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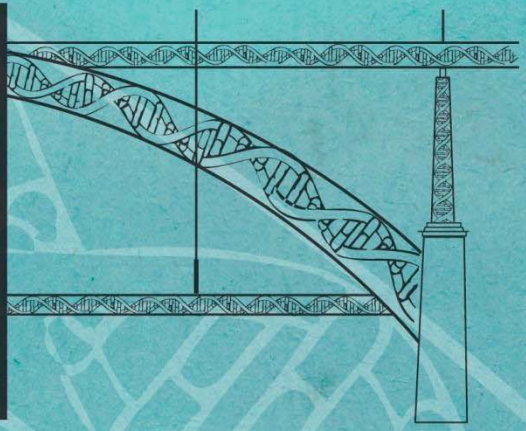
Resumos das Comunicações

XLIII Conferências de Genética
Doutor Jacinto Magalhães

28 MARÇO DE 2014

"A GENÉTICA DO X"

CGM
Centro de Genética Médica
Doutor Jacinto Magalhães



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Resumo das Comunicações

“A Genética do X” - Porto, 28 Março 2014

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NASCER E CRESCER
revista de pediatria do centro hospitalar do porto
28 de março de 2014, suplemento I

Invited speakers
Comunicações por convite

CC-01
X-CHROMOSOME: GENETIC FRAMING

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In order to discuss such a vast subject as the "genetic framing of the X-chromosome in humans" it is mandatory to approach several topics, starting with the time-frame of mammalian evolution. For many years little importance was attributed to the understanding of the evolution (divergence) of the dimorphic X and Y chromosomes, sex determination in heterogametic XY species, in species without sex chromosomes and the accurate characterization of Y and X chromosomes *per se*.

However, with the advent of cytogenetics and molecular techniques, great advances have been achieved in the knowledge of X-inactivation and gene dosage compensation in order to equalize the gene dosage between the sexes and, possibly, also between sex chromosomes and autosomes. In a relatively short time the molecular mechanisms involved in X-inactivation as an epigenetic process have been elucidated and have enabled the scientific and medical improvement of X-linked conditions, wether dominant or recessive. These achievements were followed by other scientific advances that are now routine diagnostic tools: study of the gene *XIST* and the chromosome distribution of 158 IQ-related genes, the effect of sex chromosome gene dosage on brain structure, the genetic defects and genetic-environmental interactions associated with Alzheimer's disease, fragile-X syndrome, X-linked genes and miRNA sexual dimorphism, hemophilia, Duchenne muscular dystrophy, Turner and Klinefelter syndromes.

CC-02
X-IMBALANCES BIG AND SMALL

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The X chromosome is fascinating, but the clinical interpretation of X-chromosomal aberrations are often a challenge, in particular because of (possible) mosaicism and X-inactivation.

Various abnormalities involving X will be addressed in this lecture, including whole chromosome numerical abnormalities, supernumerary marker chromosomes, X-autosome translocations as well as recurrent and non-recurrent copy number variations. For many indications, molecular techniques such as QF-PCR and genome wide array analysis are nowadays often used to test the patient samples in prenatal and postnatal genome diagnostics. For the correct interpretation of these data, however, cytogenetic knowledge is necessary and often routine cytogenetic analysis and /or Fluorescence In Situ Hybridisation (FISH) is required to further characterise the X-chromosomal abnormality.

After doing the tests, it is crucial for the clinical laboratory geneticist to not only correctly use the existing nomenclature in the test report, but also to include a clear and concise explanation of what the test result means. The requesting clinician needs to understand the meaning of the laboratory findings and the underlying genetic mechanisms in order to be able to properly counsel the patient and the parents with regard to prognosis and recurrence risk.

A variety of illustrative case examples will be presented to address the aforementioned aspects, but most likely, some questions will remain unanswered.

CC-03

UNRAVELLING THE X FILES: CHALLENGES AND DILEMMAS

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Array-Comparative Genomic Hybridization (array-CGH) has increased the diagnostic yield in patients with intellectual disability (ID), autism spectrum disorders and multiple congenital anomalies due to its improved resolution. X-chromosome has been focus of attention due to the bias in the affected male-to-female ratio and to the knowledge of X-linked genes associated with ID. With array-CGH we can either detect single gene imbalances, chromosomal region imbalances and even aneuploidies.

In a cohort of 1000 patients studied by Agilent 180K oligonucleotide array-CGH several X-chromosome imbalances were detected. Single gene deletions involving ZNF41 or IL1RAPL1 genes were equitably observed in 8 patients; DMD imbalances in 3 females and SHOX gene duplications in 1 female and 9 males. An intragenic deletion in SLC9A6 gene associated with Christianson syndrome that segregated in the family was also detected.

In 6 patients we identified Xp22.31 duplications, 3 females, 1 male with maternal inheritance and 2 males whose inheritance was not yet determined. A chromosome Xq27.1q28 interstitial duplications in 2 males, 1 maternally inherited and the other not yet determined were also identified. We also found other genomic imbalances but in single cases as for example a complex rearrangement with multiple imbalances at Xp22.33p22.2 in a male patient, maternally inherited; an Xp11.3p11.23 duplication in a female with ID whose mother is also affected and a case of triple X in an autistic female.

The challenge with X-chromosome imbalances is to, understand the biological mechanism(s) behind, interpret their impact on the phenotype, due to the presence of some alterations in the normal population and to X-chromosome inactivation in females. Clinical laboratory reporting has to use the correct nomenclature and a clear and objective interpretation of the results.

CC-04

AN INTRODUCTION TO X-LINKED IMDS

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While individually the inherited metabolic diseases are rare or very rare, overall the incidence is around 1:1,400 live births and accounts for about 15% of all single gene disorders. The vast majority of these diseases are inherited in a recessive manner with 3 or 4 being dominant conditions. However, 14 are inherited in an X-linked fashion. By my estimation there are over 200 conditions to consider, most of which are not treatable. My presentation will focus on four diseases—Anderson-Fabry disease (FD); Mucopolysaccharidosis type II (MPS II); Ornithine Transcarbamylase deficiency (OTC); and X-Linked adrenoleucodystrophy (XALD)—that illustrate several points of interest.

FD is a multi-system disease caused by a deficiency of the lysosomal enzyme alpha galactosidase. Accumulation of the substrate globotriaosylceremide (GL3) leads to a sequence of symptoms over time starting with severe neuropathic pain in the peripheries and moving on to proteinuria renal failure, cardiac and cerebrovascular disease. Without treatment death occurs by the 4th or 5th decade. Fortunately, enzyme replacement therapy is available. The clinical and therapeutic aspects of the disease will be discussed as well as the issue of late onset disease and the fact that there is a very high incidence of symptoms in the so called female carriers.

MPS II, or Hunter syndrome, is another multisystem lysosomal storage disorder caused by a deficiency of iduronate-2-sulphatase. The main features are due to skeletal involvement and like FD there is enzyme replacement therapy. However, unlike FD it is exceptionally rare for female carriers to develop symptoms or signs of the disease.

OTC is the commonest of the urea cycle defects. The symptoms are related to the accumulation of ammonia and will be discussed. In most boys the disease presents in the neonatal period. Many do not survive and those that do are usually severely brain damaged and susceptible to destabilization throughout their lives, even with the dietary treatment currently available. Interestingly, as will be discussed, about 15% of females will develop symptoms and require lifelong treatment. One of the times of greatest risk is during pregnancy and delivery.

The presentation will also describe the various manifestations of XALD from the severe childhood presentations to the adrenal and neurological disease of the onset in older boys and young men.

In addition to the clinical aspects of the four diseases, information on diagnosis and genetic counselling implications will be discussed.

CC-05

DOENÇA HEREDITÁRIAS DO METABOLISMO: EXPERIÊNCIA CLÍNICA NA TERAPÊUTICA

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As doenças hereditárias do metabolismo (DHM) são entidades de natureza genética, causadas por mutações num ou vários genes codificantes para um determinado passo metabólico. A transmissão neste grupo de patologias pode ser mendeliana (recessiva, dominante ou ligada ao X) ou mitocondrial.

Perante a suspeita clínica de um erro inato do metabolismo, devemos considerar sempre em primeiro lugar as doenças tratáveis, uma vez que nestas, a instituição precoce de medidas terapêuticas pode alterar o prognóstico do doente.

As diversas formas de tratamento nas doenças metabólicas podem ser classificadas de acordo com o seu mecanismo de ação:

- Restrição do substrato (redução do substrato da via metabólica afetada),
- Correção da deficiência de produto,
- Diminuição da toxicidade metabólica,
- Estimulação da atividade enzimática residual,
- Tratamento enzimático de substituição,
- Transplante de órgãos ou células estaminais,

São consideradas as principais DHM ligadas ao X para as quais existe uma terapêutica específica, défice em ornitina carbamoil transferase, défice em piruvato desidrogenase, doença de Hunter e doença de Fabry, referindo a evolução dos doentes em tratamento.

CC-06

XX, DOENTE OU PORTADORA?

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A Unidade de Bioquímica Genética (UBG) é o laboratório nacional de referência para as doenças hereditárias do metabolismo (DHM), nomeadamente dos grupos das doenças lisossomais, doenças peroxissomais e doenças congénitas da glicosilação.

Nestes grupos encontram-se algumas patologias ligadas ao cromossoma X: nas doenças peroxissomais, a adrenoleucodistrofia ligada ao X (X-ALD) e nas doenças lisossomais temos a doença de Fabry, a síndrome de Hunter, a ictiose ligada ao X (XLI) e a doença de Danon.

A abordagem de estudo laboratorial destas patologias é diversa, podendo envolver diferentes tipos de metodologias bioquímicas e ainda estudos de genética molecular:

A XLI é diagnosticada laboratorialmente pela determinação da actividade enzimática;

O diagnóstico laboratorial de X-ALD é baseado no doseamento de metabolitos, sendo complementado por estudos de genética molecular;

Na doença de Fabry e síndrome de Hunter, é efetuado o doseamento de metabolitos, a determinação da atividade enzimática e estudos de genética molecular;

O estudo de genética molecular é a única metodologia laboratorial usada no nosso laboratório para o diagnóstico da doença de Danon.

Tendo sido estudada na UBG a quase totalidade dos doentes portugueses afetados por essas patologias, existem dados que permitem traçar um quadro do panorama nacional relativo às mesmas, com especial ênfase na análise da informação relativa aos indivíduos do sexo feminino.

Será apresentada a caracterização laboratorial, com os diferentes tipos de dados recolhidos, para este grupo de indivíduos e analisada a correlação com a apresentação clínica.

Serão apresentados exemplos de sucessos e dificuldades que surgiram no diagnóstico de indivíduos do sexo feminino, bem como casos com especial interesse.

É discutida também a importância da comunicação clínica/laboratório, tanto no estabelecimento do diagnóstico como nas tomadas de decisão relativamente a abordagens terapêuticas, nomeadamente nas patologias para as quais existe terapia de suplementação enzimática – doença de Fabry e síndrome de Hunter – face à avaliação custo-benefício.

CC-07

ANTISENSE-MEDIATED EXON SKIPPING FOR DUCHENNE MUSCULAR DYSTROPHY – CLINICAL TRIALS AND BEYOND

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Duchenne muscular dystrophy (DMD) is a severe, progressive muscle-wasting disorder, while Becker muscular dystrophy (BMD) is milder muscle disease. Both are caused by mutations in dystrophin, a protein, which stabilizes muscle fibers during contraction by linking muscle actin to the extracellular matrix. In DMD patients mutations disrupt the open reading frame, generating prematurely truncated, nonfunctional dystrophins. In BMD patients, mutations maintain the reading frame allowing production of internally deleted, partly functional dystrophins.

The exon skipping approach uses antisense oligonucleotides (AONs) to induce skipping of targeted exons during pre-mRNA splicing, with the aim of reading frame restoration, converting of the severe DMD into the milder BMD phenotype. This approach is mutation specific. However, as mutations cluster in a few hotspots, skipping of some exons applies to larger groups of patients (e.g. exon 51 skipping applies to 13%).

After obtaining proof-of-concept in cultured patient-derived cells, this approach was further optimized in animal models. In each case AON treatment resulted in targeted exon skipping and dystrophin restoration. In animal models this was accompanied by improved muscle function and quality. Proof-of-concept in patients was achieved in a clinical trial where 4 patients received local injections with an AON targeting exon 51 (coordinated by Prosensa Therapeutics). Dystrophin was restored locally for each patient.

Towards systemic application, studies in animal models revealed that dystrophic muscles facilitated uptake of 2OMePS AONs and that subcutaneous delivery was feasible. In a subsequent clinical trial, patients were subcutaneously injected with AONS targeting exon 51. Dystrophin was restored in a dose-dependent manner. All patients were enrolled in an open label extension study and have received subcutaneous AON injections at 6 mg/kg for almost 4 years. Two phase 2 and one a pivotal, double-blind, placebo-controlled multicenter trial for exon 51 skipping have recently been completed (coordinated by GlaxoSmithKline).

In parallel, preclinical studies to further optimize treatment regimens are in progress as well as clinical trials for additional exons for exon 44 skipping (PRO044, applicable to 6% of patients), exon 45 skipping and 53 skipping (PRO045 and PRO053, both applicable to 8% of patients).

The mutation specificity of the approach poses challenges to drug development regulations. A concerted effort of academic researchers, industry, regulators and patients is needed to adapt regulations to enable application of these personalized medicine approaches to rare diseases.

CC-08

DISCOVERING "X" IN THE MYOPATHIC EQUATION

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Congenital myopathies (CM) are a heterogeneous group of diseases, generally characterized by hypotonia and muscle weakness with onset at birth or during infancy and usually with a slowly progressive disease course. Scientific and technological developments in genomics over the last two decades have contributed to the identification of genetic causes for a significant number of myopathies. However, there are still several challenges to address both in diagnostics and in research. First, there is striking genetic and clinical heterogeneity associated to CM. In fact, although muscle biopsy is paramount for the diagnostic workup, pathognomonic findings such as cores, rods, central nuclei or fibre-type disproportion, are not gene-specific. In addition, a significant subset of these patients remains genetically unsolved, requiring further investigation that may lead to the identification of new genetic causes of CM.

Our recent research in congenital myopathies has focused on the mutational profile of the myotubularin gene (*MTM1*), which is defective in X-linked centronuclear myopathy (CNM). Male patients with MTM1 mutations are usually severally affected, presenting neonatal hypotonia and inability to maintain unassisted respiration. During the development and implementation of a mutation database for *MTM1* (http://www.lovd.nl/MTM1), we noticed that no large duplications had been reported. Large duplications in *MTM1* were screened by the MLPA technique in a small group of uncharacterized CNM Portuguese patients. A large duplication spanning exons 1 to 5 was identified in a boy with a mild CNM phenotype. Further characterization revealed that this duplication causes an in-frame deletion at the mRNA level (r.343_444del). Results obtained using a low-coverage next generation sequencing (NGS) approach showed that this genomic duplication extends into the neighbouring MAMLD1 gene and subsequent analysis unveiled the presence of a *MTM1*/*MAMLD1* fusion transcript [1].

This work demonstrates that it is clinically relevant to screen large *MTM1* duplications in CNM patients since this type of mutation may account for some cases that remain genetically unanswered, as was recently validated by the publication of additional cases. It also demonstrates how different analytical approaches are often required to solve the genetic complexity of congenital myopathies; the further application of NGS technology in these disorders shall be exemplified.

References

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CC-09

FMR1 ASSOCIATED PATHOLOGIES

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Fragile X syndrome is the most common form of inherited mental retardation with a prevalence of approximately 1:2,466 men and 1:8,333 women in the Caucasian population. The molecular basis of the syndrome is predominantly a CGG expansion in the 5´- untranslated region of the *FMR1* gene. In the general population, individuals carry 6 to 55 repeats, and the triplet number is usually stably transmitted. Individuals with alleles between 55 and 200 CGG repeats are called premutated carriers and those with more than 200 CGG are considered to carry full mutations and present classical Fragile X syndrome. In the premutated range, the CGG number is unstable through transmission to the next generation and tends to expand. Diagnosis is based on the determination of the CGG number. *FMR1* premutation is much more frequent than previously thought. The most relevant pathologies associated with premutation have been described to be Fragile X premature ovarian insufficiency (FXPOI) and Fragile X tremor ataxia syndrome (FXTAS). Other clinical manifestations, associated with this premutation, were later identified as thyroid dysfunction, chronic muscle pain or fibromyalgia, among others. While FXPOI and FXTAS are definitively related, the latter manifestations require further studies. Here we revise the current knowledge of the individuals carrying *FMR1* premutation.

CC-10

GENETIC COUNSELING

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Genetic counseling is the process by which patients or relatives at risk of an inherited disorder are advised of the nature and consequences of the disorder, the probability of developing or transmitting it, and the options open to them in management and family planning.

This complex process can be separated into diagnostic and supportive aspects.

Establishing a correct diagnosis is crucial, otherwise erroneous information will likely be given with potentially tragic consequences. Reaching a diagnosis involves three fundamental steps: taking a history, carrying out an examination and undertaking appropriate complementary investigations.

An etiological diagnosis allows precise risk estimation. Sometimes, even in the absence of a molecular diagnosis, a pattern of Mendelian inheritance may be clear from the family tree allowing the calculation of a recurrence risk. However, in many instances it is not possible to arrive to an accurate diagnosis and it is necessary to resort to empiric risks, derived from family studies rather than theoretical calculations. In all cases, recurrence risks should not only be quantified but need also to be qualified and placed in context.

The supportive aspects of the counseling process involve both communication and educational skills. Only an appropriately trained professional can help the individual or the family gain enough knowledge of the disorder and the options available for risk management to allow fully informed decisions without undue pressure or stress, in a way that promotes health, minimizes psychological distress and increases personal control.

These concepts will be illustrated with relevant clinical examples.

CC-11
ÉTICA E GENÉTICA

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O conceito de ética, tal como o entendemos atualmente, resultou da evolução do pensamento filosófico durante centenas de anos e remonta à antiguidade grega e romana. Com a introdução da "bioética", termo proposto por Van Rensselaer Potter em 1970, reconheceu-se a necessidade de conciliar os conceitos ancestrais da moralidade com os conflitos éticos resultantes do evoluir da biomedicina e da tecnologia científica, sob pena de comprometer os destinos da vida humana. Nos cuidados de saúde em geral, pela sua grande aceitação, os princípios éticos enunciados por Beauchamp e Childress – autonomia, beneficência, não-maleficência e justiça – constituem a base de reflexão para a atuação de muitos profissionais de saúde, nos quais se incluem os que trabalham em genética médica e humana; nenhum destes princípios éticos deverá prevalecer sobre os outros, procurando-se habitualmente um consenso.

Na prática diária, os princípios éticos aplicam-se em genética antes da execução de uma técnica ou do estabelecimento de um diagnóstico, desde logo com uma consulta de aconselhamento genético não-diretivo, a obtenção do consentimento informado (se necessário, oral ou escrito e adequado a cada situação) e a estrita manutenção da confidencialidade dos dados pessoais e clínicos recolhidos. A partir desta consulta os problemas éticos que surgirem em cada situação serão resolvidos caso a caso, tanto em situações pré-natais como pós-natais. Em qualquer circunstância, a multidisciplinaridade das equipas e o rigor científico dos profissionais, fundamentais para o avanço do conhecimento e dos ganhos em saúde, não deverá descurar a legislação vigente e, sobretudo, compreender a necessidade de haver uma normalização razoável de procedimentos, pelo que se recomenda a leitura da seguinte documentação: 1-Lei n.º 12/2005: Informação genética pessoal e informação de saúde, DR-I SÉRIE-A, N.º 18, 26.01.2005; 2-Orientações e Princípios que orientarão a aplicação de técnicas de biologia molecular no âmbito da prestação de cuidados de saúde pelo SNS, Despacho nº 9108/97, DR-II Série, N.º 237, 13.10.1997; 3-Lei nº 67/98, 26.10.1998: Lei de proteção de Dados Pessoais, DR-I Série A, N.º 247, 26.10.1998; 4-Lei n.º 32/2006, 26.07: Procriação medicamente assistida, DR-I SÉRIE-A, N.º 143, 26.07.2006; 5-Decreto-Regulamentar n.º 5/2008, 11 de Fevereiro: Regula a utilização de técnicas de procriação medicamente assistida, DR-I SÉRIE-A, N.º 29, 11.02.2008; 6-Orientações e princípios que orientarão a estruturação do sector de DPN, Despacho nº 5411/97, DR-II Série, N.º 180, 06.08.1997; 7-Comissões de Ética para a Saúde, Decreto-Lei n.º 97/95: DR-I SÉRIE-A, N.º 108, 10.05.1995.

CC-12
UM PASSADO COM FUTURO

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O Instituto de Genética Médica Doutor Jacinto de Magalhães tem um passado que é a garantia de ter futuro; um futuro que vai poder ser criado e vivido sem destruição do passado

Porque acompanhei, de perto, a sua vida desde que o saudoso Doutor Jacinto de Magalhães o concebeu, posso apresentar um depoimento sobre todos os sucessos que marcaram a sua existência, sobre a forma como foi sendo fragilizado e sobre a esperança que se abre com o seu acolhimento no Centro Hospitalar do Porto.

Para o seu arranque foi necessário que tivesse autonomia financeira e administrativa, com um Director nomeado directamente pelo Ministro da Saúde e com ele resolvendo as decisões a tomar para que o Instituto crescesse rapidamente e sem os controlos burocráticos que tudo resolvem com lentidão.

Com a instalação e o desenvolvimento muito avançados tornou-se naturalmente necessária a integração do Instituto na estrutura geral do Ministério e a aceitação progressiva do controlo burocrático-administrativo: orçamento próprio, quadro de pessoal científico, administrativo e técnico.

Esta evolução foi-se processando, com alguns acidentes de percurso, até que uma reforma dos organismos do Ministério da Saúde, levada a cabo pelo Ministro Correia de Campos, originou a sua perda de identidade e a integração no Instituto Nacional de Saúde Dr. Ricardo Jorge. Esta integração não teve o benefício que era antecipado pelas convicções do Ministro, nem quanto às economias, nem quanto à funcionalidade, nem no que respeita ao enquadramento do pessoal nele existente. A distância física entre Porto e Lisboa, e a dificuldade em explicar as necessidades financeiras e outras para um regular funcionamento, originou um período de grande instabilidade com saída de alguns investigadores, acolhidos noutras instituições, e com uma dramática instabilidade para os que permaneceram fiéis ao espírito de Jacinto de Magalhães, de um Instituto com uma vertente clínica e uma vertente de investigação avançada ao serviço da resolução dos problemas clínicos. Tudo estava parado, em Lisboa, a aguardar decisões que não apareciam, nem boas nem más.

A decisão superior de integrar o Instituto nas actividades de investigação do Centro Hospitalar do Porto, surge como uma solução inteligente, direi mesmo sábia, para dar a esta questão uma saída honrosa para todas as partes e com um potencial de crescimento no futuro que presta homenagem à memória de Jacinto de Magalhães e à sua visão profética do futuro da Genética Clínica e de investigação.

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Oral communication
Comunicação oral

CO-01

STUDY OF THE FMR1 GENE STRUCTURE AMONG WOMEN WITH OVARIAN DYSFUNCTION FROM THE BASQUE COUNTRY

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FMR1 premutation and intermediate alleles have been associated with the development of different forms of ovarian dysfunction, being the Premature Ovarian Failure (POF) the most serious one. A group of 68 women with ovarian dysfunction of unknown aetiology and 47 control women from the Basque Country has been analyzed. Considering the number of CGG repeats, the frequency of alleles with ≥35 CGG repeats was statistically higher in the whole patient group (12.50% vs. 0%). Concerning their ovarian condition, the patient group was divided in three categories and, in the three subgroups the alleles with ≥35 CGG were also statistically higher than in controls. As the AGG interspersion pattern seems to be correlated with the instability of the alleles, the CGG repeat internal structures have been analyzed. Many of the intermediate and premutation alleles found in the patient group appeared to have two interruptions with more than 15 CGG at the 3' end (65%). Interestingly, among these alleles the predominant structure was 9+9+n, indicating a loss of AGG interruptions at the 3' end. Therefore, the data showed that among patients the alleles were more unstable and that this instability influencing the *FMR1* expansion might be related with the development of an ovarian dysfunction.

Poster abstracts
Resumos de posters

P-01

INVESTIGATION OF X-CHROMOSOME INACTIVATION PATTERNS – A VALUABLE TOOL IN GENETIC DIAGNOSIS

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To overcome the gene dosage differences between males and females, one of the X-chromosomes is epigenetically silenced in the early embryogenic process of the female foetus. The choice of which one remains active in each cell is thought to be a random process, resulting, in most cases, in a uniform X-chromosome inactivation (XCI) pattern of cells. However, studies in large cohorts of phenotipically unaffected females indicate that about 8.8% exhibit skewed profiles (>80:20). Although this skewed ratio has no clinical significance in unaffected females, it may explain disease manifestation in otherwise non-affected carries of recessive X-linked conditions. As suggested by several authors, the assessment of XCI patterns can be very useful in confirming the diagnosis of disorders involving the X-chromosome, in female patients. In our laboratory, we apply the HUMARA assay to determine the pattern of XCI. This widely used method is based on the analysis of DNA methylation and number of CAG tandem repeats at the Human Androgen Receptor (*AR*) gene locus. The *AR* gene's highly polymorphic CAG repeat enables distinction between maternal and paternal X-chromosomes, while the close proximity of cleavage sites for methylation-sensitive restriction enzymes allows the discrimination of the inactive and active X-chromosome. The work presented demonstrates the importance of performing XCI studies and determining their impact in both the clinical and the laboratory context. Several examples will be described including manifesting carriers of X-linked recessive disorders; female carriers of translocations involving the X-chromosome, carriers of newly described variants of undetermined pathogenicity and female carriers with a suspected family history of X-linked disorders associated with unilateral XCI (where no sample is available from the affected male or where no mutation has been identified). Our results corroborate previous studies and show that methylation status in the *AR* locus is a reliable method to study XCI, therefore illustrating the confidence of this approach. Nevertheless, interpretation of XCI results should be done with caution: XCI can only be ascertained in the specimen being analyzed and may not reflect the XCI patterns in other tissues; it is an age-dependent phenomenon (since skewing increases with age); in some cases, the locus under study may not be in linkage desequilibrium with the *AR* locus; other genetic factors are known to play an important role in XCI process; symptomatic females may also have other factors contributing to their phenotype.

P-02

NEXT GENERATION FRAGILE-X TESTING: GETTING AWAY FROM SOUTHERN BLOTS

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Fragile X syndrome (FXS) is the most common form of intellectual disability in the general population, usually caused by an expansion of a trinucleotide CGG repeat in the 5' untranslated region of the *FMR1* gene. In most cases, expansions over 200 repeats, termed full mutations, cause silencing of *FMR1* gene due to methylation of its promoter, and consequently loss of protein product. Expansions with 55-199 CGG repeats called pre-mutations or others even smaller (45-54 CGG repeats) named intermediate, do not cause FXS, but are frequently associated with late-onset neurological and/or reproductive disorders (FXTAS/FXPOI). Southern Blot (SB) is still considered the gold standard for molecular diagnosis of FXS, because it is able to clearly characterize size and methylation status of full and pre-mutated *FMR1* alleles (following DNA digestion with methylation sensitive enzymes). Nevertheless, SB is a very time-consuming technique and requires a large amount of intact and high-molecular weight DNA. As such, several methodologies have been developed to replace SB and overcome its disadvantages. The aim of this work was to test different techniques which can substitute totally or partially the SB, by comparing (1) their ability to quantify or discriminate normal, pre-mutated and fully mutated alleles; (2) the maximum number of CGG repeats detected; (3) their capacity to determine the DNA methylation state; (4) their power to discriminate size and methylation mosaics; and (5) the amount of DNA required for each technique. The tested techniques were High Resolution Melting Curve Analysis (currently in experimental process with prototype reagents), the FragilEase™ assay from PerkinElmer®, the Amplidex® FMR1 mPCR Protocol from Asuragen® and a multiplex assay developed by our group, for the simultaneous screening of 3 genes - *FMR1*, *AFF2* e *ARX*. For this work we tested 7 DNA samples from patients previously characterized at the molecular level in our laboratory: 6 from females and 1 obtained from a chorionic villus sample of a male fetus. These samples were previously characterized as normal (n=2), fully mutated (n=3), pre-mutated (n=1) and a size mosaic (n=1). Although using a very small number of samples, this work aims to describe and compare four different methodologies in an attempt to establish if they can adequately replace SB.

P-03

PORTUGUESE PATIENT REGISTRY FOR DUCHENNE/BECKER MUSCULAR DYSTROPHY

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Duchenne/Becker muscular dystrophy (D/BMD) collectively known as the dystrophinopathies, is one the most frequent neuromuscular diseases with onset during pediatric age, having an estimated incidence of about one in every 3500 to 5000 boys. Over the last two decades, the Molecular Genetics Unit of CGMJM has performed the genetic characterization of over 360 D/BMD patients, leading to the identification of 189 mutations, including 46 novel variants. Comprehensive analysis also involved expression studies at the mRNA level, the identification of splicing changes and ultimately providing evidence for apparent exceptions to the reading-frame rule. Considering the recent mutation-based therapeutic approaches, *DMD* gene analysis has gone beyond the molecular confirmation of the clinical diagnosis and is now also crucial for patient inclusion in disease registries and in ongoing clinical trials. In 2007, the network of excellence for the neuromuscular field - TREAT-NMD - started a global patient registry for D/BMD. This registry depends entirely on data gathered at the national level in country-specific disease registries using the same database items (mutational and clinical). This standardization enables consensus and facilitates clinical research, the development of new therapeutic approaches and clinical trials for new drugs. These trial-ready registries are also useful for phenotype/genotype correlations and epidemiological profiles of the disease. In response to this international endeavor, we developed the Portuguese D/BMD registry which is currently located in the CGMJM, Centro Hospitalar do Porto. The Portuguese registry is based on the Leiden Open Variation Database (LOVD) software and follows the TREAT-NMD charter for patient database/registry, abiding by National and European legislation concerning data. The national registry uses the clinical reporting model, where three medical coordinators from major hospital centers (Porto, Coimbra and Lisbon) were assigned to data collection (personal, clinical and pathological data) and patients' regular clinical (re)evaluation. Registry inclusion is completely voluntary and requires a specific informed consent. All the information, namely data sent by the clinician, consent and

the genetic data obtained in the laboratory, is assembled by the D/BMD registry curators and added to the database after validation. The registry was officially launched in 2012 and until now eighteen patients have been included in the database.

P-04

"ENTRE OS GENES E A MENTE" - SÍNDROMA DE TURNER E MANIFESTAÇÕES PSICOPATOLÓGICAS

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A Síndrome de Turner (ST) é uma cromossomopatia caraterizada pela monossomia total ou parcial do cromossoma X. Ocorre de forma esporádica, afetando 1 em cada 2000-5000 recém-nascidos do género feminino, associando-se habitualmente a baixa estatura, disgenesia gonadal, anomalias congénitas e adquiridas e sinais dismórficos. Estão igualmente presentes alterações neuropsiquiátricas como dificuldades cognitivas, distúrbios de perceção espacial e temporal, memória visual, atenção, reconhecimento e interpretação de emoções. São relatadas na literatura algumas doenças psiquiátricas em doentes portadoras de Síndroma de Turner, de que são exemplo as Perturbações do Humor, a Esquizofrenia e a Anorexia Nervosa. No entanto, parece não haver risco aumentado de psicopatologia grave. Verifica-se, por seu lado, maior risco de dificuldades psicossociais, dificuldades específicas de aprendizagem, problemas de comportamento e baixa autoestima. Tratando-se de uma "doença crónica", compreende-se, ainda, o possível impacto emocional. A propósito de uma adolescente com Síndrome de Turner, internada no Departamento de Psiquiatria da Infância e Adolescência do CHP, revêem-se os dados existentes na literatura sobre as manifestações psicopatológicas da Síndrome de Turner.

Palavras-chave: Síndrome de Turner; cromossomopatias; psicopatologia.

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P-05

CRANIOFRONTONASAL SYNDROME: CASE REPORT

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Background: Craniofrontonasal syndrome (CFNS, MIM #304110) is a very rare X-linked developmental malformation, caused by mutations in the *EFNB1* gene, located at Xq13.1.It was first identified as a subgroup of frontonasal dysplasia by Cohen in 1979. The incidence values that were reported ranged from 1:100.000 to 1:120.000. Heterozygous females have craniofrontonasal dysplasia (CFND) and occasionally extracranial manifestations including midline defects and skeletal abnormalities, whereas hemizygous males show no or only mild features such as hypertelorism and rarely show cleft lip or palate.

Methods: Following first description of the syndrome, approximately 180 additional cases have been published in medical literature. We report here on an additional case of a Portuguese girl with CFNS. We compared the clinical features of the previously published cases of craniofrontonasal syndrome with our case.

Results: Common findings in all reports, including our case, are coronal craniosynostosis, craniofacial asymmetry, hypertelorism, downslanting palpebral fissures, broad bifid nose, malocclusion and longitudinally grooved fingernails. Craniofrontonasal syndrome was confirmed in this patient by molecular analysis of *EFBN1* gene which was excluded in her father.

Discussion: CFNS shows a phenotypic pattern not usually seen in X-linked disorders, as heterozygous females are more severely affected than hemizygous males. Mutations in *EFNB1* are the cause of CFNS in the majority of patients, with a mutation detection rate of 92%. CNFS's clinical manifestations are sex dependent, with multiple skeletal malformations in affected females and mild or no malformations in male carriers. X-inactivation is proposed to explain the more severe outcome in heterozygous females, as this leads to functional mosaicism for cells with differing expression of *EFNB1*, generating abnormal tissue boundaries — a process that cannot occur in hemizygous males. Our report discusses a patient with clinical characteristics consistent with CNFS and in whom a de novo *EFNB1* mutation was demonstrated. Postzygotic mutation leading to somatic/germline mosaicism in the first generation is a relatively common feature of this condition and could not be excluded in the father, who had mild hypertelorism. This issue and its implications in recurrence risk were discussed with the couple.

P-06

TRANSLOCAÇÃO (X;10) APARENTEMENTE EQUILIBRADA *DE NOVO* NO SEXO FEMININO: CASO CLÍNICO

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Introdução: A translocação é uma anomalia cromossómica que envolve quebras de dois cromossomas diferentes com uma troca dos segmentos. Essa alteração pode ser equilibrada ou desequilibrada, ocorrer de novo ou ser herdada. Geralmente, um portador de translocação equilibrada não apresenta anomalias fenotípicas devidas a alteração cromossómica, exceto um risco acrescido de anormalidades reprodutivas, incluindo infertilidade, abortamentos de repetição e descendência com malformações e/ou atraso mental. A translocação equilibrada entre o cromossoma X e um autossoma, no sexo feminino, é uma situação específica. Uma vez que um dos cromossomas X das mulheres se encontra inativado, uma translocação envolvendo o cromossoma X num indivíduo do sexo feminino, mesmo aparentemente equilibrada, pode originar patologia por inativação do segmento autossómico localizado no cromossoma X se este estiver inativado.

Caso clínico: Apresentamos um caso clínico de uma menina de 2 anos que foi referenciada à nossa consulta de Genética por apresentar atraso do desenvolvimento psicomotor e dismorfia craniofacial e ter sido detetada uma translocação aparentemente equilibrada envolvendo os cromossomas X e 10 que ocorreu de novo. Cariótipo: 46,X,t(X;10)(p11.23;q11.21)dn. O estudo da inativação do cromossoma X no sangue revelou um desvio completo a favor do cromossoma X normal.

Discussão: Uma translocação aparentemente equilibrada entre os cromossomas X e autossoma envolve o fenómeno de inativação do X. No caso da inativação do cromossoma X envolvido na translocação, este fenómeno pode originar uma deleção funcional do segmento autossómico. O fenótipo dos doentes descritos na literatura com deleções do segmento terminal 10q é semelhante ao do nosso caso, o que aponta para que o cromossoma X derivado desta translocação possa estar inativo noutros tecidos, especialmente SNC, e seja a causa dos problemas da menina, uma vez que originaria uma deleção funcional do segmento 10q11.21 a qter.

P-07

ESTRATÉGIA UTILIZADA NO ESTUDO PILOTO PARA O RASTREIO NEONATAL DA FIBROSE QUÍSTICA

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O Programa Nacional de Diagnóstico Precoce (PNDP) realiza-se em Portugal desde 1979, e atualmente inclui o rastreio neonatal de 24 Doenças Hereditárias do Metabolismo (DHM) e do Hipotiroidismo Congénito (HC). Em Outubro de 2013 iniciou-se, um estudo piloto para o rastreio neonatal da Fibrose Quística (FQ), que deverá ser efetuado em 80000 recém-nascidos (RN) portugueses ao longo de aproximadamente um ano. A Fibrose Quística (Mucoviscidose) é uma doença metabólica genética, com transmissão autossómica recessiva, e que tem uma prevalência média ao nascimento de 1:3000 RN, na população caucasiana. Bioquimicamente deve-se à deficiência na proteína CFTR, codificada pelo gene *CFTR*, localizado no cromossoma 7. Estão descritas cerca de 2000 variantes genéticas associadas à FQ. Clinicamente é uma doença grave com atingimento multissistémico, caracterizada pela disfunção das glândulas exócrinas, incluindo o pâncreas, as glândulas sudoríparas e as glândulas mucosas dos tratos respiratório, gastrointestinal e reprodutivo. O aumento dos valores de ião cloreto no suor é típico destes doentes, sendo o "teste do suor" a principal análise de confirmação da doença. Diagnosticar precocemente a doença é uma fator decisivo no prognóstico, não só pela maior sobrevida, mas também para uma melhor qualidade de vida do doente. O aumento da concentração sanguínea da tripsina imunoreactiva (IRT) nos 1os dias de vida dos RN com FQ possibilita o rastreio neonatal desta doença. No entanto, apesar de uma boa sensibilidade, o IRT não é um marcador específico para a FQ, e um rastreio baseado unicamente neste marcador tem um número inaceitável de falsos positivos. Por esta razão, têm sido propostos vários algoritmos de rastreio, incluindo outros marcadores bioquímicos como a Proteína Associada à Pancreatite (PAP) ou o estudo molecular. Neste estudo piloto, o algoritmo de diagnóstico utilizado baseia-se na determinação do IRT e do PAP em sangue colhido em papel de filtro, sendo a amostra de sangue a mesma colhida para os restantes rastreios. No âmbito deste projeto já foram estudados cerca de 29 000 RN e identificados 6 casos positivos. No final deste estudo deverá ser avaliada a inclusão da FQ no PNDP e o algoritmo de rastreio a utilizar.

P-08

XL-EDMD - GENOTYPIC SPECTRUM AMONG PORTUGUESE PATIENTS

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Emery-Dreifuss muscular dystrophy (EDMD) is characterized by the clinical triad of joint contractures that begin in early childhood, slowly progressive muscle weakness and wasting initially in a humero-peroneal distribution that later extends to the scapular and pelvic girdle muscles, and cardiac involvement that may manifest as palpitations, presyncope and syncope, poor exercise tolerance, and congestive heart failure, that can result in sudden death. Age of onset, severity, and progression of muscle and cardiac involvement demonstrate both inter- and intrafamilial variability. Clinical variability ranges from early onset with severe presentation in childhood to late onset with slow progression in adulthood. In general, joint contractures appear during the first two decades, followed by muscle weakness and wasting. Cardiac involvement usually occurs after the second decade. The three genes in which mutations are known to cause EDMD are *EMD* (encoding emerin) and *FHL1* (encoding FHL1), which cause X-linked EDMD (XL-EDMD) and *LMNA* (encoding lamin A and C), which causes autosomal dominant and autosomal recessive EDMD (AD-EDMD and AR-EDMD). For all forms of EDMD the diagnosis is based on clinical findings and family history. The diagnosis of X-linked EDMD also relies on failure to detect emerin or FHL1 protein in various tissues and molecular genetic testing of *EMD* or *FHL1* whereas AD- and AR-EDMD diagnosis relies on molecular genetic testing of LMNA. We describe the molecular results for *EMD* gene screening, in a group of twenty-one Portuguese families, with clinical diagnosis of EDMD and presenting different clinical phenotypes, with or without cardiac involvement. Differential diagnosis of XL-EDMD was achieved in five families (eight patients). Four different mutations were identified, two of which have not been documented in the literature. In a female patient, a skewed X inactivation pattern was observed, explaining disease manifestation. In the remaining families, the *LMNA* gene was studied leading to confirmation of laminopathy in a four families. Molecular diagnosis is therefore very important for an early diagnosis, to prevent sudden deaths, and to distinguish X-linked EDMD from the autosomal forms, which is essential for a correct genetic counseling and subsequent prenatal diagnosis.

P-09

MX-LINKED CENTRONUCLEAR MYOPATHY: FROM CLINICAL DIAGNOSIS TO GENETIC COUNSELING

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Background: X-Linked Centronuclear Myopathy is a rare congenital myopathy characterized by hypotonia, muscle weakness and respiratory distress at birth, although the presentation may be delayed. It is the most severe and the most common of the three inheritance forms, which also include the autosomal dominant and the autosomal recessive centronuclear myopathies. While muscle biopsy is crucial to differentiate centronuclear myopathies from other congenital myopathies and muscular dystrophies, genetic testing is essential to establish a definitive diagnosis and to perform a precise genetic counseling.

Clinical report: We report a proband, first son of healthy non-consanguineous parents, who presented with severe congenital hypotonia, global muscle weakness and bilateral hand contractures. He was born prematurely, shortly after polyhydramnios diagnosis, at 30 gestational weeks. Ventilatory support was required since his birth. At examination, dolichocephaly was evident and he had ptosis and ophtalmoparesis, facial diparesia, as well as a weak cry. Muscle biopsy revealed fibers with variable diameter, including round atrophic fibers, with centrally located nuclei, as well as central areas of increased oxidative activity surrounded by a bright halo, which was compatible with a centronuclear myopathy. The previously reported pathogenic missense variant c.566A>G (p.Asn189Ser) was detected in the *MTM1* gene, in hemizygosity in the proband and heterozygosity in the mother, confirming the diagnosis of X-Linked Centronuclear Myopathy.

Discussion: The genetic testing of the X-linked form is warranted as a first-tier investigation in male infants with a severe phenotype and a characteristic muscle biopsy, since the autosomal forms of centronuclear myopathies present with a relatively mild phenotype in both males and females. The identification of a pathogenic *MTM1* mutation will enable preimplantation genetic diagnosis or prenatal diagnosis, as additional reproductive options for this couple.

P-10

PRENATAL DIAGNOSIS: A CASE OF PARTIAL TRISOMY 6Q

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Partial distal trisomy 6q is a rare event and is characterized by a distinct phenotype which includes microcephaly, acrocephaly, joint contractures and profound psychomotor retardation. The authors present a case of a 30-year-old pregnant woman referred to prenatal diagnosis due to ultrasound anomalies. It was the first pregnancy of a non-consanguineous couple with no familial or personal story of anomalies. Parents karyotype was performed. Cytogenetic analysis revealed a chromosome 15 with an increase p arm similar to a variation in length of heterochromatic stalks on the short arm. Both parents presented a chromosome 15 with satellites but different from the one detect at the amniocytes. Subtelomeric FISH analysis revealed a trisomy of 6q27-qter present at p arm of chromosome 15 - it was a *de novo* rearrangement.The parents decided to terminate the pregnancy and foetal autopsy was required. Several polymorphic variants were described in human chromosome 15 including increased amounts of short arm hetrochromatin (ph+), interpreted as a normal polymorphism.In the majority of cases partial trisomy 6q results from a balanced chromosomal rearrangement in one of the parents, usually of maternal origin. There have also been rare cases in which partial trisomy 6q has appeared from spontaneous (*de novo*) errors very early in embryonic development. The authors compared the cytogenetic and the foetal autopsy findings with those described in the literature. Every new case of a rare chromosomal alteration should be reported in order to establish a genotype/ phenotype correlation, improving risk evaluation and genetic counseling.

P-11

MUTATION ANALYSIS OF GENES INVOLVED IN SPERM MOTILITY: A STUDY IN PATIENTS WITH TOTAL SPERM IMMOTILITY

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Reduced sperm motility represents one of the major male causes of infertility. The axoneme (Ax) is the flagellar motor of the sperm cell and several mutations in genes involved in the assembly and regulation of the Ax have been proved to be responsible for certain cases of infertility associated with severe sperm immotility. For instance, mutations in the genes *CCDC39*, *CCDC40* (that are involved in assembly of the dynein regulatory complex and the inner dynein arm complex), *DNAI1* and *DNAH5* (that are involved in the assembly of outer dynein arms) are associated with primary ciliary dyskinesia (PCD). PCD is an inherited autosomal recessive genetic disorder whose typical diagnostic features include the absence of dynein arms and reduced sperm motility. Fibrous Sheath Dysplasia (FSD) is a flagellar pathology, which causes total sperm immotility, mainly due to hyperplasia and disorganization of the Fibrous Sheath (FS). Previous reports suggested that mutations in *AKAP3* and *AKAP4* genes (the main components of FS) might contribute to FSD. In a group of five Portuguese patients from Assisted Reproductive Medicine centres that presented totally sperm immotility, transmission electron microscopy revealed several structural defects in sperm flagellum, such as anomalies in dynein arms, microtubules and FS. Given the importance of *CCDC39*, *CCDC40*, *DNAH5*, *DNAI1*, *AKAP3* and *AKAP4* genes in sperm motility, we decided to screen these genes in our patients. To identify genetic alterations that could explain their phenotype, we initiated the analysis of the exonic regions of these 6 genes by Sanger sequencing. We have already sequenced five genes and *DNAH5* analysis is still ongoing (we have already sequenced thirty-five exons that are known to harbour a significant number of mutations, from a total of seventy-nine). Ten variants in *CCDC39*, twenty-six in *CCDC40*, two in *DHAI1*, seven in *AKAP3*, one in *AKAP4* and thirty-nine in *DNAH5* have been identified. The work's major contribution was the identification of fourteen new variants in *CCDC39*, *CCDC40*, *AKAP3* and *DNAH5* genes. With this work we expect to be able to offer a differential diagnosis to the patients and find potential genetic markers for individuals with this kind of problem.

P-12

PRENATAL DIAGNOSIS MOSAIC 45, X CASE WITH A MARKER CHROMOSOME

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Introduction: Small supernumerary marker chromosomes (sSMC) are structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics. Generally the size is about or smaller than a chromosome 20, and molecular cytogenetic techniques are necessary for a comprehensive characterization. Prenatally ascertained sSMCs occur in 0.075%, and 0,044% in subsequently studied postnatal analysis. The overall risk of phenotypic abnormalities in prenatally ascertained sSMCs has been estimated to be about 13%. sSMC in Turner syndrome (sSMC) are very rare in the common population (1:100,000) – however, they can be observed with a 45- and even 60-times higher frequency in infertile or intellectual disability patients, respectively. Even though sSMC derive from one of the sex chromosome in >99% of the cases and the majority form ring chromosome. There are also exceptional reports on sSMC derived from one of the autosomes. Thus, a detailed molecular cytogenetic marker chromosome characterization is needed to provide information on sSMC.

Methods: A 29-year-old primigravida woman underwent amniocentesis at 16 weeks of gestation due to a positive maternal biochemistry screening for trissomy 13 and 18, and the presence of a single umbilical artery. Karyotype (GTL-banding), aCGH (4x180k Agilent Human CGH Microarray) and FISH analysis (CEPX Spectrum Green) were sequentially performed in cultured amniocytes to better characterize this sSMC.

Results: Amniocentesis revealed a karyotype 45,X[22]/46,X,+mar[8]. Among 30 cultured amniocytes colonies, 8 contained the sSMC, whereas the remaining 22 colonies were 45,X. Using DNA extracted from cultured amniocytes, aCGH showed that the sSMC was originated from chromosome X and revealed a 19,81-Mb gene dosage increase at Xp11.21-Xq21.1. FISH analysis showed 41% (41/100) mosaicism for sSMC in cultured amniocytes and confirmed the identification of the sSMC as derivate from chromosome X.

Discussion: Althought sSMC are rare, this is a well-known cytogenetic entity. So far, a detailed molecular cytogenetic characterization of sSMC by aCGH was only perfomed in a few cases. In this case, we could conclude that sSMC was derived from Xp11.21-Xq21.1 probably as a ring [r(X)(p11.21q21.1)] due to absence of telomeric regions.

This sSMC (X) includes XIST region, allowing the inactivation of this chromosome. Once that it is very small being all the short arm and part of the long arm absent, it is expected that it is preferential inactivated instead of occurring random inactivation. This is in accordance with the absence of ultrasound abnormalities. Nevertheless, the inactivation pattern is not predictable. For the better characterization of this kind of sSMC (X), aCGH should always be performed allowing a more accurate genetic counseling.

P-13

X-LINKED ICTHYOSIS – A METABOLIC ETHIOLOGY FOR "DRY SKIN"

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Introduction: X-linked ichthyosis is a keratinization genetic disorder characterized by a generalized desquamation of large, adherent, dark brown scales involving trunk and limbs, but sparing palms and soles. It is often associated with other clinical symptoms, such cryptorchidism (20%), social communication deficits, attention deficit hyperactivity syndrome (40%) or autism (25%). XLI has an incidence of 1 in 6000 births and differs from other types of ichthyosis by transmission mode, clinical manifestations and age of onset. Biochemically, the disorder is due to deficiency in steroid sulfatase (STS), an enzyme localized in the endoplasmic reticulum and responsible for hydrolysis of cholesterol sulfate to cholesterol. Cholesterol sulfate accumulation in patient´s epidermis leads to barrier instability and inhibits the desmosomal degradation which is required for normal desquamation, thereby leading to corneocyte retention.

Aims: report the etiological identification of XLI, among all genetic disorders, an entity that shows one of the highest ratios of chromosomal deletions (found in up to 90% of patients).

Methods: Diagnosis is based on STS enzymatic activity determination as the fraction of total arylsulfatase C activity which is inhibited by dehydroepiandrosterone sulfate. Patients present undetectable levels of STS activity when compared with normal controls.

Results: Since 1984, 28 affected males were diagnosed with XLI, some of them within the same family in three different generations. Icthyosis was present as the first clinical signal.

Conclusions: ICX is usually identified as a disease with mild clinical impact and with satisfactory therapeutic response. However, the accurate diagnosis of this disease is crucial to offer patients and affected families proper guidance, regarding attention deficit hyperactivity with predominantly inattentive symptoms. Prenatal diagnosis is available and would be advocated for those cases which have Xp22.3 larger deletions encompassing neighboring genes. These patients may present mental retardation, or features of X-linked chondrodysplasia punctata , in addition to XLI. Severe XLI forms may thus represent contiguous gene deletion syndromes.

P-14

MOSAICISM WITH TWO X CHROMOSOME DIFFERENT REARRANGEMENTS AND A TURNER-*LIKE* PHENOTYPE: CASE REPORT

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The frequency of Turner Syndrome (TS) has been reported as 1/5,000 live female births. This pathology is most commonly associated with a 45,X karyotype but in approximately 25% of the patients the karyotype shows both a normal X and a structurally abnormal X chromosome. These abnormalities, which include deletions, duplications, inversions, translocations and ring chromosomes, imply chromosomal breaks and significant imbalance of gene content; they are generally benign because of the preferential inactivation of the abnormal X. Six to 15% of patients with TS are mosaics for an X ring chromosome [r(X)] line; however, in these cases the incidence of mental disability and other congenital abnormalities may be significantly higher. Some authors report that severe r(X) phenotypes can be seen in patients with active r(X) chromosomes lacking the X-inactive specific transcript gene (XIST gene).The authors present a female patient aged 3 with clinical features of Turner syndrome. Cytogenetic studies revealed a novel mosaicism with two different abnormal cell lines: 1- the major line with a normal X chromosome and another X with a rearrangement corresponding to a deletion of the distal region of the short arm (Xp) and duplication of the long arm (Xq13->qter); 2- the other with a normal X chromosome and a r(X)(p22.3q13). FISH studies confirmed: in the line containing a rearranged X chromosome a deletion of the Xp subtelomeric region (Xp22.3) and a duplication of the Xq subtelomeric region (Xq28); in the line with r(X) a deletion of both subtelomeric regions. The presence of the XIST gene was demonstrated both in normal and abnormal X chromosomes, in the two cell lines.The authors will present a complete cytogenetic characterization of the patient and discuss all the factors that play an important role in determining the phenotypic outcome.

P15

DISTAL XQ27->Q28 DUPLICATION AND FUNCTIONAL DISOMY: CLINICAL AND CYTOGENETIC CHARACTERIZATION

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Distal Xq duplications are intrachromosomal disorders that constitute the main cause of functional disomy in males. Most cases are inherited from heterozygote mothers. These duplications vary in size, location and gene content of the correspondent segment. Hemizygous male descendents have a functional partial Xq disomy and are phenotipically abnormal. A male proband aged 7 months was referred for cytogenetic studies due to psychomotor delay, coarse features and cardiopathy. Both the child and the mother's karyotypes were obtained from peripheral blood lymphocyte cultures using standard techniques and chromosomes were analysed with GTG banding. The child's mother was studied using fluorescence in situ hybridization (FISH) with a whole chromosome painting probe for the X chromosome (wcpX, Cytocell), to exclude the involvement of any other chromosome and X inactivation pattern techniques. Karyotypes were requested for the mother's parents. The cytogenetic analysis revealed extra material on the long arm of the X chromosome both in the proband and in the mother. Maternal grandparents had normal karyotypes. X inactivation studies in the mother showed that the abnormal X was always late replicating and therefore inactive. No further testing was possible in the child, since he deceased of pneumonia at the age of 8 months. The extra material observed in the distal segment of the long arm of the X chromosome in this family was interpreted as a duplication of chromosome X terminal region (q27.3->q28). Child's final karyotype: 46,Y,dup(X)(q27.3-q28)mat.Large cytogenetic visible duplications of Xq are rare, the most common being the Xq27->q28 region and there are only about 40 cases described in the literature. The prevalence of Xq duplications is still unknown but the clinical outcome is a well recognized phenotype. The proness to infections in individuals with this condition is almost invariably the cause of death in childhood. The clinical history and cytogenetic findings of this case are in agreement with similar cases previously reported. Parents were given appropriate genetic counselling and offered the possibility of prenatal genetic diagnosis in future pregnancies. Since then, two healthy 46,XX daughters were born.

P-16

CLINICAL, BIOCHEMICAL AND MOLECULAR STUDIES: STEPWISE TO ACHIEVE DIAGNOSIS OF FABRY DISEASE

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Introduction: Fabry disease (FD, OMIM # 301500), a treatable X-linked storage disorder, is caused by deficient activity of the lysosomal enzyme ?-galactosidase A (?-Gal A). Over 400 pathogenic mutations in *GLA* gene have been associated with FD. Enzymatic deficiency leads to lysosomal accumulation of globotriaosylceramide (Gb3) and lyso-Gb3. The first clinical manifestations of FD (pain in the extremities, corneal changes and angiokeratoma) develop in childhood. Progressive renal insufficiency and cardiovascular involvement are the main causes of premature death.

Aims: This work provide evidence for the needful of combining biochemical and molecular tests to diagnose hemizygotes, heterozygotes and symptomatic female carriers of FD.

Methods: FD diagnosis methodologies underlay in three approaches:?-Gal A activity: measured in capillary Dried Blood Spots (DBS), peripheral blood plasma and total leukocytes, and in cultured skin fibroblasts;GB3: urinary excretion measured in 24 hour urine;Genotype analysis: *GLA* gene mutations identified by sequencing of exons and exon-intron boundaries.

Results: This work report clinical, biochemical and molecular data of 105 patients from 51 unrelated Portuguese families. Partial reduction or absence of ?-Gal A activity confirmed diagnosis in all male patients. Mutated alleles associated with ?-Gal A pseudodeficiency may also result in low/reduced ?-Gal A activity. Wide phenotypic variability in clinical manifestations and biochemical parameters was observed in these cases, which remain to be classified as Fabry patients. In female carriers, ?-Gal A activity may range from zero to control values, thus, FD diagnosis in females can only be made through molecular genetic tests.

Conclusions: FD diagnosis is not straight forward through ?-Gal A enzymatic activity, and frequently requires a combination of different technical approaches, even in male patients due to ?-Gal A pseudodeficiency. X chromosome inactivation can mask obligate carriers, leading to normal ?-Gal A activity, so molecular analysis is the only effective approach to overcome this obstacle. Identification of a ?-Gal A mutation associated with a clinically relevant phenotype would be extremely useful for disease progression evaluation, as well as for enzyme replacement therapeutic decisions.

P-17

HUNTER SYNDROME, THE MOST PREVALENT MUCOPOLYSACCHARIDOSIS IN PORTUGAL

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Introduction: Lysosomal storage diseases (LSD) is a group of rare diseases which involves more than 50 inherited metabolic diseases, being Hunter disease - Mucopolysaccharidosis type II (MPS II)- MIM 309900, an inherited X-linked LSD. MPS II is due to iduronate-2-sulfatase (IDS) enzymatic deficiency, that leads to impaired hydrolyses of terminal iduronate 2-sulfate esters into heparan and dermatan sulfate. More than 300 mutations have been reported in *IDS* gene, located at Xq28, and MPS II has an estimated prevalence of 1 in 170 000 male live births. As well as in other MPS disorders, there is a wide clinical variability, ranging from mild to severe clinical phenotype. The availability of enzyme replacement therapy improves many of the symptoms and signs of the disease.

Aim: To report the MPS II prevalence in the Portuguese population and present data from enzyme replacement in treated patients.

Methods: MPS II diagnosis as a three steps analytical approach: screening for quantitative and qualitative urinary glycosaminoglicans accumulation, definitive diagnosis iduronate-sulphatase activity determination in blood or cultured fibroblasts and genotype identification by *IDS* gene sequencing to ascertain causal mutations.

Results: MPS II is the most prevalent MPS in Portugal. Since 1984, 33 index patients, belonging to 28 families, were diagnosed and 8 of them were submitted to enzyme replacement therapy. Apparently clinical variability among MPS II patients is a mere reflection of molecular heterogeneity, as patients with an *IDS* gene complete deletion seem to have a more severe form of the disease. A more profound clinical evaluation is required as, until now, no female patients have diagnosed.

Conclusions: Some LSD have a overlapping clinical phenotype with MPS. However, clinicians faced with male affected members in different family generations, should consider MPS II as the first hypothesis in the differential diagnosis. Once the genotype has been ascertained, genetic counseling should include female carrier identification in the affected families. Although the incidence of these genetic diseases is quite low, their combined incidence is 1 in 7000 births, which is in the range considered to be feasible for a newborn screening.

P-18

A ADRENOLEUCODISTROFIA LIGADA AO CROMOSSOMA X EM PORTUGAL

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Introdução: A Adrenoleucodistrofia ligada ao cromossoma X (X-ALD) é a mais frequente das doenças hereditárias do metabolismo de causa peroxissomal.

O défice no funcionamento da proteína transportadora membranar ABCD1 - codificada pelo gene *ABCD1* que se localiza em Xq28 – impede o transporte dos ácidos gordos saturados de cadeia muito longa (AGCML) para o lúmen do peroxissoma, e a sua beta-oxidação, o que se traduz numa acumulação em todos os tecidos. As principais manifestações surgem no córtex adrenal, mielina do sistema nervoso central e células de Leydig dos testículos. Clinicamente há um espectro de apresentação, variando tanto quanto à idade de surgimento como no que respeita ao tipo de manifestações, podendo ocorrer dentro da mesma família. O diagnóstico laboratorial baseia-se na quantificação dos AGCML. Uma percentagem de mulheres com mutações causais de X-ALD apresenta valores normais nestas determinações pelo que o estudo molecular do gene *ABCD1* deve ser realizado nos casos de indivíduos do sexo feminino com forte indicação clínica.

Objetivo: Neste trabalho pretende-se apresentar a caracterização dos indivíduos com mutações no gene *ABCD1* estudados no nosso laboratório.

Métodos: O doseamento dos AGCML é feito por cromatografia gasosa em amostras de plasma, soro ou fibroblastos de pele cultivados.

O estudo molecular do gene *ABCD1* inicia-se por PCR e sequenciação das regiões codificantes do gene e respetivas zonas flanqueantes.

Resultados: São apresentados os dados bioquímicos e moleculares, bem como a informação clínica disponível, relativamente a 118 indivíduos - 57 do sexo masculino e 61 do sexo feminino - portadores de mutações no gene *ABCD1*, pertencentes a 43 famílias diferentes.

Foram encontradas 40 mutações diferentes no total das 43 famílias portuguesas com indivíduos com X-ALD, pelo que praticamente cada família tem a sua mutação "privada".

Conclusões: A grande maioria dos indivíduos do sexo feminino estudados foi-o em resultado do estudo familiar e aconselhamento genético, tendo sido poucos os encaminhados por suspeita clínica. Pode ser o resultado tanto de uma apresentação mais atenuada da patologia como da frequente confusão dos sintomas com os de outras patologias - situação que também ocorre em indivíduos do sexo masculino - entre as quais a esclerose múltipla.

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