with non-obstructive azoospermia, oligozoospermia, or unknown semen quality (based on clinical evaluation and/or sperm counts) was retrospectively selected. Conventional karyotype was performed in all samples; Y microdeletion screen was performed in 247 samples.

Forty two abnormal karyotypes (10.2%) were found, indicating an elevated frequency of chromosome abnormalities among the selected infertile men, as compared to that of newborn populations (≈0.4%). This frequency is higher than that reported in most similar studies that pointed to frequencies ranging from 2.2%-14.3%.

Klinefelter´s syndrome was the most common chromosome disorder (4.9%). There were 18 cases with 47,XXY karyotype and 2 cases of mosaicism involving lines 47,XXY and 46,XY. Reciprocal translocations were identified in 10 cases (2.4%), particularly in men with unknown semen quality. Overall, reciprocal translocations have been found in approximately 1% of the infertile men and more commonly in azoospermics than in oligozoospermics. However, this type of association was not found in the present study.
On the other hand, Y microdeletions were identified in 16/247 cases (6.5%), more frequently in azoospermics (13.3%, corresponding to 8/60 azoospermics). Among these 8 cases, 7 presented deletions at the AZFc region.

The marked presence of chromosomal abnormalities and Y microdeletions emphasizes the relevance of studying both factors in infertile men to improve genetic counseling, to allow the development of appropriate therapies, and to expand the knowledge about the etiology of male infertility.

P 2 | Clinical Genetics

MOLECULAR CHARACTERIZATION OF PORTUGUESE PATIENTS WITH DILATED CARDIOMYOPATHY – PRELIMINARY RESULTS

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Introduction Dilated cardiomyopathy (DCM) is a heart muscle disease that affects 1:2500 adults, characterized by ventricular dilation and impairment of systolic function. Familial forms account for 30-50% of all the cases, most presenting autosomal-dominant inheritance pattern of transmission. Mutations were identified in several genes. Molecular diagnosis may have implications in clinical practice, in genetic counseling and risk stratification. We intend to estimate the frequency and molecular basis of familial and idiopathic cases of DCM in Portugal.

Methods Multicentric study of unrelated DCM patients (pts), recruited between 2013 and 2014. All pts fulfilled established criteria for the diagnosis of DCM. Detailed clinical data were obtained. Search of mutations in LMNA/C, MYH7, MYBPC3, TNNT2, ACTC1, TPM1, CSRP3, TCAP, SGCD and PLN, MYL2, MYL3, TNNI3, TAZ, LDB3 genes was performed, using PCR technique with direct-sequencing (next-generation sequencing with at-least a 30-fold coverage combined with Sanger sequencing).

Results 108 pts were included, 63 (59%) men, mean age at diagnosis 38±13 years, with 49 (45%) familial cases. In total, 35 rare variants in 8 genes (MYBPC3 28%, TNNT2 20%, LMNA 14%, MYH7 11%, TCAP 9%, PLN 9%, LDB3 6% and TPM1 3%) were identified, in 29 pts (27%). Four pts had 2 mutations (in the same or different genes) and one patient presented 3 mutations in LMNA gene. Diagnosis yield was higher in familial cases (33%vs22%). Only one variant has been previously described in association with DCM (LMNA) and 8 with hypertrophic cardiomyopathy (2 in MYH7 gene and 6 in MYBPC3).

Discussion Our results reflect the complexity and diversity of DCM genetics. For better interpretation of pathogenicity of the mutations found and their causative role in DCM, cascade molecular screening of families is under way. This will allow better elucidation of the results and further insight in genotype-phenotype correlations and risk stratification in DCM.

P 3 | Clinical Genetics

INTRA-FAMILY PHENOTYPIC HERETOGENEITY OF 16P11.2 DELETION CARRIERS IN A THREE-GENERATION PORTUGUESE FAMILY

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Introduction The chromosome 16p pericentromeric region has been reported as susceptible to chromosomal rearrangements. One of the recently recognized microdeletion syndromes is the “16p11.2 microdeletion syndrome” associated with developmental delay, autism spectrum disorder, epilepsy and obesity. This region exhibits extensive phenotypic variability and diverse clinical features, including normal phenotypes.

Case report The authors report a three-generation family with a 16p11.2 microdeletion presenting with significant phenotypic heterogeneity. The 16p11.2 microdeletions were detected by a commercial MLPA kit (Salsa®MLPA®KitP343-C2 Autism-1, version 07). We describe in detail the phenotype of five patients: 1- the proband, a 14 years-old girl with mild mental retardation, behavioral problems, social impairments, obesity and minor dysmorphic facial features; 2- her father, aged 43, with learning difficulties, difficulties in expressive language, anxiety and overweight; 3- the grandfather, 67 years-old, presenting with learning difficulties, difficulties in expressive language and obesity; 4- a first male cousin (father’s side), aged 12, with mild mental retardation, facial dysmorphism and skeletal problems; 5- a paternal aunt, aged 41, with epilepsy, obesity and learning difficulties.

Conclusion The full extent of phenotypic features associated with the 16p11.2 microdeletion disorder and the genetic modifiers of phenotype are currently unknown. A well clinically characterized three-generation family offers unique opportunities for exploring the heterogeneity of this microdeletion syndrome. This report shows the different developmental pathways and variable phenotypes among family members with the same deletion.

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LUJAN-FRYNS SYNDROME: CLINICAL INVESTIGATION VERSUS MED12 SEQUENCE ANALYSIS

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Introduction Lujan-Fryns Syndrome (LFS; OMIM #309520) is a syndromic X-linked form of intellectual disability associated with marfanoid stature, facial dysmorphism and behavioural problems. Schwartz et al. (2007) suggested that the LFS designation may be used only for those cases with a compatible clinical phenotype and mutations in the MED12 gene.

Methods A retrospective study of 25 unrelated patients with clinical hypothesis of LFS referenced to our Medical Genetics Center was made and a review of phenotypic features was performed. The inclusion criteria were: presence of clinical features according to Lyons M. (2013) and available DNA samples at our Molecular Genetics Laboratory. Ten patients were included. All exons and the respective flanking regions of the MED12 gene were sequenced by the conventional Sanger sequencing method.

Results None of the previously reported mutations and known as causative of LFS were identified. In two cases, variants of unknown significance were found and the respective families were studied. The variant c.397-39G>A, located in intron 3, was identified in one case and also in one brother and mother. Preliminary RNA studies did not allow to unravel the possible causative effect of this variant. In the other case, we found an insertion in intron 31 - c.4416-74_4416-73insCCTCTTCTCTTCTCT - and also the duplication c.6223_6228dup located in the exon 42. The sister and mother of this patient were further analyzed and both are heterozygous for both of the variants. We did not find any potential pathogenic mutation.

Conclusion Combination of intellectual disability and main phenotypic features in a male patient is not sufficient for the diagnosis of LFS. In a recent study, Hackmann et al. (2015) reinforce the need of more detailed clinical criteria as specific facial features, marfanoid habitus and obvious X-linked segregation of the disorder or an unambiguously pathogenic mutation in the MED12, for a definitive diagnosis of LFS.

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PRIMARY CONGENITAL GLAUCOMA WITH MEDICAL TREATMENT ONLY

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Introduction Primary congenital glaucoma (PCG) is a rare condition and an important cause of childhood blindness. It is characterized by an increased intraocular pressure, buphthalmos, opacification and oedema of the cornea with Haabs striae and large corneal diameter. Surgical treatment is used in 94% of the cases. Two genes (*CYP1B1* and *LTBP2*) responsible for PCG have been identified and several other loci have been mapped. *CYP1B1* is the most frequent form, and is inherited in an autosomal recessive manner. In two cohorts of Portuguese patients mutations in this gene have been identified in 28.57% and 69% of the cases.

Methods and Results We report the case of a 31-year-old man with diagnosis of bilateral congenital glaucoma at 10 days of life, without surgical therapy. The intraocular pressure values are normal with medical treatment only. There are no signs of glaucomatous optic neuropathy. The molecular analysis of *CYP1B1* gene revealed a compound heterozygous mutations c.535delG (p.Ala179Argfs*18) and c.1159G>A (p.Glu387Lys), which confirmed the diagnosis of PCG. The parents are heterozygous for one of the mutations.

Discussion PCG is genetically and clinically heterogeneous with inter and intrafamilial variability. Several studies report some genotype-phenotype correlation with bilaterality, early age at diagnosis and a more severe disease in the group with biallelic pathogenic variants in *CYP1B1*. Our case of PCG, with compound heterozygous mutations for two mutations in *CYP1B1* gene, has a mild phenotype. PCG with the same biallelic mutations has been described in the literature, with surgical treatment. We can conclude that, *CYP1B1* gene must be analyzed even in non-severe phenotypes. This report enhances the possibility that other genetic and/or environmental factors can contribute to the phenotype, thereby explaining the different clinical manifestations.

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PERINATAL DIAGNOSTIC APPROACH TO FETAL SKELETAL DYSPLASIAS - THREE CLINICAL CASES AS PARADIGM

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Introduction Osteochondrodysplasias or skeletal dysplasias (SD) are a genetically heterogenous group of disorders that affect growth morphology of the chondro-osseous tissues. Many SD can be identified by ultrasound (US) evaluation prenatally, but differential diagnosis may be challenging due to the large number of SD and their phenotypical variability with overlapping features. The confirmation of diagnosis usually relies on molecular testing, postdelivery radiographic studies and autopsy, including histomorphic analysis of cartilage and bone.

Methods We report and characterize three clinical cases of fetal SD as a paradigm of the difficulty in diagnosing SD by US.

Results All cases had shortening of fetal femora and humeri (<5th centile or -2SD) and invasive prenatal testing. In cases 1 and 2, SD was identified in 1st trimester US and microarray for SD (9 genes, 47 mutations) was normal, but pregnancy was terminated due to severity of phenotype. Case 3 was identified in the 2nd trimester and a female was born prematurely. In case 1, fetal phenotype and histological findings are suggestive of Achondrogenesis 1A; in case 2, the possible diagnosis is Thanatophoric Dysplasia; in case 3, neonatal clinical and radiographic phenotype and increased urinary levels of phosphoethanolamine point to Hypophosphatasia. In all cases, DNA was stored and sequencing of TRIP11, FGFR3 and ALPL genes respectively is still ongoing.

Discussion The molecular confirmation of the suspected clinical diagnosis is important to improve postnatal management of fetuses with SD and accurately determine recurrence risk and enable at-risk couples to access prenatal or preimplantation genetic testing in future pregnancies. We hope to enlighten the role of the medical geneticist in the diagnosis of SD, integrating clinical, radiographic and histopathological findings and keeping in mind that multidisciplinary approach is the key factor in the diagnosis of these disorders during perinatal period.

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KLEEFSTRA SYNDROME: CASE REPORT OF A PATIENT WITH AN INTRAGENIC EHMT1 MUTATION

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Introduction Kleefstra syndrome (KS) is an autosomal dominant disorder characterized by developmental delay, childhood hypotonia and distinctive facial features. Cardiac defects, hearing problems and behavioral disorders are also common. KS is caused by either microdeletion at 9q34.3 or loss of function pathogenic variants in the euchromatin histone methyltransferase (EHMT1) gene. Almost all reported cases have been de novo. Recurrence has been observed due to a parental balanced translocation or somatic mosaicism.

Case presentation We describe a 4 year-old girl, who is the child of non-consanguineous parents. She was born at term with weight on the 90th centile and length and head circumference on the 75th centile. Hypotonia was noted in the neonatal period. On follow-up, she presented mild psychomotor delay with poor speech development. Audiological testing showed mild to moderate hearing loss; cardiac evaluation identified mild valvular pulmonar stenosis; and there were non-specific findings on brain MRI imaging. On observation, brachycephaly with posterior plagiocephaly, flat facies, frontal bossing, synophris, arched eyebrows, malar hypoplasia, Cupid's bow upper lip and protruding tongue were noticed. ArrayCGH did not identify pathogenic CNVs. MLPA analysis of the EHMT1 gene was normal. Targeted gene analysis of clinical exome data revealed a heterozygous pathogenic mutation in EHMT1 gene, c.3502C>T (p.Arg1168*). Parental analysis is ongoing.

Discussion According to the literature, comparison between 9q34.3 microdeletion and EHMT1 mutation patients revealed only a few phenotypic differences. High birth weight, overweight, recurrent infections and behavioral problems were more frequent among mutation patients, while microcephaly and short stature were less common in these patients. Our patient's phenotype is consistent with the main clinical features of KS, and fits with the clinical features of EHMT1 mutation individuals.

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PRENATAL DIAGNOSIS OF IDIC(9)

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Tetrasomy of the short arm of chromosome 9 is a rare chromosome imbalance that may result from a supernumerary isochromosome 9 with the most recurrent breakpoints being 9p10, 9q12 and 9q13.

On ultrasound, it usually presents with intrauterine growth restriction (IUGR), abnormal facial profile and ventriculomegaly.
However, few reports establish a correlation between fetal features and the size of isochromosome or the presence of isodicentric 9.

We report the clinical case of a 32-year-old pregnant woman, G2P1, underwent amniocentesis at 13 weeks of gestation with fetal increased nuchal translucency (7mm). The fetus also presented IUGR, cystic higroma, generalized subcutaneous edema, cardiac malformations, facial anomalies and fetal death.

The karyotype was performed by standard in situ methods. Fluorescence in situ hybridization (FISH) was performed using centromeric probe CEP9.
Conventional cytogenetic and FISH analyses revealed a supernumerary chromosome idic(9)(q12) in all cells examined.

After counseling the couple opted for termination of pregnancy. The post-mortem analysis revealed a single umbilical arteria, IUGR, cystic higroma, facial dysmorphism with cleft lip and palate, hypertelorism and low set ears. These findings are in accordance with other reports.

Nevertheless, the hypertelorism is not commonly described and such an early detection of a cardiac anomaly is uncommon. Additionaly the fetal death occurred early than in the most cases described in literature.

Although breakpoint position effect on the severity on the phenotype is not consensual it has proposed that cases presenting with breakpoints on p10, on q12 or on q13 show a similar phenotype. However, cardiac defects seem more frequent on cases in which the abnormality includes 9q material.

This work aims to contribute to a better karyotype-phenotype correlation in cases with tetrasomy 9p and isodicentric chromosomes idic(9).

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DEVELOPMENT AND VALIDATION OF AN IN-HOUSE DATABASE FOR ARRAY COMPARATIVE GENOMIC HYBRIDIZATION RESULTS

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Introduction The classification and interpretation of Copy Number Variants (CNVs) is performed by consulting available public databases and resources. In order to help this interpretation is also important to analyse CNVs already classified by the laboratory. We developed an in-house relational Database (DB) to store results and an application which allows feed and querying the DB.

Methods A set of software requirements and functionalities was defined, using different techniques such as brainstorming, role playing and observation. Use Case Diagrams (UCD) were defined in order to model the dynamic behavior of the system and an Entity-Relationship Model (ERM) and a Relational Model (RM) were developed to model the database. When the software design was finished, a LAMP (Linux, Apache, MySQL and PHP) server was set up.

Results An in-house database (DB) was modeled, developed and populated with 3254 CNVs records from several Clinic Cases from our laboratory. In order to validate the functionalities of the DB, several queries were made on the results stored in the database which allowed the evaluation of the CNVs distribution for several attributes, for example alteration type, size, classification and chromosome.

Discussion The arrayCGH DB proved to be an efficient way for uploading spreadsheets of arrayCGH results performed at our laboratory, and also for CNV matches executed with CNVs from arrayCGH DB and/or UCSC Genome Browser. For reports management, the arrayCGH DB allows laboratory clinical geneticists to keep track and edit their arrayCGH results. After this first pilot validation the DB will be populated with more CNVs allowing this tool to be efficiently used by the laboratory clinical geneticists.

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BOLDMULTIPLE CONGENITAL DISLOCATIONS – DOMINANT VS RECESSIVE "LARSEN SYNDROME"

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Introduction Genetic syndromes that present as Multiple Congenital Dislocations are a growing group of disorders which require careful clinic-radiological characterization and differential diagnosis discussion (8 causative genes and more than 10 disorders are known). We present two cases with distinct molecularly confirmed conditions.

Case Reports Patient 1 is a 2 year-old girl who presented prenatally with bilateral club feet; congenital dislocation of the hip, elbow and fingers; spatulated distal phalanges; depressed nasal bridge and malar flattening. Her X-rays, at 4 months-old, showed normal hand bone age and a supernumerary tarsal ossification centre. Length and audition were normal. Filamin B gene molecular analysis confirmed the diagnosis of Larsen Syndrome (dominant/classic form).
Patient 2 is a 2 year-old girl with congenital dislocation of the right knee, hip and both elbows; metatarsus adductus; normal thumbs and disproportionate prenatal short stature. She had atrial septal defect that spontaneously closed in the first month. Her X-rays showed a jump in interpedicular distance from T11 to L2; vertebral bodies notching on lateral view; mild generalized epiphyseal dysplasia; and normal bone age. This clinic-radiological picture was suggestive of CHST3 deficiency, previously known as recessive form of Larsen syndrome, which diagnosis was molecularly confirmed.

Discussion An early and accurate diagnosis allowed a precise genetic counseling and a personalized multidisciplinary follow-up, not only focused on the joint treatment. In Larsen Syndrome, the recurrence risk for the couple is low (de novo mutation) and the management should include prevention of cervical spine and audition problems. In CHST3 deficiency syndrome, the recurrence risk for the couple is 25% and the management is more complex with expected progression of the spondyloepiphyseal dysplasia, short stature with frequent development of spinal kyphosis, and recommended cardiac surveillance.

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SPONDYLOENCHONDRODYSPLASIA AND SLE: REPORT ON TWO UNRELATED ADULT CASES

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Introduction Spondyloechondrodysplasia (SPENCD) is a rare immune-osseous dysplasia caused by biallelic mutations in *ACP5* gene. We report on two unrelated patients in which this diagnosis was achieved in adulthood.

Clinical Reports Patient 1 is a 26-year old female who was followed-up since age 5 months for recurrent upper airway respiratory infections and postnatal short stature. In adolescence, she had several episodes compatible with undifferentiated connective tissue disease (Raynaud phenomenon, inflammatory arthralgia, antinuclear antibodies 1:160). A brain CT revealed intracranial calcification. When she was 19 years-old, she began to have autoimmune hemolytic anemia; 4 years later, nephrotic proteinuria, fulfilling systemic lupus erythematosus (SLE) classification criteria.
Patient 2 is a 42 year-old male with short stature and a long-term history of complex immune dysregulation: recurrent episodes of severe autoimmune haemolytic anaemia and SLE since third decade of life.
SPENCD was suspected in both patients after clinical and radiological re-evaluation. Lateral spine radiograph in adulthood and re-assessment of radiographs done in childhood identified enchondroma-like widespread metaphyseal and vertebral abnormalities with platyspondyly.
ACP5 sequencing confirmed both diagnoses: patient 1 is homozygous for the variant 791T>A (p.Met264Lys) and patient 2 is homozygous for c.325G>A (p.Gly109Arg). Both missense mutations were previously reported in patients with SPENCD.

Discussion ACP5 mutations in SPENCD cause a loss of tartrate-resistant phosphatise (TRAP) activity. This condition is considered to be underdiagnosed, associated with a progressively wider phenotypic spectrum and with few available data on its natural history. The present reports constitute some of the few described cases with long term follow-up. A precise diagnosis allows a more personalized management and accurate genetic counseling.

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INTRAFAMILIAL PHENOTYPIC VARIABILITY OF 22q11.2 DELETION SYNDROME

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Introduction 22q11.2 deletion syndrome, the most common microdeletion syndrome, is characterized by dysmorphic craniofacial features (including ear abnormalities, hypertelorism, upslanting palpebral fissures, prominent nasal root with fullness superior to the nasal tip, hypoplastic alae nasi and malar flatness), congenital heart disease, palatal anomalies, immune deficiency, hypoparathyroidism, renal malformations, behavioral anomalies, psychiatric disorders and possible intellectual disability of varying severity. One of its hallmarks is a wide phenotypic spectrum, both intra- and inter-familial. Most deletions (over 90%) occur de novo.

Methods We present a family with classical 22q11.2 deletion syndrome and review the clinical findings in affected members.

Results The proband is a 3 year-old boy, referred to our Medical Genetics Department for moderate to severe global developmental delay and bilateral symmetric frontal polymicrogyria/pachygyria. His facial features suggested del22q11.2 and the diagnosis was confirmed at 2 years and 5 months of age by array-CGH. His mother and brother were thought to have the same condition, and this was subsequently confirmed by FISH analysis directed to the 22q11.2 region. His mother shows similar craniofacial features, nasal voice and has a history of sleep disturbance, depression, speech delay, cleft palate, feeding difficulties and respiratory infections as a child; she had an older brother born with cleft palate and deceased shortly after birth. The proband's younger brother, currently 9 months old, has a ventricular septal defect and left ventricular false chordae tendinae; so far his psychomotor development appears to be normal.

Discussion This family illustrates well the phenotypic variability of 22q11.2 deletion syndrome, and serves as an example of why clinicians must maintain a high degree of suspicion for this relatively prevalent condition, particularly in patients who do not have the classical clinical picture.

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7q11.23 DUPLICATION SYNDROME: REVIEW OF FOUR CASES AND FURTHER DELINEATION OF A CRITICAL REGION

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Introduction Classic 7q11.23 duplication syndrome is caused by a 1.4 Mb duplication of contiguous genes lying within the critical region for Williams-Beuren syndrome. The phenotype is characterized by dysmorphic craniofacial features (macrocephaly, brachycephaly, broad forehead, dysplastic ears, straight eyebrows, broad nasal tip, columella anomalies, short philtrum and thin upper lip) leading to a recognizable facial gestalt, speech and language delay usually with normal intellectual development (although varying degrees of intellectual disability are possible), Autism Spectrum Disorder, Attention Deficit/Hyperactivity Disorder, behavioral problems (including anxiety and oppositional disorders), neurological signs (hypotonia, gait and station abnormalities) and central nervous system anomalies.

Methods We reviewed four cases of patients diagnosed with 7q11.23 duplication syndrome and compared our findings with the latest reports in the medical literature.

Results All four patients presented a clinical phenotype compatible with previous reports on 7q11.23 duplication syndrome. Craniofacial characteristics included typical dysmorphic features. All had some degree of speech and language impairment. Three had intellectual disability (from mild to profound), while the fourth had borderline intellectual functioning. Three had anomalies on brain magnetic resonance imaging, namely ventriculomegaly. Two had pyelocalyceal dilation. Interestingly, two patients showed a shorter duplication on array comparative genomic hybridization than classically reported, one of them not comprising the *ELN* gene.

Discussion The finding of shorter duplications within 7q11.23 in patients with a classical 7q11.23 duplication syndrome phenotype helps delineate the critical duplication region. Moreover, genotype-phenotype correlations are likely to emerge, such as the putative association between aortic dilation and duplication of *ELN*.

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THE PHARMACOGENOMICS IN THE PERSONALIZATION OF ISONIAZID TREATMENT IN PATIENTS WITH TUBERCULOSIS

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Introduction Isoniazid (INH) is a pivotal agent in the treatment of tuberculosis and is also the most prevalent cause of drug-induced hepatotoxicity (DILI), an adverse effect occurring in 10-36% of patients and that may be fatal. The major aim of the project was to identify genetic and non-genetic factors contributing to INH-induced DILI. Apart from NAT2, responsible for genetic variability of INH metabolism, we also assess the role of other candidate genes like CYP2E1, GSTM1 and GSTT1, encoding detoxifying enzymes, and ABCB11, encoding a protein involved in bile salt transport.

Methods Two groups of tuberculosis patients, 111 without analytical evidence of hepatotoxicity and 56 with hepatotoxicity, were compared. Functional polymorphisms of the 5 genes were characterized by sequencing (9 SNPs from NAT2), RFLPs (rs6413432 and rs2031920 from CYP2E1 and rs2287622 from ABCB11) and PCR-multiplex (for variants GSTM1*0 and GSTT1*0).

Results and discussion Clinical variables such as gender, age and weight, were not associated with the occurrence of INH-induced DILI. Slow acetylators (SA), identified by NAT2 genotyping, were at increased risk ($p < 0.005$; OR = 3; 95% CI = 1.23-7.35), as well as homozygous for variant Ala of ABCB11 ($p< 0.01$; OR = 2.5; 95% CI = 1.26-5). The presence of both risk genotypes' was also significantly associated with increased susceptibility to hepatotoxicity ($p < 0.001$; OR=3.96; 95% CI = 1.84-8.63). Risk genotypes were frequent among patients: 52% of SA (NAT2), 32% of Ala/Ala (ABCB11) and 21% with both genotypes'. Contrarily to INH plasma concentrations, with more than 80% of population variability explained by NAT2 SNPs, INH-induced DILI is a complex phenotype, depending on multiple low penetrance multidimensional variables. Yet, as the risk variables identified are frequent in Portuguese population, prospective studies should be performed to evaluate if genotyping is cost-effective.

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NSHL STUDY IN SÃO TOMÉ AND PRÍNCIPE POPULATION

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Introduction Since 2011 an otolaryngologist team with 2 doctors, 2 nurses and 1 audiologist began humanitarian missions in São Tomé and Principe. São Tomé and Principe (STP) is an archipelago in western equatorial Africa, near Gabon, Equatorial Guinea, Cameroon and Nigeria, with Portuguese as official language. Before these missions audiological evaluation was not performed in these islands. Upon the first mission, a high prevalence of SNHL in the population was identified and it was observed upon subsequent missions.
After conducting a clinical investigation to identify risk factors of HL, otoscopy and diagnostic tests (pure tone audiogram or brainstem evoked potentials) to quantify the loss, we began a study about possible etiologies associated to SNHL.

Methods The genetic etiology associated with DFNB1 was assessed by analysing a sample of 92 bilateral SNHL patients and 179 normal-hearing individuals, both by sequencing the coding region of GJB2 gene and by investigating the presence of the two large deletions described for GJB6 gene (del1830 and del1854). The remaining individuals (n=45) from the total sample, who present unilateral hearing loss, have also been assessed regarding DFNB1.

Results Sequencing of the coding region of the GJB2 gene was performed in 66 bilateral HL patients, 35 unilateral HL patients and 148 controls. Some pathogenic and likely pathogenic GJB2 mutations have been identified in this study. No GJB6 deletions were identified.

Conclusions This is the first study aiming at identifying genetic causes for deafness in the population of STP. The results obtained suggest a mixture of genetic influences on the STP population. The most common GJB2 mutations identified in these study were all previously identified in the Portuguese population. These mutations are also common in Euro-Asiatic populations and variants already described in Africa have also been identified suggesting also its genetic contribution for the genetic background of STP population.

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TP63-RELATED DISORDERS: REPORT OF THREE CASES

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Introduction The TP63 gene is a master regulator of embryonic development and differentiation of ectodermal cells. Several mutations in TP63 have been associated with distinct human developmental syndromes, characterized by common features such as limb abnormalities, ectodermal dysplasia, and facial clefts. These syndromes are: the ectodermal dysplasia–ectrodactyly–cleft palate (EEC, MIM: 129900), the ankyloblepharon–ectodermal dysplasia–clefting spectrum including Rapp-Hodgkin syndrome (AEC, MIM: 106260), the limb–mammary syndrome (LMS, MIM: 603543), the acro–dermato–ungual–lacrimal–tooth (ADULT, MIM: 103285) and non-syndromic split-hand/foot malformation type-4 (SHFM-IV, MIM: #605289).

Methods We describe three patients with mutations in the TP63 gene. Two patients were father and son. The child had ectodermal dysplasia, cleft palate and hydronephrosis, the father had only ectodermal dysplasia. The other patient presented multiple malformatios including ectrodactyly, oligosyndactyly, ectodermal dysplasia, cleft lip and palate, blepharophimosis, lacrimal duct atresia, teeth anomalies, conductive hearing loss due to narrow ear canals and radial hypoplasia.

Results In the familial case the clinical diagnosis was Rapp-Hodgkin syndrome (RHS) and molecular analysis of the TP63 gene revealed a new pathogenic heterozygous mutation c.1963_1970dup8 (p.Glu657AspfsX50).
In the other patient the EEC3 syndrome was suspected and molecular analysis of the TP63 gene revealed a pathogenic heterozygous mutation c.1028G>A (p.Arg343Gln) which has previously been reported in other patients with EEC3.

Discussion Based on clinical features, RHS and EEC3 syndromes were suspected and confirmed by molecular analysis. Hydronephrosis has already been described in other patients with RHS and is considered a very rare feature of this syndrome. These cases provide additional evidence of the variability seen in TP63-related disorders and further delineate genotype-phenotype correlations.

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IMPROVEMENT OF THE ANTIOXIDANT POTENTIAL OF FANCONI ANEMIA CELLS WITH N-ACETYLCYSTEINE AND A-LIPOIC ACID

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Fanconi Anemia (FA) is a genetic disorder caused by mutations in one of 16 genes that function in a pathway for the maintenance of genomic stability. At cellular level FA is characterized by hypersensitivity to DNA crosslink agents, such as diepoxybutane (DEB), which results in chromosome instability (CI). The hypersensitivity of FA cells to DEB is a consequence of its oxidative stress (OS)-related mechanism of cytotoxicity, involving glutathione (GSH) depletion. Recently, it was demonstrated that FA mutations result in deregulation of the endogenous antioxidant defense. Therefore, FA cells may benefit from an antioxidant therapy independent of the FA pathway.

We have previously investigated the ability of several antioxidants to counteract the DEB related OS and two were selected: N-acetylcysteine (NAC) and α-lipoic acid (ALA). Subsequently, we observed that NAC and ALA improved genetic stability, decreasing CI both in lymphocyte cultures from FA patients and DEB-induced lymphocyte cultures from healthy donors. The aim of the present study was to evaluate if NAC and ALA, besides the genomic improvement, can also ameliorate the cellular antioxidant potential.

The antioxidant potential was evaluated measuring GSH and GSH-Px activities in red blood cells (RBC) from 4 FA patients and 9 donors obtained from blood cultures with and without exposure to NAC and ALA.

Our results showed that exposure to NAC and ALA increased GSH levels in FA RBC, pointing to an improvement of the antioxidant defense. In fact, it is known that NAC might increase GHS levels by supplying cysteine and ALA preserves the integrity of RBC by adjusting the redox disturbances. No effect was observed concerning GSH-Px levels, suggesting that no requirement of GSH-Px was involved in the antioxidant improvement with NAC and ALA.

Based on these results and on the previous CI studies, we can speculate that NAC and ALA have potential to be a first choice exogenous antioxidant therapy to apply to FA patients.

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ISODICENTRIC Y CHROMOSOMES IN PRENATAL DIAGNOSIS: TWO DIFFERENT CASES

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Introduction Isodicentric Y chromosomes - idic(Y) - are the most commonly detected aberrations of human Y chromosomes, rarely reported in the context of prenatal diagnosis. They are mitotically unstable and may be lost during fetal development, resulting in patients with mosaic karyotypes, mostly with a 45,X cell line and, consequently, with a wide phenotypic diversity ranging from Turner syndrome to mixed gonadal dysgenesis and phenotypic normal males.
The most likely mechanism of formation of an idic(Y) chromosome is by an isochromatid break, followed by a U-type exchange. Breakpoints in the short arm leads to a duplication of the entire long arm and a part of the short arm - idic(Yq), while those in the long arm originate a duplication of the entire short arm and part of the long arm - idic(Yp).

Methods The authors report two prenatal cases referred to our Centre for conventional cytogenetic studies: one because of a previous child with Spinal Muscular Atrophy Type 1 and another for increased nuchal translucency and cystic hygroma. Chromosomal analysis using GTL and CBG banding was performed according to standard procedures. Fluorescence *in situ* hybridization (FISH) was carried out using commercially available subtelomeric Yp and Yq probes (Vysis-Abbott®).

Results In both cases the karyotype revealed a mosaicism involving a 45,X line and an idic(Y) chromosome: one with an idic(Yp) and the other with an idic(Yq).

Discussion The authors will approach the mechanisms of formation of idic(Y) chromosomes and compare these findings with similar cases described in the literature. They will also discuss the dynamic nature of the mitotic instability, its role in phenotypic variability and the difficulties in prenatal genetic counselling.

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DIAGNOSIS OF MICRODUPLICATION SYNDROMES: THE CGMJM EXPERIENCE

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Microduplications are chromosomal abnormalities that are too small to be detected using conventional cytogenetics methods. The exact size and location of a microduplication that may be considered responsible for a syndrome may vary, but a specific chromosomal "critical region" is consistently involved.

Several new microduplication syndromes are emerging as disorders that have been proven to cause multisystem pathologies frequently associated with intellectual disability, multiple congenital anomalies, autistic spectrum disorders and other phenotypic findings.
The phenotype of a particular microduplication syndrome is often less evident and less well defined than the corresponding microdeletion syndrome. In adittion, some microduplication syndromes may be inherited from apparently normal parents raising concerns regarding incomplete penetrance and ascertainment bias in these newly described clinical entities.

MLPA techniques were performed using kits (MRC-Holland) P245 Microdeletion Syndromes–1, P250 DiGeorge and P343 Autism-1, in patients referred to our Cytogenetics Laboratory, showing great phenotype heterogeneity and suggesting a chromosomal disorder.

A total of 13 microduplications were detected in chromosomes 5, 7, 16, 17, 22. Using panel P245, in a total of 205 patients, we detected 10 microduplications; with panel P250, in a total of 17 patients, we found one microduplication and with panel P343, in a total of 176 patients, two microduplications.

The utility of MLPA techniques used as a second approach in patients with normal karyotype but with a phenotype that suggests a chromosomal disorder is discussed and, in situations of cost constrains, MLPA is therefore a good option when compared with more expensive investigations, such as microarray analysis.

The authors enhance the advantage of a strong collaboration between clinical and laboratory geneticists to ensure best new phenotype recognitions, particularly in case of these small genomic aberrations.

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X-LINKED ID CAUSED BY A MICRODUPLICATION IN Xp11.22 INVOLVING *HUWE1* GENE

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Introduction Intellectual disability (ID) is more prevalent in males, probably as a consequence of genetic flaws involving chromosome X. It has been estimated that up to 200 genes contribute to X-linked intellectual disability (XLID). Many such conditions are non-syndromatic, so that NGS XLID gene panels and X-exome have emerged as valuable diagnostic approaches. Instead, we present a case solved by array-CGH.

Methods The index case was a male adult patient with non-syndromic ID. He had a maternal cousin and a maternal uncle with ID, compatible with X-linked transmission. Samples were studied by array-CGH (NimbleGen CGX 135K, Perkin Elmer); genomic analysis was performed with Genoglyphix software (Signature Genomics).

Results Array-CGH revealed a microduplication with 680.82kb in Xp11.22 region (53,316,172-53,996,993; hg19), that encloses *HUWE1* gene.

Discussion *HUWE1* gene, codes for E3 ubiquitin ligase, an enzyme involved in the ubiquitination of several proteins, thus promoting their degradation. Tp53 and n-Myc are among the target proteins of huwe1, both of which play a role in neurogenesis. Tp53 has been implicated in maintaining the balance between the continuous generation of neuroblasts and their elimination through apoptosis; reduced levels of tp53 result in development abnormalities in the nervous system. Huwe1 operates upstream of the N-Myc-DLL3-Notch pathway to control neural stem cell activity, balancing proliferation and neurogenesis in the developing brain. Increased expression of *HUWE1*, as a consequence of microduplication in Xp11.2, has been previously identified as the cause of non-syndromatic ID in a scarce number of familiar and sporadic cases. Our case helps delineate the phenotype of this still relatively new cause of XLID. Additionally, this diagnosis allowed carrier testing in female relatives at risk, with major impact in genetic counseling.

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16P11.2 PROXIMAL MICRODUPLICATION: GENOTYPE-PHENOTYPE INTERPRETATION IN A TWO-GENERATION FAMILY

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Introduction Genomic rearrangements involving 16p11.2 are rare and complex events that have been associated with a wide phenotypic spectrum. The most common clinical findings for proximal dup 16p11.2 are developmental delay/intellectual disability (ID) and a range of neurobehavioral problems. Microcephaly and minor dysmorphic features are frequent among carriers, although no clinically recognizable pattern is apparent. We present a family with three known carriers of dup 16p11.2 and compare their phenotypes.

Methods The index case is a 14 year-old boy refered to the Genetics Department presenting mild to moderate ID, microcephaly and minor facial dysmorphism. The mother did poorly in school but is otherwise healthy and functional. The maternal uncle has mild ID and epilepsy. The three have a facial resemblance that is in no way distinctive, and microcephaly is present only in the boy. The degree of functional impairment is also different among them. Samples were studied by array-CGH (180K CGX-HD; Signature Genomics, PerkinElmer). Genomic analysis was performed with Genoglyphix software (Signature Genomics).

Results A ~600 Kb (29,657,190-30,192,347; hg19) inherited copy gain at 16p11.2, classified as clinically significant was identified on the boy. This CNV is also present in his mother and maternal uncle, who is also the carrier of a 15q11.2q13.1 duplication.

Discussion 16p11.2 is a clear example of a susceptibility locus, previously identified as dosage sensitive. Several genes included in this region are candidates for neurodevelopmental disorders, namely TAOK2, TBX6, SEZ6L2, DOCC2A, QPRT, MVP and KCTD13. The estimated penetrance of dup 16p11.2 is 17.4-40.7%. Incomplete penetrance and variable expressivity hamper genetic counseling.

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ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (aCGH) IN PRENATAL DIAGNOSIS: A 3 YEAR EXPERIENCE

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Introduction The use of array Comparative Genomic Hibridization (aCGH) in prenatal diagnosis can elucidate the genetic etiology in fetuses with ultrasound (US) abnormalities and characterize fetal karyotype findings to enable proper genetic counseling, adequate prenatal care, and informed decision making.

Methods CGH analysis was performed using NimbleGen CGX 135K, 180 CGX-HD, CGX - prenatal filter 37K (Perkin Elmer), according to the manufacturer's instructions. Genomic analysis used the Genoglyphix software (Signature Genomics).

Results From 2012 to 2015 our department performed 70 prenatal aCGH analyses.The indications were intra uterine growth retardation, structural malformations, and karyotype abnormalities (in fetuses with US and/or biochemical markers of aneuploidy).

Pregnant women were referred between 12 and 26 weeks gestational age.
We found 5 (7.14%) pathogenic variants. In 3 cases aCGH was performed due to karyotype findings:
Case 1 (karyotype 46,XY,t(4;19)(p16.1;q13.1): arr[hg18] 4p16.3p16.1(33,860-6,847,395)x1dn,19q13.11(38,339,094-38,625,866)x1dn;
Case 2 (karyotype 47,XY,+mar): arr[hg19] 1q21.1q21.2(146,531,538-147,390,104)x1,21q11.2q21.1(15,484,314-21,704,253)x3;
Case 3 (twin pregnancy: karyotype of fetus 1 46;XX; karyotype of fetus 2 46,XY,del(10)(q26.1)[20]/46,XY[10]): fetus 1 arr[hg18] 3q29(197,048,483-197,497,164)x1, fetus 2 arr[hg18] 10q26.13q26.3(123,359,561-135,253,240)x1~2.
Cases 4 and 5 were performed due to US malformations:
Case 4 arr[hg18] 18p11.32q23(131,491-76,114,684)x3;
Case 5 arr[hg19] 22q12.3q13.33(32,254,840-51,178,150)x3.

Discussion Although we have a small sample, our results are in keeping with those reported in the literature. Our experience confirms the power of aCGH in clarifying the clinical significance of uncertain karyotype findings.Furthermore, it strengthens the indication of aCGH as a first line approach in pregnancies with abnormal US findings, saving time and providing useful information for adequate decisions.

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A CASE REPORT OF PARENTAL TRANSMISSION OF KOOLEN DE VRIES SYNDROME

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Koolen-de Vries Syndrome (KdVS) is a rare disorder caused by haploinsufficiency of KANSL1 gene, either by heterozygous mutation of KANSL1 or microdeletion on chromosome 17q21.31. Major clinical features include delayed psychomotor development, hypotonia and characteristic facial features. To this day, all individuals reported with KdVS were identified as having the syndrome as a result of a de novo microdeletion/mutation event. Even though it is not known whether KdVS affects fertility, it is considered that parent-to-offspring transmission can take place in an autosomal dominant manner.

However, no individual with KdVS has been reported to have had children of their own.
We report two novel patients with KdVS, in which the microdeletion pattern associated with this syndrome was vertically transmitted, from mother to son. To our knowledge this is the first case report of a parental transmission of KdVS.

Array-based comparative genomic hybridization (array-CGH) was performed on an Affymetrix platform, Cytoscan 750K. Data analysis was performed on ChAS Software, Affymetrix (NCBI hg19 reference).

Proband was tested at age 7 by array-CGH which revealed a 477 Kb interstitial microdeletion at 17q21.31 (Chr17:43,710,150 44,187,492) and KdVS diagnosis was concluded. The mother of the proband was studied at age 32, after the son diagnosis, by array-CGH and a 503 Kb interstitial microdeletion at 17q21.31 (Chr17: 43,710,150-44,213,434) was found and diagnosis was also concluded as KdVS. Both microdeletions resulted in complete loss of 9 genes, of which KANSL1 and MAPT are known to be associated to human disease. Other protein and non-protein genes are unknown or have not been related to human disease and, therefore, it is unclear to which extent they are involved in the clinical phenotype. Future studies will be focused on testing further members of the family to investigate the origin of the microdeletion pattern.

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ARRAY AND NGS BASED CHARACTERIZATION OF TRANSLOCATION BREAKPOINTS OF THE t(2:7)(q23;q32),t(5;6)(q23,q26)dn

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Introduction Congenital anomalies, namely caused by chromosome rearrangements, are a leading cause of infant mortality in European countries. The elucidation of the causal relationship between rearrangements and clinical phenotypes requires an efficient approach for identification of breakpoints at nucleotide resolution.

Methods In the last decade we went from conventional FISH based positional mapping of chromosomal breakpoints to sorting and amplification of derivative (der) chromosomes followed by array painting based mapping. Currently we are moving towards the application of Next-Generation Sequencing (NGS) for the identification of chromosome rearrangement breakpoints at nucleotide resolution. By means of these comprehensive molecular techniques we unveil the structural chromosomal alterations at nucleotide resolution in a proband with t(2:7)(q23;q32),t(5;6)(q23,q26)dn. Expression profiling of the proband's LCLs was also carried out.

Results Array painting identified the breakpoints of two balanced chromosome translocations. The disruption of the *PRPF40A* and *SND1* genes by the t(2;7) was identified both by array and NGS analysis. While array analysis identified only t(5;6) breakpoints and the affected *PACRG* gene, NGS revealed further complexity of the breakpoint region. Indeed, der(6) is a complex chromosomal rearrangement (CCR) with three additional breakpoints resulting from an inversion and a *PTPRK* gene excision/insertion.

Discussion Because of the complexity of this rearrangement we are not yet able to establish the candidate genes for the observed clinical phenotype. As shown by the CCR, NGS is currently the only methodology able to identify the full spectrum of balanced structural alterations. Thus, the introduction of NGS technology for high-throughput delineation of chromosomal rearrangements is presently underway.
Study supported by FCT projects PTDC/SAU-GMG/118140/2010 and HMSP-ICT/0016/2013.

P 25 | **Cytogenetics and Genomics**

DUPLICATION OF 17q24.3q25.1 RESULTING FROM AN INTRACHROMOSOMAL INSERTION ON 17p: THE IMPORTANCE OF HIGH RESOLUTION CYTOGENETICS IN UNREVEALING CRYPTIC REARRANGEMENTS

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Intrachromosomal insertions are uncommon rearrangements, in which a chromosomal segment is intercalated into another part of the same chromosome. The cytogenetic recognition of these structurally rearranged chromosomes can be difficult to ascertain. In last years the application of array-CGH in the investigation of patients with intellectual disability and congenital malformations increased substantially the detection of cryptic chromosome imbalances. However, sometimes the rearrangement underlying the imbalance could be missed, when we only perform molecular techniques.

We report a 10 year-old girl which presented: prenatal-onset microcephaly, aplasia cutis congenital, phalangeal synostosis of the first finger, shortening of the fourth metacarpal in right hand, long first toe, pectus excavatum, dysmorphic features, short stature, hypertrichosis, intellectual disability and attention deficit hyperactivity disorder. The proband was referred by the Pediatric Endocrinology for high resolution cytogenetic analysis, a subtle alteration on 17(p13) was observed, apparently a duplication. To characterize the imbalance oligonucleotide array-CGH was performed and showed a 2,4 Mb duplication of the region 17(q24.3q25.1) encompassing 10 genes. Molecular cytogenetics with specific BAC clones proved to be an intrachromosomal insertion.

Parents were not available to study, so we failed to prove the origin of the rearrangement.

Apparently the more relevant genes involved in the imbalance are *SOX9* (OMIM: 608160) and *SLC39A11* (OMIM: 616508). SOX9 is a transcription factor essential for both sex and skeletal development and SLC39A11 is a Zinc transporter. Overlapping reports of duplications result in abnormal digit and nail development.

This report emphasis the contribution of high resolution cytogenetic analyses and molecular cytogenetics to a better understanding of the mechanism in the origin of the imbalance and providing a more accurate genetic counseling.

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FISH SCREENING IN PRENATAL DIAGNOSIS: WHAT IS ITS VALUE?

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FISH screening in uncultured amniocytes is a standard procedure for the rapid detection of the most common chromosomal aneuploidies in prenatal diagnosis. It is easy to perform, don't need cell culture, the risk for misdiagnosis is low (~0.4%) and, principally, allow results in less than 24 hours.

We report the case of a young pregnant woman with an abnormal ultrasound scan, revealing a foetus with multiple anomalies, including cardiopathy, short femur and clenched hands. It was the first pregnancy of this young, health, non-consanguineous couple with a normal family history.

Amniocentesis was performed and FISH aneuploidy technique, in uncultured amniocytes, was requested. FISH result for the aneuploidies involving chromosomes 13, 18, 21, X and Y were normal. Cytogenetic analysis of amniotic fluid revealed an unbalanced translocation involving chromosome 11 and 18, with deletion of 11q24-qter and trisomy of 18q11.2-qter, confirmed by FISH.

The foetus had several anomalies consistent with trisomy 18 phenotype.

The FISH rapid prenatal aneuploidy test is, taken in account its possibilities and limitations, a powerful tool for the clinician in the care of pregnant women. However it has several limitations, it cannot detect cytogenetic abnormalities such as mosaics, translocations or rare aneuploidies. So, this technique should not be used as an independent stand-alone technology and must be performed in conjunction with standard cytogenetic testing for clinical diagnosis.

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AN INHERITED COMPLEX TRANSLOCATION DETECTED AT PRENATAL DIAGNOSIS

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We report a case of an unbalanced rearrangement, in a fetus with ultrasound abnormalities, as a result of malsegregation of a maternal complex translocation.

A 27 years old primigravida was referred to amniocentesis at 19 weeks of gestation after the detection of agenesis of corpus callosum, holoprosencephaly and spina bifida. The fetus had a duplication of 6pter-p25 and a deletion of 7q36.3-qter. Chromosome analysis of the parents showed a complex balanced translocation in the mother, involving chromosomes 5, 6 and 7. These results were confirmed by fluorescence hybridization (FISH) with subtelomeric and painting probes.

The post mortem examination confirmed a fetus with a multiple malformation syndrome including nuchal edema and hydrops; craniofacial anomalies with severe microcephaly, single nasal cavity, anomalous philtrum and cleft palate; alobar holoprosencephaly; bilateral cystic renal dysplasia and severe pulmonary hypoplasia.

The described anomalies are consistent with the phenotype previously reported for deletion of distal 7q and duplication of 6pter-p25. Haploinsufficiency of the sonic hedgehog gene at 7q36 probably accounts for the occurrence of holoprosencephaly.

It is very important in an apparently simple translocation, to further confirm by FISH or molecular analysis, since a complex rearrangement may be present, especially in the presence of ecographic anomalies in prenatal diagnosis.

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EXCLUSION OF inv(2)(p16.1;q14.3) AS THE CAUSE OF A SEVERE CONGENITAL DISEASE BY NEXT-GENERATION SEQUENCING

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Introduction Congenital anomalies, a leading cause of infant mortality in developed countries, are usually caused by genomic and/or chromosome rearrangements. Such rearrangements, like inversions, disrupt the genomic architecture at the breakpoint regions and can be either subclinical or pathogenic. Currently, the lack of a fully annotated genome hinders the prediction of phenotypical consequences of these anomalies.

Methods We report a familial pericentric inversion, inv(2)(p16.1;q14.3), in a proband presenting multiple psychomotor and developmental anomalies, dismorphism and autistic features, with phenotypically normal parents. Traditional analysis methods are labor intensive and of low resolution. Here we employed Next-Generation Sequencing (NGS) to identify breakpoints at nucleotide resolution in the proband, followed by familial segregation analysis by Sanger sequencing. Genomic and transcriptome array analysis were performed, for exclusion of further genomic alterations and for gene expression profiling.

Results The inversion breakpoints, at chr2:55,707,929 and chr2:123,010,109 (GRCh38), did not disrupt any gene or regulatory element and are flanked by *PNPT1* and *EFEMP1*, and *TSN* and *CNTNAP5*, respectively. No significant alteration in the expression level of possible candidate genes were observed. Aside from a polymorphic duplication, inherited from his father, no other pathogenic genomic imbalances were identified in the proband.

Discussion Based on these data, the causal relationship between clinical phenotype and the inversion is most likely excluded, as the inversion probably is nonpathogenic. It was yet not possible to establish the cause of the observed phenotype. The introduction of NGS represents a hallmark in the characterization of congenital disorders associated with chromosomal rearrangements.

This study was supported by FCT projects PTDC/SAU-GMG/118140/2010 and HMSP-ICT/0016/2013.

P 29 | Molecular Genetics

COMPLEX GENETIC STRUCTURE OF SOUTHEAST ASIAN POPULATIONS USING GENOME-WIDE DATA

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The region of Southeast Asia (SEA) harbours one of the highest levels of genetic diversity in the world. Its complex demographic history, tangled with multiple Holocene migrations and population displacements following the first Late Pleistocene settlement around 50-60ka, has led to the establishment of unique admixed populations. To deepen our knowledge about the genetic diversity of this region, which has been so far poorly surveyed for the nuclear DNA diversity, we analysed an extensive genome-wide dataset comprising almost 50 East/Southeast Asian populations (both new populations and published populations in the 1000 Genomes database and the Human Genetic Diversity Project database). Several methods were employed to evaluate the genetic structure of the populations (ADMIXTURE, STRUCTURE and PCA), the gene flow between populations (TreeMix, $f3$ statistics), and to estimate the admixture time between key populations in the region (ALDER). Overall, our genome-wide analyses revealed that SEA populations have a highly complex genetic structure, translated in several layers of genetic admixture most probably as a result of thousands of years of extensive gene flow and adaptations to new environments. Nevertheless, two main clusters are identifiable, dividing the genetic composition of continental and islands of SEA, which is consistent with the different migratory and demographic histories among those populations. Our results also contribute to clarify the genetic bases of many complex traits.

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PREVALENCE OF CF AND SPECTRUM OF *CFTR* MUTATIONS IN THE PORTUGUESE POPULATION

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Introduction In the Portuguese population the prevalence of Cystic Fibrosis (CF) is unknown as well as the spectrum of Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene mutations.
The main objective of this work was to evaluate the prevalence of CF and to establish the type and frequency of *CFTR* mutations in the Portuguese population.

Methods DNA was extracted from buccal mucosa cells of 512 Portuguese children, using a commercial kit. CFTR gene analysis was performed by Sanger Sequencing in 206 samples and by Next Generation Sequencing in 306 samples.

Results A total of 124 CF- and CFTR-related disorders (RD) mutations were identified. From de 512 samples analyzed, 90 had one mutation (17.6%) and 10 had two (1.9%). Taking into account only mutations included in the CFTR2 mutation database classified as CF-mutations or with varying consequences, the carrier frequency rate was 2.3%, being the most frequent mutation the p.Phe508del (0.98%). From the mutations detected, only the p.Phe508del is included in the screening commercial kits. The majority of mutations detected have been associated to CFTR-RD. The 5T allele (c.1210−12[5T]) was detected with an allelic frequency of 3.4%, followed by the complex allele p.Gly576Ala-p.Arg668Cys with 1.1%.

Discussion A wide spectrum of *CFTR* mutations was identified, confirming the highest *CFTR* allelic heterogeneity previously reported in Mediterranean countries. These results highlight the low power detection of the commercial kits for *CFTR* gene screening in our population. Additionally, this emphasizes the importance of the implemented Neonatal Screening Program last year in Portugal, as it facilitates the early diagnosis of symptom-free-newborns carrying mutations.

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GENETIC DEFECTS OF MITOCHONDRIAL DISEASES: A NEXT GENERATION SEQUENCING APPROACH

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Mitochondrial diseases are a group of rare inherited disorders characterized by extreme phenotypic heterogeneity that can be transmitted by any mode of inheritance, with hitherto no effective therapy options. Defects in mitochondrial energy production often have multisystemic effects and tend to preferentially affect organs with high energy requirements, such as the central nervous system, cardiovascular system, and skeletal muscle. It is estimated that 1:5,000 individuals will develop a mitochondrial disease. The molecular diagnosis in mitochondrial disorders is a great challenge and in the face of recent sequencing technological advances, time is ripe to facilitate the molecular investigations and to shed light on possible new etiologies.

Since 1993, our group, pioneer in the study of these disorders in our country, has been studying more than 2500 patients with suspicious diagnosis of mitochondrial disorders. The biochemical and molecular approach used, allowed the characterization of the majority of these patients, however, some of them still remain without molecular diagnosis.

The overall aim of our project, supported by FCT (PTDC/DTP-PIC/2220/2014), is to develop a Next Generation Sequencing strategy to identify nuclear disease causing-mutations in the cohort of uncharacterized patients. In a first approach we will create a custom mitochondrial genes panel that after validation will be used in patients with unknown molecular etiology. The patients that after this first approach remain undiagnosed will be further selected for Whole Exome Sequencing. With this approach we will provide comprehensive molecular characterization for mitochondrial disorders by using cutting-edge technology that will expand the mutational spectrum in the etiology of these disorders. The development of this panel will be innovative in our country strengthening our center as a national reference for the study and research of mitochondrial disorders.

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HUMAN UPF1 TRANSLATION INITIATION IS REGULATED BY A CAP-INDEPENDENT MECHANISM

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Gene expression is a very intricate process comprising several tightly regulated steps. One of those is translation initiation that, under normal circumstances, is mostly cap-dependent. However, some proteins can initiate translation via a cap-independent mechanism. This allows the maintenance of protein synthesis under conditions that reduce global protein synthesis. Human up-frameshift 1 (UPF1) has a key role in several cellular processes such as nonsense-mediated mRNA decay, telomere replication and homeostasis, and cell cycle progression, suggesting a tight regulation in order to prevent abnormal proliferation. These data suggest UPF1 might initiate translation in a cap-independent way, allowing the cell to overcome stress conditions that impair cap-dependent translation.

To test this hypothesis, we cloned the UPF1 5'UTR in a dicistronic vector and transfected cervical and colorectal cancer cell lines with either this construct or the control counterparts. We observed a 15- to 25-fold increase in relative luciferase activity of the UPF1 5'UTR-containing construct compared to the levels obtained from the empty counterpart in all tested cell lines, suggesting a cap-independent translation initiation. Cells transfected with in vitro transcribed mRNAs resulted in a 2-fold increase in protein levels, confirming translation can occur in a cap-independent way. This is maintained under conditions of global protein synthesis inhibition. Deletional analysis of the UPF1 5'UTR revealed that the minimal core required for cap-independent activity is present either within the first 100 nucleotides or within the last 125.

Further experiments are being undertaken to understand the biological role of a cap-independent mechanism for the translation of UPF1 and how it contributes to the roles UPF1 plays in the cell.

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CTNNA3 DELETIONS – SUSCEPTIBILITY TO AUTISM SPECTRUM DISORDERS?

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Introduction Autism spectrum disorders (ASD) are neurodevelopmental conditions showing extreme genetic heterogeneity. The *CTNNA3* gene (*alpha T-catenin*) maps to 10q21.3 and codes for a protein with multiple cellular roles including cell adhesion. The *CTNNA3* gene is one of the largest genes in the human genome and is located in a common fragile site (FRA10D). Fragiles sites are considered hot spots for genomic instability, associated with neuropsychiatric diseases including autism.

Methods 560 patients were analysed using the Agilent 4x180K microarrays and cytogenomics 2.9.2.4 software. The main clinical indications were intellectual disability, ASD, epilepsy, and multiple congenital abnormalities.

Results Partial deletions of *CTNNA3* were found in 7 of the 560 patients studied and were classified as VOUS. The CNVs sizes ranged between 23 Kb and 76 Kb with genomic intervals between 68,087,319 and 69,235,883 (GRCh37), involving exons 9 to 10, and intron 6, 11 and 13 of *CTNNA3* gene. Additionally, 2 out of 7 cases showed other relevant CNVs that were reported as pathogenic or presumably pathogenic.

Discussion Recent studies from Bacchelli *et al.* (2014) showed that heterozygous exonic deletions in the *CTNNA3* gene are not pathological however, homozygous or heterozygous compound exonic deletions/mutations affecting α-catenin function may contribute to ASD pathogenesis. Only 3 of 7 patients with *CTNNA3* deletions presented ASD, both involving deletion of exons 9 and 10. Screening for additional mutations in *CTNNA3* is warranted to elucidate the phenotype in these cases.

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POLYCYSTIC KIDNEY DISEASES (PKDS) – NGS APPROACH

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Introduction Polycystic kidney diseases (PKDs) are a group of inherited disorders mainly characterized by the development of renal cysts and progressive kidney dysfunction. PKD1 and PKD2 gene mutations account for most cases of autosomal dominant polycystic kidney disease (ADPKD). PKHD1 gene mutations cause autosomal recessive polycystic kidney disease (ARPKD). So far genetic testing for PKDs has mostly relied on laborious molecular tools using haplotyping, PTT and most recently Sanger. PKD1 mutation analysis is particularly challenging due to the complexity of its genomic structure. Although next-generation sequencing (NGS) is also challenging, we developed and validated a new assay for PKD1, PKD2 and PKHD1 gene analysis using NGS.

Methods The validation step involved a total of 4 patients with PKD with previously known PKD1, PKD2 and PKHD1 mutations. Three pools of 4 samples were sequenced with the Personal Genome Machine (PGM, Ion Torrent Thermofisher). For each sample, a total of 291 amplicons covering the entire PKHD1 coding sequence (CDS), 99.7% of PKD2 and 93,7% of PKD1 were amplified using an optimized Ion AmpliSeqTM Custom White Glove Panel.

Results Bioinformatic analyses were performed using an in-house developed pipeline. We were able to detect three mutations on patient 1 (PKD1: p.Leu117Val and p.Pro579Leu; PKD2: p.Asn375Ser), two mutations on patient 2 (PKHD1: p.Arg3240Gln and p.Ile222Val), one mutation on patient 3 (PKD1: p.Thr1993SerfsX56) and one mutation on patient 4 (PKD2: p.Gln61Ter).

Discussion With our Ampliseq panel we were able to detect all the seven previously known mutations, which allow us to conclude that NGS technology is effective for gene mutation screening of the three genes associated to PKD. This strategy significantly reduces the cost and turnaround time for simultaneous PKD1, PKD2 and PKHD1 sequence analysis, facilitating PKDs genetic diagnosis.

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KRABBE DISEASE: AN INTERESTING PATIENT WITH TWO LYSOSOMAL ENZYME DEFICIENCIES

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Introduction Krabbe disease (KD, MIM#245200) is an autosomal recessive lysosomal storage disease caused by galactocerebrosidase (GALC) enzyme deficiency, that leads to progressive white matter disease due to an impaired degradation of β-galactocerebroside, the major myelin lipid, and galactosylsphingosine. KD is a classic globoid cell leukodystrophy, one of the two classic genetic leukodystrophies, together with Metachromatic Leukodystrophy. The disease occurs among infants and takes a rapidly fatal course, but rarer late-onset forms also exist. Mutations in the GALC gene are the cause for non-functional enzyme. To date, 130 mutations in the GALC gene are reported worldwide, and an attempt to establish genotype-phenotype correlations has been reported in some molecular defects. The severity of signs and symptoms is influenced by causal mutations and corresponding residual enzyme activity.

Methods Laboratorial KD diagnosis was performed by GALC enzyme activity determination in leukocytes and GALC gene sequencing, to ascertain genotype characterization. Urine qualitative evaluation of undegraded lipids excretion was also performed.

Results This work reports biochemical and molecular data of an atypical Krabbe patient with both reduced GALC and arylsulfatase A activities in leukocytes. Marked reduction of arylsulphatase A activity was due to the presence of the pseudodeficiency allele p.N350S. Two causal pathogenic mutations, previously described, were found in exons 5 and 14 of GALC gene.

Discussion Genetic leukodystrophies may have an overlapping clinical phenotype, but when there is a clinical suspicion and the laboratory performs the assay, it should always include not only enzymatic assays in blood or cultivated fibroblasts but also urine excretion of undegraded lipids and molecular analysis of DNA samples, in order to detect causal mutations and pseudodeficient alleles. Once the genotype has been ascertained, genetic counseling can be offered to the family

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STROKE RISK IN CHILDREN WITH SICKLE CELL ANEMIA – THE IMPORTANCE OF GENETIC MODULATORS OF HEMOLYSIS

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Sickle cell anemia (SCA) is an autosomal recessive disease, caused by the mutation HBB:c.20A>T, originating hemoglobin (Hb) S that, upon deoxygenation, polymerises inside the erythrocyte, deforming it and leading to premature hemolysis. The disease presents high clinical heterogeneity, stroke being the most devastating manifestation. It occurs in 11% of patients by 20 years of age. In this study we aimed to identify genetic modulators of stroke risk in SCA.

Sixty six children with SCA were categorised according to their degree of cerebral vasculopathy: Stroke (n=13), Risk (n=29) and Control (n= 24). Relevant data were collected from patients' medical records. We characterized 23 polymorphic regions in genes related to vascular cell adhesion (VCAM-1, THBS-1, CD36), vascular tonus (NOS3, ET-1), and inflammation (TNF-α, HMOX-1) as well as in known globin expression modulators (HBB cluster haplotype; HBA and BCL11A genotypes). Data analyses were performed using R software.

VCAM-1 rs1409419 allele C and NOS3 rs207044 allele C were associated to stroke events, while VCAM-1 rs1409419 allele T was found to be protective. Allele 4a of NOS3 27 bp VNTR appeared to be associated to stroke risk and the 4b allele to protection. HMOX-1 longer STRs seemed to predispose to stroke. Higher HbF levels (associated to Senegal haplotype or BCL11A rs11886868 allele T) were found in Control group, and higher lactate dehydrogenase levels were found in Risk group.

The genetic variants above modulate cerebral vasculopathy development due to their quantitative effect on gene expression, their corresponding protein products and biological activities. Our findings reinforce the relevance of vascular tonus, vascular cell adhesion, and ultimately NO bioavailability and hemolysis rate in modulating SCA stroke development and provide the first evidence of a protective role of HbF against stroke occurrence.

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GENETIC VARIANTS IN ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE ARE MODIFIERS OF THE HEMOLYSIS PHENOTYPE IN SICKLE CELL ANEMIA

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Sickle Cell Anemia (SCA) is an autosomal recessive hereditary anemia characterized by the presence of hemoglobin S (Hb S). This disease is caused by a single mutation in beta-globin gene with a corresponding amino acid substitution at the sixth position of the beta-globin chain. The easily ability of Hb S to polymerize in deoxygenated conditions gives rise to abnormal sickled red blood cells. Vaso-occlusion and hemolytic anemia are the major features of this disease, however SCA patients present clinical and hematologic variability that cannot be only explained by the single mutation in the beta-globin gene. Others genetic modifiers and environmental effects are important in the clinical phenotype.

We have studied the association between hematological and biochemical parameters (Hb S, total Hb, red cell distribution width (RDW), neutrophils, transmembrane reductase, methemoglobin reductase, serum lactate dehydrogenase (LDH), total bilirubin and reticulocyte count) and some genetic variants, from several candidate genes, in 26 paediatric SCA patients.

Our results show a significant statistical association between two *endothelial nitric oxide synthase* (*eNOS*) single nucleotide polymorphisms (SNPs) and two haemolysis parameters. Both the rs2070744_TT and the rs1799983_GG genotypes are associated with an increased reticulocyte count ($p = 0.02$ and 0.01, respectively) and higher serum LDH level ($p = 0.04$ and 0.04, respectively).

Our findings suggest that polymorphisms in the *eNOS* gene may act as genetic modifiers of the haemolysis process that could provide utility for the prediction of increased susceptibility to haemolysis-related complications. Furthermore, our results reinforce the importance of nitric oxide (NO) bioactivity in SCA. We presume that NO, and possible its precursors such as L-arginine or L-citrulline, might be used as pharmacological tools to improve the quality of life of these patients.

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CONTRIBUTION OF WESTERN BLOT STUDIES IN THE MOLECULAR DIAGNOSIS OF MUSCULAR DYSTROPHIES

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Introduction Muscular dystrophies are a group of inherited disorders of muscle, generally characterized by progressive muscle damage and weakness. In diagnosis, to reach a precise genetic defect is often complex due to the large number of candidate genes and the occurrence of overlapping clinical presentations. In an attempt to overcome these difficulties, our laboratory implemented a multiplex western blot (WB) approach to study different muscular proteins (dysferlin, calpain-3, dystrophin, sarcoglycans, ...). This methodology has been used to analyse protein abundance and/ or to check functional activity.

Methods We present examples of three patients subjected to WB studies. Patients 1 and 2, clinically and histologically compatible with a Becker muscular dystrophy (BMD), were negative for dystrophin gene (DMD) testing. Multiplex WB was conducted to evaluate the abundance of 8 proteins. Patient 3 had a clinical presentation compatible with calpainopathy and presented a single heterozygous missense variant in the calpain-3 gene (CAPN3). Calpain-3 abundance and autolytic activity was assessed in order to determine its causal nature.

Results Patient 1 presented absence for dystrophin as well as for alfa-sarcoglycan. Since alfa-sarcoglycan gene (SGCA) sequencing showed no pathogenic variants, DMD testing proceeded to mRNA studies, revealing a deletion in the 3´end of the gene. Patient 2 had normal dystrophin but an unexpected absence of the full-length dysferlin band. These results prompted dysferlin gene (DYSF) mutation screening, where a homozygous mutation was detected. Patient 3 presented loss of calpain-3 autolytic activity, despite its normal expression in quantitative terms, thereby confirming the variant´s damaging effect.

Discussion The protein analysis/testing exemplified in this work highlights the importance of WB studies in this group of muscle disorders, orientating the molecular genetic testing and helping to predict the effect of novel mutations.

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INSIGHTS FROM THE STUDY OF A FAMILY WITH FABRY DISEASE – GOOD AND BAD NEWS

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Introduction Fabry disease (FD, MIM#301500) is a lysosomal storage disorder caused by deficient or absent α-galactosidase A (α-GalA) enzymatic activity, leading to accumulation of neutral sphingolipids, mainly globotriaosylceramide (Gb3). α-GalA is encoded by the GLA gene, located at Xq22.1. The gold standard for FD diagnosis in males is α-GalA enzymatic activity, usually performed in peripheral blood leukocytes and plasma, dried blood spots (DBS) or cultured skin fibroblasts. In females, mostly due to possible skewed X chromosome inactivation, α-GalA enzymatic activity is unreliable and, for that, molecular analysis of GLA is mandatory. Presently, about 780 mutations have been described in GLA gene, most of them are private mutations. Mutations leading to major structural changes (nonsense, splicing, frameshift and large indels) are easily accepted as disease causing. However, for others, namely missense mutations not affecting the catalytic site, it requires careful evaluation of the role they play in protein folding.

Materials and methods Peripheral blood and 24 hours urine from the index patient and nine relatives was studied, for α-GalA enzymatic activity, GLA analysis and Gb3 measurement.

Results The biochemical phenotype segregated with the c.827G>A (p.S276N) mutation in the family. The plasma α-GalA enzymatic activity was normal in all males carrying this mutation, while it was reduced in their leukocytes and DBS.

Discussion To our knowledge, this is the second family carrying the c.827G>A (p.S276N) mutation worldwide, adding some evidence regarding its pathogenicity. Moreover, it allowed us to conclude that this mutation doesn't affect α-GalA enzymatic activity in plasma. Therefore, caution must be taken in FD screenings based on plasma α-GalA enzymatic activity as this leads to false negative results and prevent patients to benefit from therapeutic options presently available.
A hypothesis explaining this dual behavior of α-GalA is presented.

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GENETIC DIAGNOSIS OF EPILEPTIC ENCEPHALOPATHY USING AN EXTENDED NGS PANEL

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Epileptic encephalopathies (EE) are a phenotypically and genetically heterogeneous group of severe epilepsies accompanied by intellectual disability and other neurodevelopmental features. Here, we describe the implementation of a NGS gene panel into diagnostic routine, containing 45 EE-associated genes, including MECP2 and UBE3A. Mutations in MECP2 are responsible for Rett syndrome (RTT), which is a rare X-linked neurodevelopmental disorder affecting 1/10,000 to 1/15,000 females and that can cause a more severe neonatal encephalopathy with seizures in male patients.

In a group of patients, we amplified all exons of the 45 genes with conventional primers, pooled and sheared during library preparation, and sequenced on the Ion Torrent PGM with a minimum coverage of 40x. Sequencing data was analysed from FASTQ files using JSI SeqNext software. All pathogenic variants were confirmed by Sanger sequencing.

Here, we report a positive case of a 2 years old girl with tonic-clonic seizures, acquired microcephaly, severe development delay with absent speech and unilateral hand stereotypies, clinically suspected as having Angelman syndrome but without 15q11.2-q13 deletion. Using NGS, we found one nonsense mutation in exon 4 of the MECP2 gene (c.763C>T; p.Arg255*), previously described as a Rett syndrome-causing mutation. The c.763C>T is a common nonsense mutation which substitutes a conserved arginine (CGA) by a stop codon (TGA) leading to a truncated MeCp2 protein.

Angelman syndrome can be one of the differential diagnoses of RTT, when the main features are acquired microcephaly, epilepsy and speech impairment. In atypical clinical cases, one of the tools for differential diagnosis can be an extended gene panel, given the genetic and phenotypic heterogeneity in EE. Therefore, our extended panel proved to be a useful diagnostic tool to this patient, thereby allowing a better disease management and improved genetic counselling for this family.

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MULTI-EXON DUPLICATION OF F9 GENE FOUND BY MLPA IN A PREVIOUSLY UNSOLVED CASE OF SEVERE HEMOPHILIA B

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Introduction Hemophilia B is an X-linked bleeding disorder caused by molecular defects in the Factor IX gene (F9), leading to qualitative and/or quantitative deficiency of Factor IX (FIX). Over 1000 different mutations have been reported, with point mutations being predominant, but including insertions, deletions and complex mutations. Complete or large deletions in the F9 gene lead to severe phenotypes and account for ~2% of the alterations.

Subjects and Methods Two brothers with severe hemophilia B and a sister with low FIX:C levels were studied. Genomic DNA was extracted from peripheral EDTA blood samples. Molecular analysis was initially performed in the two affected brothers by PCR amplification followed by Sanger sequencing of fragments including the entire coding region, flanking intronic sequences, untranslated leader sequence and a segment of the putative promoter of F9. Copy number analysis was performed in DNA samples from the three individuals using a MLPA (Multiplex Ligation-dependent Probe Analysis) kit including probes specific for the eight F9 exons.

Results and Discussion Sequencing analysis did not show any mutation in F9 that could cause the severe hemophilia B in the two patients, with one of them being analyzed twice, from independently collected blood samples. As such, subsequent copy number MLPA analysis was performed, revealing a large duplication spanning exons 2 to 6 of F9. MLPA also allowed to state the heterozigosity for the duplication in the sister, to whom this information is particularly relevant for genetic counseling.
Although it was not possible to establish the breaking points leading to this duplication, the fact that it segregates with the FIX deficiency in the family support the hypothesis of a dysfunctional gene due to the fact that duplication is within F9. This study highlights the utility of MLPA to elucidate the molecular basis of rare FIX deficiency cases unsolved by sequencing.

P 42 | Cancer Genetics

THE VALUE OF MACROH2A1 AS BIOMARKER FOR PROSTATE CANCER

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Introduction Prostate cancer (PCa) is the most common noncutaneous malignancy in men and a major cause of cancer-related morbidity and mortality worldwide. Understand the genetic and epigenetic mechanisms support the development of new and improved diagnostic and prognostic tools and, therefore, most effective treatment. H2AFY gene encodes two isoforms of the H2A histone variant macroH2A1. MacroH2A1.1 inhibits cell proliferation and cell migration, whilst macroH2A1.2 has opposite functions. Previous studies associated this histone variant with various cancers, but none with PCa. Thus, we investigated whether macroH2A1 is implicated in prostate carcinogenesis and assessed its putative diagnostic/prognostic value.

Methods Transcripts levels of macroH2A1 isoforms and splicing regulators from 15 morphologically normal prostatic tissues (MNPT), 197 primary PCa and 45 high-grade prostatic intraepithelial neoplasias (PIN) were assessed using RT-qPCR.
Histopathological assessment was performed blinded to molecular results and relevant patient data were collected from clinical charts. Protein levels of macroH2A1.1 were assessed in the same series by immunohistochemistry.

Results MacroH2A1.1 mRNA was downregulated in PIN and primary PCa compared to MNPT ($p=0.003$ and $p<0.0001$, respectively). Interestingly, a similar result was found for QKI, a splicing regulator that induces macroH2A1.1 expression. Moreover, both genes efficiently identified PCa, with 90% sensitivity and specificity. QKI and macroH2A1.1 expression levels were significantly associated with Gleason score. MacroH2A1.1 transcript levels were also associated with serum PSA levels. Regarding MacroH2A1.1 protein levels, PCa samples showed significantly lower imunoexpression than PIN lesions.

Discussion MacroH2A1.1 and QKI transcript levels downregulation is associated with malignant transformation in prostate. Moreover, they might be useful as diagnostic biomarkers for PCa.

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CHARACTERIZATION OF BODY COMPOSITION, QUALITY OF LIFE, DAILY PHYSICAL ACTIVITY, ANXIETY AND DEPRESSION IN PATIENTS WITH HEAD AND NECK CANCER: PRELIMINARY RESULTS

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Introduction Head and neck cancer (HNC) patients experience unintentional weight loss, dysphagia and fatigue with impact on quality of life and survival rate. We aimed to characterize and correlate total body composition, quality of life, daily physical activity, anxiety and depression in HNC patients.

Methods Eight consecutive HNC patients (7 males and 1 female; age, 61.6±10.2 years old; weigh, 65.5±10.4 kg; height, 1.66±0.09 m; body mass index, 24.1±5.7 kg/m2) were recruited from Maxillofacial Surgery Department, Coimbra Hospital and University Centre before any treatment. Outcome measures included demographic and medical variables, body composition (assessed by bioimpedance), daily physical activity (International physical activity questionnaire), quality of life (QLQ30–H&N35 module), anxiety and depression symptoms (Hospital Anxiety and Depression Scale-HADS).

Results and Discussion Patients presented 23.4±13.5% of fat mass, 77.8±11.5% of fat free mass, 22.3±4.3kg of muscle mass in the upper and lower limbs, 55.9±9.0% of body water. In general patients spent a total of 247.5±203.9 min/day in sitting activities, and performed a total of 5323.9±9227.1 MET-min/week of physical activity; being 2520.0±6748.1 MET-min/week spent in vigorous activities, 465.0±667.9 MET-min/week in moderate activities and 2338.9±2810.6 MET-min/week in walking. Two patients showed anxiety and four presented depression symptoms. The global heath quality of life of the patients was 46.9±34.5 points, scoring 74.4±23.6 on functional scales and 12.3±8.3 on symptom scales. A significant correlation was found between sedentary time and symptom scales ($r=0.818$, $p=0.0139$), i.e. more time spent in sitting activities is associated with higher symptom scales score. In conclusion, from questionnaires patients showed good functionality and low symptoms scores, although 50% showed depression symptoms. These preliminary findings highlight the need of physical activity for better symptoms management.

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A CANCER MODEL DISEASE IN A NEW NETWORK: THE PORTUGUESE FANCONI ANEMIA RESEARCH NETWORK (PFARN)

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Fanconi anemia (FA) is a heterogeneous genetic disorder, characterized by mutations in one of the 17 FA genes described so far. The FA proteins function in a common pathway maintaining genomic stability. The clinical manifestations in FA patients are heterogeneous although all share progression to bone marrow failure (BMF). Importantly, all the FA patients show increased predisposition to cancer and some of the FA genes are well characterized cancer suppressor genes, such as FANCS (or BRCA1) or FANCD1 (or BRCA2). This makes FA an attractive model for cancer research. At the cellular level, chromosome instability (CI) is the hallmark of FA cells due to defects in DNA repair (the main function of FA proteins). This hallmark is expressed through the hypersensitivity of FA cells to DNA crosslinking agents, such as diepoxybutane (DEB), providing a unique diagnostic marker.

We present the results from the DEB induced CI studies performed at the Laboratory of Cytogenetics, ICBAS, from 1992 to 2015. Blood samples from 485 patients were obtained for confirmation/exclusion of FA. Until now, a total of 65 FA patients were diagnosed. The first aim after diagnosis is to study the gene affected in each Portuguese patient. For 25% of the patients this study was already performed. In order to expand it for the whole population, complex molecular and cellular assays are being implemented at i3S. Further competitive studies about FA will be performed inside a new network recently created: the Portuguese Fanconi Anemia Research Network (PFARN). This network will organize FA informative programs and will give to the Portuguese FA patients the possibility to be included in innovative therapies that PFARN has already access via international collaborators (for more information see http://www.eurofancolen.eu).

In summary, our aim is to introduce PFARN, and to share our purposes and actions with all the clinic and scientific community interested in FA diagnosis and research.

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PRIMARY TONGUE TUMOR AND SECOND PRIMARY TUMOR IN THE FLOOR OF THE MOUTH IN THE SAME PATIENT - THE DISCRIMINATORY POWER OF THE GENES

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Introduction In Oral Cavity tumors recurrences are frequently diagnosed within the first two or three years after initial treatment and the majority are local and regional. After the third year, the diagnosis of a second primary tumor becomes an important cause of morbidity and mortality.

Methods We identified the genomic and epigenetic profile of a primary tongue tumor and of a second primary tumor in the floor of the mouth of the same patient, diagnosed four years later. We analyzed tumor and macroscopically tumor free tissue from surgical margin, of both tumors. The genomic and epigenetic study was conducted using array-Comparative Genomic Hybridization and Methylation-Specific Multiplex Ligation-dependent Probe Amplification.

Results and Discussion The patient was 49 years old male at the time of the primary tumor diagnosis and a heavy smoker (≥20 cigarettes/day). The primary tumor was already in advanced stage when diagnosed (IVa), with lymph nodes invaded. The treatment was radiotherapy and chemotherapy. Regarding the genomic and epigenetic profile, we identified methylated genes and copy number alterations in tumor and macroscopically tumor free tissue of both tumors. Some alterations presented in the macroscopically tumor free tissue were also present in the tumor. In general there are several genomic and epigenetic differences between both tumors. In the second primary tumor we identified more aberrations than in the primary tumor. Moreover, we also observed in the second primary tumor methylation and copy number alterations in specific chromosomal regions and genes related with worse prognosis. In conclusion, genomic and epigenetic profile of tumors in different anatomic subsites seems to be different, even in the same patient exposed to the same risk factors. The molecular signatures seem to be pivotal to help in the early detection of these tumors and to predict tumor behavior.

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PREDICTORS OF PROGNOSIS IN ORAL SQUAMOUS CELL CARCINOMA: THE ROLE OF DNA METHYLATION

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Introduction Oral squamous cell carcinoma (OSCC) is a worldwide serious health problem due to the high mortality rates and severe impact in the survivors' quality of life. To improve OSCC outcomes is crucial the identification of molecular biomarkers driving disease initiation and progression. Methylation seems to be an early event in the development of OSCC and can thus be regarded as an early sign of cancer before the dissemination of the disease. Moreover, DNA methylation is a promising and interesting therapeutic target due to its reversibility characteristic since the genes that are silenced are still intact and consequently can be reactivated. We aimed to identify and correlate specific OSCC genetic and epigenetic profiles with the patients' clinic-pathological features.

Methods Biopsies of oral tumors were acquired from 93 OSSC patients and gingival samples from 11 healthy donors were used as controls. Methylation-Specific Multiplex Ligation-dependent Probe Amplification was conducted to screen copy number alterations and DNA methylation patterns in 54 tumor suppressor genes.

Results and Discussion We identified specific genetic and epigenetic profiles correlated with the patients' clinic-pathological features. We found methylated genes with discriminative power not only for tumors at early stage but also for tumors in advanced stage. Thus, we identified methylated genes with propensity for the development of metastasis/relapses during/after treatment, which consequently presented statistically significant association with shorter survival rate. Simultaneously, the most frequent copy number alterations were located at chromosomes 3, 9, 12, 16, 18, 17 and 19, being possible to identify different genetic profiles between smokers and non-smokers patients. The combination of genetic and epigenetic studies together with the pathological diagnosis seems to be mandatory not only to early detect these tumors and relapses but also to predict their prognosis.

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INHERITED COLORECTAL CANCER - VALIDATION OF MOLECULAR DIAGNOSIS BY NEXT GENERATION SEQUENCING

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Introduction Colorectal cancer is the third major cause of cancer related deaths worldwide. Around 5% of these cases are due to Inherited Colorectal Cancer (ICC) associated with highly penetrant single-gene mutations. Conventional molecular analysis of patients with ICC is well established and usually comprises PCR followed by Sanger sequencing of different genes with autosomal dominant inheritance - MLH1, MSH2, MSH6, PMS2 and EPCAM-3' deletions in Lynch syndrome, APC in Familial Adenomatous Polyposis (FAP), or the study of an autosomal recessive condition with colorectal polyps associated with MUTYH variants. As standard molecular methodologies have high costs and are time consuming, they are progressively being replaced by Next Generation Sequencing (NGS), which allows the analysis of multiple genes simultaneously and with lower costs compared to Sanger sequencing.
In order to validate NGS analysis for a set of genes associated with ICC, we performed NGS in 26 DNA samples from patients previously analysed by Sanger sequencing.

Methods NGS was performed using the Trusight Cancer Sequencing Panel and the MiSeq sequencer (Illumina), followed by bioinformatic analysis of the MLH1, MSH2, APC, MUTYH and STK11 genes using the MiSeq Reporter, VariantStudio and Isaac Enrichment tools.

Results Data analysis revealed 77 variants (31 unique, comprising 4 deletions, 1 insertion, 2 indels and 24 single nucleotide variants). Of these, 76 variants were previously identified by Sanger sequencing. NGS produced a false positive result associated with low coverage in STK11 (c.375-49G>A).

Discussion Results obtained by NGS are consistent with Sanger sequencing and showed high analytical sensitivity and specificity. Therefore after this initial validation, with high repeatability, conventional molecular analysis can be replaced by NGS, allowing us to offer the possibility to screen more genes, at lower costs and with a shorter turnaround time.

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ANALYSIS OF OVARIAN CARCINOMAS FOR SOMATIC AND GERMLINE BRCA1/2 MUTATIONS BY NEXT GENERATION SEQUENCING

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Olaparib is a PARP inhibitor that is indicated, in the EU, as monotherapy for maintenance treatment of patients with platinum-sensitive, relapsed, BRCA-mutated (germline or somatic), high-grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancer. In this study we aimed to test the feasibility of analyzing ovarian FFPE tumor tissue for BRCA1/2 mutations by NGS.

We used the BRCA Tumor MASTR Plus assay (Multiplicom) that provides BRCA1/2 full exon and adjacent intronic regions coverage. A total of 8 ovarian FFPE tumor samples were run on a MiSeq, 4 of them with a germline pathogenic BRCA1/2 mutation originally detected in routine diagnosis by Sanger sequencing (positive controls) and 4 with unknown BRCA mutational status. All mutations identified were confirmed by Sanger sequencing in tumor tissue and peripheral blood DNA was tested when available to determine if the mutations were somatic or germline.

We obtained 100% coverage of BRCA1/2 regions with >100x coverage and an overall mean average read depth of about 2000-fold. In the 4 positive controls we were able to confirm the presence of the mutation in the tumor tissue. In the 4 tumor samples with unknown BRCA status we identified one pathogenic BRCA1 frameshift mutation (peripheral blood DNA not yet available) and two variants of unknown significance: an in-frame deletion in BRCA2 not detected in the patient peripheral blood DNA and a missense mutation in BRCA2 also detected in the patient peripheral blood DNA. LOH could be inferred from NGS data in two tumors with the same BRCA1 pathogenic mutation.

In this study we show that BRCA1/2 mutation analysis is possible in DNA extracted from FFPE tissue using NGS. All the germline mutations from the positive controls were identified in tumor tissue and, in addition, we identified mutations in 3 out of the 4 samples with unknown BRCA mutational status, making possible the evaluation of indication for PARP inhibition therapy.

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TARGET GENE MUTATIONAL PATTERN IN LYNCH SYNDROME COLORECTAL CARCINOMAS ACCORDING TO TUMOR LOCATION AND GERMLINE MUTATION

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Introduction We previously reported that the target genes in sporadic mismatch repair (MMR) deficient colorectal carcinomas (CRC) in the distal colon differ from those occurring elsewhere in the colon. This study aimed to compare the target gene mutational pattern in microsatellite unstable (MSI) CRC from Lynch syndrome patients stratified by tumor location and germline mutation, as well as with that of sporadic disease.

Methods A series of CRC from Lynch syndrome patients was analyzed for MSI in genes predicted to be selective MSI targets and known to be involved in several pathways of colorectal carcinogenesis.

Results The most frequently mutated genes belong to the TGFβ superfamily pathway, namely ACVR2A and TGFBR2. A significantly higher frequency of target gene mutations was observed in CRC from patients with germline mutations in MLH1 or MSH2 when compared to MSH6. Mutations in microsatellite sequences (A)7 of BMPR2 and (A)8 of MSH3 were significantly more frequent in distal CRC. Additionally, we observed differences in MSH3 and TGFBR2 mutational frequency between Lynch syndrome and sporadic MSI CRC regarding tumor location.

Discussion Our results indicate that the pattern of genetic changes differs in CRC depending on tumor location and between Lynch syndrome and sporadic MSI CRC, suggesting that carcinogenesis can occur by different pathways even if driven by generalized MSI.

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DNA METHYLATION CHANGES IN HNSCC CELL LINES AFTER RADIATION TREATMENT

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Introduction Head and Neck Squamous Cell Carcinoma (HNSCC) is considered the sixth most malignant tumour worldwide and, despite clinical and technological advances, the five-year survival rate has not improved much. Radiotherapy is a common treatment for HNSCC, however it has drawbacks, such as radioresistance and tissue toxicities. Although it has been used for many years, there is not much knowledge about radiation effects on tissues. Anyhow, it is known that it causes genomic instability, such as changes in DNA methylation. Identify and understand alterations caused by irradiation are pivotal for decreasing toxicities in patients and enhance treatment efficacy.

Methods Two commercial cell lines, a metastatic and a non-metastatic, were used in this study. Both cell lines were fully characterized in our lab by cytogenetics and cytogenomics. They were exposed through different doses of irradiation, ranging from 0,5 to 15 Gy. Cell viability was assessed by clonogenic assay. Methylation profile was assessed by Methylation-Specific Multiplex Ligation-dependent Probe Amplification before and after radiation.

Results The metastatic line is more radiosensitive than the non-metastatic line since, for the same irradiation, the metastatic line showed a higher decrease in colony formation. After all doses of irradiation all the methylated genes remained methylated. The metastatic cell line suffered methylation of ATM after a 2 Gy dose. At different irradiation doses, both cell lines show methylation of MSH6.

Discussion MSH6 methylation seems to be an important alteration induced by irradiation, perchance leading to different outcomes accordingly to dose applied and cells intrinsic characteristics. ATM is essential for cell survival after irradiation, so it is possible that 2 Gy could induce an adaptive response. Further studies can improve the understanding of their involvement in radiation outcome and help developing targeting therapies, improving radiation outcome.

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GENOMIC CHARACTERIZATION OF LARYNGEAL SQUAMOUS CELL CARCINOMA

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Introduction Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignant tumour in the world and it can arise from the larynx, leading to the impairment of vital functions such as breathing, swallowing and speaking. Also, laryngeal squamous cell carcinoma (LSCC) is usually diagnosed in a late stage. Solid tumours, such as LSCC, result from a multistep process where genetic alterations are accumulated. However, they are usually studied as part of head and neck cancers and so one of the main challenges is to identify specific tumour markers that will help to distinguish laryngeal tumours from other cancers included in head and neck family and ultimately to improve survival rates and preserve the function of the larynx.

Methods DNA was extracted from eight fresh-frozen tissue samples of laryngeal tumors, collected from patients with LSCC, after surgery. Copy number variations were assessed by Array Comparative Genomic Hybridization and two sex-matched controls of palatine uvula obtained from patients without clinical history of cancer were used.

Results and Discussion The results showed several structural rearrangements which were most frequently located in the chromosomes 3, 8, 9, 11, X and Y. Among these alterations, the most common imbalances were loss of chromosomal regions 3p, 9p21.3, Yp and Yq while the most common gains were located in 3q, 8q, 11q13, 14q13.1 and chromosome X. The gain of 11q13 and 8q24.21 were observed in 75% of the patients and both of the regions code genes whose role in LSCC remains poorly understood, such as *PVT1*, *MIR1204*, *ANO1* and *PPFIA1*. Alongside, the total or partial loss of chromosome Y was observed in 85,7% of the male samples. Our study revealed several chromosomal alterations that may have a role in the development of laryngeal carcinoma.

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HNSCC METASTATIC AND NON-METASTATIC CELL LINES: CAN CYTOGENETIC ABNORMALITIES UNVEIL THEIR BEHAVIORAL DIFFERENCES?

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Introduction Head and Neck Squamous Cell Carcinomas (HNSCC) represent about 90% of all Head and Neck Cancers and are considered the sixth most malignant tumours worldwide, with an estimative of 600 000 new cases every year. Despite clinical and technological advances, the five-year survival rate has not improved much in the last years. Commercial cell lines characterization has obvious benefits, since they are one of the most used model in biomedical studies. As such, genetic characterization is a necessity to have as much information as possible about the cell lines, especially if they are used for translational research.

Methods Metastatic and non-metastatic cell lines were cultured in DMEM supplemented with 10% FBS and 1% of penicillin and streptomycin. For the non-metastatic, 1% of hydrocortisone was also added. The genetic characterization was performed by karyotyping and array Comparative Genomic Hybridization (aCGH).

Results and Discussion Our results showed the presence of copy number variations (CNV) on the non-metastatic cell line that were associated with early events and progression from a dysplasia to a carcinoma in situ stage, as the case of 11q distal loss. The metastatic cell line presented alterations at nearly every chromosome, including alterations associated with a metastatic stage, such as 1q and 3q gains. As expected, the non-metastatic line showed less CNV. Additionally, the metastatic cell line showed karyotypes with about 57 chromosomes, whereas the non-metastatic showed only 41 chromosomes. Our results between aCGH and karyotyping were concordant. We conclude that both techniques are helpful for genetic characterizations and their results are complementary. Furthermore, both lines presented CNV that were associated with the carcinogenesis model suggested for HNSCC. The characterization of these cell lines is the base for further studies we want to pursue in the area of radioresistance and pharmacogenetics.

P 53 | Cancer Genetics

VALIDATION OF NEXT-GENERATION SEQUENCING FOR THE DIAGNOSIS OF HEREDITARY BREAST AND OVARIAN CANCER

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Introduction Molecular diagnosis of hereditary breast and ovarian cancer (HBOC) has been mostly based on the identification of germline inactivating mutations in the high-penetrant genes BRCA1 and BRCA2. Although several other HBOC susceptibility genes have been identified, mutations in any of those are rare, rendering sequential genetic testing with standard methodologies time consuming and expensive. Next-generation sequencing (NGS) gene panels allow the simultaneous sequencing of multiple HBOC susceptibility genes at a lower cost. The aim of this work was to validate the use of an NGS cancer susceptibility gene panel for the identification of mutations previously detected by Sanger sequencing in the BRCA1, BRCA2 and TP53 genes.

Methods 20 samples from patients with personal/family history of breast cancer were sequenced on a MiSeq using the Trusight Cancer Sequencing Panel (Illumina). Bioinformatic analysis of NGS data included the MiSeq Reporter, VariantStudio and Isaac Enrichment tools (Illumina).

Results NGS successfully identified all 204 variants (38 unique, including 2 deletions and a splice variant) previously detected by Sanger sequencing in the BRCA1, BRCA2 and TP53 genes. Until now, no false-negative or false-positive results were obtained.
Discussion These results demonstrate the high analytical sensitivity and specificity obtained with NGS for the detection of sequence variants in 3 HBOC high-penetrant genes. These validation assays open the way to the definition of a clinically useful multigene panel for HBOC susceptibility based on the Trusight Cancer Sequencing Panel. This will allow a comprehensive and cost-effective molecular diagnosis of HBOC with a shorter turnaround time when compared to standard methodologies. In addition, with appropriate genetic counselling and specialized clinical surveillance, families with HBOC will benefit from these new technologies which have high impact in public health.

P 54 | Quality Control and Public Services

EVALUATING THE EUROPEAN UNION COMMITTEE OF EXPERTS ON RARE DISEASES JOINT ACTION (EJA)

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Introduction The EJA aims to assist the European Commission in formulating and implementing activities in the field of rare diseases (RD), to foster exchanges of relevant experience, policies and practices between the Member States and stakeholders. Within the EJA, an evaluation work package (WP) was planned in order to monitor its evolution and to evaluate EJA impact in the field.

Methods We used a Logic Model to establish the evaluation plan. This plan was developed with the involvement of all partners in a participative approach and included the definition of process, output and outcome indicators. For monitoring, specific tools - as web-based questionnares and methods - workshops, were designed to assess the activities and deliverables implementation.

Results The EJA started March 2012 and will end August 2015 and to date three monitoring moments took place. 58 milestones and 37 deliverables were planned. The majority of them (Until April 2014, 72.4% milestones and 61.8% deliverables) are fully accomplished.
Regarding the workshops, the main perception of the participants is that this method is useful and with practical applicability. Concerning the use of the contents, 31%, of the individuals stated that they will use them in three months, 19% in 6 months 32% in a year.

Discussion Process monitoring indicates that the project is on schedule or even ahead of it. In relation to outcome indicators the evaluation is still ongoing. One limitation of this evaluation is that the real impact of EJA will not be measurable during the project duration. Instead, proxies will be used to give an approximate measure. The involvement of all the partners in the early stages the monitoring plan, using a participatory methodology, is essential for the success of evaluation process. A multidimensional approach that includes process monitoring but also partners and other stakeholders' perception should be implemented to accomplish a comprehensive evaluation.

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RARE DISEASES IN EUROPE: THE PORTUGUESE FRAMEWORK

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Introduction The EUCERD Joint Action working for Rare Diseases (EJA) consists on 5 domains: national Rare Diseases (RD) plans/strategies, international RD nomenclatures, specialized social services, quality of care/centres of expertise and integration of RD initiatives.

Methods A workshop was held in Portugal, November 2014, which included participants from 8 countries. 5 sessions took place where the European state of art of every domain was counterbalanced with the Portuguese reality. The participants were European authorities, EJA's partners, researchers, health care and public health professionals and patients' representatives. A qualitative data analysis of the presentations contents was performed.

Results In relation to plans and strategies, a National Integrated Strategy for RD 2015-2020 was approved and encompasses healthcare and diagnosis, research and social dimensions. As regards to nomenclature, health professionals use different coding systems. A proposal for the ORPHA number system to be adopted in disease nomenclature was discussed. Among specialized social services, good practices examples were described. Concerning RD initiatives and quality of care, the main initiative is the development of a personal RD ID card with the patient's data. Finally, the creation of the European Reference Networks between healthcare practitioners and the Reference Centres (RC) of the Member States, exploring the possibilities of European cooperation in highly specialised healthcare fields was discussed. In Portugal, legislation has been published defining specific criteria to recognize RC.

Conclusions Regarding the different aspects related to RD, Portugal has been developing several activities but integration needs to be done. The National Integrated Strategy for RD was designed to address this gap and the implementation is ongoing. The final aim is in a progressive way, a real change in the complex conditions of the people who suffer from these diseases.

NOTAS

NOTES

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