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

Rute Pereira<sup>1,2</sup>, Jorge Oliveira<sup>3</sup>, Rosário Santos<sup>3</sup>, Ângela Alves<sup>2</sup>, Elsa Oliveira<sup>2</sup>, Luís Ferraz<sup>4</sup>, Alberto Barros<sup>5</sup>, Mário Sousa<sup>2</sup>

<sup>1</sup> Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Porto, Portugal;

<sup>2</sup> Departamento de Microscopia, Laboratório de Biologia Celular, Instituto de Ciências Biomédicas de Abel Salazar, UMIB-FCT, Universidade do Porto, Porto, Portugal;

<sup>3</sup> Unidade de Genética Molecular, Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar do Porto E.P.E., Porto, Portugal;

<sup>4</sup> Departamento de Urologia, Centro Hospitalar de Vila Nova de Gaia E.P.E., Vila Nova de Gaia, Portugal;

<sup>5</sup> Centro de Genética da Reprodução Prof. Alberto Barros, Porto, Portugal  
ruterpereira@gmail.com

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 *DNAI1*, 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.

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**PRENATAL DIAGNOSIS MOSAIC 45, X CASE WITH A MARKER CHROMOSOME**

Joel Pinto<sup>1</sup>, Maria Lina Moreira<sup>1</sup>, Ana Barbosa<sup>1</sup>, Vânia Ventura<sup>1</sup>, Ana Paula Neto<sup>1</sup>, Carla Ramalho<sup>2</sup>, Alberto Barros<sup>1</sup>, Sofia Dória<sup>1</sup>

<sup>1</sup> Genetics Department, Faculty of Medicine, University of Porto, Porto, Portugal;

<sup>2</sup> Obstetrics and Gynaecology Department, Faculty of Medicine, University of Porto, Porto, Portugal  
jpinto@med.up.pt

**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 trisomy 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 derivative from chromosome X.

**Discussion:** Although sSMC are rare, this is a well-known cytogenetic entity. So far, a detailed molecular cytogenetic characterization of sSMC by aCGH was only performed 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”

Carla Caseiro<sup>1</sup>, Jorge Sales<sup>2</sup>, Helena Ribeiro<sup>1</sup>, Elisabete Silva<sup>1</sup>, Domingos Sousa<sup>1</sup>, Lúcia Lacerda<sup>1</sup>

<sup>1</sup> Unidade de Bioquímica Genética, Centro de Genética Médica  
Doutor Jacinto Magalhães, Centro Hospitalar do Porto E.P.E., Porto, Portugal;

<sup>2</sup> Serviço de Pediatria, Centro Hospitalar de Vila Nova de Gaia/Espinho E.P.E., Vila Nova de Gaia, Portugal

carla.caseiro@chporto.min-saude.pt

**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. Ichthyosis 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.