

# SOCIEDADE PORTUGUESA DE GENÉTICA HUMANA



## 16ª Reunião Anual Livro de Resumos



22 a 24 de Novembro de 2012  
Porto, Portugal

16ª Reunião Anual  
da Sociedade Portuguesa de  
Genética Humana

Porto, 22 a 24 de Novembro de 2012

Casa do Médico, Centro de Cultura e Congressos da Ordem dos Médicos







Dear colleagues,

We would like to welcome you to the 16<sup>th</sup> Annual Meeting of the Portuguese Society of Human Genetics (SPGH), which takes place in the *Ancient, Very Noble, Always Loyal and Invincible* city of Porto, a UNESCO World Heritage Site. The SPGH Board and the Scientific Committee made every effort to organize an excellent program, with conferences by leading international and national specialists on the latest scientific developments in human genetics, as well as presentations by younger researchers.

We hope that the 16<sup>th</sup> SPGH Annual Meeting will provide a forum for discussion and update in several aspects of human genetics. This is particularly important for young researchers, medical residents, post-docs and students, who otherwise may have few opportunities to interact with leading scientists in this field of knowledge. This year's program also includes sessions coordinated by the SPGH Committee of Public Policy and Education in Genetics and the SPGH Committee on Bioethics to discuss topics of interest to our members and other professionals in the field of human genetics.

We count on your active participation to help the 16<sup>th</sup> SPGH Annual Meeting achieve its goals.

The Organizing Committee

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Caros colegas,

Gostaríamos de vos dar as boas-vindas à 16<sup>a</sup> Reunião Anual da Sociedade Portuguesa de Genética Humana (SPGH), que tem lugar na *Antiga, Mui Nobre, Sempre Leal e Invicta* cidade do Porto, Património Mundial da UNESCO. A Direcção e o Comité Científico da SPGH tentaram organizar um excelente programa para esta Reunião, com conferências por especialistas internacionais e nacionais sobre os desenvolvimentos científicos mais recentes na genética humana, bem como apresentações por cientistas mais jovens.

Esperamos que a 16<sup>a</sup> Reunião Anual seja uma oportunidade para discussão e actualização em várias áreas da genética humana. Este aspecto é particularmente importante para jovens investigadores, médicos internos, postdocs e estudantes, que de outro modo podem ter poucas oportunidades para interagir com os principais especialistas nesta área do conhecimento. O programa deste ano inclui ainda sessões coordenadas pela Comissão de Políticas Públicas e Educação em Genética e pela Comissão de Bioética da SPGH para discutirmos temas de interesse para os nossos associados e restantes profissionais na área da genética humana.

Contámos com a vossa participação activa para ajudar a 16<sup>a</sup> Reunião Anual da SPGH a cumprir os seus objectivos.

A Comissão Organizadora

## 16ª Reunião da SPHG

### **Comissão Organizadora**

Manuel Teixeira, Sofia Dória, Beatriz Porto

### **Comissão Científica**

Ana Fortuna, Carla Pinto Moura, Carla Oliveira, Filipa Carvalho, Joana Melo, Jorge Saraiva,  
Lina Ramos, Luísa Romão, Manuel Teixeira, Sofia Dória, Susana Fernandes

### **Comissão de apoio à Organização**

Ana Rodrigues, Sophie van Asch, Sérgio Ferreira, Rosa Sousa, Ana Barbosa, Diana Pereira,  
Paula Paulo, Diogo Silva, Joana Santos, Sofia Maia, Diana Mesquita

PROGRAMA CIENTÍFICO  
SCIENTIFIC PROGRAM

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## 16ª Reunião Anual da Sociedade Portuguesa de Genética Humana

Thursday, November 22<sup>nd</sup>

10.00 - <b>Registration opens</b>
10.30 - <b>Clubs</b> (parallel sections) <b>Cytogenetics</b> <b>Molecular genetics</b> <b>Medical genetics and dysmorphology</b>
14.00 - <b>Opening session</b>
14.15 - <b>Opening conference: Next generation phenotyping</b> <i>Chairs: Manuel Teixeira, Sofia Dória</i> <u>Raoul Hennekam</u> (Amsterdam University, The Netherlands) <b>Next generation sequencing demands next generation phenotyping</b>
15.00 - <b>Targeted therapy of genetic diseases</b> <i>Chairs: Jorge Saraiva, Isabel Cordeiro</i> <u>Julian Sampson</u> (Cardiff University, Wales, UK) <b>Targeted treatment for tuberous sclerosis</b> <u>Luís Almeida</u> (CNC, Coimbra University) <b>Targeted treatment for Machado-Joseph disease</b> <u>Carlos Farinha</u> (BioFIG, Lisbon University) <b>Targeted treatment for cystic fibrosis</b>
17.00 - <b>Coffee break/poster viewing</b>
17.30 - <b>Oral communications I</b> <i>Chairs: Luísa Romão, Ana Fortuna</i>
18.30 - <b>SPGH general assembly</b>

## 16ª Reunião Anual da Sociedade Portuguesa de Genética Humana

Friday, November 23<sup>rd</sup>

09.00 - <b>Human evolutionary genetics and founder effects in Mendelian diseases</b> <i>Chairs: Jorge Sequeiros, António Amorim</i>  <u>Luísa Pereira</u> (IPATIMUP, Porto University) <b><i>Mitochondrial DNA genomics: human population history, selection and pathogenicity</i></b>  <u>Manfred Kayser</u> (Erasmus University Medical Center, Rotterdam, The Netherlands) <b><i>Genes, geography and history of Europe: Genetic population substructure of Europeans</i></b>  <u>Angel Carracedo</u> (Galician Pub. Found. Genomic Med., Sant. Compostela, Spain) <b><i>The importance of populations in clinical genetics</i></b>
11.00 - <b>Coffee break/poster viewing</b>
11.30 - <b>Oral communications II</b> <i>Chairs: Lina Ramos, Hildeberto Correia</i>
12.30 - <b>Lunch</b>
14.00 - <b>Genetic heterogeneity of deafness</b> <i>Chairs: Carla Moura, Susana Fernandes</i>  <u>Karen Avraham</u> (Tel Aviv University, Israel) <b><i>Using deep sequencing to decipher the genetic heterogeneity of deafness</i></b>
14.45 - <b>Aneuploidy: Causes and consequences</b> <i>Chairs: Filipa Carvalho, Isabel Carreira</i>  <u>Alan H. Handyside</u> (Leeds University, UK) <b><i>Genome wide analysis of chromosomal abnormalities in the human preimplantation embryo in vitro</i></b>  <u>Montserrat Garcia Caldés</u> (Barcelona Autonomous University, Spain) <b><i>Aneuploidy, what we know about its causes</i></b>
16.15 - <b>Sattelite symposium by Illumina</b>

16.45 - <b>Coffee break/poster viewing</b>
17.15 - <b>Oral communications III</b> <i>Chairs: Joana Melo, Beatriz Porto</i>
18.15 - <b>Public policy and education in genetics session</b> <i>Chair: Astrid Vicente</i>  <u>Jorge Sequeiros</u> (ICBAS, Porto University), <u>Miguel Viveiros</u> (Ord. Biol.), <u>Madalena Ávila</u> (Anbioq) <b><i>Clinical laboratory genetics specialization</i></b>
20.00 - <b>Conference dinner</b>

## 16ª Reunião Anual da Sociedade Portuguesa de Genética Humana

Saturday, November 24<sup>th</sup>

09.00 - <b>Cancer genetics: germline, somatic, and something in between</b> <i>Chairs: Sérgio Castedo, Carla Oliveira</i>  <u>Helen Lindsay</u> (Yorkshire Regional DNA Laboratory, UK) <b><i>Next generation sequencing in mainstream hereditary cancer diagnostics</i></b>  <u>Luis A. Pérez Jurado</u> (Pompeu Fabra University, Barcelona, Spain) <b><i>Early onset clonal chromosomal mosaicism and the risk of cancer in adulthood</i></b>  <u>João Barata</u> (IMM, Lisbon University) <b><i>Genetic lesions and posttranslational alterations promoting T-cell acute lymphoblastic leukemia</i></b>
11.00 - <b>Coffee break/poster viewing</b>
11.30 - <b>Bioethics committee session</b> <i>Chair: Heloísa Santos</i>  <u>Célia Ventura</u> (INSA) <b><i>Bioethical aspects of genetic testing of psychiatric diseases and dementias</i></b>
12:00 - <b>SPGH award conference</b> <i>Chairs: Manuel Teixeira, Sofia Dória</i>
12:30 - <b>Basic and clinical research awards</b> <b>Closing session</b>

# PALESTRAS LECTURES

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## NEXT GENERATION SEQUENCING DEMANDS NEXT GENERATION PHENOTYPING

**Raoul C. M. Hennekam**

*Dept. of Pediatrics and Translational Genetics, AMC, Amsterdam, Netherlands; Dept. of Neurology, UCL, London UK*

The evaluation of a child with an unusual face or other abnormalities at birth has always been the field of Dysmorphology, trying to recognize the combination of the findings in the child and establishing a diagnosis this way. The same holds even stronger for the children with an intellectual disability.

The advent of rapid molecular diagnostic tests using next generation sequencing (NGS) techniques is changing our policies and it has been suggested repeatedly that Dysmorphology and other phenotyping will disappear as evaluation of patients will no longer be needed.

In this lecture first the importance of NGS and the magnificent opportunities this creates for all fields in genetics and indeed in medicine in general will be discussed. But the limitations of this technique will be evaluated as well, such as non-genetic influences on birth defects and intellectual disability, the need for excellent knowledge of natural history of disorders, and the limitations and difficulties in finding firmly established causes by NGS in itself. Therefore it seems likely that NGS can only be successful if it goes hand in hand with very careful evaluation of the patients that are investigated this way. Recognition of the limitations of NGS will minimize false expectations and foster the most fruitful investigations.

*Reference: Hennekam RCM, Biesecker LG. 2012. Next-Generation Sequencing demands Next-Generation Phenotyping. Hum Mutat 33:884-6.*



## TARGETED TREATMENT FOR TUBEROUS SCLEROSIS

**Julian R. Sampson**

*Institute of Medical Genetics, Cardiff University and University Hospital of Wales, Cardiff CF14 4XN, UK*

mTOR inhibitor therapy has been licensed for the treatment of tuberous sclerosis in the USA and in Europe. This advance represents one of the first examples of an emerging approach to treatment of inherited disorders based upon an understanding of gene function. But what were the discoveries that resulted in this advance and how did they come about? The story really started with the identification of the *TSC2* gene in 1993, a discovery that was enabled by astute observations made by clinical geneticists from Portugal. The gene encoded a previously unknown protein that was noted to contain a region of homology to GTPase activating proteins (GAPs). Several years of work in model organisms and cell culture models were required before it became clear that, through its GAP domain, *TSC2* regulates the small G protein Rheb and thereby controls the activity of the mTOR complex. *TSC1* was identified as a partner protein that was also required for this activity and that was mutated in other patients with tuberous sclerosis. These findings suggested that mTOR inhibitors might be able to effectively replace the activity of the damaged *TSC1* or *TSC2* genes to normalise mTOR signalling in patients with tuberous sclerosis.

Initial investigations were undertaken in cell culture and transgenic mouse models using the naturally occurring mTOR inhibitor rapamycin, a macrolide antibiotic originally isolated from bacteria in a soil sample from Easter Island. The drug was found to be exceptionally effective in reducing the growth of *Tsc2*<sup>-/-</sup> cells and in shrinking tumours in *Tsc2*<sup>+/-</sup> mice. Clinical trials were rapidly initiated in several different settings. These included children and adults with tuberous sclerosis-associated brain tumours (SEGA), adults with tuberous sclerosis-associated renal angiomyolipoma and tuberous sclerosis-associated and sporadic pulmonary lymphangiomyomatosis. In each of these contexts rapamycin or its derivative Everolimus proved both effective and safe. The drugs have also been investigated in rodent models of tuberous sclerosis in relation to learning and seizures – again with successful outcomes. Now clinical trials are underway in the USA and Europe to investigate whether the neurocognitive problems that are common in patients with tuberous sclerosis can also be improved by mTOR inhibitors.

Many questions remain to be answered: which patients with tuberous sclerosis should be treated and when? Is there a place for prevention as well as treatment of the manifestations of tuberous sclerosis? What doses of mTOR inhibitors should be used? Clinical geneticists can play a vital role in answering these questions by getting involved in therapeutic research.

## TARGETED TREATMENT FOR MACHADO-JOSEPH DISEASE

**Luís Pereira de Almeida**

*Center for Neurosciences and Cell Biology and Faculty of Pharmacy, University of Coimbra*

Machado-Joseph disease or spinocerebellar ataxia type 3 (MJD/SCA3) is a neurodegenerative disorder associated with aggregation of a mutant form of the protein ataxin-3 in the brain. The disease is autosomal dominantly-inherited and caused by a CAG expansion in the coding region of the *ATXN3/MJD1* gene. There is currently no therapy available that would block disease progression. However, the post-transcriptional gene silencing technique — RNA interference (RNAi) allows down-regulation of the expression of mutant genes and has potential as a therapeutic approach but raises the issue of preserving the function of the wild-type *ATXN3* allele as the loss of function of wild-type ataxin-3 (Atx3), which has been shown to play a role in ubiquitin-mediated proteolysis, could potentially be deleterious.

Therefore a strategy allowing a selective targeting of the mutant allele while preserving the expression and functions of the wild-type allele present in heterozygous patients, the large majority, was needed, a challenge toward a clinical development of RNA interference for this and other disorders. To tackle this question we developed an allele-selective silencing strategy that we validated in a lentiviral rat model of MJD. In MJD patients, an intragenic single nucleotide polymorphism (SNP) is present in more than 70% of the cases. We generated lentiviral vectors (LV) encoding short-hairpin RNAs (shRNAs) targeting this SNP, to downregulate mutant human ataxin-3 *in vivo* in a selective manner. Our results showed that this SNP can be used to selectively inactivate mutant ataxin-3, the MJD gene product. Lentiviral-mediated silencing of mutant human ataxin-3 significantly reduced neuropathological abnormalities associated with MJD *in vivo* (Alves et al PlosOne 2008).

More recently, we investigated whether this silencing strategy, when applied to the cerebellum one of the most severely attained regions in MJD, would decrease: a) the motor behaviour deficits, b) the neuropathological features of the disease, c) upon intervention at different stages of the disease. For this purpose we used the lentiviral vectors encoding short hairpin sequences specifically targeting mutant Atx3 (shAtx3) in two experimental paradigms corresponding to early and late stage of the disease, respectively: (1) simultaneous injection of lentiviral vectors encoding the silencing and the overexpressing mutant ataxin-3 sequences in the mouse cerebellum; and (2) injection of shAtx3 lentiviral vectors in a transgenic mouse model expressing a truncated form of mutant ataxin-3 (Torashima et al 2008), and presenting a marked ataxic phenotype by the time of injection at 3-4 weeks of age.

Silencing of mutant Atx3 expression led to a robust enhancement of motor coordination, gait and balance performance in both models, as compared to control groups. In the first model, quantitative analysis of rotarod performance and footprint patterns revealed nearly complete recovery of mice injected with shAtx3, and significant differences compared to controls injected with shGFP. In the second model, significant differences in motor coordination

between shAtx3 injected mice and control ones injected with shGFP were observed. Finally, an important alleviation of the typical neuropathological hallmarks of the disease was also observed, in both models, regarding the number of intranuclear inclusions, and neuronal markers loss.

These data support the therapeutic potential of gene silencing by RNA interference for therapy of Machado-Joseph disease.

*Support: Fundação para a Ciência e a Tecnologia (FCT) SAU-FCF/70384/2006, SAU-NEU/099307/2008, The Richard Chin and Lily Lock Research Fund, the National Ataxia Foundation and the Association Française Contre les Myopathies (AFM).*

## TARGETED TREATMENT FOR CYSTIC FIBROSIS

**Carlos M. Farinha and Margarida D. Amaral**

*BioFIG – Center for Biosystems, Functional and Integrative Genomics, Faculty of Sciences, University of Lisboa*

Cystic Fibrosis (CF), the most common lethal autosomic recessive disorder among Caucasians affecting ~80,000 individuals worldwide, is dominated by the respiratory disease, the main cause of morbidity and mortality. CF symptoms also include pancreatic insufficiency, male infertility and elevated saline concentration in sweat. Current therapies have considerably improved lifespan and quality of life for CF patients but treat mostly the multi-organ CF symptoms. Correcting the basic molecular/cellular defect in CF is the adequate strategy to a more definitive solution.

CF is caused by dysfunction of a single gene, encoding the CF transmembrane conductance regulator (CFTR), a 1,480 amino acid protein functioning as a chloride channel at the apical membrane of epithelial cells. Although ~1,900 mutations cause CF, one single mutation (F508del) occurs in 90% of CF patients. F508del leads CFTR protein to misfold, retention by the endoplasmic reticulum (ER) quality control and premature degradation, thus failing to reach the cell surface.

The first CFTR-modulating therapy which reached the market this year, Kalydeco (VX-770/Ivacaftor, Vertex Pharmaceuticals, USA), is only approved for one rare mutation (G551D) occurring in ~3-4% of CF patients.

To help the majority of CF patients which carry the F508del, Vertex has placed another compound under trial, VX-809 (Lumacaftor), a small molecule "corrector" rescuing F508del-CFTR trafficking to the cell surface. However, clinical trials indicate very modest efficacy in patients.

In fact, evidence accumulates that F508del-CFTR has multiple defects, which should be corrected by different therapeutic agents, possibly in combination therapy. This opens avenues for discovery of additional correctors and pathways acting on F508del-CFTR by distinct mechanisms of action from VX-809, i.e., on a different binding pocket of the misfolded protein or at another trafficking checkpoint of the secretory pathway.

As CF is a paradigmatic trafficking disorder among rare diseases, there is much confidence that successes in CF will translate into other disorders sharing the similar basic defects.

*Work supported by strategic grant PEst-OE/BIA/UI4046/2011 FCT, Portugal to BioFIG and FCT/MCTES grants PTDC/SAU-GMG/122299/2010, PTDC/BIA-BCM/112635/2009 and CFF (USA) Ref: 7207534.*

## **MITOCHONDRIAL DNA GENOMICS: HUMAN POPULATION HISTORY, SELECTION AND PATHOGENICITY**

**Luísa Pereira**

*IPATIMUP, Porto University*

The era of genomics conducted to the characterization of mitochondrial genomes (~16,6kb in mammals) in more than 2,400 species and in ~20,000 humans from worldwide populations, rendering this the best characterized genomic region. This extended amount of information has been greatly explored in population genetics, aiming to reconstruct the human history. Notwithstanding the promising insights from the recent genome-wide studies in unraveling the past, mtDNA continues to be the most reliable genome for dating the most recent common ancestors of human lineages. I will present the most recent studies and conclusions in this field.

I will also show how the knowledge we are acquiring on the worldwide population mtDNA diversity should be taken into account to help clinical geneticists to evaluate the pathogenicity of mtDNA variants in many complex diseases. We used detailed phylogenetic trees for human mtDNA, combined with pathogenicity predictions for each amino acid change, to evaluate selection on mtDNA-encoded proteins. Our approach differed from others in the sense that we had two quantitative measures (the pathogenicity score and a time scale for the emergence of all non-synonymous mutations), allowing to perform an objective statistical evaluation. We clearly showed that protein variants with high pathogenicity scores were significantly rarer in the older branches of the phylogenetic tree, meaning that human mtDNA is under the effect of purifying selection which eliminates pathogenic mutations along time. We found no measurable difference in this mtDNA purifying selection across the global population, being similar in all geographical regions.

We then tested our approach in datasets of oncogenic (cells stuffed with mitochondria) and non-oncogenic tumors, and observed that tumors escape selection, accumulating mutations at random, and that there is a positive selection leading to the accumulation of pathogenic mutations in the oncogenic tumors, which potentially render mitochondria ineffective in these tumors.

The list of pathogenicity scores for all possible human mtDNA non-synonymous mutations estimated by us can be used as a tool for assessing novel protein variations which are often reported in patients with mitochondrial disease of unknown origin, or for assessing somatic mutations acquired through aging or detected in tumors. This information is essential for oriented functional essays of mtDNA mutations.

## GENES, GEOGRAPHY AND HISTORY OF EUROPE: GENETIC POPULATION SUBSTRUCTURE OF EUROPEANS

**Manfred Kayser**

*Erasmus University Medical Center Rotterdam, The Netherlands*

Investigating genetic-geographic population substructure allows conclusions about evolutionary history but also is of practical relevance such as in disease gene mapping (where observing substructure is not appreciated to reduce false positive hits), or in forensics (where observing substructure is appreciated for allowing bio-geographic ancestry inference useful in police investigations). Various studies on the genetic-geographic substructure of worldwide populations have revealed insights into human evolutionary history such as the Out-of-Africa migration of modern humans or about settlement histories of major geographic regions. Such studies have also shown that individuals can be assigned to their bio-geographic origins in the major geographic regions based on a large number of genome-wide or a small number of ancestry-informative single nucleotide polymorphisms with implications for medicine and forensics. Studies focusing on within-continental substructure are more scarce perhaps because genetic differentiation within a more restricted geographic region is expected to be smaller than on the worldwide scale; thus, practical implication for medicine or forensics may be less strong. However, it is important for various aspects to investigate genetic-geographic substructure within a continental region such as within Europe. This lecture will provide a brief overview about the extent of genetic-geographic substructure within the European population, will touch on the underlying population history for Europe as a whole, and will also zoom into certain European regions such as the Netherlands and specific European populations such as the Roma.

## THE IMPORTANCE OF POPULATIONS IN CLINICAL GENETICS

**Angel Carracedo**

*Fundación Galega de Medicina Xenómica-SRERGAS; CIBERER-Universidade de Santiago de Compostela*

The spectrum of diseases where the contribution of clinical genetics is requested is moving from mendelian traits to complex diseases but still in both the importance of populations is key.

This fact will be illustrated with two examples related with breast and colorectal carcinoma, typical complex diseases with a mendelian subset. In this latter we will demonstrate the importance of founder effects and how this can change the diagnostic strategies and even the guidelines for genetic counseling.

The knowledge of the populations is also essential for the analysis of the genetic component of the non-mendelian subset. The search of genes involved in cancer are of importance to identify new pathways, this is to say new targets for drugs, to stratify the disease and for pharmacogenomics. Association studies using GWAS strategies has allowed us to identify a number of loci associated with cancer. In CRC from a total of 35% of genetic variance, 5% are due to mendelian genes, most of which has been identified but only an additional 8% has been identified with GWAS studies. There is a number of strategies to search for the missing variability and one of them is the use of populations different from those of European origin where the majority of the GWAS studies have been carried out. Populations with recent admixture offer the additional advantage of the potential use of admixture mapping strategies. We will here describe the efforts and the strategies we are using for the search of genes involved in CRC in Latin American populations.

## USING DEEP SEQUENCING TO DECIPHER THE GENETIC HETEROGENEITY OF DEAFNESS

**Karen B. Avraham**

*Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*

Identification of genes responsible for medically important traits is a major challenge in human genetics. While classic techniques such as linkage analysis and Sanger sequencing have led to the discovery of over 100 genes for hearing loss, more genes remain to be discovered. Exome capture and massively parallel sequencing can be exploited to address this challenge for genetically heterogeneous hereditary deafness. We developed a targeted capture pool to identify mutations in 284 human genes and human orthologues of mouse genes responsible for hearing loss. Multiplexed libraries representing 96 Israeli Jewish and Palestinian Arab patients were analyzed with paired-end sequencing, using the Illumina HiSeq 2000 platform. The increase in gene discovery from this experiment was remarkable, from 9 to 21 and 13 to 23 in the Israeli Jewish and Palestinian Arab populations, respectively. As an example, the discovery of a mutation in a new deafness gene, *SYNE4*, will be presented. This gene encodes a protein that is a member of the "linker of nucleoskeleton and cytoskeleton" (LINC) complex in the nuclear envelope. Mice lacking *Syne4* are deaf. The nuclei of the outer hair cells of these mice fail to maintain their basal localization in the cell, potentially affecting cell motility and hence response to sound. This finding has revealed an entirely new pathology that leads to deafness. Overall, this study, leading to an increase in the number of genes responsible for deafness, has implications not only for the Middle East, but worldwide, as many mutations first found in this region have turned out to be present in other populations. This strategy allows for improved diagnostics, facilitating discovery of causative mutations in an economically and temporally feasible manner, leading to improved genetic counseling and hearing loss management.



## GENOME WIDE ANALYSIS OF CHROMOSOMAL ABNORMALITIES IN THE HUMAN PREIMPLANTATION EMBRYO IN VITRO

**Alan H. Handyside**

*The Bridge Centre, London and Institute of Integrative and Comparative Biology, University of Leeds, Leeds, United Kingdom*

Chromosome aneuploidy is a major cause of pregnancy loss, abnormal pregnancy and live births following both natural conception and in vitro fertilisation (IVF) and increases exponentially with maternal age in the decade preceding the menopause. Molecular genetic analysis has shown that these are predominantly maternal in origin and trisomies most frequently occur through errors in the first meiotic division. Analysis of chromosome copy number in the three products of female meiosis, the first and second polar bodies and the corresponding zygote by microarray comparative genomic hybridisation (array CGH), in women of advanced maternal age undergoing IVF, has recently revealed a pattern of frequent multiple meiotic errors, caused by premature predivision of sister chromatids in meiosis I and a high incidence of error in meiosis II. This pattern is similar to those observed in various mouse models which implicate the gradual depletion of cohesins, which are essential for cohesion of sister chromatids, as the primary cause of age related aneuploidy in female meiosis. However, defects in other aspects of meiosis including the formation and stabilisation of chiasmata and the spindle assembly checkpoint (SAC) may also contribute. The challenge remains to explain the molecular basis of 'physiological' rather than 'chronological' female aging and the contribution of multifactorial causes from the fetal to adult ovary.

## ANEUPLOIDY, WHAT WE KNOW ABOUT ITS CAUSES

### Montserrat Garcia Caldés

*Unitat de Biologia Cel·lular i Genètica Mèdica, Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Medicina, Universitat Autònoma de Barcelona*

No fewer than 5% of all clinically recognized human pregnancies are trisomic or monosomic. These aneuploidies are mainly autosomopathies caused by errors produced during the first stages of the female meiotic process. In this sense, one might even dare to go so far as to say that female meiosis (much more than male meiosis) is error-prone and we also know that this flaw increases exponentially with age.

Female and male gametogenesis have the same objectives. Cellular proliferation, reductional division (meiosis) and cellular differentiation processes occur in both sexes. However, there are differences: in mammals, both sexes, to accomplish their objectives, use different methods, have dissimilar levels of success and the end-product they achieve is different. These differences, onset, duration and outcome in females are significant enough to constrain the gametogenesis process in females and cause that the life cycle of the human oocyte to be time-consuming and complex.

In this talk, I will analyze what we know about the methods both sexes apply to achieve gametes that are able to carry out the fertilization process with different levels of success.

I will consider the key ingredients of this complex process: synapsis, recombination and segregation, with the goal of showing the multifactorial idiosyncrasy of aneuploidy.

## NEXT GENERATION SEQUENCING IN MAINSTREAM HEREDITARY CANCER DIAGNOSTICS

**Helen Lindsay**

*Yorkshire Regional Genetics Service*

Next generation sequencing (NGS) was introduced into our laboratory in February 2010, the first diagnostic service of its kind from a CPA accredited UK provider. The first test to be introduced was familial breast cancer gene screening (*BRCA1/BRCA2*), with further services being introduced systematically. These include HNPCC, Li-Fraumeni syndrome, and familial pheochromocytoma and paraganglioma. To date, we have issued over 2500 NGS reports. Here we discuss our experience of implementing next generation sequencing workflows and ongoing developments.

All current services follow standardised protocols conducted in parallel and incorporate target enrichment by long-range PCR, robotics and automated library construction. Data is analysed using NextGENe software in combination with customised spreadsheets to assist with quality checks and variant assessment. Workflows are continually evolving, and we are currently investigating alternative targeting methods to enable the analysis of larger gene panels, for detecting dosage abnormalities, and for tumour analysis.

In our hands, the performance of NGS matches that of conventional sequencing in terms of both test sensitivity and specificity. Implementation of the new methodology has significantly improved patient pathways as turnaround times and testing costs are reduced. NGS has enabled the development of new specialised services where a range of genes underlying a particular phenotype can be sequenced in parallel rather than using a step-by-step approach. This is likely to create even more long-term cost savings in clinical pathways. The new streamline workflows have also enabled an increase in testing capacity with existing staff numbers.

**EARLY ONSET CLONAL CHROMOSOMAL MOSAICISM AND THE RISK OF CANCER IN ADULTHOOD**

**Luis A. Pérez Jurado**

*Pompeu Fabra University, Barcelona, Spain*

Abstract not available.

## GENETIC LESIONS AND POSTTRANSLATIONAL ALTERATIONS PROMOTING T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

**João T. Barata**

*Instituto de Medicina Molecular, Lisbon, Portugal*

While alterations in genes encoding key members of signaling pathways are commonly reported, there is also evidence that posttranslational modifications may significantly impact the effector capacity of a certain pathway without the requirement for direct genetic or epigenetic lesions in the elements of that pathway. Our work has provided evidence of the importance of both mechanisms in the context of T-cell acute lymphoblastic leukemia (T-ALL), an aggressive pediatric malignancy. We have shown that the majority of primary T-ALL patient samples display PI3K/Akt pathway hyperactivation more frequently as a consequence of PTEN posttranslational inactivation than from genetic alterations (J Clin Invest 2008, 118:3762). PTEN functional inactivation is mediated by CK2 – a ubiquitous serine/threonine protein kinase whose expression and activity is augmented in many cancers, including T-ALL. Treatment with CK2 or PI3K small molecule inhibitors promotes cell death in primary leukemia cells without affecting normal T-cell precursors, revealing the CK2-PTEN-PI3K axis as critical for T-ALL cell viability. On the other hand, our work indicating the importance of interleukin 7 (IL-7)-mediated signaling for T-ALL *in vitro* proliferation (J Exp Med 2004, 200:659) and *in vivo* expansion (Cancer Res 2011, 71:4780) led us to investigate whether there were gain-of-function mutations in the receptor for this microenvironmental cytokine. We found that around 9% of T-ALL cases display heterozygous somatic *IL7R* exon 6 mutations, which promote constitutive signaling independently of the ligand, resulting in increased cell cycle progression, viability, *in vitro* transforming capacity and *in vivo* tumorigenesis. We also found that JAK/STAT pathway inhibitors can be of value to eliminate mutant IL7R-expressing cells (Nat Genet 2011, 43:932).

## **ASPECTOS BIOÉTICOS DOS TESTES GENÉTICOS EM DOENÇAS PSIQUIÁTRICAS E DEMÊNCIAS**

**Célia Ventura**

*Membro da Comissão de Bioética da Sociedade Portuguesa de Genética Humana*

A definição de doença mental abrange um conjunto de sintomas ou comportamentos clinicamente reconhecíveis associados ao sofrimento e disfunção pessoal. As doenças psiquiátricas e as demências são doenças mentais altamente estigmatizantes que levam a situações de discriminação. Nas doenças psiquiátricas, incluem-se, nomeadamente, os transtornos psicóticos, de personalidade, de humor e os transtornos invasivos do desenvolvimento. Estas são, em larga maioria, doenças comuns complexas, de transmissão multifactorial. Aparentemente responsáveis pela predisposição individual ou familiar, estão hoje identificadas associações de grande número de SNPs e CNVs, em diferentes estudos e populações. No referente às demências, é importante salientar que muitas (Alzheimer precoce, Doença de Huntington, Demência fronto-temporal, etc.) têm transmissão mendeliana, sendo conhecidos os genes e principais mutações. Contudo, a variação da gravidade, penetrância e o fenómeno de antecipação complicam as conclusões obtidas por diagnóstico pré-sintomático ou pré-natal. Em síntese, os testes genéticos para as doenças psiquiátricas e demências que são hoje oferecidos são variados e, para além dos diagnósticos, são frequentes os testes preditivos de susceptibilidade (probabilísticos) ou pré-sintomáticos (determinísticos). Infelizmente, o risco genético é muitas vezes mal interpretado e sobrevalorizado. Na maioria das patologias, mal definidas e comuns, os benefícios de saber o risco genético dos indivíduos testados deverá ser cuidadosamente ponderado em relação às consequências psicológicas e sociais. Porém, o aparecimento de testes de predisposição genética, com pouco ou nenhuma validade clínica, oferecidos à população ou às famílias tem vindo a aumentar. A investigação genética das doenças mentais, além de permitir melhorar a sua classificação pode identificar anomalias funcionais e bioquímicas subjacentes à doença, facilitando a descoberta de novos fármacos. Porém, as comissões de ética deverão sempre avaliar com rigor as consequências da investigação em doentes vulneráveis (menores e/ou com capacidades cognitivas ou de comunicação diminuídas). O aconselhamento genético dos doentes e o consentimento informado, do próprio sempre que possível, e ainda a garantia de total privacidade e confidencialidade são indispensáveis na realização dos testes. Às considerações bioéticas sobre os fundamentos destas considerações, seguem-se algumas recomendações da Comissão e a sua discussão pelos elementos presentes.



COMUNICAÇÕES ORAIS  
ORAL COMMUNICATIONS

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Oral Communications - Session I		
Number	First Author	Title
OC1	Cláudia Reis	KABUKI SYNDROME: GENOTYPE CHARACTERIZATION OF 15 PATIENTS
OC2	Ana Jalles	IDENTIFICATION OF CANDIDATE THERAPEUTIC COMPOUNDS FOR MACHADO-JOSEPH DISEASE IN C. ELEGANS
OC3	Vera Lima	DISSECTING A LINK BETWEEN ANEUPLOIDY AND CHROMOSOME INSTABILITY IN HUMANS
OC4	Emília Vieira	DISTRIBUTION OF LIMB GIRDLE MUSCULAR DYSTROPHY SUBTYPES AMONG PORTUGUESE PATIENTS
OC5	Sara Berguete	CLINICAL DIAGNOSIS VERSUS MOLECULAR DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA
OC6	Joana Melo	CHROMOSOME 16p11.2 COPY NUMBER VARIANTS ASSOCIATED WITH GLOBAL DEVELOPMENTAL DELAY, INTELLECTUAL DISABILITY AND BEHAVIORAL PROBLEMS: REPORT OF FIVE PATIENTS



# **(OC1) KABUKI SYNDROME: GENOTYPE CHARACTERIZATION OF 15 PATIENTS**

**Reis CF<sup>1\*</sup>, Beleza-Meireles A<sup>1\*</sup>, Benoit V<sup>2</sup>, Rodrigues F<sup>1</sup>, Duarte C<sup>3</sup>, Amorim M<sup>1</sup>, Venâncio M<sup>1</sup>, Ramos F<sup>1</sup>, Sá J<sup>1</sup>, Ramos L<sup>1</sup>, Lederer D<sup>2</sup>, Saraiva J<sup>1</sup>**

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*\*Both authors contributed equally to this work*

**Introduction:** Kabuki syndrome (KS) is a rare autosomal dominant multi-system disorder that can result in congenital anomalies, typical dysmorphism and variable developmental delay/intellectual disability. Most cases are due to de novo point mutations and rarely intragenic deletions/duplications of MLL2. A reduced number of cases are associated with KDM6A mutations.

**Methods:** Fifteen patients with KS, 13 of which index cases, who had previously been phenotypically characterized through comprehensive clinical and psychological evaluation, were submitted to molecular diagnosis. Sequencing of the MLL2 gene was performed in all patients, followed by MLPA for detection of intragenic deletion/duplication. In cases with no mutations in MLL2, analysis of the KDM6A gene was performed.

**Results:** Mutations were found in 8 patients, 3 of which shared the same familial mutation. All were heterozygous point mutations in MLL2 gene: 4 were classified as pathogenic and 2 as probably pathogenic. The MLL2 mutation detection rate was 46.2% (6/13) for index cases in this cohort of patients. The MLL2 MLPA analysis and sequencing of KDM6A gene is still ongoing in two of these patients, as well as most of the parental studies for the identified mutations.

**Discussion:** In the present study, MLL2 mutation detection rate in the concluded molecular studies was significant (46.2%) but below the average reported in similar studies (55 to 80%). There was one case of familiar Kabuki syndrome with three family affected members with a MLL2 pathogenic mutation, which is a rare occurrence. Another patient with a frameshift mutation in MLL2 gene has characteristic facial features but no intellectual disability. Diagnosis confirmation contributes to the ongoing delineation of a possible phenotype-genotype correlation with benefits to patient's management. It is also important for accurate genetic counseling of patients and their parents as it provides the possibility of molecular prenatal diagnosis.

## **(OC2) IDENTIFICATION OF CANDIDATE THERAPEUTIC COMPOUNDS FOR MACHADO-JOSEPH DISEASE IN C. ELEGANS**

**Jalles A, Teixeira-Castro A, Martins-Araújo M, Miranda A, Bessa C, Maciel P**

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Introduction: Machado-Joseph disease (MJD) is a neurodegenerative disorder caused by the expansion of a polyglutamine (polyQ) tract within the C-terminal of the ataxin-3 (ATXN3) protein. Mutant ATXN3 has an increased tendency to self-associate and enter the aggregation process, which has several pathophysiological consequences for affected neurons. The lack of therapeutic strategies that effectively prevent neurodegeneration in MJD patients prompted us to search for compounds that modulate mutant ATXN3-related pathogenesis. Recent data from our lab have shown that many aspects of MJD can be properly modeled in the round worm *Caenorhabditis elegans*, and others have shown that can provide a suitable platform for the discovery of bioactive compounds and target identification.

Methods: We used our *C. elegans* MJD model to screen a library of ~1200 mainly FDA-approved compounds for their ability to prevent or delay the formation of mutant ATXN3 aggregates and neurological dysfunction.

Results: We excluded the small molecules that were found to be toxic or cause developmental delay to the *C. elegans* at the concentrations tested. Of the remaining, sixty compounds reduced mutant ataxin-3-mediated neurological dysfunction by 50% or more, five of which made mutant ATXN3 expressing worms perform like wild-type animals in the motility assay. Most of the effective small molecules also reduced mutant ataxin-3 aggregation.

Discussion: The hit compounds belong to different therapeutic groups, namely modulators of neurotransmission, and of nuclear hormone receptors, and also antibacterial/antifungal drugs. We are presently investigating their cellular targets, which can provide a better understanding of MJD pathogenesis. We should be able to identify efficacious compounds that can be tested in higher organisms including transgenic mouse models, and eventually enter clinical development.

### **(OC3) DISSECTING A LINK BETWEEN ANEUPLOIDY AND CHROMOSOME INSTABILITY IN HUMANS**

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*\*These authors contributed equally to the work*

**Introduction:** Aneuploidy is the most common chromosome abnormality in humans, and is the leading genetic cause of miscarriage and congenital birth defects. The effects of aneuploidy in human primary cells in the absence of chromosome instability-inducing mutations or drug treatments are unknown. Presently few evidence exists for aneuploidy-induced chromosome instability in mammalian cells. Thus, a causative link between aneuploidy and chromosome instability will be systematically addressed in human primary cells.

**Material and Methods:** We investigated the proliferation rate and cell-cycle behavior of aneuploid human primary cells isolated from prenatal diagnosis supernatant samples using long-term time-lapse microscopy to directly score individual cell behavior/fate. Primary cell cultures have been established from these samples. These included control cell cultures (from samples with normal karyotype), single-chromosome trisomies and double trisomies. Cryopreservation has been performed at each cell culture passage. Karyotype has been analyzed at passage 2 to confirm cell population homogeneity and exclude maternal contamination. Karyotyping was also performed at higher passages. The rate of chromosome missegregation was measured by FISH analysis.

**Results and Discussion:** Preliminary data suggest the existence of a mitotic delay in trisomic cells which might be indicative of spindle assembly checkpoint activation and presence of spindle/chromosome attachment defects. Karyotype and FISH analysis reveals an increased incidence of chromosome gain and/or loss in aneuploid cases when compared with control cases.

#### **(OC4) DISTRIBUTION OF LIMB GIRDLE MUSCULAR DYSTROPHY SUBTYPES AMONG PORTUGUESE PATIENTS**

**Vieira E<sup>1</sup>, Gonçalves A<sup>1</sup>, Maia N<sup>1</sup>, Oliveira ME<sup>1</sup>, Oliveira J<sup>1</sup>, Bronze-da-Rocha E<sup>2,3</sup>, Santos R<sup>1,2</sup>**

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**Introduction:** Although the limb girdle muscular dystrophies (LGMD) are collectively characterized by progressive muscle weakness, they exhibit wide clinical and genetic heterogeneity. Over 23 loci have been identified; eight are autosomal dominant subtypes (LGMD1A-1H) and fifteen are autosomal recessive (LGMD2A-2O). The prevalence and relative distribution of the different subtypes varies widely between populations. Here we present an overview of the LGMD profile in our population, as ascertained in patients referred from all over the Country over a period of 15 years, with clinical phenotypes ranging from severe childhood-onset LGMD, adult-onset LGMD and distoproximal myopathy, to barely symptomatic presenting only hyperCKemia.

**Methods:** Inclusion criteria were essentially compatible clinical presentation and/or muscle biopsy (histology and immunocytochemistry). A total of 425 families were selected, representing 525 genetic tests for different genes: CAPN3 (n=87), DYSF (n=105), SGCG (n=94), SGCA (n=46), SGCB (n=39), SGCD (n=18), TCAP (n=6), FKRP (n=55), TTN (n=4), ANO5 (n=11), MYOT (n=5), LMNA (n=46), and CAV3 (n=9).

**Results:** Differential diagnosis was achieved in 183 unrelated families, where a total of 92 different mutations were identified, 44 of which were novel. The gamma-sarcoglycanopathies were the most frequent subtype (13.4%), clearly as a result of a founder effect among families of gypsy (Roma) ethnicity and the presence of a frequent mutation thought to be of North African origin. This was followed by the dysferlinopathies (10.4%), with no particular hotspot along the 56 exons, except for two private mutations. All other forms detected each represented under 5% of the cases, and 56.9% remained genetically unresolved.

**Conclusion:** The profile of our patients differs slightly from that of other large cohorts in studies reported for the European, North American, Australian and Brazilian populations. In this group of disorders with wide genetic heterogeneity, knowledge of the subtype and mutational spectrum in our population facilitates the diagnostic approach.

## **(OC5) CLINICAL DIAGNOSIS VERSUS MOLECULAR DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA**

**Berguete S<sup>1</sup>, Alves AC<sup>1,2</sup>, Leitão F<sup>1</sup>, Medeiros AM<sup>1,2</sup>, Bourbon M<sup>1,2</sup>**

*On behalf of the investigators of the Portuguese FH Study*

*<sup>1</sup>Unidade de I&D, Grupo de Investigação Cardiovascular, Departamento de Promoção da Saúde e Prevenção de Doenças Não Transmissíveis, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; <sup>2</sup>Center for Biodiversity, Functional & Integrative Genomics (BioFIG)*

**Introduction:** Familial hypercholesterolaemia (FH) is an inherited disorder of cholesterol metabolism with increased cardiovascular risk. Clinical diagnosis is obtained from clinical history, physical signs, biochemical markers and family history. Clinical criteria for the FH diagnosis most applied worldwide were proposed by Simon Broome Register Group (SBRG) and Dutch MEDPED Program (DMP). Genetic diagnosis of FH is based on the search for mutations in LDLR, APOB and PCSK9. The AIM of this study was to compare efficacy of the application of SBRG and DMP criteria to Portuguese index patients with molecular study.

**Methods:** A sample of 607 index patients referred to Portuguese FH Study (PFHS) was classified according with SBRG and DMP criteria and classification was compared with results of molecular study.

**Results:** According to clinical criteria, several patients were classified with no FH: 334 following SBRG, 228 following DMP. PFHS didn't find mutation in 79% (264/335) patients with no SBRG diagnosis and in 83% (189/228) with no DMP diagnosis. In the group of 273 patients classified with possible/definite FH by SBRG criteria, 53% (146/273) have a LDLR mutation (missense 32% (86/273), nonsense 16% (44/273), 6% (16/273) with splicing defects), 2% (6/273) are homozygous, 2% (5/273) have an APOB mutation and 0,7% (2/273) a PCSK9 mutation. In the group of 379 patients classified with DMP as having FH, 46% (173/379) have a LDLR mutation (missense 27% (101/379), nonsense 13% (49/379), 6% (23/379) with splicing defects), 2% (7/379) are homozygous, 2% (8/379) have an APOB mutation and 0,5% (2/379) a PCSK9 mutation.

**Discussion:** Both criteria gave similar results for mutation type and misdiagnosed about 30-37% of the Portuguese patients. Definition of better international set of clinical criteria would be useful to identify FH patients to avoid unnecessary molecular studies since only genetic diagnosis give an accurate result.



**(OC6) CHROMOSOME 16p11.2 COPY NUMBER VARIANTS ASSOCIATED WITH GLOBAL DEVELOPMENTAL DELAY, INTELLECTUAL DISABILITY AND BEHAVIORAL PROBLEMS: REPORT OF FIVE PATIENTS**

**Melo JB<sup>1</sup>, Ferreira SI<sup>1</sup>, Ferrão J<sup>1</sup>, Pires LM<sup>1</sup>, Jardim A<sup>1</sup>, Monteiro R<sup>2</sup>, Neto S<sup>3</sup>, Luz A<sup>3</sup>, Nunes C<sup>3</sup>, Ramos F<sup>4</sup>, Sá J<sup>4</sup>, Ramos L<sup>4</sup>, Saraiva J<sup>4</sup>, Carreira IM<sup>1</sup>**

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**Introduction:** Microarray-based comparative genomic hybridization (array-CGH) has revolutionized the genetic diagnostic of patients with global developmental delay (GDD), intellectual disability (ID) with or without dysmorphisms, multiple congenital anomalies (MCA) and autism spectrum disorders (ASD). The diagnostic yield has improved and new syndromes have been described. One of those syndromes is the occurrence of a Copy Number Variant (CNV) both in deletion and in duplication at chromosome 16p11.2. The clinical spectrum is variable and the manifestations typically associated are: language delay, ID, GDD, ASD, dysmorphic features, abnormal head size and behavioral problems.

**Methods:** We studied a cohort of 500 patients with GD, ID, MCA and ASD using an Agilent 180K whole genome oligonucleotide array-CGH.

**Results:** We detected five patients with imbalances at 16p11.2, three males and two females, being three deletions (one de novo, one paternal and one unknown) and two duplications (one de novo and one unknown). The phenotype of the patients is variable and comprises cognitive impairment, GDD, autism, dysmorphisms in some patients and behavioral problems. The observed CNVs range in size from 445 Kb to 545 Kb, with 28 genes involved, except for a patient with a 445 Kb duplication of unknown origin, where only 26 genes are included. In this case the proximal breakpoint is different and more distal. The majority of the previously reported deletions were de novo, and we report a paternally inherited deletion from a phenotypically normal father.

**Discussion:** Recently, mirror contrasting phenotypes have been associated with gene dosage at the chromosome 16p11.2 locus. The phenotypic variability and the existence of incomplete penetrance for 16p11.2 imbalances represent a challenge for the clinical interpretation of the impact of these CNVs in the patients' phenotype and for the genetic counseling of carrier families.

Oral Communications - Session II		
Number	First Author	Title
OC7	Inês C Conceição	PUTATIVE PATHOGENIC CNVS IN AUTISTIC PATIENTS OF PORTUGUESE ORIGIN: RECURRENCE RATES, GENIC CONTENT AND AUTISTIC TRAIT INHERITANCE
OC8	Ana B. Sousa	UNUSUAL PRESENTATION OF X-LINKED SCID DIAGNOSED BY WHOLE-EXOME SEQUENCING
OC9	Fátima lopes	WHOLE EXOME SEQUENCING IN PATIENTS WITH A RETT-LIKE PHENOTYPE
OC10	José Luis Costa	VALIDATION OF NEXT GENERATION DNA SEQUENCING FOR THE MOLECULAR DIAGNOSIS OF IDIOPATHIC HYPERTROPHIC CARDIOMIOPATHY
OC11	Mónica Costa	ASSOCIATION OF HIGHLY CONSERVED MAJOR HISTOCOMPATIBILITY COMPLEX SNP HAPLOTYPES WITH IRON AND LOW CD8+ T- LYMPHOCYTE PHENOTYPES IN HEMOCHROMATOSIS PATIENTS WITH HFE C282Y HOMOZYGOSITY FROM THREE GEOGRAPHIC AREAS
OC12	Carla Valongo	CREATINE DEFICIENCY SYNDROMES: BIOCHEMICAL AND MOLECULAR ASPECTS



**(OC7) PUTATIVE PATHOGENIC CNVS IN AUTISTIC PATIENTS OF PORTUGUESE ORIGIN: RECURRENCE RATES, GENIC CONTENT AND AUTISTIC TRAIT INHERITANCE**

**Conceição IC<sup>1,2,3</sup>, Correia C<sup>1,2,3</sup>, Oliveira BA<sup>1,2,3</sup>, Coelho J<sup>1</sup>, Café C<sup>4,5</sup>, Almeida J<sup>4,5</sup>, Mougá S<sup>4,5,6</sup>, Duque F<sup>4,5</sup>, Oliveira G<sup>4,5,6,7,8</sup>, Vicente AM<sup>1,2,3</sup>**

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Recent reports by the Autism Genome Project (AGP) consortium and other groups show that Copy Number Variants (CNV), while individually rare, collectively may explain a large fraction of the etiology of Autism Spectrum Disorders (ASD). The goal of this study was to establish the relevance for ASD etiology of potentially pathogenic CNVs identified in a Portuguese population sample by the AGP whole genome CNV analysis.

We selected 1062 CNVs, present in 300 individuals, which did not overlap more than 50% with CNVs in 8000 controls. These CNVs ranged from ~5 kb to 3.7 Mb, 54% were deletions and 65% were genic. CNVs were inspected for recurrence rates and inclusion of ASD-implicated or candidate genes. Interesting CNVs were validated by qPCR, including SHANK3 and NRG1. Thirteen were “common” CNVs, defined as CNVs with a frequency of ≥1%, and were present in a total of 115 individuals (~38%). Four CNVs spanned no genes but might include regulatory elements, while the other nine included one gene or parts of it, namely ATRX (in 30 individuals), and DPYD (in 22 individuals), except for one CNV which encompassed four genes in tandem with ATRX.

We further compared data for autistic traits in the parents (using the BAPQ and SRS questionnaires) with the type of inheritance (inherited vs de novo). We observed a significant excess of autistic traits in the fathers that transmitted a CNV, mainly in the “rigid” personality, which is defined as little interest in change or difficulty adjusting to change.

Here we highlight the importance of studying “common” CNVs and CNVs located in noncoding regions for understanding the etiology of autism. Also, we re-affirm the previously suggested presence of subthreshold autistic traits in the parents of children with ASD, in particular in patients with inherited CNVs.

## (OC8) UNUSUAL PRESENTATION OF X-LINKED SCID DIAGNOSED BY WHOLE-EXOME SEQUENCING

**Sousa AB<sup>1,2</sup>, Abhyankar AV<sup>3</sup>, Albuquerque A<sup>2,4</sup>, Vieira M<sup>5</sup>, Carmo JA<sup>6</sup>, Silva SL<sup>2,7</sup>, Boisson B<sup>3</sup>, Frugoni F<sup>8</sup>, Notarangelo LD<sup>8</sup>, Sousa AE<sup>2,4</sup>, Casanova JL<sup>3</sup>, Marques JG<sup>2,9</sup>**

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Introduction: Severe combined immunodeficiencies (SCID) are a clinically and genetically heterogeneous group of diseases characterized by failure of adaptive cellular and humoral immune mechanisms leading to life-threatening infections in infancy.

X-linked SCID is caused by mutations in the *IL2RG* gene located on Xp13.1. This gene codifies the interleukin (IL) receptor common gamma chain that heterodimerises to form receptor complexes for the cytokines IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, which play a central role in lymphocyte homeostasis and function. In XL-SCID signaling through these receptors is compromised, such that the number of T cells is usually very low (T-), B cells are generally present albeit nonfunctional (B+), NK cells are low or absent (NK-).

Case Report: DMO is the first son of a young healthy consanguineous couple. He was diagnosed with SCID at 4-months following admission for severe respiratory failure due to *Pneumocystis jiroveci* pneumonia and disseminated *Mycobacterium bovis* (BCG) infection. The immunological phenotype was T-B-NK-. There was evidence of maternal chimerism. There were no maturative blocks in granulopoiesis, ADA activity was normal and there was no evidence of a DNA repair defect, excluding the usual causes of T-B- SCID. He underwent hematopoietic stem cell transplantation with adequate engraftment resulting in immunological reconstitution. At this point whole-exome sequencing (WES) was undertaken, and identified the mutation p.M145fsX21 in hemizygosity in the *IL2RG* gene. The mutation was subsequently confirmed by Sanger sequencing.

Discussion: Two lessons should be learnt from this case. Firstly, XL-SCID was not considered in the initial differential diagnosis because the immune phenotype was B- but reality does not always conform to textbook rules. Secondly, autosomal recessive inheritance was assumed because of parental consanguinity but turned out to be just a coincidence. Finally, this case illustrates the power of next-generation sequencing (NGS) approaches to produce etiological diagnosis in situations of wide genetic heterogeneity.

## (OC9) WHOLE EXOME SEQUENCING IN PATIENTS WITH A RETT-LIKE PHENOTYPE

**Lopes F<sup>1,2</sup>, Barbosa M<sup>1,2,3</sup>, Temudo T<sup>4</sup>, Sá J<sup>5</sup>, Dias Al<sup>6</sup>, Oliveira G<sup>7</sup>, Cabral P<sup>8</sup>, Calado E<sup>6</sup>, Fineza Cruz I<sup>7</sup>, Soares G<sup>3</sup>, Vieira JP<sup>6</sup>, Venâncio M<sup>5</sup>, Oliveira R<sup>5</sup>, Jonasson I<sup>9</sup>, Ameer A<sup>9</sup>, Gyllensten U<sup>9</sup>, Maciel P<sup>1,2</sup>**

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Rett syndrome (RTT) results in a large fraction of patients from mutations in the *MECP2* gene, usually a point mutation or a small insertion or deletion. Some cases with gross rearrangements involving the *MECP2* gene, usually large deletions, are also found. However, there is still a large portion of cases that clinically resemble RTT for which no *MECP2*, *CDKL5* or *FOXG1* mutations can be found. The goal of this project was to identify new genetic causes of Rett-like phenotypes using whole exome sequencing (WES), in a cohort of 20 Portuguese patients (18 female, 2 male) with a clinical presentation significantly overlapping Rett syndrome, according to the recently revised criteria. We used the SOLiD technology for sequencing, after exome capture. In the variant filtering, we excluded variants present in an in-house database of exome sequences (from controls and patients with different disorders, excluding ID), searching for de novo variants in heterozygosity, inherited variants present in heterozygosity in parents but in homozygosity or compound heterozygosity in the patient, and maternally transmitted variants in male patients. We performed bibliography searches using PubMed, and searched for functional and disease related information in the Gene and OMIM databases. We used SIFT and Polyphen to predict the impact of the variants found.

WES allowed the identification of gene mutations with a very likely contribution for the neurodevelopmental pathology observed in the studied patients. Interestingly, sequence variants in several genes related to ciliopathies, autism and other neurological disorders (including ataxias and neuropathies) were identified. Patients were often carriers of more than one potentially pathogenic mutation, in addition to heterozygous mutations in known recessive disease-causing variants (associated with unrelated phenotypes). With this work we expect to define new genetic – X-linked, autosomal recessive and dominant - disorders resembling Rett syndrome that have until recently been recalcitrant to gene identification.

**(OC10) VALIDATION OF NEXT GENERATION DNA SEQUENCING FOR THE MOLECULAR DIAGNOSIS OF IDIOPATHIC HYPERTROPHIC CARDIOMIOPATHY**

**Costa JL<sup>1</sup>, Ribeiro C<sup>1</sup>, Justino A<sup>2</sup>, Fernandes R<sup>1</sup>, Sousa S<sup>1</sup>, Canedo P<sup>1</sup>, Pina MJ<sup>2</sup>, Cirnes L<sup>1</sup>, Machado JC<sup>1</sup>**

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**Background:** The development of massive parallel sequencing (MPS) has revolutionized the field of genomics and created new avenues for basic research. However, the implementation of these technologies in a clinical diagnostic setting remains largely unexplored. Idiopathic hypertrophic cardiomyopathy (HCM) is an heterogeneous genetic disorder with causative mutations identified in 14 genes. Due to the genetic heterogeneity and low throughput of current gene-diagnostic tools, the molecular diagnosis of patients with HCM is both challenging and time-consuming. In this study, we used the benchtop Ion Torrent PGM to develop a MPS based approach for the molecular diagnosis of patients with HCM.

**Methods:** We designed a multiplex PCR-based strategy for the enrichment of all coding regions of 8 genes that together account for nearly 95% of all mutations detected in HCM. A dedicated variant prioritization pipeline (VPP) was developed for data-analysis. Constitutional genomic DNA of 30 cases was used to optimize the strategy, and to validate and estimate the power of the new methodology. All samples were studied using both the “gold standard” Sanger sequencing and MPS on the PGM.

**Results:** All 196 variants, including both non-causing and disease-causing variants, identified by Sanger sequencing were detected with our MPS approach. Disease-causing mutations were identified in 51% of the cases. Blind analysis of the data resulted in an experimental approach with a specificity of 98% and a maximum analytical sensitivity ≥98%, with a confidence of 95%. The developed workflow resulted in a turnaround time reduction of 30%.

**Discussion:** In this study we developed a faster, more comprehensive and more cost-effective methodology for the genetic screening of patients with HCM, than conventional Sanger sequencing. This approach demonstrates the potential of a combined MPS-Sanger sequencing based strategy as an effective molecular diagnostic tool for heterogeneous diseases.

**(OC11) ASSOCIATION OF HIGHLY CONSERVED MAJOR HISTOCOMPATIBILITY COMPLEX SNP HAPLOTYPES WITH IRON AND LOW CD8<sup>+</sup> T-LYMPHOCYTE PHENOTYPES IN HEMOCHROMATOSIS PATIENTS WITH HFE C282Y HOMOZYGOSITY FROM THREE GEOGRAPHIC AREAS**

**Costa M<sup>1</sup>, Cruz E<sup>1,2</sup>, Barton JC<sup>3</sup>, Thorstensen K<sup>4</sup>, Morais S<sup>5</sup>, Martins da Silva B<sup>6</sup>, Pinto JP<sup>1</sup>, Vieira CP<sup>7</sup>, Carracedo A<sup>8</sup>, Vieira J<sup>9</sup>, Acton RT<sup>3,10</sup> Porto G<sup>1,2,5</sup>**

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Over 80% of patients with Hereditary Hemochromatosis (HH), an iron overload disorder, are homozygous for the C282Y mutation of *HFE*, localized at the major histocompatibility complex (MHC) region, 4 Mb telomeric to human leukocyte antigen (HLA)-A. C282Y mutation is in strong linkage disequilibrium with particular HLA alleles and haplotypes. A phenotype of low CD8<sup>+</sup> T-lymphocyte numbers is common in HH patients and is transmitted in association with particular HLA-A alleles, supporting the postulate that MHC-class I genes influence CD8<sup>+</sup> T-lymphocyte numbers.

Analysis of 608 MHC haplotypes (using HLA-A, -B and 3 SNP markers between the HLA region and *HFE*) and of CD8<sup>+</sup> T-lymphocyte numbers in three populations of HH patients from geographically distant regions, namely Alabama (USA), Porto (Portugal) and Trondheim (Norway), showed that relative frequencies of haplotypes and their associated phenotypes are variable, probably reflecting different recombination histories. To study haplotype structure associated with low CD8<sup>+</sup> T-lymphocytes, we performed high-density SNP mapping (62 markers) of the region between *HLA-A* and *HFE* in 86 chromosomes from Portuguese HH patients and 210 control chromosomes from healthy subjects. Two major haplotype groups, defined as AA and GC according to flanking alleles of a block of 4 SNP markers, occurred in patients and controls. Further analysis of all HH chromosomes suggests that there are two independent conserved ancestral haplotypes: one (AA group) that contains HLA-A\*03-B\*07 and another (GC group) that contains HLA-A\*01-B\*08. Both conserved haplotypes are associated with low CD8<sup>+</sup> T-lymphocytes. This association is lost in non-conserved GC



haplotypes but is maintained in all AA haplotypes, suggesting that AA haplotypes arose more recently.

Our results contribute to localizing a candidate locus associated with transmission of the trait of low CD8<sup>+</sup> T-lymphocytes. Our strategy of selecting non-conserved chromosomes may help to identify individual loci contributing to low CD8<sup>+</sup> T-lymphocyte phenotypes in *HFE* C282Y homozygotes.

## **(OC12) CREATINE DEFICIENCY SYNDROMES: BIOCHEMICAL AND MOLECULAR ASPECTS**

**Valongo C<sup>1</sup>, Almeida LS<sup>1</sup>, Ramos A<sup>1</sup>, Salomons GS<sup>2</sup>, Jakobs C<sup>2</sup>, Vilarinho L<sup>1</sup>**

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Introduction: Creatine deficiency syndromes (CDS) represent a group of inborn errors of creatine biosynthesis: L-arginine-glycine amidinotransferase - AGAT and guanidinoacetate methyltransferase - GAMT deficiencies and transport (creatine transporter - SLC6A8 deficiency). Patients with CDS may present with mental retardation (MR), expressive speech and language delay, and epilepsy. Patients with GAMT deficiency or SLC6A8 deficiency may also exhibit autistic-like behavior. The common denominator of these disorders is the depletion of the brain creatine pool, as demonstrated by in vivo 1H-MRS.

Patients and Methods: The authors studied 6,600 urine samples from Portuguese autistic children and young adults for defects in creatine metabolism. We started with the determination of guanidinoacetate and creatine in urine by GC-MS-SIM. Based on these findings, enzyme assays or DNA mutation analysis may be performed. Molecular genetic analysis for GAMT deficiency and creatine transporter deficiency is also available in our laboratory.

Results: A marked excretion of guanidinoacetate in urine compatible with GAMT deficiency was observed in seven cases. Furthermore, other 15 patients showed high urinary levels of creatine/creatinine ratio what suggests a defect of SLC6A8. All GAMT deficient patients show the same mutation (c.59G>C) which suggests a founder effect in our population. Molecular genetic analysis of the SLC6A8 deficiency patients revealed a large spectrum of mutations.

Discussion: So far, 22 patients with CDS were identified in our laboratory (1:300). We believe these defects are still under diagnosed, so the possibility should be considered in all children affected by unexplained MR, seizures, and speech delay. SLC6A8 defect should also be considered in males with MR and negative fragile-X testing. GAMT deficiency is treatable with oral creatine monohydrate and ornithine supplementation with arginine dietary restriction.



Oral Communications - Session III		
Number	First Author	Title
OC13	Ana Justino	NON-OPTICAL MASSIVE PARALLEL DNA SEQUENCING FOR THE GENETIC DIAGNOSIS OF THE RAS/MAPK RELATED SYNDROMES
OC14	Rafaela Lacerda	MLH1 AND DCC, TWO GENES INVOLVED IN COLORECTAL CANCER, HAVE DIFFERENT MECHANISMS OF TRANSLATION INITIATION
OC15	Manuela Pinheiro	THE MSH2 C.388_389DEL MUTATION SHOWS A FOUNDER EFFECT IN PORTUGUESE LYNCH SYNDROME FAMILIES BUT ALSO OCCURS DE NOVO IN DIFFERENT POPULATIONS
OC16	Tiago D Baptista	EPIGENETIC MODULATING DRUGS INDIRECTLY DECREASE LEVELS OF H2A.Z BY SIRT1 UPREGULATION IN PROSTATE CANCER CELLS
OC17	Bruno Pereira	MODULATION OF CDX2 EXPRESSION BY THE RNA-BINDING PROTEIN MEX3A: IMPACT ON INTESTINAL-TYPE DIFFERENTIATION AND STEMNESS
OC18	Paula Paulo	MOLECULAR SUBTYPING OF PRIMARY PROSTATE CANCER REVEALS SPECIFIC AND SHARED TARGET GENES OF DIFFERENT ETS REARRANGEMENTS



**(OC13) NON-OPTICAL MASSIVE PARALLEL DNA SEQUENCING FOR THE GENETIC DIAGNOSIS OF THE RAS/MAPK RELATED SYNDROMES**

**Justino A<sup>1,2</sup>, Dias P<sup>3</sup>, Pina MJ<sup>1,2</sup>, Ribeiro C<sup>1</sup>, Sousa S<sup>1</sup>, Cirnes L<sup>1</sup>, Costa JL<sup>1</sup>, Sousa AB<sup>3</sup>, Machado JC<sup>1,4</sup>**

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<sup>2</sup>*Faculdade de Medicina da Universidade do Porto;* <sup>3</sup>*Serviço de Genética, Hospital de Santa Maria de Lisboa;* <sup>4</sup>*Departamento de Patologia e Oncologia, Faculdade de Medicina da Universidade do Porto*

**Introduction:** The Noonan, Cardio-facio-cutaneous, Costello and LEOPARD syndromes are members of the Neuro-cardio-facio-cutaneous syndromes group (NCFCS). Mutations in 11 genes associated with the RAS/MAPK signaling pathway have been causally linked to these disorders. Due to the high number of genes and lack of mutation hotspots it is difficult to perform molecular diagnosis of NCFCS. Moreover, the clinical overlap of the syndromes makes it difficult to define straightforward strategies of gene selection for molecular diagnosis. Although massive parallel sequencing (MPS) has the potential to offer a solution for this problem, its implementation in a diagnostic setting remains unexplored.

**Methods:** We designed a multiplex PCR-based strategy for the enrichment of all coding regions of the 11 genes, and developed a dedicated variant prioritization pipeline (VPP) for data-analysis. Two sets of samples were studied using the Ion Torrent PGM: a training set (18 cases) used to optimize the strategy; and a validation set (33 cases) used to validate and estimate the power of the methodology. Sanger sequencing was performed to confirm all variants and fill in regions with insufficient coverage.

**Results:** All variants previously identified by Sanger sequencing were detected with our MPS approach. Additionally, the analysis of new genes revealed 3 new mutations in cases previously reported as negative (p.Ala488Val and p.Pro782Leu on CBL, and p.Ser2Gly on SHOC2). The new methodology resulted in an experimental approach with a specificity of 99.7% and a maximum analytical sensitivity  $\geq 97.7\%$  with a confidence of 95%.

**Discussion:** In this study we developed, for the first time, a workflow that provides a comprehensive genetic screening in patients with NCFCS, in a fast and cost-efficient manner. This approach demonstrates the potential of a combined MPS-Sanger sequencing based strategy as an effective diagnostic tool for heterogeneous diseases.

**(OC14) MLH1 AND DCC, TWO GENES INVOLVED IN COLORECTAL CANCER, HAVE DIFFERENT MECHANISMS OF TRANSLATION INITIATION**

**Lacerda R**<sup>1,3</sup>, **Marques-Ramos A**<sup>1,3</sup>, **Teixeira A**<sup>1,2</sup>, **Romão L**<sup>1,3</sup>

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**Introduction:** Colorectal cancer (CRC) is one of the leading causes of death worldwide and its development and progression can be dependent upon regulation of gene expression. MLH1 and DCC are two genes whose expression is altered in CRC. MLH1 is a mismatch repair gene and its expression is repressed in CRC. DCC is a tumor suppressor gene that has one allele deleted in CRC. Regulation of gene expression can occur at translation level, namely at the initial step. Translation initiation can be cap-dependent or internal ribosome entry site (IRES)-dependent. IRESs are structures within the 5' untranslated region (UTR) of the mRNAs that directly recruit the ribosome without scanning the 5'UTR. This work aims to investigate whether MLH1 and DCC transcripts are translated via IRES or if their translation is exclusively cap-dependent.

**Methods:** The MLH1 and DCC 5'UTRs were cloned in the dicistronic p\_Renilla\_Firefly vector. NCM460 cells (derived from normal intestinal mucosa) and SW480 cells (derived from stage IV CRC adenocarcinoma) were transfected with such plasmids. Luciferase activity was measured by luminometry assays and compared to the negative control.

**Results and Discussion:** The levels of Firefly luciferase observed when DCC 5'UTR is present are similar to those of negative control in both cell lines, which means translation of this transcript is exclusively cap-dependent and not mediated by IRES. Regarding MLH1 5'UTR-containing plasmid, the levels of Firefly luciferase observed are 70-fold greater than that of negative control in NCM460 cells and 18-fold greater in SW480 cells, which might indicate IRES activity. However, when cells were transfected with a promoter-less plasmid containing this 5'UTR, we observed a 482-fold increase in Firefly luciferase levels in NCM460 cells and a 42-fold increase in SW480 cells, which suggests this region works as a cryptic promoter that is much more active in normal cells than in adenocarcinoma cells.

**(OC15) THE MSH2 C.388\_389DEL MUTATION SHOWS A FOUNDER EFFECT IN PORTUGUESE LYNCH SYNDROME FAMILIES BUT ALSO OCCURS DE NOVO IN DIFFERENT POPULATIONS**

**Pinheiro M<sup>1</sup>, Pinto C<sup>1</sup>, Peixoto A<sup>1</sup>, Veiga I<sup>1</sup>, Mesquita B<sup>1</sup>, Henrique R<sup>2</sup>, Lopes P<sup>2</sup>, Sousa O<sup>3</sup>, Fragoso M<sup>4</sup>, Moreira-Dias L<sup>5</sup>, Baptista M<sup>6</sup>, Marinho C<sup>7</sup>, Mangold E<sup>8</sup>, Vaccaro C<sup>9</sup>, Evans GD<sup>10</sup>, Farrington S<sup>11</sup>, Dunlop MG<sup>11</sup>, Teixeira MR<sup>1</sup>**

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**Introduction:** The MSH2 c.388\_389del mutation has occasionally been described in Lynch families worldwide. At the Portuguese Oncology Institute in Porto, Portugal, we have identified 16 seemingly unrelated families with this germline mutation.

**Methods:** In order to evaluate if this alteration is a founder mutation or a molecular defect that arose repeatedly de novo, we performed haplotype analysis in the 16 Portuguese index cases and 55 relatives, as well as in four index cases and 13 relatives reported from Germany, Scotland, England, and Argentina.

**Results:** In the Portuguese families we observed a shared haplotype of ~10 Mb and all were originated from the north of Portugal. In the reported families outside Portugal with this mutation different haplotype backgrounds were observed.

**Discussion:** Our results suggest that the MSH2 c.388\_389del mutation is a founder mutation in Portugal with a relatively recent origin. In the reported families outside Portugal this mutation has occurred de novo on multiple occasions in different haplotype backgrounds. We also conclude that the high proportion of the MSH2 c.388\_389del mutation indicates that screening for this alteration as a first step may be cost-effective in the genetic testing of Lynch syndrome suspects of Portuguese ancestry, especially those originating from the north of Portugal.



**(OC16) EPIGENETIC MODULATING DRUGS INDIRECTLY DECREASE LEVELS OF H2A.Z BY SIRT1 UPREGULATION IN PROSTATE CANCER CELLS**

**Baptista TD<sup>1,2</sup>, Graça I<sup>1,2</sup>, Sousa E<sup>1,2</sup>, Oliveira AI<sup>1,2</sup>, Costa-Pinheiro P<sup>1,2</sup>, Amado F<sup>4</sup>, Henrique R<sup>1,3,5</sup> and Jerónimo C<sup>1,2,5</sup>**

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**Introduction:** Current therapeutic approaches for advanced prostate cancer (PCa) are limited and mostly ineffective. Because epigenetic aberrations are common in PCa, the therapeutic use of epigenetic modulating drugs might provide a therapeutic alternative. The histone variant H2A.Z and its acetylated form have been previously associated with activation of oncogenes owing to their location near the TSS in PCa cells. Thus, we aimed to evaluate the effect of epigenetic modulating drugs on H2A.Z expression.

**Methods:** Three PCa cell lines (LNCaP, DU145 and PC-3) were treated with 5-aza-2'-deoxycytidine (DAC) and Trichostatin A (TSA). Transcript and protein levels of H2AFZ and SIRT1 were assessed by quantitative RT-PCR and Western blot, respectively. ChIP was performed to evaluate how treatment altered the pattern of histone marks. Subsequently, exposure to nicotinamide and resveratrol, an inhibitor and an inducer of sirt1 activity, respectively, was performed to evaluate the role of sirt1 in H2A.Z levels.

**Results:** Treatment with TSA alone and combined with DAC led to an increase of H2AFZ transcript levels, although with a concomitant decrease in protein levels. Conversely, SIRT1 transcript and protein levels increased after drug exposure. ChIP revealed an increase of activation marks within the TSS region for both genes. Remarkably, after inhibition of sirt1, H2A.Z levels increased dramatically, whereas induction of sirt1 led to an abrupt decrease in H2A.Z levels.

**Discussion:** Although epigenetic drugs are able to regulate both H2AFZ and SIRT1 transcription levels, the lack of correlation between mRNA and protein levels for H2AZ suggest the involvement of a post-translational mechanism. Indeed, sirt1 has the ability to degrade H2A.Z, as previously reported for cardiomyocytes. Thus, H2A.Z protein levels are indirectly regulated by epigenetic drugs, probably through SIRT1 upregulation. In primary PCa, overexpression of H2AFZ and downregulation of SIRT1 validate both genes as targets of epigenetic treatment.

**(OC17) MODULATION OF CDX2 EXPRESSION BY THE RNA-BINDING PROTEIN MEX3A: IMPACT ON INTESTINAL-TYPE DIFFERENTIATION AND STEMNESS**

**Pereira B<sup>1</sup>, Sousa S<sup>1,2</sup>, Barros R<sup>1</sup>, Carreto L<sup>3</sup>, Oliveira P<sup>1</sup>, Oliveira C<sup>1,4</sup>, Chartier N<sup>5</sup>, Rouault JP<sup>6</sup>, Freund JN<sup>7</sup>, Billaud M<sup>8</sup>, Almeida R<sup>1,4</sup>**

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**Introduction:** The homeobox transcription factor CDX2 plays a key role in specifying intestinal cell fate, both in intestine during normal development and in tumorigenic processes of the gastrointestinal tract, implying a need for tight regulation. Our objective was to identify new CDX2 regulatory mechanisms.

**Methods:** Through genome-wide screening of a three-dimensional culture system comprising the gastric carcinoma AGS cell line and an extracellular matrix (Matrigel), we disclosed the RNA-binding protein MEX3A as a putative CDX2 regulator. Biological relevance of this regulation was addressed by modulating MEX3A levels in various cell assays, performing RNA-immunoprecipitation and luciferase reporter experiments and assessing its expression in mouse intestine.

**Results:** We observed that CDX2 protein expression was abrogated in three-dimensional culture, lacking correlation with mRNA suggesting a post-transcriptional regulation. Transcriptomic analysis revealed an increased expression of the RNA-binding protein MEX3A in the 3D culture. MEX3A repressive function was assessed by an inverse correlation with CDX2 protein, both in gastric and colorectal cell lines. We proved interaction of MEX3A with CDX2 mRNA 3'untranslated region and defined the specific binding sequence. Phenotypic characterization of in vitro models demonstrated that MEX3A further impacts on intestinal stem cell potential, differentiation and polarity. Finally, we showed MEX3A is expressed in mouse intestine, specifically where stem, transit-amplifying and migrating post-mitotic cells are present.

**Discussion:** The recent identification of MEX3A as being part of an intestinal stem cell signature together with our observations support an in vivo role for MEX3A on intestinal stemness and CDX2 interaction. We thus provide the first demonstration of MEX3A functional conservation as a novel translational repressor in humans and of CDX2 as one of its targets, affecting processes of intestinal-like differentiation and stemness and possibly contributing to gastrointestinal homeostasis and carcinogenesis.

# **(OC18) MOLECULAR SUBTYPING OF PRIMARY PROSTATE CANCER REVEALS SPECIFIC AND SHARED TARGET GENES OF DIFFERENT ETS REARRANGEMENTS**

**Paulo P<sup>1</sup>, Ribeiro FR<sup>1</sup>, Santos J<sup>1</sup>, Mesquita D<sup>1</sup>, Almeida M<sup>1</sup>, Barros-Silva JD<sup>1</sup>, Itkonen H<sup>2</sup>, Henrique R<sup>3</sup>, Jerónimo C<sup>1</sup>, Sveen A<sup>4</sup>, Mills IG<sup>2</sup>, Skotheim RI<sup>4</sup>, Lothe RA<sup>4</sup>, Teixeira MR<sup>1</sup>**

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**Introduction:** Genomic rearrangements involving ERG and ETV1 (two members of the ETS family of transcription factors) are found in 50-70% of prostate carcinomas (PCa). The products of specific chimeric genes could be targeted therapeutically, but the nuclear localization of the aberrant ETS proteins makes them difficult therapy targets in vivo. This work aimed to evaluate whether ERG and ETV1 regulate specific or shared target genes, as some may be more amenable to targeted therapy.

**Methods:** We performed differential expression analysis on nine normal prostate tissues and 50 PCa enriched for different ETS rearrangements using exon-level expression microarrays, followed by in vitro validation using cell line models.

**Results:** We found specific deregulation of 57 genes in ERG-positive PCa and 15 genes in ETV1-positive PCa, whereas deregulation of 27 genes was shared in both tumor subtypes. We further showed that the expression of seven tumor-associated ERG target genes (PLA1A, CACNA1D, ATP8A2, HLA-DMB, PDE3B, TDRD1 and TMBIM1) and two tumor-associated ETV1 target genes (FKBP10 and GLYATL2) was significantly affected by specific ETS silencing in VCaP and LNCaP cell line models, respectively, whereas the expression of three shared candidate targets (GRPR, KCNH8 and TMEM45B) was significantly affected by silencing of either ETS. Interestingly, we demonstrate that the expression of TDRD1, the top-most overexpressed gene of our list of ERG-specific targets, is inversely correlated with the methylation levels of a CpG island found at -66bp of the transcription start site in PCa and that TDRD1 expression is regulated by direct binding of ERG to the CpG island in VCaP cells.

**Discussion:** We conclude that ETS transcription factors regulate specific and shared target genes and that TDRD1, FKBP10 and GRPR are promising therapeutic targets and can serve as diagnostic markers for molecular subtypes of PCa harboring specific fusion gene rearrangements.

## Posters

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**(P1) MICRODUPLICATION SYNDROMES - THREE CASES OF POTOCKI-LUPSKI SYNDROME**

**Louro P<sup>1</sup>, Oliveira R<sup>1</sup>, Reis CF<sup>1</sup>, Garabal A<sup>1</sup>, Duarte C<sup>2</sup>, Melo JB<sup>3</sup>, Carreira IM<sup>3</sup>, Ramos F<sup>1</sup>, Sá J<sup>1</sup>, Venâncio M<sup>1</sup>, Ramos L<sup>1</sup>, Saraiva J<sup>1</sup>**

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**Introduction:** Chromosomal microarray analysis is currently being used for the etiologic diagnosis of mental retardation such as occurs in Potocki-Lupski syndrome, a recently recognized syndrome of multiple congenital anomalies and psychomotor developmental delay.

Our purpose is to compare the phenotype of our patients with similar cases described in the literature and once more to highlight the utility of chromosomal microarray analysis in the diagnosis of microdeletion/microduplication syndromes, namely in the detection of Potocki-Lupski syndrome.

**Methods:** DNA was extracted from patients' blood and Agilent 4x180K whole genome oligonucleotide array-CGH was performed.

**Results:** The authors report three cases of psychomotor developmental delay and variable phenotype in which a *de novo* duplication was identified in the critical region 17p11.2, associated with Potocki-Lupski syndrome.

**Discussion:** Our patients have some phenotypic alterations found in individuals with Potocki-Lupski syndrome. In the last few years, array-CGH has assumed an essential part in the diagnosis of microdeletion/microduplication syndromes, which are not diagnosed with conventional cytogenetic studies, therefore allowing a more precise genetic counselling of patients and their families.

## **(P2) MICRODELETION SYNDROMES - ONE CASE OF 17q21.31 MICRODELETION SYNDROME**

**Louro P, Reis CF, Garabal A, Sá J, Ramos L, Saraiva J**

*Department of Medical Genetics, Centro Hospitalar e Universitário de Coimbra, Portugal*

**Introduction:** The 17q21.31 microdeletion syndrome is characterized by psychomotor developmental delay, dysmorphisms and congenital anomalies. It is a fully penetrant trait with variable expressivity and its prevalence in general population is estimated at approximately 1:16,000 individuals.

Our objective is to compare our patient's phenotype with other cases described in the literature and once again to underline the utility of chromosomal microarray analysis in the diagnosis of microdeletion/microduplication syndromes.

**Methods:** Extraction of DNA from peripheral blood lymphocytes. Chromosomal microarray analysis (Affymetrix CytoScan HD).

**Results:** We report a case of polymalformative syndrome (bilateral sensorineural hearing loss, convergent strabismus, ventricular septal defect, bilateral ureteropelvic dilatation, congenital hip dislocation, and bilateral clubfoot) with psychomotor developmental delay in which a deletion was identified in the critical region 17q21.31.

**Discussion:** Our patient shows phenotypic alterations common to individuals with 17q21.31 microdeletion syndrome. SNP array is presently one of the best diagnostic tools at our disposal for the diagnosis of microdeletion/microduplication syndromes, especially on those with variable clinical spectrum, namely 17q21.31 microdeletion syndrome.

### (P3) RETINOBLASTOMA: PRELIMINAR REPORT OF TEN CASES

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Introduction: Retinoblastoma is a malignant tumor of the developing retina that occurs in children, usually before age five years. It occurs in cells that have cancer-predisposing mutations in both copies of the gene *RB1* and may be unifocal or multifocal. The clinical diagnosis is usually established by examination of the fundus of the eye using indirect ophthalmoscopy. *RB1* is the only Individuals heterozygous for a cancer-predisposing mutation in one *RB1* allele are said to have a germline mutation and thus have a hereditary predisposition to RB. They also have an increased risk of developing other RB-related (non-ocular) tumors.

Method: Clinical characterization and molecular genetic testing of the *RB1* gene.

Results: The authors report ten cases of bilateral and unilateral Retinoblastoma. In six cases was identified a pathogenic heterozygous mutation in the *RB1* gene.

Discussion: Molecular genetic testing of the *RB1* gene can identify a germline mutation in 90%-95% of individuals with a hereditary predisposition to Retinoblastoma. This diagnosis allows early diagnosis and treatment in asymptomatic children at risk as well as allow couples at high risk, the use of reproductive options available (prenatal diagnosis and Preimplantation genetics diagnosis).



#### **(P4) FLOATING HARBOR: CLINICAL AND MOLECULAR DIAGNOSIS**

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Introduction: Floating-Harbor syndrome is a rare genetic disorder characterized by proportionate short stature, delayed bone age, delayed speech development, and distinctive facial features, specifically triangular face with a broad nasal bridge, wide columella and short philtrum. The speech defect is marked by impairment of expressive language and is often associated with a peculiar hypernasal voice. There are less than fifty cases described in the literature.

Method: Re-evaluated of clinical phenotype and sequencing of SRCAP gene, on chromosome 16p11.2.

Results: Identification of a pathogenic heterozygous mutation in SRCAP gene.

Discussion: The authors present a case of patient with clinical diagnosis of Floating Harbor Syndrome at 3 years-old of age in which was possible to perform molecular diagnosis at 17 years-old. The molecular characterization of this clinic diagnostic was of great importance for its autosomal dominant inheritance, allowing adequate genetic counseling and the possibility of performing molecular prenatal diagnosis or preimplantation genetic diagnosis.

**(P5) CHRISTIANSON SYNDROME IN A PATIENT WITH AN INTERSTITIAL XQ26.3 DELETION**

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Introduction: Christianson syndrome is a syndromal form of X-linked mental retardation (XLMR) characterized by microcephaly, severe to profound developmental delay and intellectual disability, ataxia and epilepsy, among other less frequent clinical manifestations.

Clinical Report and Results: We describe the case of a 21-year-old male patient with profound intellectual disability, absent speech, ataxia, microcephaly, epilepsy, kyphoscoliosis and minor skeletal abnormalities. The family history suggested a form of XLMR, with three other male family members affected and several women with a milder phenotype. Array comparative genomic hybridization (a-CGH) analysis was performed which revealed an interstitial 10,9 Kb deletion in Xq26.3 affecting SLC9C6. The diagnosis of Christianson syndrome was thus established. This deletion was inherited from his mother, who had a history of learning disabilities. The a-CGH also showed a de novo small Xq21.1 deletion encompassing the MAGT1 gene, associated with non-syndromal XLMR and an interstitial Yq11.2 4,1 Mb duplication.

Discussion: Most of the clinical features of this patient are in accordance with other patients with SLC9A6 associated Christianson syndrome, except for the skeletal findings. The majority of cases described in the literature derive from SLC9C6 point mutations unlike the case here reported. The a-CGH was extremely useful in the investigation of this patient, providing the diagnosis. The molecular diagnosis allowed ongoing family studies and the possibility of prenatal or preimplantation diagnosis in carrier women of the Xq26.3 deletion.

## **(P6) PITT-HOPKINS SYNDROME: A CASE REPORT**

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Introduction: Pitt-Hopkins syndrome (PTHS, MIM#610954) is characterized by severe intellectual disability, typical facial features and tendency to epilepsy, episodic breathing abnormalities and stereotypic movements. The cause of PTHS is de novo haploinsufficiency of the TCF4 gene (MIM\*602272) at 18q21.2.

Clinical Report and Results: A 12-year-old girl was referred for reevaluation of postnatal microcephaly, severe intellectual disability and stereotypic hand movements with no diagnosis after extensive studies. The etiologic investigation had been initiated years before by karyotyping, followed by methylation studies of the Angelman syndrome locus, metabolic disorders screening, brain MRI and MECP2 (Rett syndrome) gene sequencing, all of which showed no significant abnormalities. Sequencing of the UBE3A (Angelman syndrome) and ZFXB1B (Mowat-Wilson syndrome) genes were later conducted with no diagnostic results as well. The hypothesis of Pitt-Hopkins syndrome emerged upon reevaluation, suggested by the presence of episodic shouting and possible hyperventilation. The diagnosis was confirmed by sequencing of the TCF4 gene that revealed a de novo heterozygous mutation in exon 11 (c.886delT – p.C296VfsX11).

Discussion: The patient presented relatively unspecific clinical manifestations regarding Pitt-Hopkins syndrome: severe intellectual disability, postnatal microcephaly and stereotypic movements; she lacked the typical facial features and a sound history of episodic hyperventilation, prompting the study of other more frequent differential diagnosis. This case supports previous recommendations regarding TCF4 mutation screening in patients in whom the diagnosis of Angelman, Rett or Mowat-Wilson syndromes were suspected. The diagnosis allowed accurate genetic counseling of the parents, and the possibility of a molecular prenatal diagnosis in future pregnancies due to the 1% recurrence risk related to germline mosaicism. This case report also illustrates the importance of reevaluating patients previously observed.

**(P7) FURTHER CHARACTERIZATION OF OCULOauriculoVERTEBRAL SPECTRUM - DESCRIPTION OF 40 CASES**

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**Introduction:** Oculoauriculovertebral spectrum (OAVS) is a common but phenotypically heterogeneous craniofacial disorder, characterized by anomalies of the ear, hemifacial microsomia, and vertebral defects. Associated clinical findings include anomalies of the eye, heart and brain.

**Methods:** We have phenotypically evaluated 17 female and 23 male OAVS patients, of different ethnic backgrounds, all presenting with either isolated microtia or preauricular tags, in association with hemifacial microsomia or a positive family history as minimal diagnostic criteria.

**Results:** Most cases were sporadic (28/30). Hemifacial microsomia and/or anomalies of the ear were present in all patients. Bilateral involvement was predominant (20/40) and always asymmetrical. Hemifacial microsomia was observed in 37/40 patients, with a predilection for the right side, and associated with ipsilateral nerve weakness in 23 cases. Preauricular skin tags were observed in 21 patients (bilaterally in 10) and not correlated with the severity of the OAVS phenotype. Microtia/anotia and/or displacement of ear were observed in 29 patients, and frequently associated to ipsilateral atresia of external auditory canal (21/37). Hearing loss was confirmed in 24/40 patients including conductive hearing (11/40), mixed (12/40), and sensorineural (1/24). Cleft lip and/or palate was observed in 4 cases and associated with a more severe phenotype. Ocular involvement was rare and included epibulbarermoid, microphthalmos, and coloboma of the eyelid/iris/optic. 13 patients presented learning difficulties and/or developmental delay. Additional, craniofacial features included micrognathia and palate hypoplasia (1/40); extra maxillary incisor (1/40); left facial cleft (1/40); and hypoplasia of the corpus callosum (2/40). Congenital cardiopathies were described in 5/40 patients; genito-urinary involvement (4/40), and limb abnormalities (4/40) were also observed.

**Discussion:** The phenotypic profile in our patients was similar to previous reports. However, we found that bilateral involvement and hearing loss (conductive, mixed and neurosensorial) are more common in our group. Molecular studies are ongoing to define the genetic aetiology of OAVS.

## (P8) DESCRIPTION OF TWO PATIENTS WITH FUMARASE DEFICIENCY

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**Introduction:** Fumarate hydratase (FH) deficiency is a very rare autosomal recessive disorder characterized by early onset severe encephalopathy. Milder cases have been described.

**Methods:** We reviewed the clinical data of two girls with fumarase deficiency, diagnosed after fumaric aciduria detection in the diagnostic workup of development delay.

**Results:** Patient 1: 11 year-old girl with severe developmental delay, behavioural problems and growth retardation, presents persistent hyperlactacidaemia, with increased lactate/pyruvate ratio. Family history was irrelevant. Brain MRI scan disclosed unspecific abnormalities. NMR spectroscopy was normal. Fumaric acid in urine was 24  $\mu\text{mol}/\text{mmol creat}$  (normal: 0 - 3,76).

Patient 2: 6 year-old girl presents severe developmental delay and oppositional disorder, postnatal microcephaly, gait imbalance, epilepsy and intermittent hyperlactacidaemia. EEG showed fast rhythms and nonspecific paroxysmal activity in the right occipital region. She presents mild hypertonia and hyperreflexia of the limbs and ataxic gait. Her parents report miscarriage in their first pregnancy. MRI scan depicted mild cerebellar and subcortical brain atrophy. NMR spectroscopy was normal. Fumaric acid in urine was 55  $\mu\text{mol}/\text{mmol creat}$  (normal: 0 - 3,76).

Further biochemical screening, including mitochondrial studies, blood acylcarnitine profiles and chromosome analysis were normal. Sequencing of the coding sequence of the FH gene in the two girls did not identify any mutations.

**Discussion:** The diagnosis of the two patients was based on the clinical presentation, presence of fumaric aciduria and absence of additional specific abnormalities. No mutations were found in the coding sequence of FH. Routine sequencing method, however, does not identify splice-site mutations or large rearrangements, which together account for 16% of the cases. Although there is no specific treatment, molecular diagnosis is essential for specific genetic counseling. Furthermore, FH mutation carriers have an increased risk for leiomyomatosis and renal cell cancer. Therefore risk assessment and surveillance should also be provided to these families.

**(P9) OCULOauriculoVERTEBRAL SPECTRUM: REPORT OF THREE FAMILIAL CASES WITH EVIDENCE OF AUTOSOMAL DOMINANT INHERITANCE**

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**Introduction:** Oculoauriculovertebral spectrum (OAVS) describes a heterogeneous developmental disorder of craniofacial structures derived from the first and second branchial arches, associated to ocular anomalies and vertebral defects. The aetiology of OAVS is still unknown and likely to be heterogeneous. Most cases are sporadic. More than 40 familial cases with OAVS have been reported so far.

**Methods:** We describe the clinical phenotype of three familial cases of OAVS with an autosomal dominant mode of inheritance. We also performed a comprehensive literature review and compared our patients with other published familial cases.

**Results:** In our families the phenotype is mild, similar to those described in the literature, and bilateral involvement is common. Ear displacement and mild facial asymmetry are observed in all affected individuals. However, severe microtia, eye and vertebral involvement are not observed in our families. One of our patients has learning disabilities and bilateral hearing loss, which is not commonly described in AD OAVS. Inter- and intra-familial variable clinical expression is observed.

**Discussion:** We describe three further OAVS families with AD transmission. Familial AD cases usually present with a milder phenotype, and a higher frequency of bilateral auricular involvement, and this was the case in our families. Like many AD genetic conditions, the phenotypes show variable expression within families, and there were additional clinical features in some cases such as learning difficulties, bilateral hearing loss, severe microtia, eye and vertebral defects. Since more inherited OAVS cases are being identified it highlights the importance of a detailed family history for genetic counseling purposes, even though the genetic causes are not yet known. It is also important to identify any minor clinical signs, such as mild hemifacial microsomia and discrete preauricular tags, that may be present in apparently affected relatives as this may be relevant to their reproductive risks.

**(P10) IMPACT OF GENETIC COUNSELLING ON REPRODUCTIVE PLANNING OF COUPLES IN FAMILIES WITH MYOTONIC DYSTROPHY TYPE 1**

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Introduction: Myotonic dystrophy type 1 (DM1) is a neuromuscular, multisystem, progressive disease, with autosomal dominant inheritance. Genetic counselling is a delicate process in this disease, as reproductive decisions are difficult due to the variable clinic presentation, the variable age of onset and the phenomenon of anticipation. We planned (1) to assess the impact of genetic counselling in couples where man or the woman had DM1, their reproductive choices and factors that influenced it, (2) to consider the results of prenatal diagnosis (PND), and (3) to assess the influence of psychosocial elements in couples reproductive planning.

Methods: A retrospective study of 10 years used a questionnaire aimed at those couples who had been seen in genetic counselling for reproductive planning.

Results: The main reproductive choice was not to have children (55.6%), followed by pregnancy and PND (33.3%). In 60% of the couples who had PND, the foetus was affected and the option had been for termination of pregnancy. The main factors that have influenced reproductive decisions were (1) the high risk of having an affected child, and (2) the existence of other children prior to genetic counselling; and (3) the psychosocial impact of the disease.

Discussion: Genetic counselling had a strong impact on reproductive choices in couples from families with myotonic dystrophy. A multidisciplinary team should always be involved in the counselling process, to meet the expectations and the needs of counselees, and to provide the support they need at all stages of their decision-making process about reproductive options.

## **(P11) CASCADE SCREENING IN FAMILIAL HYPERCHOLESTEROLEMIA: IMPORTANCE IN EARLY DETECTION**

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*On behalf of the investigators of the Portuguese FH Study*

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**Introduction:** Familial hypercholesterolemia (FH) is a genetic condition mainly caused by mutations in LDLR but missense mutations in APOB and PCSK9 can cause similar phenotypes. FH is characterized by increased levels of LDL cholesterol, leading to premature cardiovascular diseases (CVD). Cascade screening (CS) allows the rapid identification of new FH cases within a family.

The main goal of this work is to perform CS of families and to understand the importance of this approach to identify prematurely individuals that are at risk to develop CVD.

**Methods:** DNA extraction was prepared from blood samples from family members of each index patient (which already had a molecular diagnosis) and then the amplification and sequencing of exons that contains mutations in LDLR, APOB or PCSK9, depending of the mutation found in each index case was performed. The sequencing results were analyzed using the software staden package®.

**Results:** In one year of work, we did the CS of 45 families, comprehending 106 relatives (23 children and 83 adults). Of these, 66 had a known mutation causing FH. However, 49,18% of these relatives weren't under medication, and so, in greater risk to develop a premature CVD.

**Discussion:** CS is the most cost effective method to identify FH patients for several reasons. First, the molecular study is done by searching the mutation that was found in the respective index case, saving time and money. Second, relative's phenotype, most of the times, is less severe and does not allow clinical identification and so CS allows premature detection of FH and cardiovascular risk stratification, leading to the reduction of morbidity and mortality by implementation of adequate counseling and therapeutic measurements. FH is still under-diagnosed in Portugal, and so, efforts must be made to enlarge adherence of relatives to CS, maybe through increase disclosure of information to the community.



**(P12) ORPHANET-PT – THE REFERENCE PORTAL FOR RARE DISEASES AND ORPHAN DRUGS IN PORTUGAL**

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In Europe, the definition of a disease is a prevalence below 1:2,000 persons, irrespective of aetiology. There are more than 7,000 rare diseases, affecting 25-30 million patients in Europe; 80% of rare diseases are of genetic origin. The small number of cases of each of these diseases, in a given geographic region, delays diagnosis and management, and creates great difficulties for service planning and clinical care, as well as for basic and clinical research, including the development and trial of new drugs.

ORPHANET ([www.orpha.net/national/PT-PT](http://www.orpha.net/national/PT-PT)) is the reference database of rare diseases and orphan drugs, which aims at providing high quality, scientifically validated information to patients, health professionals, researchers and the industry; and at fostering interaction among these groups.

ORPHANET contains detailed information about over 2,000 rare diseases, including specialized clinics, diagnostic labs, research projects, support groups, clinical trials and orphan drugs available in the participating countries.

In Portugal, Orphanet has been active since November 2002; it is based at CGPP, IBMC, since April 2009. A national scientific committee has been created. Our working group aims also at collecting all the relevant information on resources available in the field of rare disorders and orphan drugs in the country. We will present a global overview of ORPHANET, highlighting the ongoing work of the Portuguese team and the results achieved, as well as new services to be provided.

**(P13) PROGRAMA NACIONAL DE DIAGNÓSTICO PRECOCE EM PORTUGAL-  
CASUÍSTICA DE 2011**

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O Programa Nacional de Diagnóstico Precoce (PNDP) visa identificar doenças nas primeiras semanas de vida do bebé de forma a possibilitar uma intervenção precoce e a impedir a ocorrência de atraso mental, doença grave irreversível ou morte da criança. O PNDP iniciou-se em 1979 com o rastreio da fenilcetonúria tendo-se implementado em 1981 o rastreio do hipotiroidismo congénito. As novas tecnologias de espectrometria de massa, permitindo a análise simultânea de vários parâmetros numa só amostra de sangue, possibilitaram, a partir de 2004, o aumento das doenças rastreadas até às atuais 25 (24 doenças hereditárias do metabolismo e o hipotiroidismo congénito). O rastreio é feito sobre o sangue colhido por picada no pé do bebé, entre o 3º e o 6º dia de vida, para uma ficha com papel de filtro adequado, sendo a totalidade das análises efetuadas na Unidade de Rastreio Neonatal Metabolismo e Genética do INSA. A taxa de cobertura do PNDP é 100% dos recém-nascidos, sendo o tempo médio de início de tratamento de 9,87 dias. Em Portugal, durante o ano de 2011, foram rastreados 97.116 recém-nascidos tendo-se identificado 44 casos de Hipotiroidismo Congénito (1/2.207) e 31 casos de doenças hereditárias do metabolismo (1/3.133). Os programas de rastreio neonatal são sistemas dinâmicos que devem ser continuamente avaliados e atualizados. Nesta conformidade, há duas doenças cuja integração no Programa Nacional de Diagnóstico Precoce, deve ser considerada e avaliada, atendendo à realidade atual em termos demográficos e de tratamento efetivo - a fibrose quística e a anemia falciforme (drepanocitose). Serão assim integradas no projeto de desenvolvimento futuro do rastreio neonatal em Portugal.

**(P14) MPAG - A COMPETENCES-CENTRED PROGRAMME FOR GENETIC COUNSELLORS IN PORTUGAL**

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Genetic counseling is an essential area in healthcare. Educational programmes for non-physicians exist since 1969, in the USA. In Europe, the first started in 1992, in the UK (Manchester and Cardiff). In the last 5 years, new master courses began in Europe (Bergen, Marseille, Genoa, Barcelona, Groningen and Uppsala).

In 2009, we initiated a two-year full-time master programme (120 ECTS). It is limited to 5-6 students (nurses, clinical psychologists), with some previous clinical experience. Its main objective is to train professional counselors, to join multidisciplinary teams at medical genetic services and consultations.

A structured curriculum was based on the ESHG core-competences for genetic counsellors. The course consists mainly of small-group tutorials and has a large practical component. Educational areas are (1) principles and practice of genetic counseling; (2) human and medical genetics; (3) communication skills, clinical psychology, mental health, and psychosocial genetics; (4) public health, community genetics, organization of services, health policy and health economy; (5) quantitative and qualitative methods and research methodologies; and (6) bioethics and medical ethics. Nuclear disciplines in these areas are required, as are clinical rotations (prenatal diagnosis, paediatrics, neurological disorders, cancer genetics, clinical psychology); a large range of optional disciplines and clinical rotations are also offered as part of the first curricular year. The second year is fully in training at a medical genetics unit, and includes a research seminar.

We expect this to harmonize with other European programmes and help moving toward recognition of the profession of genetic counselors in Portugal and in Europe.

## **(P15) HYPOMELANOSIS OF ITO VS PIGMENTARY MOSAICISM: A CHALLENGING DIAGNOSIS**

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**Introduction:** Since 1952 several case reports and case series of Hypomelanosis of Ito (HI) have described different associations with multiple extra cutaneous manifestations, being the neurological anomalies the most frequent and important ones.

**Methods:** We report a 5-year-old girl, referred by dyschromia, minor dysmorphic features, strabism, ventricular septal heart defect, and slight learning difficulties. The peripheral blood karyotype was normal.

Hypopigmented patches along Blaschko's lines were observed. Other anomalies included a simian palm crease on right hand, persistency of fetal pads, and mild genu valgum/recurvatum. A skin biopsy for histopathological and cytogenetic studies was performed on a hypopigmented patch and a magnetic resonance imaging (MRI) of central nervous system (CNS) was requested.

**Results:** Histopathological study of the skin was inconclusive. However, the cytogenetic study revealed the existence of mosaicism: one normal cell line and one abnormal cell line with monosomy of chromosome 18 and presence of a marker chromosome (46,XX,-18,+mar[22]/46,XX[28]). Fluorescence in situ hybridization (FISH) technique identified the marker as a derivative of chromosome 18, comprising the centromere and a small portion of euchromatic material. CNS MRI was normal.

**Discussion:** Some authors suggest that HI would be better designated as "pigmentary mosaicism", following the hypothesis that the chromosomal abnormalities reported specifically disrupt pigmentary genes. Although numerous cases of mosaicism concerning chromosome 18 were previously described, this specific cytogenetic anomaly was not yet reported. The mild phenotype presented in this case suggests that mosaicism may have a low expression. Nevertheless, the lost genetic material in the abnormal cell line raises several questions about future outcomes, especially regarding the theoretical concern of a predisposition to certain malignancies and the difficulty of genetic counseling. This case illustrates the challenge of a diagnosis when facing a variety of phenotypes and reinforces the importance of karyotyping other tissues besides peripheral blood.

**(P16) TRÊS REARRANJOS DIFERENTES, TRÊS FENÓTIPOS DIFERENTES: ESTUDO FAMILIAR DO CROMOSSOMA 14**

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**Introdução:** Cromossomas derivativos são o resultado de rearranjos estruturais que tanto podem ocorrer num só, como entre dois ou mais cromossomas. Estes rearranjos dão origem a cromossomas estruturalmente anormais, podendo resultar um fenótipo normal ou mais ou menos grave, dependendo do tipo de anomalia encontrada.

**Materiais e métodos:** Caso índice: homem de 55 anos, referenciado para estudos de citogenética clássica (cariótipo com bandas GTG de alta resolução) e molecular (MLPA – kits P036 e P070 e FISH com sonda subtelomérica específica para o cromossoma 14) por apresentar um quadro clínico de atraso mental. Posteriormente realizaram-se estudos citogenéticos a uma irmã com atraso cognitivo e baixa estatura, e a mais quatro familiares com fenótipos normais.

**Resultados:** O cariótipo do caso índice revelou a existência de uma anomalia cromossómica estrutural desequilibrada num dos cromossomas 14, sugerindo uma deleção da banda 14q32, e uma duplicação do braço curto localizada na parte terminal do braço longo. Nos estudos de citogenética molecular, a técnica de MLPA identificou uma deleção da região subtelomérica no braço longo do cromossoma 14, em ambos os kits e, posteriormente, a técnica de FISH comprovou essa deleção.

Após estudos familiares, concluiu-se que dois dos irmãos apresentavam anomalias cromossómicas distintas do caso índice, envolvendo igualmente o cromossoma 14. Apesar de não ser possível efetuar o cariótipo à mãe (falecida), presume-se que estas alterações tenham tido origem numa anomalia cromossómica materna, uma vez que o pai deste indivíduo apresentava um cariótipo normal.

**Conclusões:** Os autores apresentam os resultados citogenéticos dos vários indivíduos estudados, e realçam a raridade da existência de três rearranjos diferentes (um deles aparentemente equilibrado e dois desequilibrados), envolvendo o cromossoma 14, encontrados numa mesma família.

**(P17) DE NOVO ROBERTSONIAN TRANSLOCATION UNMASKED: TRISOMY 13 MOSAICISM.**

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**Introduction:** If a de novo non-homologous Robertsonian translocation is identified prenatally, the risk of phenotypic abnormality is very low and arises from the small risk of UPD14 or 15, which has been estimated at 0.65% (Silverstein et al. Prenat Diagn 2002; 22: 649-51). In our clinical case another justification was found for structural congenital cardiopathy and developmental retardation.

**Clinical Case:** The patient is a seven years old boy born from a 34-year-old G3P2 mother at 38 weeks gestation via normal spontaneous vaginal delivery. Ventricular septal defect and aortic coarctation were diagnosed and chirurgic correction was performed. Laryngomalacia and right inguinal hernia were also diagnosed. The parents were healthy, and unrelated. Family history was unremarkable. There was one healthy sister. The mother had no known exposure to teratogens.

On physical examination, weight was at 75–90th centile, stature was at 75th centile, occipitofrontal circumference was at 25th centile. Facial phenotype was essentially unremarkable. Severe global developmental delay and ataxic gait were present.

**Results:** Chromosome analysis from peripheral blood leukocytes using GTG banding technique revealed a de novo Robertsonian translocation; 46,XY,der(13;14)(q10;q10)dn. Uniparental disomy 14 was excluded. Magnetic resonance imaging with spectroscopy suggests small multiple subcortical perinatal hypoxia sequelae. Metabolic screen reveal no significant alterations. Comparative genomic hybridization results were compatible with trisomy 13 mosaicism. A new chromosome analysis from peripheral blood leukocytes confirmed the results obtained. Final result was: mos45,XY,der(13;14)(q10;q10)[18]/46,XY,+13,der(13;14)[7]dn.

**Discussion:** Cardiac abnormality is present at 80% of cases with trisomy 13, ventricular septal defect is the most frequent anomaly. Cases with trisomy 13 mosaicism most often show a less severe clinical phenotype with every degree of variation, from the full pattern of malformation seen in trisomy 13 to a near-normal phenotype. The degree of mental deficiency is variable (Smith's Recognizable Patterns of Human Malformation, 6th Ed.).

This case could illustrate a recommendation for search for trisomy mosaicism when a de novo non-homologous Robertsonian translocation is detected. We found no other cases at the literature.

It was possible to provide accurate diagnosis and genetic counseling – less than 1% recurrence risk – for this child bear willing couple.

**(P18) A COMPLEX REARRANGEMENT INVOLVING CHROMOSOMES 5, 6 AND 15 WITH A TERMINAL DELETION OF CHROMOSOME 5P: CASE REPORT**

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Introduction: The cri-du-chat syndrome (CdCS) is a genetic disease that results from a deletion of contiguous genes in the short arm of chromosome 5 (5p); this deletion may be very variable in size. The main clinical features of CdCS include a cat-like cry, growth retardation, microcephaly, broad nasal bridge, epicanthal folds, micrognathia, abnormal dermatoglyphics, severe mental retardation and psychomotor delay.<sup>1</sup> The cat-like cry has been considered the hallmark for the diagnosis.<sup>2</sup> Phenotypic and cytogenetic variability are common.

Clinical Case/Material and methods: We present a 5-year-old boy referred to our Clinical Genetics consult due to developmental delay in which complex translocation was previously found.

The clinical observation revealed minor non-specific facial dysmorphisms and developmental delay of which speech was most affected. In the newborn period he presented with low-birth weight (<P5), laryngomalacia with cat-like cry, patent foramen ovale, bilateral inguinal hernia and repeated ear infections.

Results: High resolution GTG banding complemented with FISH analysis of the subtelomeric region and CdCS critical region of chromosome 5 detected an unbalanced complex chromosomal rearrangement, involving chromosomes 5, 6 and 15, with a subtelomeric deletion 5p15.33→5pter, occurring de novo.

Discussion/Conclusion: The gene hTERT (human telomerase reverse transcriptase) is located in the deleted region (5p15.33). It has been suggested that this gene is essential for normal cell growth and prevention of the end-to-end chromosome fusion in humans, during early development. Cat-like-cry critical region isn't consensually established yet, although it appears to involve the chromosomal segment 5p15.2→5p15.3 which is apparently conserved in our patient. This finding could further contribute to a better characterization of the "critical region" for cat-like cry.

At the present moment we cannot exclude additional imbalances involving other breakpoints in our patient. Techniques with higher resolution such as aCGH, might help to accurately define the breakpoints and the genome dosage imbalances in this case and therefore further contribute to the genotype-phenotype correlation.

Mainardi PC et al (2006). *Orphanet Journal of Rare Diseases*; Wu Q et al (2005). *Eur. J. of Human Genetics* 13:475-485; Zhang A et al (2003). *Am. J. Hum. Genet.* 72:940-948; Zhang X et al (2005). *Am. J. Hum. Genet.* 76:312-326



**(P19) CLINICAL, CYTOGENETIC AND MOLECULAR FINDINGS OF A “DE NOVO” inv dup del (6q)**

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**Introduction:** Complex rearrangements resulting in inverted duplications contiguous to a terminal deletion (inv dup del) were first reported for the short arm of chromosome 8 in 1976. Since then this type of structural anomaly has been described for an increasing number of chromosomes. In these rearrangements, the concomitant presence of a deletion and a duplication has important consequences in genotype-phenotype correlations. The authors describe the clinical findings and the cytogenetic characterization of a rare inv dup del involving the long arm of chromosome 6.

**Material and methods:** A girl aged 5 was referred for subtelomeric studies with the indication of psychomotor retardation, autistic features and stereotypies. Chromosome analysis with high resolution GTL-banding was performed on metaphases obtained from cultured peripheral blood lymphocytes. Molecular studies included MLPA (Kits P036 and P070, MRC-Holland), FISH with subtelomeric and whole chromosome painting probes specific for chromosome 6, and cCGH techniques.

**Results:** Initial MLPA studies detected a subtelomeric deletion in the long arm of chromosome 6; the subsequent karyotype revealed a structurally abnormal chromosome 6 with additional material in the end of the long arm. FISH analysis showed the deletion and demonstrated that the extra material was derived from chromosome 6; cCGH techniques defined the extension and confirmed the breakpoints of the duplicated segment. Thus this rearrangement was interpreted as an inv dup del (6q). Since parental karyotypes were normal, this anomaly was considered “de novo”.

**Discussion:** As far as we know this is the first description of a patient presenting with a “de novo” inv dup del (6q). We compare the clinical features in this child with the previously reported cases with either an isolated terminal deletion or a duplication of distal 6q. The authors enhance the importance of the combination of high resolution banding with molecular studies in the characterization of this rare rearrangement.

**(P20) TRÊS REARRANJOS DIFERENTES, TRÊS FENÓTIPOS DIFERENTES: ESTUDO FAMILIAR DO CROMOSSOMA 14**

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**Introdução:** Cromossomas derivativos são o resultado de rearranjos estruturais que tanto podem ocorrer num só, como entre dois ou mais cromossomas. Estes rearranjos dão origem a cromossomas estruturalmente anormais, podendo resultar um fenótipo normal ou mais ou menos grave, dependendo do tipo de anomalia encontrada.

**Materiais e métodos:** Caso índice: homem de 55 anos, referenciado para estudos de citogenética clássica (cariótipo com bandas GTG de alta resolução) e molecular (MLPA – kits P036 e P070 e FISH com sonda subtelomérica específica para o cromossoma 14) por apresentar um quadro clínico de atraso mental. Posteriormente realizaram-se estudos citogenéticos a uma irmã com atraso cognitivo e baixa estatura, e a mais quatro familiares com fenótipos normais.

**Resultados:** O cariótipo do caso índice revelou a existência de uma anomalia cromossómica estrutural desequilibrada num dos cromossomas 14, sugerindo uma deleção da banda 14q32, e uma duplicação do braço curto localizada na parte terminal do braço longo. Nos estudos de citogenética molecular, a técnica de MLPA identificou uma deleção da região subtelomérica no braço longo do cromossoma 14, em ambos os kits e, posteriormente, a técnica de FISH comprovou essa deleção.

Após estudos familiares, concluiu-se que dois dos irmãos apresentavam anomalias cromossómicas distintas do caso índice, envolvendo igualmente o cromossoma 14. Apesar de não ser possível efetuar o cariótipo à mãe (falecida), presume-se que estas alterações tenham tido origem numa anomalia cromossómica materna, uma vez que o pai deste indivíduo apresentava um cariótipo normal.

**Conclusões:** Os autores apresentam os resultados citogenéticos dos vários indivíduos estudados, e realçam a raridade da existência de três rearranjos diferentes (um deles aparentemente equilibrado e dois desequilibrados), envolvendo o cromossoma 14, encontrados numa mesma família.

**(P21) IDENTIFICATION OF A MOSAIC NON-INHERITED SMALL SUPERNUMERARY RING CHROMOSOME 2: CYTOGENETIC-MOLECULAR STUDIES AND GENOTYPE-PHENOTYPE CORRELATION**

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**Introduction:** The identification of supernumerary marker chromosomes (SMCs) derived from all the autosomes is currently possible, but rarely by conventional cytogenetics alone. Supernumerary ring chromosomes (SRCs) account for about 10% of these cases. SRCs derived from chromosome 2 are unusual, and there are only a few cases reported in the literature. The severity of the phenotype depends on the type of the mosaicism, the percentage of cells affected by the genetic change and the chromosome involved.

**Methods:** The authors report the case of a boy aged 8 referred for cytogenetic studies, presenting with behavior and learning problems, mental retardation with uncoordinated speech, attention deficit and hyperactivity (PHDA), as well as small slanting palpebral fissures.

The karyotype was obtained from peripheral blood lymphocyte cultures using high resolution GTL banding and standard techniques. Fluorescence in situ hybridization (FISH) was performed using specific probes for the centromeric regions of all chromosomes (Chromoprobe Multiprobe - ISystem).

**Results:** Cytogenetic analysis revealed two cell lines: one with a supernumerary marker ring chromosome, 47,XY,+r (52%), and a normal cell line, 46,XY (48%). The SRC was identified by FISH with the chromosome 2 centromeric probe. Since the parents had normal karyotypes, this abnormality was "de novo". Final karyotype of the proband was: mos 47,XY,+r[26]/46,XY[24].ish r(2)(D2Z2+)+dn.

**Discussion:** The clinical description of this patient is in agreement with other reports of the literature. Molecular characterization by FISH analyses is an useful way of investigating the presence of euchromatin contained in a SMC and establishing new chromosomal syndromes. However, to better characterize this ring, in order to establish a more accurate genotype-phenotype correlation, more studies involving other technologies should be performed, thus allowing suitable genetic counseling.

**(P22) DELEÇÃO CROMOSSÓMICA INTERSTICIAL EM 14q “DE NOVO”:  
APRESENTAÇÃO DE UM CASO**

**Ribeiro MC<sup>1</sup>, Mota Freitas M<sup>1</sup>, Ribeiro J<sup>1</sup>, Manuel Lopes M<sup>2</sup>, Oliva Teles N<sup>1</sup>, Correia H<sup>1</sup>,  
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**Introdução:** As deleções intersticiais são anomalias cromossómicas estruturais, desequilibradas, resultantes de dois pontos de quebra, frequentemente associadas a quadros clínicos anormais devido à perda de material genético ativo (eucromatina). As consequências fenotípicas dependem do segmento cromossómico perdido e do número de genes aí localizados.

**Material e Métodos:** Os autores apresentam o caso de um indivíduo do sexo masculino, de 11 anos de idade, referenciado para estudo citogenético por apresentar um quadro clínico de atraso de desenvolvimento psicomotor, défice cognitivo e problemas de comportamento. Realizaram-se culturas sincronizadas de linfócitos de sangue periférico, bandas GTG de alta resolução e, posteriormente, estudos de hibridação *in situ* por fluorescência (FISH) com sondas de pintura cromossómica total e subtelomérica, específicas para o cromossoma 14.

**Resultados:** A análise das metáfases revelou a presença de uma anomalia estrutural no cromossoma 14, interpretada como uma deleção intersticial do segmento compreendido entre as bandas 14q24.3 e 14q32.1. A análise por FISH permitiu confirmar esta deleção intersticial. Como os cariótipos dos pais foram normais, conclui-se que esta anomalia cromossómica é “de novo”, estabelecendo-se o cariótipo do doente como:

46,XY,del(14)(q24.3q32.1).ish del(14)(wcp 14+,SHGC36156+)dn

**Discussão:** A deleção intersticial encontrada no cromossoma 14 implica uma monossomia do segmento 14q24.3→14q32.1. As alterações descritas mais comuns, associadas a esta deleção, incluem ADPM e algumas malformações *minor*. Os autores apresentam este caso pela raridade da anomalia citogenética encontrada e comparam-no com a literatura atual.

**(P23) INTERSTITIAL DELETION 15q21 AND PRADER-WILLI LIKE SYNDROME PHENOTYPE: CASE REPORT**

**Pires S<sup>1</sup>, Oliva Teles N<sup>1</sup>, Ribeiro J<sup>1</sup>, Mota Freitas M<sup>1</sup>, Marques B<sup>2</sup>, Reis G<sup>3</sup>, Correia H<sup>2</sup>, Fonseca e Silva ML<sup>1</sup>**

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**Introduction:** Chromosome 15q interstitial deletions not involving the Prader-Willi/Angelman region are uncommon and poorly characterized. Very few cases of different segmental losses involving the 15q21 region have been reported at cytogenetic level. All the described patients present with moderate to severe mental retardation and characteristic facial dysmorphic features. Some authors compare the similarity between the phenotype of these patients with some features of Prader-Willi syndrome (PWS).

**Methods:** We report the case of a girl aged 8 referred for conventional cytogenetics and fluorescence in situ hybridization (FISH) for the PWS region, presenting with mental retardation, almond-shaped eyes, obesity, small hands with short fingers and diminished pigmentation of the hair.

**Results:** The chromosomal analysis revealed an interstitial deletion of the long arm of chromosome 15, apparently between 15q21 and 15q22. Deletion at 15q11.2 (Prader-Willi/Angelman critical region) was excluded by FISH.

To establish the exact breakpoints molecular studies were performed using bacterial artificial chromosome (BAC) clones spanning the 15q21.3 region. The absence of signal in this region defines the proband's final karyotype as:

46,XX,del(15)(q21.3q21.3).ish del(15)(q21.3q21.3)(bA74K1-)

**Discussion:** The authors emphasize the importance of complementary FISH and molecular studies in chromosomal abnormalities and compare the proband's phenotype with similar cases described in the literature.

**(P24) BACK TO THE KARYOTYPE: A CASE OF MOSAIC MARKER CHROMOSOME 11 DETECTED BY ACGH**

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Array comparative genomic hybridization (aCGH) is currently being used in many cytogenetic laboratories as a first-line approach to detect chromosome imbalances associated with development delay, mental retardation and congenital anomalies. However some abnormalities are better elucidated by conventional techniques such as chromosomal mosaicism is suspected.

Here we report the clinical and cytogenetic findings of a 4-year old girl with intellectual disability, mild dysmorphism and macrocephaly with a 5,9 Mb gain in chromosome 11 detected by aCGH and a previous normal prenatal karyotype.

The size and pattern of the aCGH showing a three and four copies profile suggested a complex rearrangement involving the 11q11-q12.2 region, compatible with a supernumerary marker chromosome (SMC). The case was then reevaluated by karyotyping, revealing a *de novo* mosaic SMC in approximately 70% of the cells analyzed.

Although aCGH accurately identified the chromosome and gene content of the SMC in the patient presented here, karyotype was necessary to determine the presence and structure of the marker and to established the associated abnormal cell line.

We compare our patient with other reported cases of SMC(11), to determine the respective contributions of this rearrangement to the phenotype.

**(P25) OLIGONUCLEOTIDE ARRAY-CGH IN A COHORT OF 500 PATIENTS WITH INTELLECTUAL DISABILITY, MULTIPLE CONGENITAL ANOMALIES, LEARNING DIFFICULTIES AND AUTISM SPECTRUM DISORDERS**

**Carreira IM<sup>1,2</sup>, Ferreira SI<sup>1</sup>, Pires LM<sup>1</sup>, Ferrão J<sup>1</sup>, Jardim A<sup>1</sup>, Matoso E<sup>2</sup>, Melo JB<sup>1</sup>**

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**Introduction:** Microarray-based comparative genomic hybridization (array-CGH) is nowadays the first genetic test offered to detect genomic imbalances in patients with intellectual disability with or without dysmorphisms, multiple congenital anomalies, learning difficulties and autism spectrum disorders. It allows the possibility to screen the whole genome at once and with high resolution, increasing the diagnostic yield and identifying Copy Number Variants (CNVs) sometimes challenging to interpret.

**Methods:** In a 20 month period, 500 patients were analysed with Agilent 180K whole genome oligonucleotide microarrays. The observed imbalances were classified in five main classes depending on the genetic content, overlapping with known microdeletion/duplication syndromes and existence of previous described CNVs in normal individuals.

**Results:** In 195 patients of the studied cohort, 269 imbalances were identified, 120 deletions, 147 duplications and even two tetrasomies, that belong to a class of pathogenic or potentially pathogenic imbalances. 62.5% of these imbalances had a genomic size between 1.985 Kb and 400 Kb, and 16% between 1-5 Mb, with the remaining distributed by different genomic ranges up to 53 Mb. Of the imbalances whose origin is known, 41.8% were maternal in origin, 35.2% paternal, 0.6% inherited from cousin progenitors, corresponding to one of the tetrasomies, and 22.4% de novo. In the studied cohort, 22 patients revealed to have known microdeletion or microduplication syndromes, eleven de novo, three paternal, one maternal and seven of unknown origin.

**Discussion:** We detected a total of 291 pathogenic or potentially pathogenic imbalances in 500 patients, with known microdeletion or microduplication syndromes included. We concluded that inherited CNVs have a smaller genomic size than de novo CNVs. The great majority, almost 77%, of all the inherited CNVs identified were smaller than 500 Kb, while 35% of the de novo imbalances had a genomic size between 1- 3 Mb.

**(P26) 2p14 DE NOVO DELETION: A MOSAIC DETECTED BY ACGH IN A FEMALE PATIENT ASSOCIATED WITH MICROCEPHALY, ABNORMAL GROWTH AND GLOBAL DEVELOPMENT DELAY**

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**Introduction:** Microarray-based comparative genomic hybridization (array-CGH) is actually used as the first tier test to detect genomic imbalances in patients with intellectual disability and multiple congenital anomalies. It has improved the diagnostic yield and the identification of Copy Number Variants (CNVs). Array-CGH has also proven to be a powerful technology for the detection of mosaicism, whose severity depends on the genes involved, the proportion of abnormal cells and tissue distribution.

**Methods:** An Agilent 180K whole genome oligonucleotide array-CGH was performed in a 18 month patient with microcephaly, abnormal growth and global developmental delay.

**Results:** A chromosome 2p14 interstitial de novo deletion was identified by array-CGH in a 50% mosaic status. The proband and the parents were studied by fluorescence in situ hybridization, ruling out the existence of any rearrangement. The deletion in the proband was confirmed to be present in 50% of the analyzed cells: arr 2p14(64,273,917-65,845,421)x1~2 dn.

**Discussion:** To our knowledge only two patients have been reported with deletion in chromosome 2p14, but not in a mosaic situation. There are some overlapping phenotypic characteristics with our patient, like minor dysmorphisms, absence of congenital anomalies and microcephaly. The common deleted region in the three patients includes 12 genes, three of which have a high rank for being likely haploinsufficient: PELI1, SERTAD2 and RAB1A genes. RAB1 gene is involved in vesicle trafficking and neurite growth and the other common deleted genes are also involved in processes like vesicle trafficking, regulation of the actin cytoskeleton, neuronal differentiation and regulation of axon guidance. Despite the mosaicism situation, that can be explained by a post-zygotic mitotic error, this case helps to reinforce the pathogenic effect of 2p14 deletions. The report of additional patients will be necessary to further delineate a critical region and to establish the genes responsible for the phenotype.



**(P27) 6q25.3 DELETION ENCOMPASSING *ARID1B* IN A PATIENT WITH INTELLECTUAL DISABILITY AND AGGRESSIVE BEHAVIOR**

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We report a *de novo* deletion at 6q25.3 citoband in a male patient with intellectual disability (ID), hirsutism, sparse and coarse hair, coarse face, bushy eyebrows, squint, bulbous nose, large mouth, thin upper lip, short stature, brachydactyly, umbilical hernia, and borderline hepatosplenomegaly (on ultrasound). He had mild ID, with worse performance in the language area, was hyperactive and aggressive. There was familial history of ID, namely in a 1<sup>st</sup> grade female cousin.

The molecular karyotype was determined by aCGH analysis on a human genome CGH Agilent 180K custom array, with a mean resolution of 17 Kb (Agilent, Santa Clara, CA) and the confirmation studies and inheritance analysis were carried out by qPCR by amplification of a segment inside the altered region.

The patient had a *de novo* 2.7 Mb deletion at chromosome region 6q25.3 containing 14 genes. This region was previously found to be deleted in patients with development delay, microcephaly, dysmorphic features and hearing loss together with sacral/anorectal malformations. Among the genes affected in the patient, *ARID1B* (AT-RICH INTERACTION DOMAIN-CONTAINING PROTEIN 1B) was recently found to be mutated in patients Coffin-Siris syndrome. The authors also described copy number variations affecting *ARID1B* gene to be associated with the syndrome (Santem GW, et al, 2012). Mutations in *ARID1B* and low expression levels of this gene have also been described in patients with intellectual disability, speech impairment and autism. This data suggests that haploinsufficiency of *ARID1B* is responsible for a spectrum of neurodevelopmental phenotypes and might explain the intellectual and speech impairment also featured in the syndrome. This case brings new insight to the delineation of the 6q25 microdeletion and *ARID1B* haploinsufficiency- associated phenotypes.

**(P28) 7q33 DELETION IN A FAMILY WITH INTELLECTUAL DISABILITY, DYSMORPHIC FEATURES AND BEHAVIORAL CHANGES**

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In this work we report a maternally inherited 7q33 deletion in a 19 year old boy with mild intellectual disability (ID) (IQ= 53), familial history of ID (mother and sister affected; a second-grade cousin with ID, dysmorphic features and epilepsy), seizures, reduced vision, hypotony, dysmorphisms, aggressiveness and hyperactivity. The same alteration was present in his sister that displays mild ID (IQ=62), short stature, facial dysmorphisms (similar to the ones present in the brother) and behavioral changes (disinhibition). The mother cannot read or write and has facial dysmorphisms similar to the ones present in the daughter.

The molecular karyotype was determined by aCGH analysis using a human genome CGH Agilent 180K custom array with a mean resolution of 17 Kb (Agilent, Santa Clara, CA) and the confirmation studies and inheritance analysis were carried out by qPCR using a fragment designed inside the altered region.

The deletion encompassed 2 Mb deletion at chromosome region 7q33 and included 15 genes (*AGBL3*, *AKR1B1*, *AKR1B10*, *AKR1B15*, *BPGM*, *C7orf49*, *CALD1*, *CNOT4*, *EXOC4*, *LRGUK*, *NUP205*, *SLC35B4*, *STRA8*, *TMEM140*, *WDR91*). One of the most interesting genes covered by the deletion is the *CNOT4* (CCR4-NOT transcription factor complex, subunit 4) gene. The CNOT4 protein plays both positive and negative roles in transcriptional regulation and a positive role in transcriptional elongation. In yeast, the ortholog of CNOT4 (Not4) regulates the expression of Jhd2 (the yeast ortholog of *JARID1C*, for which mutations have been described in patient with X-linked ID).

Comparison with similar cases, expression and functional studies should help us clarify the relevance of the deleted genes for ID.

**(P29) 400 REASONS TO PERFORM ARRAY CGH**

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The advance of new technologies in Human Cytogenetics resulted in a paradigm shift in the technical approach of genetic diagnosis.

Chromosomes are no longer big strands of black and white bands analyzed by the human eye, with the help of a microscope, but can be now screened at molecular level.

Using *arrayCGH* we can detect copy number variations (CNVs) with a resolution as low as 10kb.

This powerful tool allows us to go from genotype to phenotype instead of a more conventional approach. This represents a shift in Human Genetics in the sense that currently technology overpasses knowledge.

International guidelines recommend the use of array CGH as the first line approach in cases of autism, developmental delay, intellectual disability and/or congenital anomalies.

Following this recommendations, the laboratory of Serviço de Genética Médica of HSM introduced this technique in the routine procedures since the end of 2010.

Consequently the laboratory is witnessing an inversion in the approach to ID/CA/DD cases.

Accurate interpretation and classification of CNVs is fundamental to establish the co-relation between genotype and phenotype, helping clinicians in their counseling to affected individuals and their families.

We present a critical overview of CNV classification approach in nearly 400 array results, discussing frequencies of clinically significant and uncertain findings in 20% abnormal results.

### **(P30) FISH ANALYSIS OF X CHROMOSOME LOW LEVEL MOSAICISM**

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Introduction: Fluorescence in situ hybridization (FISH) allows the study of numeric/structural chromosome aberration in large amount of cells and as an adjunct to karyotype analysis provides a sensitive and cost-effective technique to identify of minor cell population. Until now, there is no concern about which percentage is acceptable to define low level mosaicism(1). This study evaluates the percentage of X chromosome mosaicism in patients with low level mosaicism karyotype (45,X/46,XX or 45,X/47,XXX, <10%) relatively a one control group.

Methods: After karyotype, 112 female patients were selected for this study: 54/112 had normal karyotype (control group), and 58/112 patients (test group) had low level mosaicism, involving at least 45,X cell line. Cytogenetic analysis, after 72h culture, was performed by GTL banding. For FISH were study 200 cells analyzed by epifluorescence microscopy using centromeric probes for X and 18 chromosome (control probe). In order to determine the differences between those groups, t-student test was performed. (SPSS, v20.0).

Results: Significant increase in percentage of low level mosaicism were observed in group test relatively to control group ( $4,20 \pm 1,89\%$  vs  $2,33 \pm 1,41\%$ ,  $P<0,001$ ).

Discussion: As expected, significant increase in percentage of low level mosaicism were observed in group test relatively to control group. FISH as an adjunct to karyotype analysis provides a sensitive and cost-effective technique to identify low level mosaicism. Our percentage of mosaicism for control group ( $2,33 \pm 1,41\%$ ) should be linked with culture artifacts or errors associated to hybridization, therefore mosaicism values below 3% should not be considered.

(1) Wiktor AE et al. (2005) *Am J Med Genet* 138A(3):259-61

### **(P31) TRISOMY 4 CONFINED TO PLACENTA IN A PRENATAL DIAGNOSIS CASE**

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Introduction: Confined placental mosaicism (CPM) is diagnosed when some trisomic cells are detected on chorionic villus sampling (CVS) and only normal cells are found on a prenatal test, such as amniocentesis. CPM is detected in approximately 1-2% of ongoing pregnancies that are studied by CVS at 10 to 12 weeks of pregnancy.

Most pregnancies that are diagnosed with CPM continue to term with no complications and the children develop normally. However, some pregnancies with CPM have complications, due to placental dysfunction, uniparental disomy of the fetus and undetected mosaic trisomy of the fetus. The risks for these settings depend of the origin of error, level of mosaicism and chromosome involved.

The study of CVS in normal karyotype fetus with ultrasound abnormalities may be considered, since in some cases, can help in the diagnosis.

Material and Methods: A 31 years-old primigravida was referred to amniocentesis at 25 weeks of gestation after the detection of ultrasound abnormalities and intrauterine fetal death (IUFD). Amniotic fluid (two cultures) and placenta (three cultures) were performed according the protocols establish in the laboratory. Cytogenetic analysis followed the standard cytogenetic guidelines.

Results: Cytogenetic analysis of amniotic fluid revealed a 46,XY karyotype. The post mortem examination showed fetal death with a maturity corresponding to 24 weeks, IUGR and short femur, coexisting micrognathia, nuchal edema (2.1mm), mild hydrocephalus, cerebellar vermis hypoplasia, bilateral pyelectasis and pulmonary hypoplasia. Cytogenetic analysis of placenta sampling showed a karyotype of 47,XY,+4 in all cells examined.

Discussion: Mosaicism of trisomy 4 is a rare condition, with 8 cases reported in the literature, and usually associated with anomalies. The authors compare the cytogenetic and the fetus(s) clinical findings with those described in the literature.

**(P32) DIZYGOTIC MONOCHORIONIC TWINS WITH 46,XY/46,XX CHIMERISM**

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**Introduction:** Infertile couple who recurred to intracytoplasmatic sperm injection treatment cycle with transfer of two embryos. After confirmation of pregnancy, ultrasound identified one monochorionic/biamniotic twin pregnancy and it was assumed that only one embryo had implanted with posterior division. The ultrasound follow-up showed that both fetuses had gender discordance. At 31 weeks a cesarean-section was performed with two newborns of different sex.

**Methods:** After birth, peripheral blood samples, buccal cells and skin biopsy were taken to perform zygosity studies, by quantitative fluorescent multiplex PCR amplification of STRs and for FISH and karyotype analysis.

**Results:** STRs profile showed that both twins were carriers of Y chromosome sequences and had a tetra-allelic profile similar for several markers. The karyotype identified 80% of cells 46,XY in both twins. FISH observed 14% and 16% of XX cells in the phenotypically male and female twin, respectively. In buccal cells, 15/27 STRs were different between the two twins (but they were already present in the tetra-allelic profile from the peripheral blood). Y chromosome sequences were also detected in both twins, although residual in the female. FISH technique verified the presence of XX[1]/XY[41] and XX[20]/XY[20] cells in the male and female, respectively. After multidisciplinary assessment it was decided to perform a skin biopsy from both twins. By STRs analysis we did not observe anymore the presence of Y chromosome sequences in female twin. FISH analysis and karyotype revealed only XY cells in the male and XX in the female.

**Discussion:** The presence of the Y chromosome sequences or 46,XY cells in the phenotypically female twin and 46,XX cells in the male, was, most likely, due to a phenomenon of vascular anastomoses which resulted in hematopoietic chimerism among dizygotic twins, resulting from the very close implantation of the two transferred embryos.

### **(P33) PRENATAL DIAGNOSIS: THE IMPACT OF NEW LABORATORY TECHNOLOGIES**

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Introduction: Rapid common aneuploidy testing (RAT) (13, 18, 21, X, Y) are widely used in prenatal diagnosis (PD) as a complement of karyotyping. FISH and MLPA can be used for that purpose. Although designed for detection of specific chromosomes, both enable faster results, allowing earlier intervention and reducing parental anxiety.

The aim of this work was to evaluate the impact of karyotyping substitution, by RAT, in PD as a stand-alone test, with costs and time benefits.

Materials and Methods: The 7035 amniotic fluid (AF) samples received in the laboratory (2006 - 2010), were reviewed. The concordance between results that would have been obtained, by karyotyping and MLPA, if both were applied, successfully, to all the AF samples was determined.

Discussion: In the study where the concordance between both techniques was determined, all samples considered, showed that the karyotype would have identified 241 chromosome alterations. The MLPA would have detected 117 (48,5%) of these and, in 8,3% of cases, a suspicion of alteration would have existed. The concordance between the two technologies was 98,5%, with 1,5% of false negative results, having 41,3% of the latter a high/ unknown clinical significance. The referrals for the prenatal study, analysed individually, revealed variable values of false negative results: from 1% (in advanced maternal age) to 53,1% in cases where one of the parents was a carrier. These results were expected and are in agreement with those previously reported.

Conclusion: The RAT techniques are highly sensitive for the common aneuploidias, are economic and provide faster results. Although, their application in substitution of traditional karyotyping would lead, in some cases, to a wrong diagnosis, with emotional and economic prejudice for not only the affected child's family but also for the State. These techniques are useful and they should be applied as a diagnostic complement.

**(P34) A RETROSPECTIVE STUDY OF DOWN SYNDROME IN PRENATAL DIAGNOSIS. DID CHORIONIC VILLUS SAMPLING ALLOW A BETTER PREVENTION?**

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**Introduction:** Down syndrome (DS) is the most common single genetic cause of human moderate mental retardation, with an estimated prevalence of 9.2 cases per 10,000 live births. We aimed at analyzing changes in prenatal diagnosis (PND) over time, namely the referral reasons for chromosome analyses and the introduction of chorionic villus sampling (CVS), and its influence on the results obtained in DS cases.

**Methods:** We retrospectively evaluated the PND results from samples analyzed between 1987 and 2011 (25 years) in our cytogenetic laboratory taking into account the referral reasons, type of sample, karyotype and reporting time.

**Results:** 263 fetuses with a karyotype compatible with DS were identified in a total of 18,107 karyotypes (1.5%). The highest frequencies of DS were found among cases referred because of ultrasonography findings (namely increased nuchal translucency) or positive first trimester screening and when one parent carries a chromosomal rearrangement. The frequency of recurrence was found to be 1/72.

The increasing use of CVS led to an earlier response in terms of gestational age (mean at diagnosis- 13+4 weeks). In addition, an increased percentage of karyotypes with SD was detected (8.4% of CVS samples). On the other hand, implementation of molecular rapid aneuploidy detection in part of the samples allowed a better report time in DS cases, from 23 days in 1987 to 2 days in 2011.

**Discussion:** DS detection remains the most important reason for performing PND. The collecting of CVS has been rising over the last years, which has resulted in an increased number of trisomy 21 cases identified in a lower gestational age, allowing a better karyotype-phenotype correlation in earlier pregnancies. Moreover, the use of complementary molecular techniques for the detection of common aneuploidies reduced the mean reporting time and allowed an earlier decision of the couple concerning the future of gestation.



**(P35) Y-CHROMOSOME DETECTION IN TURNER SYNDROME**

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Introduction: Turner syndrome (TS) is a chromosomal disorder characterized by the presence of a single normal X chromosome in women. Additionally to the X chromosome monosomy, other cell lines could co-exist, containing the Y chromosome or part of it. The presence of Y chromosome in patients with TS represents an increased risk (15-30%) of developing gonadoblastoma. In this study it was checked the absence/presence of different genes mapped on Y chromosome, not detected by conventional cytogenetic techniques.

Material and Methods: DNA Samples from 98 TS patients: 81 peripheral blood, 2 amniotic fluid and 15 miscarriages samples. Karyotype was performed. DNA was extracted from cells fixated in methanol:acid acetic and analyzed by polymerase chain reaction (PCR) for the presence of 4 genes - SRY and TSPY (short arm) / DDX3Y and HSFY (long arm).

Results: From peripheral blood and miscarriages samples, we found 22 with 45,X karyotype, 65 with mosaic cell lines (45,X or 47,XXX) and 9 with structural abnormalities (involving 21 or X chromosome) with or without mosaic cell lines. All amniotic fluid samples presented 45,X karyotype. SRY sequence was detected in 3,06% (3/98), TSPY in 2,04% (2/98), DDX3Y in 1,02% (1/98). The PCR revealed hidden mosaics not detected by cytogenetic analysis in 4,08% (4/98) of cases with the Y chromosome material present in the minority cell line.

Conclusions: Detection of Y chromosome mosaicism is clinically important, due to the increased risk of tumor formation, especially gonadoblastoma in TS patients with hidden Y chromosome. The prophylactic gonadectomy is the procedure of choice to exclude gonadal malignancy in TS patients carrying Y chromosome sequences. This type of analysis should be a complementary diagnostic method contributing to a more accurate clinical diagnosis.

**(P36) AGE-AT-ONSET VARIATION IN A LARGE GROUP OF FAP ATTRV30M KINDREDS: GENDER DIFFERENCES AND THE PHENOMENON OF ANTICIPATION**

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Introduction: Familial amyloid polyneuropathy (FAP) ATTRV30M is an autosomal dominant systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene. More than 100 mutations have been found in the TTR gene but V30M is by far the most common. A wide variability in age-at-onset (AO) has been uncovered, including among Portuguese patients [17-82 yrs]. Early ( $\leq 40$ ) and late-onset ( $\geq 50$ ) cases are not separate entities, often coexisting in the same family, with offspring showing anticipation - a much earlier AO than their affected parent.

Methods: Our aim was to study anticipation in a larger number of kindreds than assessed before and to gain more insight into parent-of-origin effects.

From the UCP-registry, we analysed 926 parent-offspring pairs, both clinically observed and with well-established AO.

Results: Women had a statistically significant higher AO than men, either for daughters (mean, SD - 33.7, 6.84) vs. sons (29.43, 6.08) or mothers (39.57, 11.75) vs. fathers (35.62, 11.62). Also, 291 parent-offspring pairs showed marked anticipation ( $\geq 10$  years) and the transmitting parent was the mother in 203 pairs. Conversely, among the 22 offspring showing a 10 years higher AO, 19 had a transmitting father.

Mother-son pairs showed larger anticipation (10.43, 9.34) while the father-daughter pairs showed only residual anticipation (1.23, 9.77). Both offspring and parent's gender were highly significant factors (with no interaction). Noteworthy, no parent with AO  $\leq 40$  had an offspring with AO  $\geq 50$ .

Discussion: These findings confirm anticipation as true biological phenomenon. Furthermore, both parents and offspring's gender were found to be highly significant factors for anticipation. We also found that mother-son pairs showed higher anticipation.

The study of genetic modifiers should focus on families, aiming to unravel mechanisms of anticipation that may have important clinical implications.

### **(P37) LMX1B MUTATIONS IN NAIL-PATELLA SYNDROME (NPS)**

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**Introduction:** Nail-Patella syndrome (NPS; MIM 161200) is a rare (1/50000 births) autosomal dominant disorder characterized by dysplastic nails, absent or hypoplastic patellae, elbow dysplasia, iliac horns, glomerulopathy, and glaucoma. NPS is caused by mutations in the LMX1B gene (LIM homeobox transcription factor 1-beta), mapped at chromosome 9q34. The main pathogenic mechanism underlying NPS, particularly of the skeletal defects, is the haploinsufficiency of LMX1B.

**Methods:** Genomic DNA was extracted from 4 patients with the clinical diagnosis of NPS and an unequivocal autosomal dominant pattern of inheritance. The 8 exons of LMX1B were amplified by PCR and sequenced in an ABI Prism 3500 genetic analyzer. MLPA technique (SALSA P289-A1 LMX1B) was used for detection of deletions and/or duplications.

**Results:** Four different mutations were detected: two nonsense mutations – p.Q60X, on exon 2, and p.C142X, on exon 3; one missense mutation – p.R223Q, on exon 4; and a duplication of exon 3 (dup Ex.3). Mutations p.C142X and dup Ex.3 are novel.

**Discussion:** The genetic diagnosis of NPS was confirmed by the identification of a pathogenic LMX1B mutation in all four patients. Three of those mutations are located in the LIM domains (exons 2\_4) and the remainder in the homeodomain of LMX1B (exons 4\_6), the former causing a decrease in transcriptional activity and the latter leading to loss of DNA binding activity.

# **(P38) FRAXE MOLECULAR DIAGNOSIS IN INDIVIDUALS REFERRED FOR FRAXA SCREENING**

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Introduction: FRAXE mental retardation is a form of mild to moderate intellectual disability generally associated with learning difficulties, communication deficits, attention problems, hyperactivity and autistic behavior. The folate-sensitive fragile site FRAXE (AFF2/ FMR2 gene) is ~600 kb distal to FRAXA (FMR1 gene), which is the most common cause of inherited mental retardation. Normal individuals present 4–39 copies of the polymorphic CCG repeat in AFF2, while individuals expressing the fragile site have >200 copies and the CpG island is fully methylated.

Goal and methods: Reports of full expansions and pre-mutations in AFF2 are rarely documented. In this respect, it has been very difficult to determine to what extent the alleles with CCG repeats in the range of 36 to 199, have a pathogenic effect. Intellectually disabled individuals are primarily referred for FRAXA screening and individuals who are negative for FRAXA are possible candidates for FRAXE screening. In order to complement our PCR analysis with Southern blot and hybridization, we cloned a segment of the AFF2 gene that could be used as a labeled probe to determine more accurately the extent of expansion of the CCG repeats.

Results and discussion: We have developed a probe to be used for Southern blot analysis that reliably detects the AFF2 CCG triple repeat amplification. We present validation data and results of AFF2 molecular analysis in a subpopulation of 5000 individuals originally referred for FRAXA screening. The presence of pre-mutated and fully expanded alleles in either gender was confirmed by Southern blot analysis, which also enabled evaluation of methylation status and exclusion of repeat number mosaics or PCR failure. We recommend the use of this probe as a method of choice for the detection of AFF2 pre-mutations, full mutations and mosaics, specifically in individuals found to be negative upon FRAXA screening.

**(P39) REPORT ON A NOVEL MUTATION, P.ASP171ASN, AFFECTING WOLFRAMIN, IN A NONSYNDROMIC SENSORINEURAL LOW-FREQUENCY HEARING LOSS PATIENT**

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**Introduction:** Nonsyndromic sensorineural hearing loss (HL) affecting high frequencies is a genetically heterogeneous common disorder on the contrary of low frequency sensorineural HL (LFSNHL), an unusual type in which frequencies at 2000Hz and below are predominantly affected. Mutations in WFS1 gene, coding for wolframin, a membrane glycoprotein localised in the endoplasmic reticulum (ER), are responsible for Wolfram Syndrome type1 (WFS1), being a common cause of nonsyndromic LFSNHL. The expression of wolframin in the human cochlea remains unknown but its localization in the ER suggests a role in ion homeostasis maintained by the canalicular reticulum, a specialized form of ER.

**Methods:** A Portuguese patient presenting bilateral nonsyndromic progressive LFSNHL was audiotically evaluated by pure tone audiometry and blood sample was collected after written informed consent was signed. Screening for mutations in the GJB2 gene and for the common GJB6 deletions was first performed by automatic sequencing and by multiplex PCR, respectively. Automatic sequencing of exons 5 and 8 of WFS1 gene was later performed. One hundred hearing Portuguese controls were sequenced for the exon 5 of WFS1 gene.

**Results:** A novel mutation, p.Asp171Asn, was found in exon 5 of WFS1 gene, in heterozygosity. One intronic variant previously reported, IVS4 – 9 A>G, was also identified in homozygosity in this patient. No mutations were found in GJB2 and GJB6 genes. The novel mutation p.Asp171Asn, at codon 511, changes an aspartic acid to an asparagine in the extracellular N-terminus domain of the protein. This novel mutation wasn't present in 100 Portuguese hearing controls.

**Discussion:** The auditory phenotype of this patient might probably be due to the novel mutation p.Asp171Asn. Its functional characterization should be performed in order to assess its effect on the protein and to clarify in which way the introduced change in the residue 171 leads to low frequency HL.

**(P40) FBN1 MUTATIONS IN PORTUGUESE PATIENTS WITH MARFAN SYNDROME**

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Introduction: Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder, characterized by a widely variable combination of ophthalmic, pulmonary, cardiovascular and skeletal anomalies. Symptoms may appear at any age and vary greatly between patients, even within the same family. The major manifestations include tall stature, long thin limbs and arachnodactyly, pectus excavatum/carinatum, myopia, mitral valve prolapse and regurgitation, and dilatation of the aortic root (occasionally leading to dissecting aneurysm and death). The majority of patients fulfilling the clinical criteria for MFS carry a pathogenic mutation on the FBN1 gene, encoding fibrilline-1, an extracellular glycoprotein essential for connective tissue. Mutations have also been found in TGFBR1 and TGFBR2 in patients previously shown not to have mutations in FBN1.

Methods: We studied 6 patients with a clinical phenotype suggestive of MFS for mutations in the FBN1 gene. All exons and flanking intronic regions were amplified by PCR and then sequenced bidirectionally. MLPA was also performed to detect large gene rearrangements in patients in whom no mutation was found.

Results: Mutation screening revealed the presence of three novel mutations in three of our patients: one indel mutation, c.2422delinsCTGTTT, leading to a frameshift and a premature stop codon (p.Ile808LeufsX41); one missense mutation, p.Tyr2149Asn (c.6445T>A); and another missense mutation, p.Met1? (c.3G>A), probably resulting in the loss of the start codon. Until now, we did not find any large rearrangements.

Discussion: Our results show that FBN1 mutations seem also to be the main cause of MFS in Portuguese patients. Regarding the three patients in whom no mutations were found, clinical information must be reviewed, in order to evaluate the need to pursue with mutation screening in the TGFBR1 and TGFBR2 genes. FBN1 testing is useful to confirm the clinical diagnosis of Marfan syndrome, and to allow proper genetic counselling to patients and families.

#### **(P41) OSTEOGENESIS IMPERFECTA TYPE I CAUSED BY COL1A1 GENE MUTATION**

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Introduction: Osteogenesis imperfect (OI) comprises a heterogeneous group of diseases characterized by susceptibility to bone fractures, specific extra-skeletal manifestations and, in most cases, presumed or proven defects in collagen type I biosynthesis. About 90% of the individuals with OI type 1 are heterozygous for causative variants in the COL1A1 and COL1A2 genes. Usually frameshift, nonsense and splice-site alterations in one COL1A1 allele that leads to mRNA instability and haploinsufficiency are found in these patients.

Our aim was to establish the molecular diagnosis, by screening the 51 exons of COL1A1 and search for mutations in patients with clinical indication.

Methods: Mutation screening was performed by PCR amplification of all coding and flanking regions, followed by bidirectional direct sequencing.

Results: So far, we have performed mutational analysis in two patients clinically diagnosed with OI, and found one nonsense mutation in exon 9 (c.658C>T; p.Arg220X) in one of the patients, previously described as causing OI. The clinical findings in this patient included fractures of the lower limbs after minimal trauma and bluish sclera, with disease onset in the childhood. The second patient showed no mutations in COL1A1 and presented a disease onset in adulthood, with early osteoporosis as the first clinical symptom suggestive of OI.

Discussion: Early onset symptoms of OI are probably more associated with COL1A1 gene mutations. We do need to study additional patients and the mutation analysis of COL1A2 is currently being performed.

Mutation screening in these genes can be invaluable to confirm and establish an early diagnosis, and to allow proper genetic counseling, including the offer of prenatal diagnosis, whenever appropriate.

**(P42) FRAGILE X SYNDROME: INTERGENERATIONAL ALLELE INSTABILITY AND ASSOCIATED PHENOTYPES IN FAMILIES**

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**Introduction:** Fragile X syndrome (FXS) is the most common hereditary form of intellectual disability with an estimated frequency of 1/4000 males and 1/8000 females. This disease is caused by a (CGG)<sub>n</sub> expansion in the 5'UTR of the FMR1 gene, which as a result is methylated and gene silenced. Four classes of alleles can be found based on CGG repeat length: normal (5-44), intermediate (45-54), premutation (55-200) and full mutation (>200). In premutation carriers, both FMR1-related primary ovarian insufficiency (FXPOI) and fragile-X associated tremor/ataxia syndrome (FXTAS) have been described. To gain insights into instability of FMR1 CGG repeats and associated phenotypes, we studied 541 individuals from 128 FXS Portuguese families.

**Methods:** DNA samples were genotyped by PCR and Southern blot analysis. Additional clinical evaluation was performed in premutation carriers.

**Results:** Among FXS families, 5.3% intermediate, 29.9% premutation and 26.6% full mutation alleles were found. Normal and intermediate alleles were stable upon transmission. For 115 maternal premutation transmissions, 26 (23%) with alleles ranging 60-98 CGGs remained in premutation size with an average expansion of 17 repeat units, whereas 89 (77%) with alleles ranging from 66-199 CGGs expanded to full mutation. In 44 transmissions of maternal full mutation, the offspring inherited alleles in the full mutation range. For 10 paternal transmissions of premutations, ranging 56-120 CGGs, all daughters inherited a premutation allele, with an average expansion of 7 repeat units. After clinical evaluation of 7 premutation carriers, 1 male with FXTAS and 2 females with FXPOI were identified; however the remaining premutation individuals were not yet examined.

**Discussion:** In Portuguese FXS families, allele instability upon transmission is in agreement with previous reports. The risk of premutation to full mutation expansion increases with maternal premutation size.



#### (P43) MOLECULAR DIAGNOSIS OF TUBEROUS SCLEROSIS

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**Introduction:** Tuberous sclerosis complex (TSC) is an autosomal dominant disorder, characterized by hamartomas in multiple organ systems, including the brain, skin, heart, kidneys and lungs. Approximately 30% of the described cases of TSC are due to mutations in TSC1: frequency of cases due to mutations in TSC2 is consistently higher and these tend to be associated with a more severe phenotype. TSC1 encodes hamartin, which interacts with tuberlin, the protein encoded by TSC2. Both are tumor-suppressor genes. TSC1 mutations are mostly small deletions/insertions and nonsense mutations; TSC2 mutations may also include large gene rearrangements.

**Methods:** We have clinically ascertained 11 patients and performed TSC1 and TSC2 mutation analysis, by PCR amplification of all exons and flanking intronic regions, followed by direct sequencing. In patients in whom no mutation was found, we have also performed MLPA to detect large gene rearrangements.

**Results:** We have identified three different mutations in TSC1 and five in TSC2. In TSC1, we found 2 missense mutations (one novel) and a deletion of 2 bp resulting in a frameshift. In TSC2, we identified two frameshifts (resulting from a deletion and a duplication), one nonsense and one splice-site mutation; all novel. By MLPA, a large deletion, encompassing 7 exons in TSC2, was identified in a child with epileptic crises since infancy. We have confirmed the diagnosis in 8 of the 11 cases studied: 3 patients with TSC1 and 5 with TSC2 mutations.

**Discussion:** We have confirmed that TSC2 was more common than TSC1, similar to the reported in other populations. Both genes explained ~70% of our cases. Also, as most of the mutations are novel reinforces the fact that TSC mutations are mostly private. Molecular confirmation of a clinical diagnosis in patients with TSC allows confirmation of diagnosis and proper genetic counseling to patients and their relatives.

**(P44) SCREENING FOR MUTATIONS IN CLCN1: MYOTONIA CONGENITA IN PORTUGUESE PATIENTS**

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Introduction: Myotonia congenita is an inherited muscle disorder caused by mutations in the CLCN1 gene, a voltage-gated chloride channel, expressed in the skeletal muscle. It is the most common channelopathy.

Genetically, two modes of inheritance have been described: autosomal dominant (Thomsen's myotonia) and autosomal recessive (Becker's myotonia). Both are caused by mutations in CLCN1. The autosomal recessive form is more frequent, and is often associated with more severe stiffness of muscles, transient weakness and generalized muscular hypertrophy. In these two forms, age-at-onset is variable: usually in infancy or early childhood in autosomal dominant and somewhat later in the autosomal recessive form. Mutations in CLCN1 include missense (the most frequent), nonsense, insertions, deletions and splice-site mutations. Recently, large rearrangements have also been described.

Methods: We have clinically ascertained 19 Portuguese patients with a clinical diagnosis of myotonia congenita and performed CLCN1 mutation analysis. PCR amplification of all exons and flanking intronic regions was performed, followed by bidirectional direct sequencing.

Results: Using this approach we have identified nine different mutations: 6 missense (one novel), 2 splice-site (one novel) and 1 (novel) deletion resulting in a frameshift. The latter involves the deletion of 44 bp, leading to a truncated protein.

We have confirmed the diagnosis in 8 out of the 19 cases: 7 with an autosomal recessive form and 1 with autosomal dominant inheritance.

As large rearrangements were recently described in CLCN1, we are currently performing MLPA in the cases where no mutations were found by sequencing.

Discussion: In this group of patients, we found CLCN1 mutations in almost 50% of cases, which suggest this gene to be a common cause of myotonia congenita in Portugal. Additionally, molecular confirmation of the clinical diagnosis in patients with myotonia congenita allows proper genetic counselling to patients and their relatives.

**(P45) MUTATION SCREENING IN GENES CAUSING LEUKOENCEPHALOPATHY WITH VANISHING WHITE MATTER**

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**Introduction:** Leukoencephalopathy with vanishing white matter (VWM) is an autosomal recessive disorder that mainly affects children and young adults. It is characterized by cerebellar ataxia, spasticity, variable optic atrophy and diffusely abnormal cerebral hemispheric white matter. VWM has been found to be caused by mutations in five genes (EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5) encoding for five subunits of the eukaryotic translation initiation factor 2B (eIF2B), essential for protein synthesis. The gene most frequently mutated is EIF2B5; point mutations are the most frequent. Our aim was to perform mutation analysis of the five genes mentioned above, in patients with clinical and imaging findings suggestive of VWM.

**Methods:** Four Portuguese patients were selected for mutation screening for the eIF2B encoding genes. All coding regions and intron-exon boundaries were PCR- amplified, followed by bidirectional direct sequencing.

**Results:** In one patient, we identified two heterozygous missense mutations in the EIF2B5 gene: c.338G>A (p.Arg113His), in exon 3, and c.943C>T (p.Arg315Cys), in exon 7. No mutations in the five genes studied, were found in the remaining three patients. Two additional cases are currently under study.

**Discussion:** We identified one compound heterozygote at the EIF2B5 locus. Both mutations have been previously described in VWM patients; in fact, c.338G>A is the most frequent mutation reported. This allowed us to confirm the clinical diagnosis of VWM in this patient. Although 90% of the patients with VWM show mutations in one of the five referred genes, further genetic heterogeneity has been proposed, which is reinforced by our study. As VWM can present with a variable phenotype, mutation screening in these genes can be invaluable to establish a genetic diagnosis, in addition to allowing proper genetic counselling, including the offer of prenatal diagnosis.

**(P46) MOLECULAR CHARACTERIZATION OF METHYLMALONYL COA MUTASE DEFICIENCY IN PATIENTS IDENTIFIED THROUGH NEWBORN SCREENING**

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Methylmalonyl CoA mutase (MCM) deficiency due to mutations in MUT gene is a rare metabolic disorder with autosomal recessive inheritance.

Based on the complete or partial absence of functional apoenzyme, two distinct biochemical phenotypes can be associated with MCM deficiency: mut0 and mut- forms, respectively. Patients presenting the mut0 form often develop, already in the first days of life, severe clinical symptoms resulting from rapidly progressing metabolic acidosis.

From the genetic point of view this is an heterogeneous condition, with most mutations present in single families. In spite of this heterogeneity, several mutations were found to be frequent, some of them among specific ethnic groups.

Since the inclusion of MCM deficiency in the Portuguese Newborn Screening in 2005, approximately 715 000 newborns have been tested and only three were found to have MCM deficiency, thus confirming the low frequency of this disease (1:238 333). These patients were identified through elevated C3 (propionylcarnitine) and C3/C2 ratio, and they all presented a severe clinical phenotype. Molecular study was done by sequencing whole coding sequence and exon-intron flanking regions, after PCR amplification from genomic DNA.

Four different mutations were identified in these patients. One of them (p.G717V) was reported to be frequent among black patients and two other (R108C and c.1022dupA) were previously found among patients of Hispanic origin. Mutation p.G626Efs\*18 was found in two different patients, although their families don't seem to be related.

MCM deficiency is often a life threatening disease with neurological manifestations difficult to prevent with traditional therapies. Molecular characterization of MCM deficient patients is important, not only to elucidate the genetic epidemiology of the disease in Portugal, but also because novel therapies based on the genotype have recently been proposed for MCM deficiency.

**(P47) A FAMILIAL PARTIAL AZFB/C MICRODELETION ASSOCIATED WITH DIFFERENT FERTILE PHENOTYPES**

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After the Klinefelter syndrome, Y chromosome microdeletions are the second most frequent genetic cause of spermatogenic failure resulting in male infertility. Y chromosome microdeletions, encompassing one or more of the three AZF regions, are associated with diverse testicular histology, ranging from Sertoli-cell-only syndrome (AZFa del), maturation arrest (AZFb del) to hypospermatogenesis (AZFc del). The molecular screening of these regions is routinely performed in the work-up of infertile patients with azoospermia or severe oligozoospermia as each one has different prognostic values, both in terms of clinical decision-making and appropriate genetic counselling as well as for understanding the etiology of spermatogenesis impairment. Different partial AZFc deletions were already described, although it is still controversial if these are truly a genetic risk factor for spermatogenesis impairment or a deletional variant without phenotypic consequences.

Here we present the molecular results obtained after AZF analysis of two infertile brothers (both diagnosed with oligoteroastenoazoospermia), and of their fertile father. Several multiplex-PCR assays were performed with distinct sets of STS markers, specific for the three AZF regions.

The molecular analysis revealed that all three men presented the same partial AZFb/c microdeletion, namely the absence of the sY1197, sY1291 and sY1192 STSs. This microdeletion probably results from the recombination of amplicons b1/b3, reducing the gene copy number of *PRY*, *BPY*, *DAZ*, and *RBMY*.

The b1/b3 deletion is rare and its influence on spermatogenesis is still not clear since it can be found in men with severe oligozoospermia or with normal sperm counts. Our result suggests that b1/b3 del is most likely a risk factor predisposing to spermatogenic failure, but is not sufficient alone. The different (in)fertile phenotypes associated with it, a fertile father opposed to his two infertile sons, can be possibly influenced by genetic background, environmental and epigenetic factors, contributing to different phenotypic expressions of individual/specific genomes.

**(P48) GLA MUTATION P.F113L FOUND IN APPARENTLY UNRELATED INDIVIDUALS REVEALED THE SAME ORIGIN.**

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Introduction: The lysosomal alpha-galactosidase  $\alpha$ -Gal) is the enzyme deficient in Fabry Disease. The  $\alpha$ -Gal gene (GLA) localises to Xq22.1 and has 7 exons. There are over 650 pathogenic GLA mutations associated to Fabry disease, most of them private to single families.

The GLA p.F113L mutation is associated with the later-onset, cardiac variant of Fabry disease and was identified in four apparently unrelated Portuguese probands, from different locations in Portugal and in a Portuguese immigrant in France. Two of the patients share a very common Portuguese family name but all the others have different family names.

The aim of this study was to test whether or not these mutations have arisen independently or shared a common haplotype.

Methods: Genomic DNA samples of each patient were haplotyped with microsatellite markers close to the  $\alpha$ -Gal locus, including DXS8020, DXS8034, DXS8089, DXS8063 and DXS8096. A healthy granddaughter of one of the patients was used as negative control and to check for the informativity of the selected microsatellite markers. Microsatellites were analysed with an ABI Prism 3500 Genetic Analyzer using GeneMapper Analysis Software (Version 4.1).

Results and Discussion: All four probands share the same microsatellite haplotype, spanning ~3cM around the  $\alpha$ -Gal locus. The healthy control, who is heterozygous for all the markers, has only a single allele of one of them in common with the patients, including her grandfather. These results suggest that the four patients have a common ancestor and that the Portuguese GLA p.F113L mutation resulted from a single mutational founder event.

**(P49) CADASIL: MUTATIONAL STUDIES IN THE PORTUGUESE POPULATION**

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Introduction: CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leucoencephalopathy) is a genetic disorder associated with stroke in young adults, typically caused by mutations involving cysteine residues in EGF-like domains of the NOTCH3 protein. We screen for NOTCH3 mutations using a two-tier approach, first sequencing exons 4, 11\_12, 18 and 19, which are the highest-yielding in Portuguese patients with CADASIL.

Methods: NOTCH3 gene exons 4, 11, 18\_19 were sequenced in 732 Portuguese patients with clinical and/or neuroimaging signs suggestive of CADASIL; exon 12 was also sequenced in 375 patients. Screening of all other relevant exons was selectively performed in 51 cases.

Results: A total of 19 different mutations involving cysteine residues were found in 83 cases (11%), 4 of which had not been reported before. Mutation p.R558C, in exon 11, was identified in 39 apparently unrelated patients. Five patients had mutations outside the high-yielding exons and one such mutation (p.C1099Y), in exon 20, was identified in two apparently unrelated patients. Sixteen missense mutations not involving cysteine residues were identified in 63 patients, including 5 known polymorphisms and 11 sequence variants of unknown significance. Three of the latter (p.R163W, p.T575M, p.W1028S) were predicted pathogenic by in silico analysis and were not found in more than 200 healthy subjects.

Discussion: Clinical criteria used to screen for NOTCH3 mutations will have to be optimized. Exon 20 should be added to the first-tier mutational screening for CADASIL in our population. The significance of NOTCH3 mutations not involving cysteine residues remains uncertain.

**(P50) STUDY OF THE PREVALENCE OF CFTR GENE SEQUENCE VARIATIONS IN THE PORTUGUESE POPULATION**

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Introduction: Cystic Fibrosis (CF) is defined as the most frequent autosomal recessive disease in the Caucasian population. In the European population, the CF carrier frequency is 1 in 25, with significant variations according to ethnic group and geographical location. In Portugal, the prevalence of CF is unknown. The aim of the present study is to determine the type and frequency of Cystic Fibrosis Transmembrane Conductance Regular (CFTR) mutations in the Portuguese population and to evaluate the prevalence of CF in our population.

Methods: CFTR gene analysis was performed in DNA samples extracted from buccal mucosa cells of 100 children. CFTR gene screening consisted in the analysis of the 27 CFTR exons by direct sequencing.

Results: A total of 10 different mutations were identified. Mutation L997F was identified in 3 samples (3%), R668C and G576A in 2 samples (2%) and R75Q, R170H, D443Y, F508del, V754M, L976S and S1235R in 1 sample (1%). Two well-known complex alleles, G576A-R668C and G576A-R668C-D443Y, were identified in 2 samples. A total of 21 polymorphisms were detected, 2 of them were identified for the first time. The IVS8-T5 variant was identified in 8 samples, with an allelic frequency of 4%.

Discussion: The carrier frequency detected in this preliminary analysis of 11% is lower than that previously reported for the Caucasian population (25%). The frequency of the IVS8-T5 allele (4%) is similar to that described for the general population (5%). Two CF mutations were not identified simultaneously in any sample, however, the association of the L967S mutation with the IVS8(TG)12T5, which may cause congenital absence of the vas deferens, if in compound heterozygosity, was identified in a sample from a boy. These results are expected to contribute to a better knowledge of the Portuguese CFTR gene mutations spectrum as well as to improve the CF diagnosis in Portugal.



## **(P51) MOLECULAR GENETICS OF HUMAN NOCICEPTION**

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**Introduction:** Pain is an unpleasant sensory and emotional experience with an important protective function. In most developed countries 8-30% of the adult population suffers from chronic pain with important negative economical and social consequences. Several common diseases also have important pain burdens such as in cancer and diabetes. Nevertheless, pain management of chronic pain is inefficient. In this project we take advantage of naturally occurring mutations in the human population to identify genes relevant to the sensation of pain.

**Methods:** We have whole-exome sequencing of individuals suffering from rare genetic inherited disorders of pain sensing, having either an absence of pain with or without associated neuropathy, or excess pain. We will identify candidate genes by combining whole-exome data with publically available data relating to gene expression and gene function, as well as proprietary expression data in the nociceptive system. We will then primarily use in vitro model systems, such as established cell-lines, to determine the cellular effects of the genetic changes found.

**Results:** This approach has led to the identification of several previously unknown genes involved in pain such as SCN9A, and NGF. We have already identified a strong candidate in one of our patients and will be undertaking the evaluation of the mechanistic contribution of the mutation found in the patient. If the mutation proves true there is a strong potential for immediate clinical benefits due to the existence of drugs developed to manage other conditions acting upon the identified protein.

**Discussion:** We expect to find genes involved in different biological and physiological processes whose commonality will be the disruption of the pain sensing neuronal network. Those genes will most likely be involved in the function, plasticity and/or development of the nociceptive neuronal system. The pharmaceutical industry is currently developing antagonists to molecules discovered through approaches such as this.

**(P52) COPY NUMBER VARIANTS INVOLVING COMPONENTS OF THE GLUTAMATERGIC SYNAPTIC PATHWAY IN ASD PATIENTS**

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Copy Number Variants (CNVs) may play an important role in susceptibility to Autism Spectrum Disorders (ASD), in particular when deleting or duplicating genes involved in synaptic structure and function such as glutamatergic synapse genes.

Identifying CNVs of etiologic relevance for ASD that include glutamatergic genes may contribute to the understanding of glutamate-related pathogenic mechanisms in this disorder. For this purpose, we crossed the information available in public databases on glutamatergic pathways (KEGG database) and the results of a large genomic screening for CNVs, carried out by the Autism Genome Project (AGP) in 2184 ASD patients. We identified a total of 67 CNVs, including 45 deletions and 22 duplications, encompassing genes involved in glutamatergic neuronal excitability and synaptic plasticity processes at postsynaptic level (SHANK1/2/3; GRM5/7; GRIN3A/B; GRIA2; GRID2; GRIK2/3/4; GRIN3A; SLC1A1; SLC1A7; ADCY2/5/7/8; RGS4/7), that were not present in 8000 control subjects from available databases. In a subset of 342 Portuguese ASD probands, CNVs were validated by quantitative or Long Range PCR, breakpoints determined by sequencing analysis and clinical correlations further explored. We identified two de novo microdeletions of GRIN3A and SHANK3 genes, and maternally or paternally inherited microdeletions of GRID2 and RGS7 genes; two duplications of the ACDY7 gene, one de novo and another present in both parents, and a duplication of the RGS4 gene with maternal inheritance. The probands with CNVs in ACDY7, SHANK3 and RGS4 genes had a positive family history of ASD and learning difficulties. Three patients presented intellectual disability, but none had epilepsy, although probands with the RGS4 and ACDY7 duplications revealed an altered neurological exam and MRI, respectively. Overall there was no distinct clinical phenotype associated with these alterations.

The present results show an excess of structural alterations encompassing glutamatergic genes in this patient sample, reinforcing the putative role of the glutamate pathway in ASD.

# **(P53) THE ROLE OF ADH1B IN ALCOHOL CONSUMPTION AND STROKE SUSCEPTIBILITY**

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Moderate alcohol consumption is thought to be protective against cardiovascular disease, through the mediation of rising HDL cholesterol levels. The study of genetics variants of the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) genes involved in alcohol metabolism is important to understand the patterns of drinking habits and its effects in stroke susceptibility. The functional single nucleotide polymorphism (SNP) rs1229984 (A/G) of the gene ADH1B is strongly associated with alcohol metabolism and its A allele plays a protective role against heavy drinking because it confers an higher alcohol metabolic rate and consequent accumulation of toxic acetaldehyde. Hence we analyzed if a possible association of this variant with stroke susceptibility is mediated by the patterns of alcohol consumption.

SNP rs1229984 was genotyped using a TaqMan Drug Metabolism Genotyping Assay in 569 stroke patients with extended clinical and lifestyle information (age 20-64, mean 52.0; Nmale=362 and Nfemale=207) and 433 controls with matching clinical and lifestyle information (age 54-80, mean 63.9; Nmale=201 and Nfemale=232). Logistic regression analysis was used to determine the effect of the genetic variable on stroke susceptibility.

Preliminary results show an association between this SNP and stroke susceptibility (P=0.006), with the A allele showing a protective effect for stroke. Further analysis is ongoing to explore additional information on drinking patterns and lipid profiles available in this population.

**(P54) SPRY2 AND SPRY4 INTERACTION: A NOVEL GENE-GENE INTERPLAY IN TOOTH AGENESIS**

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Introduction: Tooth agenesis affects 20% of the world population and agenesis of maxillary lateral incisors (MLIA) is one of the most frequent subtypes, characterized by the absence of formation of deciduous or permanent lateral incisors.

Odontogenesis is a complex mechanism regulated by sequential and reciprocal epithelial-mesenchymal interactions, controlled by activators and inhibitors involved in several pathways, such as the fibroblast growth factor (FGF) pathway. Disturbances in FGF signaling can lead to abnormalities in odontogenesis, resulting in alterations in the formation of the normal number of teeth. Sprouty family members functioned as a negative feedback regulator of FGF signaling.

Therefore, our aim was to study for the first time the involvement of SPRY2 and SPRY4 genes in susceptibility to MLIA and to explore a possible gene-gene interaction.

Methods: A case-control study, in a total of 306 individuals was performed; with a case-control ratio of 1:2 to increase statistical power of the study. We selected 10 tagging single nucleotide polymorphisms (SNPs), which were genotyped by SNaPshot, using a multiplex approach.

Results: We found that the GA genotype of rs504122 in SPRY2 gene presented a higher risk for individuals with MLIA ( $p = 0.008$ ), that remained significant after Bonferroni correction.

We also performed a haplotype-based analysis for SPRY4 gene. The T-G-G-A-T-C haplotype revealed a nominal significant association ( $p=0.016$ ) with an increased MLIA susceptibility; however, this did not remain significant after multiple testing correction ( $p>0.05$ ).

Importantly, we have uncovered a strong synergistic interaction between SPRY2 and SPRY4, associated with MLIA liability ( $p=0.012$ , after a 1,000-fold permutation test).

Discussion: Although the molecular mechanisms involved in tooth agenesis remain unknown, our results provide the first evidence of the involvement of sprouty genes in MLIA susceptibility, leading to a better understanding of the genetic mechanisms underlying this trait.

**(P55) MULTIPLEXING TEST FOR MOLECULAR DIAGNOSIS OF SKELETAL DYSPLASIA  
(BY CGC MUTATION PANEL)**

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*CGC Genetics*

**Introduction:** Skeletal Dysplasias (SD) account for more than 450 genetic diseases with bone involvement but variable clinical characteristics, whose diagnosis is based on clinical examination, radiological findings, histo-pathological and molecular analysis. They represent approximately 5% of genetic diseases of the newborn and are a major cause of problems for families and patients due to its morbidity, high lethality and complex medical problems. Genetic testing gives an early clinical diagnosis and is essential for a differential diagnosis. The molecular characterization of genes responsible for SD, is extremely important for establishing a precise diagnostic evaluation, namely during the prenatal period.

**Method:** We developed a multiplex mutation panel that tests 47 point mutations on genes FGFR3, SLC26A2, COL2A1, CRTAP, LEPRE1 and SOX9. This approach allows the molecular diagnostics of the most frequent and severe forms.

**Results:** We tested 132 cases (48 amniotic fluids, 24 peripheral bloods, 36 DNA samples, 16 cell cultures, 6 CVS, 1 umbilical cord blood, 1 paraffin block, and 1 tissue sample from a kidney fetal biopsy; one case was tested by CVS and fetal biopsy, confirming the previous result) and 22 had a positive result. 18 cases were positive for heterozygous mutations in FGFR3 gene [8 with c.742C>T, 4 with c.1138G>A, 3 with c.1118A>G, 2 with c.1948A>G and 1 with the c.2420G>C mutation], 3 cases with mutations on SLC26A2 gene [2 with c.835C>T mutation in homozygous and 1 with both c.532C>T and c.835C>T mutations in heterozygous] and 1 case with a homozygous mutation IVS1+2C>A in CRTAP gene.

**Discussion:** The multiplex mutation panel detects the most common mutations of SD, drastically reducing turnaround time (one week after DNA extraction), maintaining accuracy and liability. Results are independent from the type of sample. Faster and earlier molecular diagnostic is achieved, allowing an early decision-making process in patient management and conduct, being particularly relevant in prenatal situations and future pregnancies.

**(P56) GENETIC DIAGNOSIS OF MATURITY ONSET DIABETES OF THE YOUNG (MODY) IN PORTUGAL**

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**Introduction:** Maturity onset diabetes in the young (MODY) is a heterogeneous autosomal dominant form of diabetes mellitus with typical onset before age 25 and a primary defect in pancreatic beta-cell function. Patients with MODY may erroneously be classified as having type1 diabetes or type2 diabetes and MODY is thought to explain about 2% of all diabetes but its true prevalence in many populations is still not clear. It is estimated that in Portugal has 600.000 diabetic patients and about 12.000 could be MODY. MODY2 patients have mild, asymptomatic, and stable hyperglycaemia that is present from birth. In contrast, patients with MODY3 have a progressive defect in insulin secretion frequently resulting in severe and progressive hyperglycaemia in adult life. MODY2 and MODY3 are the most common forms in Europe. The different MODY types can only be determined by molecular diagnosis. A genetic diagnosis often changes patient management, since patients with GCK mutations rarely require pharmacological treatment and HNF1A/4A mutation carriers are sensitive to sulfonylurea. The aim of this work was to characterize the MODY gene defect associated to each patient to improve patient management.

**Methods:** We analysed 18 patients with clinical diagnosis of MODY using direct sequencing and MLPA techniques for GCK (MODY2), HNF1A (MODY3), HNF4A (MODY1) and HNF1B (MODY5) genes.

**Results and Discussion:** We found 9 different mutations, 8 missense and 1 frameshift, 2 of these have not been described before. The mutations found are located in GCK gene (c.544G>A (V182M), c.214G>A (G72R), c.1358C>T (S453L), c.757G>C (V253L) and c.372C>A (D124E)) and in HNF1A gene: (c.92G>A (G31D), c.864G>C (G288G), c.391C>T (R131W), c.872delC (p.Pro291fs). Molecular genetic testing is useful in patients with MODY because it confirms a diagnosis of monogenic diabetes, predicts clinical course, defines risk for relatives and determines treatment according to their condition, minimising the effects of the disorder.

**(P57) THE HUMAN ERYTHROPOIETIN TRANSCRIPT IS REGULATED AT THE TRANSLATIONAL LEVEL BY AN UPSTREAM OPEN READING FRAME**

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Introduction: Among the various cis-acting elements present in the 5' leader sequence of mRNAs there are upstream open reading frames (uORFs). Although their function is still poorly understood, they are known to downregulate the main ORF expression of several human transcripts that code for key regulatory genes.

The human erythropoietin (EPO) is a glycoprotein initially characterized as a hormone mainly synthesized and released from the kidney, with a key role in hematopoiesis. However, many reports have implicated EPO in several non-hematopoietic functions and have shown its production in other organs. Consequently, it might be used as a therapeutic target for the treatment of several human disorders. Our aim is to study a natural occurring 14-codon-uORF on the human EPO transcript.

Methods: To explore the mechanisms by which EPO uORF controls translational efficiency, HepG2, HEK293 and REPC cells were transfected with several constructs carrying the luciferase reporter gene with the intact or disrupted EPO uORF, with or without the EPO 3' untranslated region (3'UTR). Luciferase activity was measured by luminometry and the mRNA levels were quantified by RT-qPCR. Furthermore, we also analyzed its response to several cell stress stimuli.

Results: Results show that the EPO uORF can decrease the main ORF translation efficiency in about 3-fold. In addition, our data support the conclusion that reinitiation, and in less extent leaky scanning, are responsible for the main ORF translation. In addition, the 3'UTR does not affect the role of the uORF, but it increases the luciferase levels. Specifically in REPC cells, translational inhibition mediated by the EPO uORF is overridden in response to chemical hypoxia, which is due to less uAUG recognition.

Discussion: Our finding of a functional uORF revealed a new level of EPO regulation. Our belief is that understanding the molecular mechanisms through which the EPO uORF controls translation may be valuable in the determination of new therapies.

## **(P58) MOLECULAR INVESTIGATION OF PEDIATRIC PORTUGUESE PATIENTS WITH SENSORINEURAL HEARING LOSS**

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Introduction: Sensorineural hearing loss (SNHL) is one of the most common disabilities in human, and genetics is an important aspect in research and clinical practice for SNHL. One in 1000 children is born with bilateral SNHL, and 50-70% of them have monogenic causes for their deafness.

Hereditary hearing loss can be classified into syndromic and nonsyndromic depending on the associated features. Whilst over 400 genetic syndromes have been described in association with mono- or bilateral deafness, syndromic conditions account for about 30% of hereditary congenital hearing loss whereas the relative contribution to all deaf people is much higher (>70%) for nonsyndromic subtypes.

The understanding of the molecular genetics in SNHL has advanced rapidly during the last decade but the molecular etiology of hearing impairment in the Portuguese population has not been investigated thoroughly.

Methods: We analyzed the whole mitochondrial genome in 95 unrelated children with SNHL (53 non-syndromic and 42 syndromic) and searched for variations in two frequent mutated genes, GJB2 and GJB6, in the non-syndromic patients.

Results: Mutations in mtDNA were detected in 4.2% of the cases, including a hitherto undescribed change in the mtDNA-tRNATrp gene (namely, m.5558A>G). We also identified mono- or bi-allelic GJB2 mutations in 20 of 53 non-syndromic cases and also detected two novel mutations (p.P70R and p.R127QfsX84).

Discussion: Our data suggest that analysis of the GJB2 gene may have clinical implications in the diagnosis of deaf Portuguese children. Also, it would make feasible early rehabilitation and prevention in affected families. The relatively higher incidence of mtDNA mutation also suggests that screening for variations in the mitochondrial genome should always be considered unless mitochondrial inheritance can be excluded for certain. The molecular diagnosis will permit more accurate genetic counseling for family members, monitor possible multisystem complications, and avoid usage of aminoglycosides if infections occur.



**(P59) A NOVEL MISSENSE MUTATION IN SUCLA2 ASSOCIATED WITH MILD METHYLMALONIC ACIDURIA**

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Introduction: Succinyl CoA synthase is a mitochondrial matrix enzyme that catalyzes the reversible synthesis of succinate and ATP or GTP from succinyl-CoA and ADP in the tricarboxylic acid cycle (TCA). This enzyme is made up of two subunits,  $\alpha$  and  $\beta$ , encoded by SUCLG1 and SUCLA2, respectively.

The clinical features of patients with mutations in SUCLA2 include early childhood hypotonia, developmental delay, and almost invariably, progressive dystonia and sensorineural deafness. Mutations in SUCLA2 and SUCLG1 cause an encephalomyopathic form of infantile mtDNA depletion syndrome. A useful diagnostic clue in succinyl CoA synthase disorders is a “mildly” elevated urinary methylmalonic acid (MMA), and presence of TCA intermediates.

To date, two patients with SUCLG1 mutations have been reported, whereas mutations in SUCLA2 have been reported in 17 patients. We here present an additional patient with a novel SUCLA2 mutation.

Methods: We report a 17-month-old-boy, who presented severe muscular hypotonia, failure to thrive, developmental delay, weight loss during a gastroenteritis crises, dysmorphisms and muscular atrophy. A clinical investigation disclosed hyperlactacidemia together with moderate excretion of MMA and elevated C4-dicarboxylic carnitine. Sequencing analysis of SUCLA2 and SUCLG1 was performed using standard methods.

Results: Mutation analysis of SUCLA2 revealed a homozygous c.985A>G mutation in exon 8 (p.M329V). This missense mutation affects an amino acid that is highly conserved in different species and was not found in controls. The analysis by bioinformatics tools also confirmed a pathogenic mutation.

Discussion: The clinical and biochemical phenotype of our patient is strikingly similar to other reported patients with SUCLA2 mutations. In addition, the mildly elevated levels of methylmalonate and lactate raised the suspicion of this disease.

Our study contributed to expand the spectrum of patients with SUCLA2 mutations, and will be important for an accurate genetic counseling and a prenatal diagnosis to the affected family.

## **(P60) *IL10* SEQUENCING FOR BEHÇET'S DISORDER IN THE PORTUGUESE POPULATION**

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Behçet's disease (BD) is a chronic inflammatory disorder classified as a vasculitis, and may involve several organs, such as skin, mucocutaneous membranes (oral and genital aphtae), eyes, joints, lungs, gastrointestinal and central nervous systems. Common variants in *IL10* have been recently associated with BD in two independent genome-wide association studies (GWAS) in the Turkish and Japanese populations and replicated by our group in an Iranian dataset. Despite the GWAS success in identifying single nucleotide polymorphisms (SNPs) that contribute to some complex diseases, the majority of genetic variants contributing to disease susceptibility are yet to be discovered. In fact, it has been argued that these variants are not likely to be captured in current GWAS that focuses on common SNPs. Sequencing enables the identification of new rare variants of small/moderate effect and/or mutations that can be related to the BD phenotype, which in turn promise to help explain the missing heritability after the GWAS era.

50 Portuguese cases with BD were sequenced using the traditional Sanger sequencing for the entire *IL10* ORF including also 1Kb upstream and downstream of *IL10*. Additionally, two conserved regions located in the *IL10* promoter were also sequenced.

We identified so far 24 known single nucleotide variants (SNVs) in our sample with minor allele frequencies (MAF) ranging from 0.01 to 0.40, and three new SNVs in our BD cases. These new SNVs were present in a heterozygous state. One of these new variants is located at intron 3 and was detected in two individuals while the other two are located in the promoter region and were identified in one individual each. The absence of these three novel variants has been confirmed in 50 healthy Portuguese individuals and genotyping of 100 additional healthy Portuguese controls is ongoing to assess if these SNVs are specific of Behçet's disease patients or if they are rare variants not yet reported. Genotyping of these three new variants will also be performed in 300 Iranian BD patients and controls to investigate if they are specific to Portuguese BD patients.

**(P61) EVALUATING SELECTION IN HUMAN NUCLEAR-ENCODED GENES FOR PROTEINS INTERVENING AT THE MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION PATHWAY**

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**Introduction:** The project “1000 genomes” [1] is rendering worldwide human genomes freely available. This is a powerful tool to check diversity and demographic events in many genes. We have previously proven that purifying selection acts on mitochondrial genes intervening in the oxidative phosphorylation pathway, by removing deleterious mutations [2]. We also showed [3] that somatic mutations appearing in oncocytic tumors seemed to be positively selected towards high pathogenicity scores. But most of the proteins involved in the oxidative phosphorylation pathway are coded by the nuclear DNA and transferred afterwards to mitochondria. In this work we will evaluate if the nuclear-encoded genes also show signs of selection as its mitochondrial-encoded counterparts.

**Methods:** We extracted the genetic information for the 120 nuclear-encoded genes from the “1000 genomes” website, from four population groups (246 individuals from Africa, 379 from Europe, 286 from Asia and 181 from America). Haplotypes were inferred by using the FastPhase algorithm [4]. Genetic diversity, population comparisons and deviations from neutrality were evaluated in the software Arlequin [5]. The pathological status of non-synonymous mutations was inferred by using the algorithm MutPred [6].

**Results:** We already implemented all the scripts to transform data to be used in the diverse softwares and employed Linux scripts to automatically run analyses in a considerable amount of files. We confirmed that the work flow is functioning by testing 20% of the 120 genes. These first results are allowing to identify signs of selection in some genes.

**Discussion:** The nuclear and the mitochondrial genomes have different controls and rates of replication but as proteins coded by both must cooperate in the major pathway used by cells to obtain energy, they must in some way be coordinated. This work, by evaluating selection acting both at nuclear- and mitochondrial-encoded genes from the oxidative phosphorylation pathway can shed light on the cross-talk between the two genomes.

*References and websites:*

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- 2- Pereira et al. (2011)
- 3- Pereira et al. (2012)
- 4- [http://c4c.uwc4c.com/express\\_license\\_technologies/fastphase](http://c4c.uwc4c.com/express_license_technologies/fastphase)
- 5- <http://cmpg.unibe.ch/software/arlequin3>
- 6- <http://mutpred.mutdb.org>

**(P62) EXOME SEQUENCING OF FAMILIAL HYPERCHOLESTEROLAEMIA PATIENTS: UNIQUE ALTERATIONS IN CHOLESTEROL RELATED GENES ANALYSIS**

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Familial hypercholesterolemia (FH) is a monogenic condition caused, in most cases, by mutations in LDLR, APOB and PCSK9. In the Portuguese FH Study only 40% of clinical FH patients have an identifiable mutation so, other mutations in these genes or other gene defects must exist to explain the cause of hypercholesterolemia in the remaining families.

The main aim of this project was the whole-exome sequencing of 5 index patients with clinical diagnosis of FH (4 without a detectable mutation and one patient (P1) heterozygous for a LDLR mutation but with a severe phenotype) in order to identify the genetic cause of the hypercholesterolemia in these patients.

The exome sequencing was performed in an Illumina sequencer and data analysis was performed by annovar software. Alterations in 33 cholesterol related genes previously described in linkage or GWAS studies were annotated for analysis.

Most of the alterations found were common variants described in dbSNP. In the 5 samples a mean of 14726 variants in 12223 genes, was registered. The majority of unique undescribed alterations found are nonsynonymous (1897), followed by synonymous (1190), splicing (33), frameshift (176), indel (159) and stopgain/loss (54).

A total of 53 unique alterations were found in 33 cholesterol related genes under analysis. From these 10 have not been described previously in dbSNP: 4 nonsynonymous, 4 synonymous, 1 is a frame deletion and 1 is a frameshift. Variants in CELSR2, CPT1A and OSBPL1A genes are the most promising unknown alterations causing disease in these patients. Family studies are being conducted to observe co-segregation of the alteration with the phenotype.

Identification of novel genes causing FH will improve patient identification and prognosis and can lead to the identification of novel lipid lowering drugs.

**(P63) POLIMORFISMO DOS GENES GSTT1 (RS56106137) E GSTM1 (RS72989301) DA GLUTATIONA S-TRANSFERASE EM PACIENTES COM ANEMIA FALCIFORME**

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**Introdução:** O processo mutacional que dá origem à hemoglobina S (HbS) é a causa das profundas alterações fisiopatológicas da anemia falciforme (AF) que são desencadeadas principalmente por polimerização, degradação oxidativa da HbS e geração de radicais livres oxidantes. Tem havido considerável interesse na determinação dos polimorfismos destes genes associados a doenças inflamatórias crônicas, como a AF, pela relação das variações genótípicas e fenotípicas das GSTs em poder modificar o passo-a-passo do processo carcinogênico, incluindo a biotransformação de radicais livres, reparo do DNA, apoptose e controle celular.

**Métodos:** Foram analisadas amostras de DNA genômico de 278 pacientes com AF para correlacionar os genótipos nulos T1 e/ou M1 da GST com as principais manifestações clínicas da doença a partir da técnica de PCR multiplex utilizando primers forward e reverse para os tais genes.

**Resultados e Discussão:** Foi observado um elevado percentual de pacientes com genótipos nulos, onde 27,3% dos pacientes tiveram somente o gene GSTM1 nulo e 14,7%, somente o gene GSTT1. Ambos os genes ausentes foram vistos em 11,1% da população estudada. Síndrome torácica aguda (STA) e necrose asséptica da cabeça do fêmur (NACF) foram as manifestações clínicas que apresentaram maior taxa de risco com o genótipo GSTT1 nulo, 10,34 e 6,33, respectivamente. Diferente do resultando encontrado com o gene GSTT1, pacientes com nulidade genômica GSTM1, apresentaram 3,864 vezes mais chances de desenvolver acidente vascular cerebral (AVC) e alto risco de apresentar úlcera maleolar e STA (OR=6,91 e 4,23, respectivamente). Ao analisar a associação dos dois genes nulos, somente STA, úlcera maleolar e NACF apresentaram valores significativos ( $P < 0,05$ ) e taxas de riscos importantes. Portanto, a ausência dos genes GSTT1 e/ou GSTM1 foi avaliada como um fator de risco importante para o agravamento da AF, podendo ser utilizada como ferramenta adicional para identificação e monitoramento de indivíduos suscetíveis ao agravamento dessa hemoglobinopatia.

## **(P64) REVERSING THE EFFECT OF THE IDUA GENE W402X MUTATION?**

**Ribeiro D, Amaral O**

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Introduction: Mucopolysaccharidosis type I (MPS I; OMIM #252800) is an autosomal recessive disorder, which results from the defective activity of the lysosomal enzyme  $\alpha$ -L-iduronidase (IDUA, EC 3.2.1.76). The gene encoding  $\alpha$ -L-iduronidase (IDUA; OMIM #252800) maps to chromosome 4p16.3 and contains 14 exons.

The W402X mutation is the most common in patients of European Caucasian origin, appearing in over 45% of alleles in unrelated patients of various western European origins. In addition, this mutation has been considered to play an important role in terms of the pathophysiology of MPS I.

The main objective of this work was to functionally evaluate the susceptibility of the nonsense mutation W402X of the IDUA gene to mechanisms of nonsense suppression with two individual compounds.

Methods: Nonsense suppression therapy experiments were carried out in fibroblast cell lines from individuals homozygous and heterozygous for the nonsense mutation W402X of the IDUA gene, as well as in normal control cell lines, by using variable concentrations of distinct substances and different incubation times.

Results: The results obtained with the different treatments in a W402X homozygous cell line showed that it was possible to obtain an increase in the levels of expression. It was found that the increase of the IDUA gene expression led to levels of expression comparable to those of the control cell line without treatment.

Discussion: The IDUA gene mutation W402X not only results in nonsense mediated decay, but is also susceptible to suppression of nonsense with different compounds. Further experiments are underway in order to determine the possible implications of this type of approach in the recovery of protein function.

*Additional information:*

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**(P65) METHYLCROTONYL CoA CARBOXYLASE DEFICIENCY: DISORDER OR JUST A BIOCHEMICAL PHENOTYPE?**

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Introduction: 3-methylcrotonyl-CoA carboxylase deficiency (MCCD) was considered extremely rare before newborn screening (NBS) was undertaken but is now found in a number of asymptomatic babies or sometimes their mothers. This disorder of leucine metabolism, is the commonest organic aciduria found by screening, with a incidence of about 1:32 392 in our country.

The clinical phenotype has been shown to vary considerably, ranging from entirely asymptomatic to death in infancy. A review of the literature on 37 individuals indicates that only 27% developed normally and stayed completely asymptomatic. Approximately 30% were reported to suffer from muscular hypotonia and psychomotor retardation, and almost half suffer from various other neurological symptoms. Even a lethality of 11% was observed.

The metabolic phenotype characterizing MCCD is the elevated excretion of the diagnostic compounds 3-methylcrotonylglycine and 3-hydroxyisovaleric acid, and the presence of abnormally elevated blood levels of 3-hydroxyisovalerylcarnitine (C5-OH), as determined by tandem mass spectrometry (MS/MS).

Patient and methods: The authors present a symptomatic case with an increase of C5-OH in the acylcarnitine profile who have a developmental delay.

Blood spot samples from newborns are collected between day 3 and 6 in Watman 903 filter paper. Acylcarnitines in samples are analysed by MS/MS. Genes *MCCA* and *MCCB* that encodes the enzyme 3-MCC were studied by reported methods.

Results: The molecular study has allowed the identification of the *compound heterozygous* in this patient: the frameshift mutation p.S173FfsX25 and the missense mutation p.V339M. Both mutations are described in the literature.

Discussion: The newborn screening identification of a patient which developed symptoms seems to indicate that this disease should be included in NBS programs. More studies are needed to find genetic and/or biochemical markers that explain why a relatively small number of individuals are at risk of developing a severe disease phenotype.

Another important reason to include MCCD in our panel is that other disorders are also detected by the marker C5OH; for example deficiencies of holocarboxylase synthetase, and 3-hydroxy- 3-methylglutaryl-CoA lyase.

**(P66) MECHANISMS OF GENOMIC REARRANGEMENTS LEADING TO LARGE PARK2 DELETIONS**

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**Introduction:** Autosomal recessive juvenile Parkinson disease (AR-JP) is an early-onset form of Parkinsonism that is usually clinically indistinguishable from idiopathic PD forms. Mutations in PARK2 are the most common cause of Parkinsonism with early-onset (<40 years). All types of mutations have been described in PD patients, but large PARK2 deletions account for 50% of the mutations identified in Portuguese patients with AR-JP. This high frequency can be explained by the large introns of this gene and by the localization of PARK2 within a fragile site, FRA6E.

**Methods:** In order to determine the breakpoints of fourteen deletions we previously found in Portuguese patients we took several approaches before developing a successful one to narrow down deletion breakpoints. We used the SNaPshot technique to genotype SNPs and search for loss of heterozygosity or complete loss of both alleles. Real-time PCR analysis was performed to confirm the homozygous state. Long-range PCR were carried out and deletion breakpoints were narrowed down by primer walking and direct sequencing. Finally, the PARK2 sequence was analyzed using RepeatMasker.

**Results:** Our results showed that are at least three mechanisms responsible for parkin deletions. The mechanism that mediated most PARK2 deletions is the Non-homologous end joining, the major pathway that repairs double-strand DNA breaks. We have also found Alu-mediated non-allelic homologous recombination (NAHR) and LINE-mediated NAHR. NAHR usually requires two low copy repeats with sufficient length of high homology to act as recombination substrates.

**Discussion:** This task proved to be particularly laborious due to the PARK2 extremely large introns. Interspersed repetitive sequences, such as LINE and Alu elements, are transposable sequences involved in recombination events that are commonly associated with breakpoints, and are reported to originate genomic deletions by promoting recombinational instability. This study allows us to a better understanding of the genomic mechanisms underlying gene rearrangements in PARK2.



## (P67) MOLECULAR DIAGNOSIS OF FRONTOTEMPORAL DEMENTIA IN PORTUGUESE PATIENTS

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Frontotemporal dementia (FTD) is the second most common form of dementia in individuals under age 65 years. FTD is an autosomal dominant disorder, caused by frontotemporal cerebral atrophy, covering a heterogeneous group of sporadic and familial cases. The common presentation is an early change in personality and social behavior, and language dysfunction. Several genes have been implicated, although most FTD patients present mutations in the microtubule associated protein tau (*MAPT*) and granulin (*GRN*) genes (17q21-22). The prevalence of this disease in Portugal is still unknown.

We performed mutation analysis of *MAPT* and *GRN*, by PCR amplification of all exons and flanking intronic regions, followed by bidirectional direct sequencing, and MLPA analysis whenever appropriate, in a group of 52 Portuguese patients with a clinical diagnosis of FTD. Mutation screening of *VCP* is ongoing (26 patients already screened).

No pathogenic mutations described in the literature were found. Instead, several novel nucleotide variants were found in *MAPT* (c.1561+165 G>A, c.1827+15 C>T, c. c.1561+119 G>A) and *VCP* (c.811+2T>C, c.351G>T, 2160+32A>G) genes. A bioinformatics approach was performed to determine the pathogenicity of these variants, suggesting all but one are probably benign. The mutation c.811+2T>C in the *VCP* gene is probably pathogenic, as it is predicted to disrupt the splicing consensus of exon 7.

The molecular diagnosis of FTD is important for confirmation of its clinical diagnosis and, even more importantly, for the purpose of genetic counseling of at-risk relatives.

**(P68) CASK AND MIGRAINE: A NEW RISK FACTOR IN MEN**

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**Introduction:** Migraine is a chronic disease characterized by episodes of headache associated with other neurological symptoms. Genetic studies and a 3-fold higher prevalence in females compared with males suggest a possible influence of the X chromosome in migraine susceptibility. The CASK gene (Calcium / calmodulin-dependent serine kinase), located on the X chromosome, is involved in the regulation of calcium channels that are important in the release of neurotransmitters, and may play a role in the pathophysiology of migraine. The aim of this study was to evaluate common variants in CASK to understand the differences in the frequency of disease among men and women.

**Methods:** The study was based on a case-control strategy, comprising 188 cases and 287 controls. Eleven tagging SNPs were selected and their genotyping was performed using SNaPshot. Allelic, genotypic and haplotypic frequencies were compared between cases and controls, stratifying by gender and correcting for multiple testing.

**Results:** In the group of men, allelic analysis revealed an increased risk for the C allele of rs2998250 and for the A allele of rs5918209 (OR = 2.31, 95% CI: 1.00-5.37) in migraine susceptibility (OR = 3.00, 95% CI 1.29 - 6.96). These results remained statistically significant after correction for multiple testing. No differences in haplotype frequencies were found. Regarding women, both in the allelic analysis and in the logistic regression no significant results were found.

In the haplotype analysis, the haplotype A-C-G-G-C-A-T-C-A-A was more cases in controls (OR: 2.38, 95% CI 1.15-4.96), withstanding permutation correction.

**Discussion:** Our results point to the involvement of CASK as a risk factor, particularly in men. The study of these genetic variants related to gender help to increase the knowledge of the genetic basis of this disease and may contribute to a better diagnosis.

**(P69) MOLECULAR CHARACTERIZATION OF THE NEURONAL CEROID LIPOFUSCINOSIS PATIENTS IN PORTUGAL**

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Introduction: The neuronal ceroid lipofuscinoses (NCL) are a group of severe and untreatable inherited neurodegenerative diseases, considered the most common among the childhood onset ones, although it may have onset at later ages. The hallmark is the lysosomal accumulation of autofluorescent lipopigment, which can be displayed in distinctive patterns. Currently there are 13 genes involved, and most of the encoded proteins have unknown function. NCL shows an autosomal recessive mode of transmission, except for the CLN4 - DNAJC5 gene – which is autosomal dominant.

Methods: Clinical suspects were tested for PPT1 and TPP1 enzyme activity (testing for CLN1 and CLN2, respectively), and the patients with deficiency were tested for mutations in the affected gene. The rest of the patients were tested for the very prevalent mutation in CLN3 gene by specific PCR and the exome sequencing of the other genes, following criteria based on clinical symptoms, age of onset and accumulation patterns.

Results: Data on the NCL patients is presented, both clinical and laboratorial, updated with the newly described genes testing and including first CLN7 (MFSD8 gene) patient diagnosed in Portugal, as well as unpublished mutations.

Discussion: Highlight is put in phenotype/genotype (gene and mutation) correlation. Evolution of the diagnostic algorithms is discussed. Implications on genetic counseling are also discussed.

**(P70) C. ELEGANS AS A BIOLOGICAL PLATFORM IN THE FUNCTIONAL STUDY OF GENES ASSOCIATED WITH NEURODEVELOPMENTAL DISORDERS**

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**Introduction:** In the past years, a great scientific effort has been made in the identification of genes associated with neurodevelopmental disorders such as intellectual disability (ID), autism spectrum disorders (ASD) and epilepsy. These disorders are very heterogeneous, but frequently co-exist. Patients with ID, both syndromic and non-syndromic, often display autistic features and epileptic events, as for example, in Rett or Fragile X syndrome. However, apart from the genetic association, studies addressing the functional impact of these genes/mutations to the nervous system and their role in the disease are oftentimes lacking. We are currently using the nematode *C. elegans* as a biological platform for the functional validation of genetic findings of neurodevelopmental disorders.

**Methods:** We are currently analyzing a set of 66 *C. elegans* mutants for genes associated with the above mentioned disorders: 24 ID-associated genes (29 strains); 7 ASD-associated genes (13 strains); and 16 epilepsy-associated genes (24 strains). These mutants are being characterized in terms of neurological phenotype and seizure susceptibility.

**Results:** So far, partial analysis of the experimental set shows that several strains display altered neurological phenotypes, such as motility defects (35%), altered chemotaxis response to isoamyl alcohol (70%) and NaCl (40%), as well as susceptibility to GABA antagonist PTZ (80%).

**Discussion:** Currently, the strains with overt phenotypes have been selected for further characterization in terms of neuronal architecture and synaptic processes. These and further studies may reveal some of the molecular and cellular mechanisms underlying neurological dysfunction in these disorders.

**(P71) GENETIC VARIATION IN SNAP25 IS INVOLVED IN COMMON MIGRAINE SUSCEPTIBILITY**

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Introduction: Migraine is a complex disorder resulting from the interaction of genetic and environmental components. Information regarding the genetics of the common forms of migraine is still scarce. To unravel genetic factors of complex diseases case-control association studies are the most common strategy and candidate genes for these studies are usually involved in the disorder's pathophysiology. Regarding migraine, several pathways may be considered, among them the neurotransmission systems.

An association between STX1A – which encodes syntaxin-1A, a member of the SNARE complex, essential for neurotransmission – has been found in three distinct populations (Portuguese, Catalanian and German). SNAP25 encodes a homonym protein that is also part of the SNARE complex and therefore a reasonable candidate as a susceptibility gene.

Methods: We have recently finished the genotyping of 14 SNPs, covering most of the common genetic variation (>10%) in the SNAP25 gene, in a sample of 475 individuals – 188 patients and 287 controls, matched by age and geographical location. The genotyping methodology chosen was SNaPshot, a powerful high throughput technique specifically used to genotype SNPs.

Results: A statistical analysis of the results has unraveled several significant associations, namely two allelic – with the rs363050 (OR = 1.45; p = 0.006) and rs6108463 (OR = 1.63; p = 0.003) SNPs – as well as two haplotypic associations responsible for an increased migraine susceptibility.

Discussion: The results of this study point toward SNAP25 as a susceptibility gene for migraine. It is important that further studies clarify the role of these associated genetic variants, as the SNAP-25 protein is already a candidate biochemical target for migraine therapy.

## (P72) ANALYSIS OF VERY LARGE COPY NUMBER VARIANTS IN AUTISM SPECTRUM DISORDER

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Autism Spectrum Disorder (ASD) is a neuropsychiatric condition characterized by impairments in social interaction and communication, restricted/repetitive behaviors and a strong genetic etiology partly due to the occurrence of rare Copy Number Variants (CNVs). In this study we selected very large CNVs (>750 kb) for further characterization, because they encompass and/or disrupt more genes, therefore having a higher probability of being pathogenic.

From the CNVs identified by the Autism Genome Project (AGP) in a Portuguese patient subset (N=342), we selected potentially pathogenic CNVs based on very large size, absence or low overlap with controls (<50%) and brain gene content. Validation of the CNVs, identified by the Illumina 1M in the AGP study, was performed by quantitative PCR (qPCR) and breakpoint mapping by Long-Range PCR, in patients and parents. Clinical phenotype and family history of patients were correlated with genetic findings.

Five large CNVs in five patients (on 3p22.1-22.2, 9q31.1, 16p13.11-13.12, 20p13) were validated, while a single CNV on 11q14.3 was a false positive in two patients. Two ASD children showed inherited duplications, on 3p22.1-22.2 and 20p13. Deletions were identified in three patients: two on 16p13.11-13.12, one inherited and one de novo and one de novo on 9q31.1. Clinical presentation in these patients was heterogeneous. Inherited CNVs co-segregated with autistic traits or psychopathology in the transmitting parent in two families, and three patients had family history of psychiatric disorders or cognitive disability in other first or second-degree relatives. Breakpoint mapping showed that in all cases these CNVs encompassed brain-expressed genes relevant for ASD susceptibility, namely SCN11A, MOBP, SLC44A1, GRIN3A, NDE1, NTNA1, PDYN, SIRPA.

These results indicate that very large CNVs are rare events with heterogeneous behavioral consequences but, given their genic content, co-segregation with psychopathology and family history, of likely etiological relevance. Functional analysis is required to confirm their pathogenicity.

**(P73) LENTIVIRAL-MEDIATED EXPRESSION OF MUTANT ATAXIN-3 IN THE MOUSE CEREBELLUM INDUCES ATAXIA AND CEREBELLAR NEUROPATHOLOGY**

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**Introduction:** Machado-Joseph disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3) is a fatal, dominant neurodegenerative disorder caused by the polyglutamine-expanded protein ataxin-3. Clinical manifestations include cerebellar ataxia and pyramidal signs culminating in severe neuronal degeneration. Currently, there is no therapy that allows the modification of disease progression.

**Material and methods:** In the present study, we aimed at investigating whether expression of mutant human ataxin-3 in one of the most severely affected brain regions in the disorder - the cerebellum would reproduce the behavioral defects associated with the neuropathology in this region. For this purpose we injected lentiviral vectors encoding full-length human mutant ataxin-3 cDNA in the mouse cerebellum of three weeks old C57/BL6 mice.

**Results:** We show that circumscribed expression of human mutant ataxin-3 in the cerebellum mediates within a short time frame - 6 weeks, the development of a behavioral phenotype including reduced motor coordination, ataxic wide-based gait and hyperactivity. Furthermore, expression of mutant ataxin-3 resulted in the accumulation of intranuclear inclusions, neuropathological abnormalities and neuronal death.

**Discussion:** This data shows that lentiviral-based expression of mutant ataxin-3 in the mouse cerebellum induces localized neuropathology, which is sufficient to generate a behavioral ataxic phenotype. Moreover, this approach provides a physiologically relevant, cost- and time-effective animal model to gain further insights into the pathogenesis of MJD and for the evaluation of experimental therapeutics of MJD.

**(P74) APCS AND RBP4 GENES AS MODIFIERS OF AGE-AT-ONSET IN FAMILIAL AMYLOID POLYNEUROPATHY (FAP ATTRV30M)**

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Introduction: Familial amyloid polyneuropathy (FAP ATTRV30M) is an autosomal dominant inherited disease, due to a point mutation in the TTR gene (chr18q11.2-q12.1). Remarkable differences in mean age-at-onset (AO) have been described in different clusters, including within Portuguese population.

Among Portuguese families, FAP shows a wide variation in AO (17-82 yrs) and asymptomatic carriers aged 95 can be found; this variation is also often observed between generations.

A previous study in Portuguese patients (Soares et al., 2005) found a modifier effect in AO for APCS gene and RBP4 gene, when comparing classic and late-onset patients with controls. However variation between generations was not taken into account.

Our aim was to investigate if these two candidate-genes have a modifier effect in AO variation from parent to offspring in FAPATTRV30M families.

Methods: We collected a sample of 36 FAP families with at least 2 generations affected. We selected 5 tagging SNPs through the degree of linkage disequilibrium (LD) existing between SNPs and also the 5 SNPs previously described. These SNPs were analysed by SNaPshot and RFLP, respectively. Samples' genotyping is currently underway and results are being analyzed with the GeneMapper™ v.4.0 software.

Results and Discussion: Preliminary results in 5 FAP families showed that although for RBP4 gene we found different genotype's frequencies in patients for rs7079946 and rs17484721 from HapMap, no striking differences were found between generations in the families analyzed for the two genes.

In the total sample of Portuguese families ascertained, we expect to find or exclude the potential role of these candidate-genes as modifiers of FAP ATTRV30M AO. The study of genetic modifiers is crucial to understand the mechanisms involved in AO variability within and between families and may have an important impact in genetic counselling.



**(P75) NEUROPATHOLOGY OF A TRANSGENIC MOUSE MODEL OF MACHADO-JOSEPH DISEASE**

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**Introduction:** Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder caused by the expansion of a polyglutamine tract (polyQ) in the C-terminus of the *ATXN3* gene product, ataxin-3. In this study, we proposed to target pharmacologically ataxin-3 misfolding and aggregation, thought to underlie the disease, through treatment with the Hsp90 inhibitor 17-DMAG.

**Methods:** Here, CMVMJD135 mice were chronically treated with 17-DMAG in the attempt to revert their neurological phenotype.

**Results/discussion:** 17-DMAG-treated animals presented less foot dragging and this symptom appeared later in life comparing to vehicle-treated animals. 17-DMAG treatment also improved motor performance of the animals in the rotarod, balance beam and motor swimming tests. Other motor deficits observed in CMVMJD135 mice, namely loss of limb strength and limb clasping, were not improved by 17-DMAG treatment.

Pathological analysis demonstrated a reduction of shrunken hyperchromatic nuclei upon 17-DMAG treatment, in affected brain areas, such as pontine and dentate nuclei, whereas no differences were observed in spared areas, such as the striatum and dentate gyrus. While in vehicle-treated animals 22% of pontine nucleus cells showed ataxin-3 intranuclear inclusions, in 17-DMAG treated mice only 6% contained these inclusions. Interestingly, the protein (but not mRNA) levels of mutant human ataxin-3 were dramatically reduced in 17-DMAG treated animals, suggesting that this treatment is promoting mutant ataxin-3 degradation rather than refolding. The beneficial effects of 17-DMAG decreased with age, becoming non-significant from the age of 30 weeks. Moreover, acute 17-DMAG treatment failed to induce the expression of molecular chaperones in 20 weeks old mutant ataxin-3 but not wild-type mice. This suggests that the mechanisms of induction of the heat shock response may become impaired during ageing in MJD animals. Our results suggest HSP90 inhibition as a good strategy for MJD therapy but also indicate that complementary strategies may be needed to avoid blunting of the HSR.

**(P76) “BRITISH MUTATION” FOUND IN A PORTUGUESE PATIENT WITH HEREDITARY SENSORY NEUROPATHY TYPE IA**

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**Introduction:** Hereditary sensory neuropathy type IA (HSN1A) is an autosomal dominant disease, characterized by progressive degeneration of dorsal root ganglia and motor neurons, with onset in the second or third decades. Clinically, it involves an early sensory loss in the feet followed by distal muscle wasting and weakness. HSN1A is often associated with progressive sensorineural deafness and the loss of pain and temperature sensation, leading to chronic skin ulcers and distal amputations. HSN1A is caused by mutations in the SPTLC1 gene, mapping to chromosome 9q22.1-q22.3 locus and composed by 15 exons. SPTLC1 gene encodes a serine palmitoyltransferase long-chain base subunit 1 (SPTLC1 subunit), the key enzyme in sphingolipid biosynthesis.

**Methods:** We have ascertained two Portuguese patients with a clinical diagnosis of HSN1A and performed SPTLC1 mutation analysis, for diagnostic testing. Mutation screening was performed by PCR amplification of all exons and flanking intronic regions, followed by bidirectional direct sequencing.

**Results:** Mutation screening performed in these two unrelated patients allowed us to detect the disease-causing mutation in one of them. We have found a T-to-G transversion, at nucleotide 399 of the SPTLC1 gene, which results in a missense mutation (p.C133W).

**Discussion:** Our sample of patients, albeit small, allowed the confirmation of the clinical diagnosis in one patient with HSN1A, and additionally, will allow proper genetic counseling to this family. In this patient (originating from Aveiro), we have found the so-called “British mutation” (p.C133W), although this has already been found in families from Canada and even from China. This mutation has been found to result in massive cell death during neural tube closure, raising the possibility that neural degeneration in HSN1A is due to ceramide-induced apoptotic cell death.

**(P77) LARGE SCALE FUNCTIONAL RNAI SCREEN IN C. ELEGANS TO FIND MOVEMENT ENHANCERS IN THE CACNA1A MUTANT**

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**Introduction:** Mutations in the CACNA1A gene that encodes the pore-forming  $\alpha 1$  subunit of human voltage-gated Cav2.1 (P/Q-type) Ca<sup>2+</sup> channels cause several autosomal-dominant neurologic disorders, including familial hemiplegic migraine type 1 (FHM1), episodic ataxia type 2 (EA2), and spinocerebellar ataxia type 6 (SCA6).

In order to identify modifiers of uncoordination in movement disorders, we performed a large scale functional RNAi screen using the *C. elegans* strain CB55, which carries a truncating mutation in the *unc-2* gene, the worm ortholog for the human CACNA1A.

**Methods:** The screen was carried out by the feeding method in a 96-well liquid culture format using the ORFeome v1.1 feeding library of ORFs (Source Bioscience) as previously described. We used time-lapse imaging of worms in liquid culture to assess changes in thrashing behaviour. Raw imaging data was analysed with open source Image J, and the thrashing analysis results were loaded on CellHTS2 for further exploration.

**Results:** We looked for genes that when silenced ameliorated the slow and uncoordinated phenotype of *unc-2*. Raw data has already been collected for the full library and 55% of the primary screen has been analysed by CellHTS2. So far we found 42 candidate genes improving CB55 motor function with an overrepresentation of genes involved in cell signalling, signal transduction and vesicle mediated transport, according to gene ontology.

**Discussion:** We fully expect that completion of the analysis of the remaining 45% of the library will also reveal enrichment for the same cellular pathways. We are now following with more detail the genes that already scored in the first screen by expanding the panel of behavioural and neurodegeneration assays. Overall the *unc-2* mutant is a very attractive model to study neuronal dysfunction and movement disorders as mutations in the human gene give rise to at least three different disorders with overlapping phenotypes.

**(P78) PHYLOGENETIC ANALYSIS OF THE MIRANDA DO DOURO POPULATION: INSIGHTS FROM THE Y-CHROMOSOME**

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Miranda do Douro municipality, located at the North-Eastern portion of Portugal, bears an interesting linguistic event. In addition to the Portuguese, its habitants speak a particular linguistic variant, known as Mirandês. However, this dialect is not a variant from the Portuguese language, but instead, belongs to the Astur-Leonese linguistic family. The persistence of Mirandês language in Miranda do Douro reflects historical relationships with the neighbouring Kingdom of Leon, nowadays part of Spain. In fact, the geographic location of Miranda do Douro, favoured interactions between populations living near the Portuguese-Spanish border, strengthening their affinities and likely influencing the genetic landscape of the municipality.

A random sample of 58 unrelated males from Miranda do Douro municipality was typed for 26 Y-chromosome bi-allelic and 17 microsatellite markers. Available data from surrounding populations was compiled, with the aim to investigate which populations may have contributed to the current male genetic pool of Miranda do Douro.

The obtained results showed that the population from Miranda do Douro presented a typical Western European genetic composition. In a microgeographic perspective, results revealed signs of some degree of admixture between three geographical-related Iberian populations: Portuguese, Leonese and communities of Crypto-Jewish origin. On one hand, the high prevalence of shared haplotypes, within the R1-M173 haplogroup, and the analysis of the genetic distances, corroborated the influence of some Portuguese and Leonese populations in the Miranda do Douro gene pool. On the other hand, the presence of J-12f2a and T-M70 haplogroups, found at high frequencies in Crypto-Jewish populations, demonstrated a probable contribution from these small communities that remain for a long period close to the Miranda municipality. Overall, Miranda do Douro shows up as a region prone to gene flow, having absorbed historic, linguistic and genetic intakes from its neighbor populations.

**(P79) THE SURVEY OF HEREDITARY ATAXIAS AND SPASTIC PARAPLEGIAS IN PORTUGAL: A POPULATION-BASED PREVALENCE STUDY**

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**Background:** Epidemiological data on the hereditary ataxias (HCA) and spastic paraplegias (HSP) are scarce. The precise clinical and genetic diagnosis of many affected families is uncertain. Our aim is to estimate the prevalence of HCA and HSP and describe some of the main clinical groups found in a Portuguese population-based survey.

**Methods:** This study covered all Portuguese regions, sequentially, throughout 1994-2004. Independent prevalence days were defined for each region. Multiple sources were used to identify patients, including clinical files search, active call for the collaboration of neurologists and geneticists, but relying mainly in the active survey of general practitioners. Neurologists from the research evaluated all the patients using defined inclusion criteria. The clinical and genetic investigation of the included families extended until 2010.

**Results:** During the study 368 health centres were contacted, 86.1% cooperated. From 2724 suspected patients, 1336 patients were diagnosed with HCA/HSP. The estimated overall prevalence was 12.9/105 for a population of 10,322 millions. The prevalence of HCA was 5.6/105 for dominant and 3.3 for recessive. The HSP are less prevalent, 2.4 for dominant and 1.6 for recessive. Friedreich ataxia and ataxia with oculomotor apraxia (AOA) were the most prevalent recessive HCA, 1.0/105 and 0.4/105 respectively. The most prevalent HSP forms were SPG4 and SPG3, 0.91/105 and 0.14/105 respectively. The genetic cause has not been identified in 39.6% of the patients.

**Conclusion:** This population-based study is probably the largest ever performed in the field of HCA and HSP. Several novel genes were discovered. New disease clusters and multiple undiagnosed families were identified, setting the basis for assistance, prevention and counselling programs.

**(P80) ANALYSIS OF 15 AUTOSOMAL STR LOCI IN THE POPULATION OF THE STATE OF ACRE, NORTHERN BRAZIL**

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The history of Acre's population is distinct from other regions of Brazil, since it was the last region to be inhabited by non-indigenous individuals. The "Acrianos" are mostly descendants of Northeasterners and indigenous groups who lived there. The aim of this research was to build a database specific to the population of Acre and to compare Acre with other populations.

The genetic characterization of each region of the state (Baixo Acre, Juruá, Purus, Tarauacá-Envira and Alto Acre) was performed by determining the frequencies for 15 autosomal STRs (short tandem repeats) contained in the amplification kit AmpF!STR® Identifier® Plus (Applied Biosystems) in a sample of 503 non-indigenous individuals.

Based on the allele frequencies, it was found that the population does not show significant deviations from the Hardy-Weinberg equilibrium. Comparing the regions of the state locus to locus, based on observed values of  $F_{st}$ , it was verified that there were no significant differences between them. The Nei's genetic distances calculated between Acre, the five regions of Brazil, several African and European countries and Mexico confirmed that Acre is integrated in the Brazilian context. In calculating the Nei's genetic distances between Acre and other states and countries bordering with Brazil, it showed that the populations of Bolivia and Peru are the only ones that show a larger distance. There was also no correlation between genetic and geographic distances between Acre and other Brazilian states. Relative to the comparison of this database with the other two used in forensic testing in Acre (from Brazil and from Amapá state), low values of Nei's distances for the 12 loci considered were found. This, together with the fact that the three databases have very similar values of genetic diversity, indicates that all are suitable for genetic testing involving individuals in this population, for the markers analyzed.

**(P81) ASSESSMENT OF GENETIC RISK FOR DYSLIPIDEMIA AND CARDIOVASCULAR DISEASES IN THE AZOREAN POPULATION**

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In recent years, large studies have unraveled several loci implicated in dyslipidemia, which is one of the most important risk factors for cardiovascular diseases (CVD). These diseases show a higher mortality rate in Azores when compared to mainland Portugal. In order to investigate this question, we characterized 19 SNPs in 4 genes associated with dyslipidemia: 4 on PCSK9 (rs11206510, rs11591147, rs562556, rs505151), 5 on USF1 (rs10908821, rs3737787, rs2516839, rs1556259, rs2774279), 6 on LDLR (rs6511720, rs2228671, rs688, rs5927, rs1433099, rs2738466) and 4 in APOE (rs405509, rs429358, rs7412, rs439401). Genotyping was performed by real time PCR using TaqMan assays, in a sample of 170 healthy blood donors. The results demonstrate that, although allele frequencies in Azoreans were similar to those reported for the HapMap CEU population, haplotype and genotype analysis revealed some differences important for assessing genetic risk. For example, haplotype analysis revealed that LDLR showed the highest number (19) of different haplotypes, being GCTGGA (2 risk alleles) the most frequent (29.4%). For PCSK9, the haplotype with 3 risk alleles – TGAG – showed a frequency of 2.9%. Based on the reported phenotypic associations and the OR values for populations of European ancestry, a risk profile analysis was performed. Three categories were defined: i) high risk for myocardial infarction (MI), ii) high risk for increased levels of plasma total cholesterol (TC), and iii) protective/low risk CVD. Interestingly, the data show that males have 2.3x higher risk than females for MI; however, females present a higher risk for increased levels of TC. Moreover, the data suggest that Azoreans from western group are ~2x more protected to dyslipidemia and CVD when compared to the other two groups. Taken together, the results constitute a valuable resource for future CVD case control studies in Azoreans, as well as for developing strategies to CVD prevention, health promotion and population education.

**(P82) THE RELATIONSHIP BETWEEN TASTE-RELATED POLYMORPHISMS AND LIFESTYLE: A STUDY IN AFRICAN POPULATIONS**

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Human evolution was characterized by significant dietary changes believed to have represented selective pressures driving genetic changes. The most recent dietary shift occurred 10,000 years ago, when humans began to abandon hunting and gathering in favour of agriculturalist and pastoralist lifestyles. The alterations in food behaviours possibly entailed modifications in biological processes, mainly in food metabolism and taste sensitivity. Concerning taste perception, five taste qualities are widely recognized: bitter, sweet, salty, sour and umami. Variability in taste sensibility is in part genetically determined.

To obtain insights on the relationship between diet and the distribution of taste-related genetic variations, African populations with distinct lifestyles, agriculture (Angola, Mozambique and Equatorial Guinea) and pastoralism (Uganda), and a control sample from Portugal were characterized for eleven SNPs located in genes TAS1R1 and TAS1R3, involved in the sensitivity to umami and sweet, and TAS2R16 and TAS2R38, implied in bitter perception. For the genotyping, two PCR and Single Base Extension Multiplexes containing the eleven SNPs were optimized.

Concerning the variation G516T at TAS2R16, a remarkable parallelism emerged in Africa between its frequency distribution and the Bantu expansion. For this variation as well as for the sweet-related SNPs at the TAS1R3, a clear differentiation between African and non-African populations was found. AMOVA results further showed that geography plays a main role in the patterns of genetic diversity of the tested variations. Despite that, geography did not seem enough to explain the worldwide distributions of all of them. Especially at TAS1R1 and TAS1R3, some signs suggest that they have been under selective forces, although their nature and strength is still uncertain. In this study, no clear association emerged between lifestyle and taste-related polymorphisms, but the scarcity of data to address the issue does not allow excluding lifestyle as a factor contributing to shape diversity at the studied variations.



**(P83) INSIGHTS INTO SOUTH-AMERICAN COLONIZATION THROUGH MTDNA ANALYSIS IN NATIVE COLOMBIAN POPULATIONS**

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Aiming to add some clues on the colonization of the American continent, more precisely the entrance points and dispersion routes taken, studies with lineage DNA markers such as mitochondrial DNA (mtDNA) and Y chromosome have been performed, which allow tracing back the history of populations because they are transmitted without recombination to the descendants.

In the present study we determined the matrilineal ancestry of samples from two regions in Colombia through mtDNA analysis. Based on the observation of Native American haplogroups in both populations, we also intended to perceive if there are differences that could indicate different migrations towards the South of the continent.

The complete mtDNA control region was sequenced for 98 samples from the two groups (38 Emberá from Antioquia and 60 samples from various ethnic groups from Cauca) and compared with the revised Cambridge Reference Sequence. Haplogroup frequencies were calculated and phylogenetic analyses were performed.

The vast majority of haplogroups found in both Colombian populations are typically Native American. Our results show that while in the Antioquia region, the Emberá population presents a very reduced number of haplotypes, all belonging to haplogroups A, B and D, the Cauca region is more diverse and has a significant percentage of C haplogroup lineages. When dividing the Cauca group into smaller speaking groups it is visible that they are obviously distinct and behave as small populations that have suffered evolutionary forces along time such as genetic drift and bottlenecks. When comparing with other populations from literature, there is a notable proximity between Chibchan speaking groups, whereas non-Chibchan remain differentiated. Regarding a geographic separation, there is no visible substructure. Instead, distinct patterns are visible both in northern and southern populations within Colombia which may result from distinct ancient routes.

**(P84) THE NEW EUROPEAN STANDARD SET OF STR MARKERS IN THE PORTUGUESE POPULATION AND A COMPARATIVE PERFORMANCE OF TWO NEXT GENERATION MULTIPLEX SYSTEMS**

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Standardisation of DNA profiling throughout Europe was proposed by European Working Groups (ENFSI and EDNAP), since differences exist between countries in their choice of loci for national forensic DNA databases. Thus, apart from the previously established seven STRs, a new European Standard Set (ESS) of five additional loci (D1S1656, D2S441, D10S1248, D12S391 and D22S1045) was proposed. In this way, new multiplex STR systems, like ESSplex Plus (Qiagen) and NGM kit (Applied Biosystems), were developed, fulfilling the European instructions and needs for improved sensitivity and robustness, also for degraded DNA analysis. In this work, these kits were used to characterize the five new markers in a Portuguese sample and an evaluation of their performance was also carried out.

A total of 246 unrelated donors living in Portugal were sampled. DNA was extracted and amplified by PCR with ESSplex Plus and NGM kits. Each of the five new markers was characterized in terms of allele frequency estimation, Hardy-Weinberg equilibrium, parameters of forensic interest, as well as a segregation analysis. Moreover, genotype concordance was evaluated since ESSplex Plus and NGM share the same loci. Performance was compared in terms of sensitivity and in situations of degraded DNA analysis. Regarding sensitivity, serial dilutions from different sources of samples were made to obtain quantities from 500pg to 5pg per reaction. For degraded DNA analysis, tests were conducted on artificially (UV-light) degraded DNA samples.

For the five new loci, no deviations from Hardy-Weinberg equilibrium were observed and no significant allele frequency differences were detected comparing our sample with other European samples, available in the literature. Also, no genotyping inconsistencies were observed between both multiplex kits. Concerning performance, ESSplex Plus kit revealed to be slightly more sensitive when compared to NGM and also demonstrated a higher capability in obtaining genetic information from degraded DNA samples.

**(P85) Y-CHROMOSOME LINEAGES IN CENTRAL PORTUGAL: SURNAMES THAT MEAN TREES AND JEWISH ANCESTRY**

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Introduction: It is commonly said that in Portugal surnames that mean trees are an evidence of Jewish ancestry. If this assumption is correct a higher than expected frequency of Y-haplogroups associated to Jewish communities will be found in men carrying these specific surnames in comparison with men without this type of surname.

The objective of this study was to test whether it would be possible to discriminate between two population samples of Portuguese men with and without surnames derived from tree names, using Y-haplogroups.

Methods: The most common haplogroups described in the Portuguese population were assessed in a total of 116 subjects from Central Portugal: 53 men carrying surnames meaning trees and 63 without this type of surname. Fourteen biallelic markers were studied by PCR-RFLP (M9, M269, M304, M35, M78, M81, M123, M410, M12, M70) or sequencing (M267, M170, M201, M22), using primers previously described.

Results and Discussion: The typed SNPs allowed identifying 12 different Y-haplogroups. The typical Western European haplogroup R1b1b2-M269 was the most common in the two analysed sub-populations, reaching a 0.604 frequency among the surnames derived from trees, and 0.635 in the reference sample. When a pairwise differentiation test based on haplogroup frequencies were computed between the two sub-populations the difference is not statically significant ( $F_{ST}$  p-value =0.729). Taking J-M304 lineage as a putative signal of Jewish ancestry, only two chromosomes (0.038 frequency) were found in the sub-population of Portuguese men carrying surnames that mean trees (0.143 frequency in the general population). In conclusion, this genetic study of surnames in Central Portugal does not corroborate a Jewish descent for men carrying surnames that mean trees, which is in concordance with most historians believing that this hypothesis is just a legend regarding Portuguese Jews when they were forced to choose between conversion or expulsion in 1497.

**(P86) GEOGRAPHIC DISTRIBUTION OF THE LACTASE PERSISTENCE -13910C>T POLYMORPHISM ACROSS PORTUGAL**

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Introduction: Lactase persistence (LP) during adulthood is a common autosomal-dominant trait in Europe, which shows a tendency to increase from the southeast (~50%) to the northwest (80%–95%). Among individuals of European descent, the single nucleotide polymorphism (SNP) -13910C>T (rs4988235), located ~14 kb upstream of the lactase (LCT) gene, has been associated with the lactase enzyme activity. The frequency of the persistence -13910\*T allele do vary across Europe, being 70–80% in North and 5–10% in South, in broad agreement with that expected from distribution of the LP phenotype. However, within countries such gradients for the LP polymorphism have not yet been studied extensively. The aim of this study was to assess the allelic distribution of the -13910C>T polymorphism into the three main macro regions of continental Portugal.

Methods: A total of 199 Portuguese subjects of European descent, were sampled in different locations clustered into Northern (above Douro river; n=64), Central (between Douro and Tagus rivers; n=70) and Southern (below Tagus river; n=65) Portugal. The -13910C>T polymorphism was genotyped with TaqMan probes by real-time PCR.

Results and Discussion: We found an overall frequency of 0.349 for the -13910\*T allele, but with a substantial difference in frequency between North/Centre and South: the -13910\*T allele ranges similar frequencies of 0.383 and 0.393 in Northern and Central Portugal, respectively, whereas it reaches values of 0.269 in the Southern region. These differences between North/Centre and South regions are close to being statistically significant in population differentiation tests: exact p-value of 0.05 and 0.06 for North vs. South and Centre vs. South, respectively. This finding is in general agreement with the overall tendency for a North-South decreasing gradient of the -13910\*T frequency in Europe. In conclusion, our study suggests the existence of apopulation stratification in Portugal concerning the -13910C>T polymorphism.

**(P87) NOVEL GENE FUSIONS IDENTIFIED IN PROSTATE ADENOCARCINOMA**

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**Introduction:** Rearrangement of TMPRSS2 with ERG has been recurrently found in around 50% of prostate adenocarcinomas (PCa). Other members of the ETS family of transcription factors, namely, ETV1, ETV4, ETV5 and more recently FLI1, have also been found to be involved in gene fusions in a smaller proportion of PCa.

The prostate-specific androgen regulated gene TMPRSS2 is the main 5' fusion partner in the majority of PCa with ERG rearrangement, and has also been found fused to other ETS genes. In the present study we have focused on the characterization of PCa samples with confirmed ETS rearrangement in order to unveil the 5' fusion partner genes involved.

**Methods:** We studied a set of 18 prostate tumor samples with extreme outlier overexpression of ETV1 (n=14), ETV4 (n=3) and ETV5 (n=1), subsequently validated for genomic rearrangement of the overexpressed ETS by FISH. These samples were selected from a cohort of 200 patients with clinically localized PCa consecutively diagnosed and treated with radical prostatectomy that were previously typed for ETS rearrangements. The presence of the most common 5' fusion partners was assessed by RT-PCR. Additionally, we performed 5' Rapid Amplification of cDNA Ends followed by subsequent sequencing of the cloned PCR products to unveil the 5' fusion partners.

**Results:** RT-PCR confirmed the presence of TMPRSS2-ETV1 in three out of 14 PCa with ETV1 rearrangement. 5'RACE allowed us to identify two novel 5' fusion partners (PSGR and UBTF) of ETV1 and ETV4, respectively, as well as two novel gene fusion combinations between two previously described genes (SLC45A3-ETV4; HERVK17-ETV4). This approach also allowed us to identify previously described fusion transcripts (C15orf21-ETV1 and EST14-ETV1).

**Discussion:** This study is an important contribution to the characterization of gene fusions in PCa. We describe a prostate-specific androgen-regulated gene (PSGR) as a ubiquitously expressed gene (UBTF) as two novel 5' fusion partners in PCa. Additionally we describe novel gene fusion combinations between genes known to be involved in PCa gene fusions

## **(P88) PARTIAL TRISOMY 1q IN A CASE WITH MYELOYDYSPLASTIC SYNDROME**

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**Introduction:** The myelodysplastic syndromes (MDS) are clonal hematopoietic disorders characterized by cytopenia and bone marrow dysplasia. This syndrome is characterized by profound heterogeneity in morphologic presentation, clinical course and cytogenetic features. Approximately 50% of patients display clonal chromosome abnormalities like del5q, del20q and trisomy8.

**Materials and Methods:** The authors present a case of an 85-year old man with MDS. Bone marrow cell cultures and GTL banding were performed according to optimized protocols. Cytogenetic analysis followed the standard guidelines. Fluorescence in situ hybridization technique (FISH) was made to clarify the rearrangement detected

**Results:** It were analyzed 22 metaphases and eleven presented an der(16) from an unbalanced translocation between chromosomes 1 and 16 resulting a trisomy 1q21-qter. The result was confirmed by FISH technique.

**Discussion and Conclusions:** Partial trisomy 1q is a rare event, just 29 cases reported, and usually detected as a secondary chromosomal abnormality in a complex karyotype. There are only 3 cases and the present one, with partial trisomy 1q as a sole abnormality.

This case reveals the importance of cytogenetic study since confirms the diagnosis and offers critical information regarding to the prognosis and therapy to apply

**(P89) THE VALUE OF A PANEL OF DNA METHYLATION BIOMARKERS IN DETECTING UPPER URINARY TRACT UROTHELIAL CELL CARCINOMA**

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**Introduction:** Upper urinary tract urothelial cell carcinoma (UUT-UCC) accounts for about 5% to 10% of all urothelial tumors, and is more prevalent in adult males, peaking in incidence in the seventh decade. UUT-UCC are biologically and histologically similar to those of the bladder but more prone to muscle invasion, entailing a worse prognosis. We have previously identified a panel of epigenetic biomarkers (GDF15, TMEFF2 and VIM), that accurately identifies bladder cancer in urine. Herein, we aimed to evaluate the performance of this methylated gene panel for UUT-UCC detection in tissue and urine samples.

**Methods:** Methylation levels of the reference and target genes were assessed in phenol chloroform extracted and bisulfite modified DNA of 50 paraffin-embedded tissues of UUT-UCC, 40 normal upper urinary tract mucosas (NUUTs), 22 urine samples from UUT-UCC suspects, and 22 urine samples from controls, using real-time quantitative MSP. Histopathological classification was performed by an experienced pathologist, blinded to molecular results, and relevant patient data were collected from clinical charts. Molecular analysis and clinicopathological data were compared using appropriate statistical tests.

**Results:** Methylation levels of GDF15, TMEFF2 and VIM were significantly higher in tumors than in NUUTs ( $P < 0.001$ ,  $P = 0.001$  and  $P < 0.001$ , respectively). Remarkably, the three-gene panel accurately identified UUT-UCC, with a sensitivity and specificity of 100% in tissue samples, and a sensitivity over 80% in urine samples. No significant correlations were apparent between gene methylation levels and any of the clinicopathological parameters.

**Discussion:** Similar to urinary bladder urothelial tumors, the combined assessment of GDF15, TMEFF2 and VIM promoter methylation accurately identifies UUT-UCC. Because UUT-UCC are difficult to detect clinically and imagiologically at an early stage, this gene panel might provide a useful tool for early detection of UUT-UCC in urine samples.

**(P89) SERRATED POLYPOSIS WITH FAMILY HISTORY OF POLYPS AND/OR COLORECTAL CANCER: A DISTINCT CLINICAL AND MOLECULAR ENTITY DIFFERING BETWEEN THE PROXIMAL AND THE DISTAL COLON**

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**Introduction:** Serrated polyposis (SPP) is characterized by the development of multiple colorectal serrated polyps and increased predisposition to colorectal cancer (CRC). We aimed to characterize at clinical and molecular level a cohort of SPP patients with or without family history of polyps (multiple or diagnosed at a young age) and/or CRC in first degree relatives (SPP-FHP/CRC).

**Methods:** We analyzed 62 serrated or adenomatous lesions from 11 SPP-FHP/CRC families and 6 sporadic SPP patients for microsatellite instability (MSI), hypermethylation of MGMT and mismatch repair (MMR) genes, and somatic mutations in WNT and RAS/RAF genes.

**Results:** SPP-FHP/CRC patients presented an older mean age at diagnosis ( $p=0.027$ ), a more heterogeneous histological pattern of lesions ( $p=0.032$ ) in comparison with sporadic SPP. Two forms of SPP-FHP/CRC appear to exist, according to the molecular alterations and to the preferential location of early lesions, proximal/whole colon and distal. Notably, MMR gene methylation was detected exclusively in the former [10/29 (34%) vs 0/18,  $p=0.0039$ ]. Proximal/whole colon SPP-FHP/CRC presented also a higher frequency of MSI and WNT mutations [15/26 (58%) vs 2/15 (13%),  $p=0.006$ ; 14/26 (54%) vs 4/20 (20%),  $p=0.02$ , respectively] but a lower frequency of BRAF mutations [12/20 (60%) vs 7/30 (23%),  $p=0.009$ ], when compared with the distal form. Two groups of patients were identified in each form, whose lesions harboured preferentially KRAS or BRAF mutations, respectively. CRC was more frequent in proximal/whole colon SPP following a KRAS (alternate) pathway [4/4 vs 1/8 (12%),  $p=0.01$ ].

**Conclusions:** We conclude that SPP-FHP/CRC appears to be a distinct clinical and molecular entity, presenting two different forms, proximal/whole colon and distal, the former with an early MMR deficiency. CRC risk appears to be higher in proximal/whole colon SPP-FHP/CRC following an alternate (KRAS) pathway.



**(P90) IDENTIFICATION OF PREVIOUSLY UNRECOGNIZED FAMILIAL ADENOMATOUS POLYPOSIS IN CHILDREN WITH DESMOID TUMORS**

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**Introduction:** Desmoid tumors, may occur sporadically or as result of inherited predisposition (as part of familial adenomatous polyposis - FAP) and often present B-catenin overexpression, which may result from activating somatic mutations of the CTNNB1 gene (encoding the B-catenin) or from biallelic inactivation of the APC gene.

**Material and methods:** We analyzed two patients under one year of age with Gardner fibroma by chromosome banding analyses, comparative genomic hybridization (CGH), fluorescence in situ hybridization (FISH), germline and somatic APC mutation analyses, immunohistochemistry for B-catenin and somatic CTNNB1 exon 3 mutation analyses.

**Results:** Chromosome banding analysis of the tumor of the first infant revealed the karyotype 46,XY,del(5)(q11q35),der(11)(11pter->11q14::?:11q23->11qter)[16]/46,XY[4], and loss of one copy of the APC gene was confirmed by FISH. APC germline mutation analysis revealed the c.4687dup (p.Leu1563Profs\*4) mutation.

The second infant presented the c.5826\_5829del (p.Asp1942Glufs\*27) APC germline mutation, but the tumor revealed no copy number alterations in APC by CGH or FISH. We searched for somatic APC mutations in the tumor and the c.1678A>T (p.Lys560\*) mutation was found.

Both tumors showed nuclear staining for B-catenin with absence of nuclear expression of B-catenin in normal cells, and sequence analysis of CTNNB1 exon 3 revealed no somatic mutations.

**Discussion:** This work allowed us to suggest the following diagnostic strategy of FAP-associated pediatric desmoids tumors (including Gardner fibromas): tumors should first be tested for B-catenin expression by immunohistochemistry and, if nuclear expression is detected, somatic mutation analysis of CTNNB1 exon 3 should be performed. If no mutations in CTNNB1 are found, genetic counseling and germline APC mutation analysis should be offered. Irrespective of whether or not a genetic diagnosis of FAP is confirmed, somatic APC copy number losses or point mutations may be investigated in order to fully identify the pathogenetic mechanism of a given desmoid tumor.

**(P91) *POU1F1* IS A NOVEL FUSION PARTNER OF *NUP98* IN ACUTE MYELOID LEUKEMIA WITH t(3;11)(p11;p15)**

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**Introduction:** NUP98 gene rearrangements have been reported in acute myeloid leukemia and are thought to be associated with poor prognosis. More than 20 different partner genes have so far been identified, giving rise to fusion proteins that seem to function as aberrant transcription factors.

**Methods:** A 57-year-old female was diagnosed with therapy-related AML. The bone marrow sample was cultured for 24 hours and chromosome banding analysis of the bone marrow was performed after Leichmann's staining. Dual color, dual fusion FISH analysis using BAC generated probes was performed on chromosomal metaphases. Molecular genetic analysis for the detection of fusion transcripts was done by RT-PCR and the genomic breakpoints were cloned by PCR followed by sequencing analysis.

**Results:** The bone marrow karyotype presented a t(3;11)(p11;p15) as the only cytogenetic abnormality. FISH and molecular genetics analyses identified a class 1 homeobox gene, POU1F1, located on chromosome 3p11, as the fusion partner of NUP98. Additionally, a FLT3-ITD mutation was identified in the bone marrow sample.

**Discussion:** We have identified POU1F1 as the NUP98 fusion partner in therapy-related AML with a t(3;11)(p11;p15). In addition, we have found that the patient harbored an FLT3-ITD mutation, which most likely collaborated with the NUP98-POU1F1 fusion gene in malignant transformation. This is the first POU family member identified as a fusion partner in leukemia.

**(P92) ORAL CANCER: TRANSLATING MOLECULAR GENETIC DATA INTO CLINICAL PRACTICE - THE CONTRIBUTION OF ARRAY-CGH**

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**Introduction:** Oral cancer is a worldwide serious problem of health. For this type of tumors, alterations in almost all chromosomes has been reported, nevertheless there are some chromosomal regions described in the literature as consistently altered. However, only a few genes associated with oral cancer have been identified. Additionally, the knowledge regarding the association between the genotype, the behavior and the progression of these tumors is very scarce. In light of the above, the main goal of this study was the characterization of the genomic profile of oral tumors from patients with oral cancer diagnosis through the application of whole genome array-Comparative Genomic Hybridization (aCGH).

**Methods:** Biopsies of tumor were acquired from 8 patients. Healthy donors were used as controls. The aCGH was performed using an Agilent oligonucleotide microarray 4x180K.

**Results:** With this whole genome approach we detected imbalances in all chromosomes. We identified 1409 genes with gain and 8815 genes with loss. Some of these genes are already associated with oral cancer and others play an important role in tumorigenesis development.

**Discussion:** With this approach we identified the most prevalent chromosomal regions previously reported in literature as altered in oral cancer. Additionally, we also identified imbalances in other chromosomal regions that might contain important genes related to oral cancer initiation and progression. This study also highlights some putative new biomarkers with possible diagnostic and prognostic value. In order to validate these putative biomarkers further studies are needed.

**(P93) SOMATIC MUTATIONS AND DELETIONS OF THE E-CADHERIN GENE PREDICT POOR SURVIVAL OF GASTRIC CANCER PATIENTS**

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**Introduction:** The prognosis of gastric cancer (GC) is poor and the molecular pathogenesis players vastly unknown. Surgery remains the primary option in GC treatment. The aim of this study was to investigate the impact of somatic CDH1 alterations in prognosis and survival of GC patients.

**Methods:** A series of sporadic and familial GC cases (diffuse and intestinal) (n=246) were analyzed for somatic CDH1 mutations, promoter hypermethylation and loss of heterozygosity (LOH) by PCR-Sequencing. E-cadherin protein expression was determined by immunohistochemistry. Associations between molecular, clinicopathological and survival data were analyzed.

**Results:** CDH1 somatic alterations were found in ~30% of all GC cases. Both histological types of sporadic GC displayed: LOH in 7.5%, mutations in 1.7% and hypermethylation in 18.4% of the cases. Primary tumors from Hereditary Diffuse Gastric Cancer (HDGC), lacking germline CDH1 mutations, showed exclusively CDH1 promoter hypermethylation in 50% of the cases. Familial Intestinal Gastric Cancer (FIGC) tumors showed LOH in 9.4% and hypermethylation in 17.0%. CDH1 alterations did not associate with a particular pattern of E-cadherin expression. Importantly, the worst patient survival rate among all GC analyzed was seen in patients with tumors carrying CDH1 structural alterations, preferentially those belonging to FIGC families.

**Discussion:** CDH1 somatic alterations exist in all clinical settings and histotypes of GC and associate with different survival rates. Their screening at GC diagnosis may predict patient prognosis and is likely to improve GC patient management.

**(P94) GENOMIC CHARACTERIZATION OF TWO LARGE ALU-MEDIATED REARRANGEMENTS OF THE BRCA1 GENE**

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**Introduction:** In order to determine whether a large genomic rearrangement is actually novel and to gain insight about the mutational mechanism responsible for its occurrence, molecular characterization with breakpoint identification is mandatory.

**Methods:** We here characterize two large deletions involving the BRCA1 gene by Multiplex Ligation Probe Amplification, semi-quantitative multiplex PCR and long distance PCR.

**Results:** The first rearrangement harbored a 89 664 bp deletion comprising exon 7 of the BRCA1 gene to exon 11 of the NBR1 gene (c.441+1724\_oNBR1:c.1073+480del). The second harbored a 23 363 bp deletion encompassing BRCA1 exons 11-15 with an Alu element inserted at the breakpoints of the deleted region (c.671-319\_4677-578delinsAlu).

**Discussion:** Regarding the deletion of exon 7 of BRCA1 to exon 11 of NBR1 gene, two highly homologous Alu elements were found in the genomic sequences flanking the deletion breakpoints. Furthermore, a 20-bp overlapping sequence at the breakpoint junction was observed, suggesting that the most likely mechanism for the occurrence of this rearrangement was nonallelic homologous recombination. Concerning the BRCA1 exons 11-15 deletion, the observed rearrangement could be due to an insertion-mediated deletion mechanism caused by Alu retrotransposition, since the Alu element inserted belongs to a still active AluY family. To conclude, we describe the breakpoints of two novel large deletions involving the BRCA1 gene and analysis of their genomic context allowed us to gain insight about the respective mutational mechanism.

**(P95) VALIDAÇÃO DA TÉCNICA DE PCR EM TEMPO REAL NA DETECÇÃO DO REARRANJO PML-RAR $\alpha$  DA LEUCEMIA AGUDA PROMIELOCÍTICA**

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**Introdução:** A Leucemia Aguda Promielocítica está relacionada em 90% dos casos com uma alteração genética, a translocação recíproca balanceada t(15; 17) (q22; q21) resultando no oncogene híbrido PML-RAR $\alpha$  responsável pela expressão da proteína anormal denominada PML-RAR $\alpha$ . Este marcador molecular é útil no diagnóstico e para acompanhamento da evolução da doença - estudo da doença residual mínima (DRM). Para tanto, é fundamental que as técnicas de análise molecular sejam submetidas à validação, sobretudo os métodos *in house*. Este estudo teve por objetivo validar a técnica molecular da PCR em Tempo Real (RQ-PCR) *in house* para a detecção do rearranjo PML-RAR $\alpha$ .

**Material e Métodos:** o sistema TaqMan® foi utilizado para RQ-PCR. A eficiência do ensaio foi medida através da curva padrão com kit comercial. Foram analisadas acurácia e precisão da técnica RQ-PCR através de experimento de diluição seriada utilizando células NB4 por comparação interlaboratorial. A análise da sensibilidade entre as técnicas RQ-PCR e RT-PCR (Transcrição Reversa seguida da Reação em Cadeia de Polimerase) foi realizada por ensaio intralaboratorial.

**Resultados e Discussão:** A sensibilidade alcançada pela técnica de RQ-PCR variou de 10<sup>-3</sup> a 10<sup>-4</sup> e para a RT-PCR foi de 10<sup>-2</sup>, mostrando assim que para a detecção do rearranjo PML-RAR $\alpha$  a técnica RQ-PCR foi mais sensível. O ensaio de RQ-PCR apresentou alta especificidade e repetibilidade. A reprodutibilidade apresentou variação de um log na análise interlaboratorial em relação à sensibilidade do teste. Para a RT-PCR a medula óssea alcançou maior sensibilidade (10<sup>-5</sup>) quando comparada com o sangue periférico (10<sup>-2</sup>). Em relação ao tipo de transcrito a sensibilidade do bcr1 foi de 10<sup>-3</sup> e do bcr3 variou de 10<sup>-2</sup> a 10<sup>-3</sup>. Este estudo permitiu determinar a acurácia e a precisão das técnicas RQ-PCR e RT-PCR na detecção dos transcritos PML-RAR $\alpha$  para pacientes com LAP, possibilitando estabelecer um ensaio confiável para diagnóstico e estudo da DRM.

**(P96) 5' UNTRANSLATED REGION OF THE MAMMALIAN TARGET OF RAPAMYCIN MRNA HARBORS AN INTERNAL RIBOSOME ENTRY SITE**

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Introduction: mammalian Target of Rapamycin (mTOR) is a highly conserved kinase that is responsive to several cellular stimuli. 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. According to its particular role in controlling general mRNA translation, it is expected that mTOR expression itself is tightly regulated. In stress conditions such as hypoxia and mitosis, overall protein production is reduced with the exception of mTOR and some other stress-responsive proteins. The presence of internal ribosome entry site (IRES) in some transcripts allows mRNA translation without the requirement of some translation initiation factors that tend to be inactivated in stress conditions. 5' Untranslated regions (5'UTRs) with IRESs usually have high GC content, pyrimidine-rich tracts, Y-shaped and stable hairpins, features that are present at the mTOR 5'UTR. Therefore, we hypothesized that mTOR 5'UTR harbors an IRES allowing cap-independent synthesis of mTOR protein in stress conditions.

Methodology: In order to test if mTOR 5'UTR displays IRES activity, Hek293T and Hela cells were transfected with a dicistronic reporter plasmid carrying the mTOR 5'UTR upstream of the second cistron. To study the mTOR IRES activity in colorectal cancer (CRC), NCM460 (normal intestinal mucosa), Sw480 (stage IV Adenocarcinoma) and HCT116 (metastatic cell line) were used.

Results and Discussion: mTOR 5'UTR displays IRES activity. In Hek293T cells, IRES activity of mTOR is 2.5 and 1.5 fold higher than the observed in the positive controls, c-myc and EMCV IRESs, respectively. In Hela cells, the IRES activity of mTOR, c-myc and EMCV is similar (3 fold the empty counterpart). Since mTOR signaling is hyper-activated in primary CRC tumors and RNAi-mediated knockdown of mTOR diminish tumor growth in vitro and in vivo, we tested if CRC malignization is accompanied with changes at mTOR IRES activity. We have observed that in NCM460, in Sw480 cells and in HCT116 cells mTOR show similar IRES activities. This suggests that mTOR IRES activity is constant during CRC malignization.

**(P97) POSSIBLE ASSOCIATION BETWEEN TGF-B1 POLYMORPHISM AND ORAL CANCER**

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**Introduction:** Oral squamous cell carcinoma (OSCC) is a world-wide health problem because is the most prevalent tumor among head and neck cancer and is a great cause of cancer morbidity and mortality. Growth factors are involved in tumorigenesis in many different tissues. In this context, transforming growth factor B1 (TGF-B1) is involved in the regulation of numerous immunomodulatory processes and works as a multi-functional cytokine. **Introduction:** The development of oral squamous cell carcinoma (OSCC) is a multistep process. TGF-B1 is involved in the regulation of numerous immunomodulatory processes and works as a multi-functional cytokine. **Objectives:** The aim of this study was to investigate the possible association between the TGF-B T869C polymorphism and oral cancer.

**Methods:** Genomic DNA from 62 male smoker patients diagnosed for oral squamous cell carcinoma and 62 smokers without cancer blood donors were analyzed for TGF-B polymorphism by polymerase chain reaction (PCR).

**Results:** The C allele was significantly more prevalent in the oral cancer group than in the controls and allele C carriers presented an estimated 2.73-fold greater relative risk of develop cancer when compared with allele C noncarriers (OR = 2.73, 95% CI = 1.19 to 6.28). Although the T allele was not statistically significant among controls, when consider the genotypic analysis, the TT homozygous genotype showed protector for oral cavity cancer (OR = 0.37, 95% CI = 0.16 to 0.84).

**Conclusion:** Assuming that the variation TGF-B1 869 T/C affects secretion of the protein, we hypothesized that the C allele could increase secretion of TGF-B secretion which suppresses antitumor immune responses and may alter the OSCC risk. Further studies are needed to characterize the molecular mechanisms by which TGF-B1 is involved in susceptibility to oral neoplasm and to confirm the true role of the T and C TGF-B alleles in the carcinogenesis



**(P98) PROGRESSIVE POSITIVE SELECTION OF NORMAL CELLS IN PERIPHERAL BLOOD LYMPHOCYTES FROM A FA PATIENT AFTER BMT PRESENTED WITH CHIMERISM**

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Introduction: Fanconi anemia (FA) is a rare recessive disorder characterized by chromosome instability (CI), several congenital malformations and increased predisposition to cancer. Clinically and genetically is a highly heterogeneous disease, therefore, the diagnosis is performed using a cellular hallmark that is common to all patients, the hypersensitivity to the clastogenic effect of DNA cross-linking agents, in particular to diepoxybutane (DEB).

Hematological abnormalities are the most important features during evolution of the disease. They usually result in early cell death, with a higher risk of aplastic anemia, myelodysplastic syndrome and acute myeloid leukemia. The only long-term cure for the blood defects is bone marrow transplant (BMT). One of the problems related with BMT can be the presence of chimerism, i.e., the presence of a significant number of FA cells inside the expected donor population.

The aim of this work is to perform a case study of the chimerism evolution in a male FA patient, after BMT from a female related donor.

Methods: A total of 5 DEB induced lymphocytes cultures were performed, one before BMT, and five after BMT (3, 6, 12, and 18 months after BMT). Cultured lymphocytes were evaluated, according to the CI parameters. A chimerism evaluation was performed, sustained on the identification of the sexual chromosomes.

Results and Discussion: Our results clearly show that, after the presentation of a 46% chimerism, there is a progressive positive selection of cells from the donor, and this selection is proportional to the time elapsed after BMT. These results are supported by the progressive decrease of CI in the lymphocytes and disappearance of the chimerism. This study suggests that the presence of chimerism, after BMT, in the peripheral blood of FA patients may not be a long term problem, once a positive selection of normal cells occurs along the time.

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