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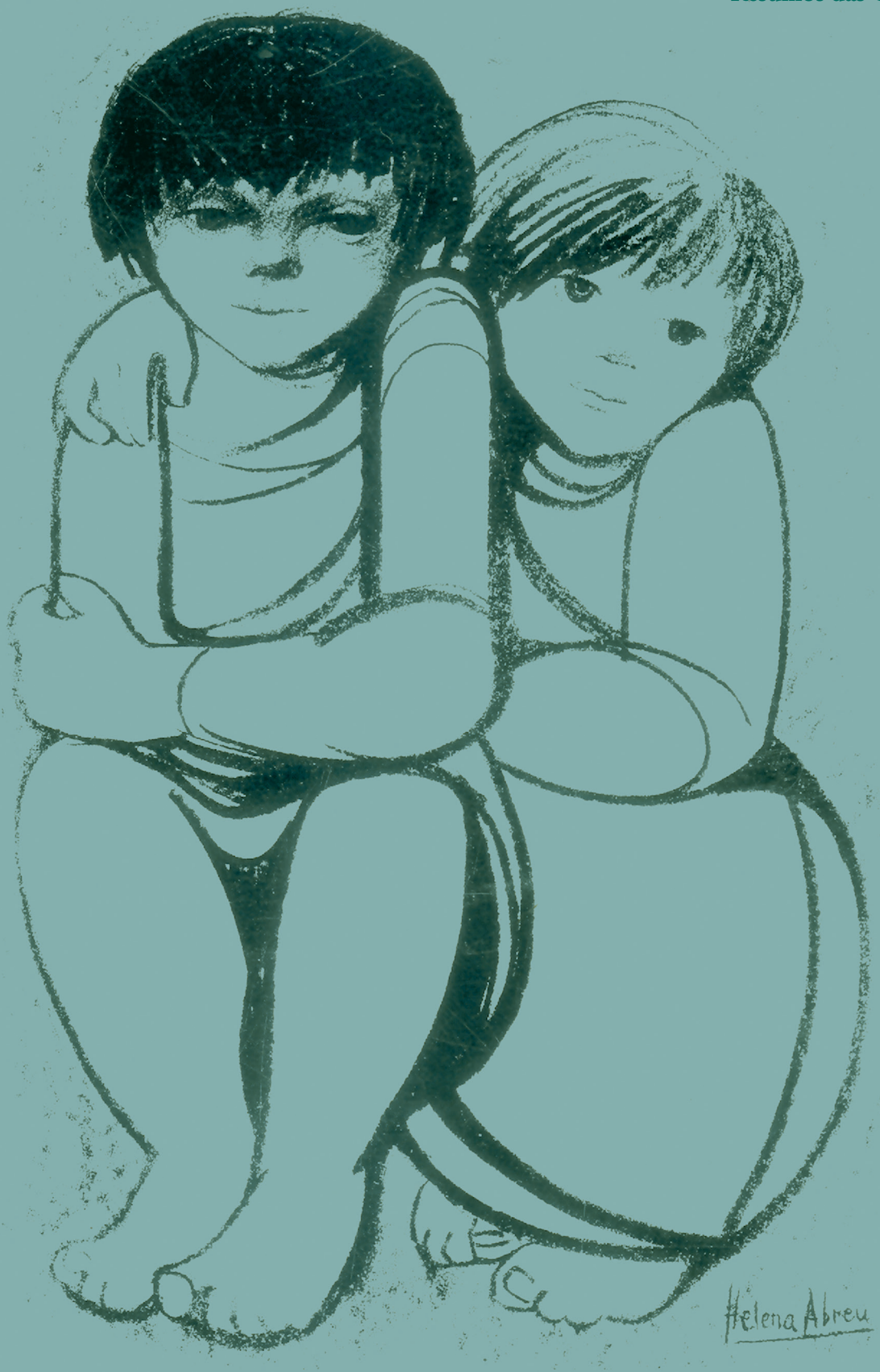
Resumos das Comunicações



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Mobile Phone: (+351) 915 676 516
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Invited speakers
Comunicações por convite

Neuronal Migration | Migração Neuronal

CC_01
NEURONAL MIGRATION: A CRITICAL JOURNEY FOR A HEALTHY BRAIN

Ricardo Rodrigues
Centro de Neurociências e Biologia Celular de Coimbra, Universidade de Coimbra
ricardojrodrigues@gmail.com

Brain development comprises a tightly controlled sequence of events from neurogenesis to cell migration and synapse formation and stabilization, in order to establish a normal neural network. Neuronal migration is a fundamental process during brain development essential for the formation of a proper brain citoarchitecture and the establishment of neuronal/synaptic networks. Indeed, impairment in neuronal migration is one of the major causes of cortical malformation, which has been shown to be associated to several neurological and psychiatric disorders (*Neuron 60:273-841*). Hence, it is of upmost importance to unravel the mechanisms governing neuronal migration.

It has been previously shown in mice that adenosine, through the activation of A_{2A} receptors (A2ARs), controls tangential migration of interneurons (*Sci. Trans. Med. 5:197ra104*). We have now found that A_{2A}Rs also controls the radial migration of mice cortical principal neurons originated from the ventricular zone since: 1) embryos lacking the A_{2A}R showed a delayed migration of cortical principal neurons at embryonic day 17 (E17) in comparison to their wild-type littermates;

2) embryos exposed to the A_{2A}R antagonist SCH58261 (daily 0.1 mg/kg *i.p.* injection in pregnant females from E13 to E16) have shown delayed migration when compared with embryos exposed to vehicle; and 3) the knockdown of A2ARs in migratory neurons through the *in utero* electroporation of plasmid encoding shRNA specific for A_{2A}R at E14 also delayed migration. In each experimental approach, the antagonism of A2ARs led to an accumulation of neurons in the intermediate zone, where it is required a transition from a multipolar to a bipolar shape and the establishment of an axon-like leading process in order for neurons to proceed their migration into the cortical plate (*Nat. Neurosci. 12:1693-700*). Accordingly, we found that ~50% of mice primary cortical neurons cultured in the presence of the selective antagonist of A_{2A}Rs, SCH58261 (50 nM),(applied at DIV 0) could not form axons (evaluated at DIV 3), showing that A_{2A}R is required for proper cortical principal neuronal migration, in particular for the transition from the intermediate zone into the cortical plate, by controlling the establishment of neuronal polarity. This is particularly relevant due to the well-known impact of caffeine in adenosine receptors.

Furthermore, we could also find that purinergic receptors are also critical in the tangential migration of cortical interneurons. Besides A_{2A}Rs, we have now found that P2 receptors (P2Rs) are also necessary for interneurons migration, in particular those derived from medial ganglionic eminence (MGE). In MGE explants cultures, the selective blockade of P2Y1R (MRS2179, 10 μM) significantly decreased the migration of interneurons from the MGE explant, while the pharmacological activation of P2Y1R (MRS2365, 100 nM) did not modify cell migration. However, in the presence of apyrase, an enzyme that removes the extracellular ATP and ADP, the selective activation of P2Y1R (MRS2365, 100 nM) increased significantly the migration of MGE cells, confirming that P2Y1R promotes MGE-derived interneurons migration. On the other hand, the Bz-ATP (a preferring agonist for P2X1 and P2X7 receptors) inhibited in a dose-dependent manner the MGE-interneurons migration. This effect was prevented by the P2Rs antagonist PPADS (10 μM), but not by selective antagonists of P2X7R. These findings show that P2Rs can regulate interneurons migration in a bidirectional manner, promoting it through the activation of P2Y1R and inhibiting it through the activation of P2X1R. Furthermore, we could observe by immunohistochemistry a predominant localization of P2Y1R in the progenitor pools, whereas P2X1R was mainly expressed in the migratory pathways throughout the telencephalon but absent in the proliferative regions. These findings support a two-stage model for the role of P2Rs in cortical interneurons migrations. At an initial stage, ATP/ADP through the activation of P2Y1R functions as a motogenic factor necessary to push interneurons away from the progenitor pools. Once outside, the expression of P2X1R ensures that interneurons do not enter again the progenitor pools, thus playing a crucial role in the guidance of the cells towards the neocortex.

Neuronal Migration | Migração Neuronal

Altogether, these findings identify a novel signalling system critically involved in neuronal migration and corticogenesis.

Supported by FCT (EXPL/NEU-NMC/0612/2012;PTDC/NEU-MC/3567/2014), EU (M.Curie:cycle4-2013-PT07) and Alzheimer's Association (NIRG-15-361884).

CC_02

DPN DAS ANOMALIAS CEREBRAIS

Maria Céu Rodrigues
Centro Materno Infantil do Norte - Centro Hospitalar do Porto
mcpprodrigues@gmail.com

As anomalias do Sistema Nervoso Central (SNC) são, a seguir às cardiopatias congénitas, as anomalias fetais mais comuns.

A avaliação ecográfica básica do SNC inclui três planos da cabeça fetal – transtalâmico, transventricular e transcerebeloso – e três planos da coluna vertebral – axial, coronal e sagital. Se as estruturas visualizadas nestes diferentes cortes são normais, podemos concluir pela normalidade do SNC do feto. Se, pelo contrário, anormalidades são encontradas, há que prosseguir o estudo imagiológico, com recurso à Neurosonografia e/ou Ressonância Magnética.

Assim, quando o exame ecográfico do SNC do feto não é normal, há que fazer o diagnóstico das alterações encontradas, pesquisar lesões associadas e estabelecer o prognóstico.

Aborda-se o diagnóstico das anomalias da linha média, da fossa posterior, do manto cerebral e as anomalias da calote craneana.

Neuronal Migration Anomalies | Anomalias da Migração Neuronal

CC_03
NEURONAL MIGRATION DISORDERS: A NEUROIMAGING APPROACH

José Eduardo Alves
Hospital Santo António - Centro Hospitalar do Porto
zeedualves@gmail.com

Recent advances in the genetic basis of cortical development and malformations improved our knowledge of the biological pathways underlying these disorders and offer a new approach to their classification. Boundaries between disorders of neuronal proliferation, migration or subsequent cortical organization are fading as we have come to realize that the same genes are implicated in different developmental stages. Disorders of microtubule function are a newly recognized group of malformations, with similar genetic underpinnings and sharing some characteristic brain imaging findings.

Although genotype-phenotype correlation is still a challenge, neuroimaging plays an essential role in the pre- and post-natal diagnosis of brain malformations and guiding further investigation.

CC_04
GENETIC DIAGNOSIS

Maria João Sá
Centro de Genética Médica Doutor Jacinto Magalhães - Centro Hospitalar do Porto
m.joao.sa@chporto.min-saude.pt

Cortical malformations associated with an abnormal neuronal migration frequently result in intellectual disability and refractory epilepsy. Genes that cause periventricular nodular heterotopia, lissencephaly and subcortical band heterotopia have been defined. They are often, although not exclusively, involved in the function of the actin and microtubule cytoskeletons. Genetic testing may be straightforward in some neuronal migration disorders with well-known genotype-phenotype correlations. However, it may require a complex diagnostic algorithm, including the use of next-generation sequencing techniques, to assess overlapping phenotypes caused by pathogenic variants in different genes. A multidisciplinary approach is thus recommended in etiologic diagnosis of neuronal migration disorders.

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CC_05

THE GENETICS OF EPILEPSIES

Carla Marini
Meyer Children's Hospital - UF, Florença, Itália
carla.marini@meyer.it

Epilepsies are a highly heterogeneous group of neurologic disorders that encompass several syndromes with variable severity ranging from benign to progressive and catastrophic. Epilepsy are characterized by an heterogenous background in terms of seizure types, age of onset, clinical features, electroencephalographic expression, response to treatment and most of them have a strong genetic component. This genetic component has been demonstrated for a variety of epilepsies and epilepsy syndromes including some early-onset epileptic encephalopathies (EOEE), a group of the severe epilepsies, for which *de novo* mutations have been identified in a plethora of genes. The genetic etiology has also been elucidated for some benign epilepsies with neonatal-infantile onset and for some focal epilepsies that might carry mutations in some genes involved in brain functions. At present, from 100 to 200 genes are tested for diagnostic purposes using Next-Generation Sequencing (NGS) and gene panels. This landscape is further complicated by the genetic heterogeneity often observed in epilepsy syndromes in which several genes may be involved, each with a low mutation rate. Most mutations are *de novo* dominant but some have an autosomal recessive inheritance with homozygous or compound heterozygous alleles. Autosomal dominant mutations with parental mosaicism, or an X-linked inheritance with unbalanced X-inactivation, should also be taken into account. Ion channel genes have long been considered to be the only significant group of genes implicated in the genetic epilepsies, but a growing number of non-ion-channel genes have more recently been identified. Thus the scenario of genetic diagnostic testing has significantly changed. This presentation will focus on genetics of epilepsies also considering its impact on the improvement of the clinical management, avoiding time consuming investigations, and guiding treatment in some cases.

CC_06

DISORDERS OF INTELLECTUAL DEVELOPMENT: BEYOND GENETICS AND GENOMICS

Cristina Dias
Francis Crick Institute, Londres, Reino Unido
cristina.dias@sanger.ac.uk

Intellectual disability (ID), or intellectual developmental disorder, is a common disorder that affects 1 to 2.5% of individuals. It is clinically and aetiologically heterogeneous. Up to 30% of individuals with ID also have Autism Spectrum Disorder (ASD). The prevalence of ID in patients with ASD has been estimated to be as high as 40 to 68%. Overlap between ID and ASD is further evidenced by the overlap in genetic aetiologies of the two conditions.

Whereas external non-genetic factors can adversely affect early brain development manifesting as ID, we estimate that 60% of individuals with severe ID have a genetic or chromosomal abnormality underlying their condition. The increase in diagnostic yield afforded by next generation sequencing technologies has increasingly identified genetic causes of sporadic and familial ID. Over 900 genes are currently thought to be associated with ID and related disorders.

The identification of novel genetic causes of ID and ASD has revealed that while each genetic cause may be individually rare, genes involved in transcriptional regulation are overrepresented in cohorts of individuals with neurodevelopmental disorders, specifically genes controlling chromatin modification and covalent modifications of histones. We have focused our research on BAF swi/snf ATPase chromatin modifiers and related genes. Notably, mutations in BAF complex gene *ARID1B* is one of the most common causes of ID and is implicated in ASD. Using animal and cellular models, we can further investigate the molecular underpinnings of these disorders and convergent epigenetic mechanisms involved in ID. I will review the current understanding of the genetic aetiologies of ID, and discuss the advances in translational research in "epigenetic" neurodevelopmental disorders.

LIPOFUSCINOSES

CC_07

THE NEURONAL CEROID LIPOFUSCINOSES OR BATTEN DISEASE

Sara E Mole

MRC Laboratory for Molecular Cell Biology, UCL GOSH Institute of Child Health, Department of Genetics, Evolution and Environment, University College London, Londres WC1E 6BT, Reino Unido

s.mole@ucl.ac.uk

The neuronal ceroid lipofuscinoses (NCL, Batten disease) are a group of inherited monogenic neurodegenerative diseases characterised by the accumulation of autofluorescent lipofuscin-like (age pigment) material in lysosomes, and neuronal loss. Those affected suffer seizures, visual failure, declining mental and motor skills, and die prematurely. The age of onset is usually in childhood, but ranges from birth to late in adulthood. Thirteen genes are known to cause NCL, with over 440 mutations listed in the NCL Mutation Database (http://www.ucl.ac.uk/ncl). These genes are named *CLN1/PPT1, CLN2/TPP1, CLN3, CLN4/DNAJC5, CLN5, CLN6, CLN7/MFSD8, CLN8, CLN10/CTSD, CLN13/CTSF*, as well as *CLN11/GRN*, *CLN12/ATP13A2* and *CLN14/KCTD7* described in a few families. There is a characteristic disease phenotype recognised for most NCL genes that is associated with complete loss of gene function, as well as disease with a later age of onset or more protracted that arises when partial gene function remains. Some mutations in NCL genes cause a distinct disease phenotype. Some adult cases carry mild mutations in genes that usually cause NCL in childhood and others in genes that cause onset only in adulthood. Recent advances in DNA sequencing technologies provide the means to identify the genetic basis of disease in single families and is changing diagnostic practice. A new nomenclature for NCL has been developed that is gene-based. There is still a need to understand the molecular and cellular basis of the NCLs as well as to develop new therapies.

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Oral communications
Comunicações orais

CO_01

SANFILIPPO SYNDROME REGISTRY PROJECT AND NATURAL HISTORY STUDIES: AN EXAMPLE OF PATIENTS, PARENTS AND RESEARCHERS COLLABORATING FOR A CURE

Jill Wood[1,2], Stuart Siedman[3], Cara O'Neill[4], Paul Levy[5], Kyle Brown[6], Megan Donnell[7], Raquel Marques[8], Arleta Feldman[9], Belen Zafra Garcia[10], Guilhain Higonnet[11], Sean Ekins[2], Robert Pleticha[12]

[1] *Jonah's Just Begun, P.O. Box 150057 Brooklyn, NY11215, USA*
[2] *Phoenix Nest, Inc. P.O. Box 150057, Brooklyn NY11215, USA*
[3] *Ben's Dream, Sanfilippo Research Foundation, P.O. Box 81268, Wellesley, M0248-0002*
[4] *Cure Sanfilippo Foundation, PO Box 6901 Columbia, SC2926*
[5] *The Children's Hospital at Montefiore, MMCCHAM, 3415 Bainbridge Avenue, Bronx, NY10467*
[6] *Patient Crossroads, 4West 4th Avenue, Penthouse C, San Mateo, CA94402*
[7] *Sanfilippo Children's Foundation, PO Box 475, Freshwater NSW2096, Australia*
[8] *Associação Sanfilippo Portugal, Apartado 544, 2821-901 Lisboa, Portugal*
[9] *Mały Macieki Wielcy Czarodzieje OS, Mozarta 1/32, 31-232Krakow, Poland*
[10] *Asociación Sanfilippo Barcelona, Parc Gernika15, Barcelona, Spain*
[11] *Associacion Sanfilippo Sud, Les Planes 82210 Saint Arroumex, France*
[12] *Rare Connect.org, A Partnership of Eurordis and NORD, Spain*
sanfilippoportugal@gmail.com

In the past years, clinical trials, for Sanfilippo type A, have been conducted, namely Lysogene's gene therapy and Nationwide Children's Hospital started gene-therapy clinical trials for type A and is aiming to start it for type B. For other Sanfilippo types C and D the research is at preclinical stages including gene-therapy (Type C) and developing and characterizing a knock-out mouse model (Type D). These recent scientific advancements towards treatments for Sanfilippo Syndrome indicate that it is time for us collect and analyze information on Sanfilippo patients in a single centralized registry as part of the Patient Crossroads CONNECT website (https://connect.patientcrossroads.org/?org=SanfilippoRegistry). In addition it is important we understand how the disease progresses and what differences there may be between the different types. This requires natural history studies (NHS) which can help us in determining the clinical outcome measures, identify potential surrogate endpoints via defined assessments including standardized clinical, biochemical, neuro-cognitive, behavioural, developmental, and imaging measures. From our experiences such data collected from NHS studies are not shared between researchers except when published as papers at a much later date. Sanfilippo Syndrome has a very small patient population and the participation in multiple NHS (which may be occurring simultaneously) places an unrealistic burden on patients and families. Sanfilippo Syndrome is ultra-rare and patients are geographically diverse. Providing patients and families with an outlet to find pertinent information pertaining to Sanfilippo, such as where Natural History Studies and clinical trials are taking place, or making themselves known by participating in a centralized registry, is essential. With the use of RareConnect platform we hope to bring families from around the world closer together and give them access to information that they may not have access to otherwise. We will describe how the data collected from the NHS studies for Types A and B performed at Nationwide Childrens Hospital and for Type C at The Children's Hospital at Montefiore will be available to other qualified institutions to prevent repetition. Such NHS studies and registries can also help in identifying participants for clinical trials. We will illustrate how close collaborations between parent/patient led disease organizations and clinical researchers, is essential to ensure our limited funding and time is well spent as we try to identify treatments.

CO_02

OVERCOMING THE DIAGNOSTIC CHALLENGES IN NEUROLOGICAL DISORDERS: THE ROLE OF NEXT-GENERATION SEQUENCING

Sofia Gouveia[1], Ana Fernández-Marmiesse[1], Francisco Laranjeira[2], Iria Roca[1], Juan A Cocho[1], Daisy Castiñeiras[1], José M Fraga[1], María Luz Couce[1]

[1] Unidad de Diagnóstico y Tratamiento de Enfermedades Metabólicas Congénitas, Hospital Universitario de Santiago de Compostela, Spain

[2] Unidade de Bioquímica Genética, Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal

sofiabsg@gmail.com

Rare genetic diseases affect at least 1 in 50 individuals. There are an estimated 6000-7000 of these diseases, at least half of which are characterized by dysfunction of the central or peripheral nervous system. These clinical presentations involve the full range of the neuroaxis and include monogenic forms of brain malformations, ataxias, encephalopathies, myopathies and muscular dystrophies, neuropathies, movement disorders, metabolism defects, epilepsies, ciliopathies and dementias. Identifying the mutation(s) responsible for any of these varied clinical presentations can be extremely challenging. The chief difficulty resides in the genetically heterogeneous nature of neurological disease. The diagnostic process is often long and complex, without a conclusive molecular diagnosis. The arrival of next-generation sequencing (NGS) coupled with advanced bioinformatics processing is changing the face of rare disease diagnosis by offering faster, less expensive, and higher-resolution genetic testing. Although it is becoming increasingly more common for clinicians to use genomic data in their daily work for disease prevention, diagnosis, and treatment, the process of integrating genomic data into the practice of medicine has been a slow and challenging one. Genomic medicine programs are currently under way at several academic medical centers and large integrated health systems, but it is challenging to identify which genomic applications have robust evidence supporting their use in the clinic to improve patient outcomes.

The present work is centered on a 4-year (2013-2016) cohort study that enrolled over 600 patients who were investigated for putative neurologic and/or metabolic conditions. We present a comprehensive description of the results from the application of an NGS-based platform (NeuroMeGen) to the diagnostic workflow of these patients. The platform consists of several customized gene panels optimized for different groups of disorders clustered by clinical and/or biochemical overlapping. We assess the power of the NeuroMeGen tool and look at its overall and panel-specific diagnostic yield (the epilepsy and neuromuscular panels offered the best results) and the involvement of *de novo* mutations and copy number variations in the mutational load of these diseases. Finally, we discuss the possible causes of non-diagnosis and review the challenges and knowledge gaps identified during the development of NeuroMeGen. This work represents the first broad-scale approach to the implementation of genomic evaluation for the public health system in the Iberian Peninsula.

CO_03

AUTISM SPECTRUM DISORDERS – CLINICAL USEFULNESS OF aCGH

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

[1] Unidade de Genética, Departamento de Patologia, Faculdade de Medicina, Universidade do Porto, Porto, Portugal

[2] I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

[3] MMED - Mestrado Integrado em Medicina, Universidade do Porto, Porto, Portugal

[4] Consulta de Genética - CHSJ, Porto, Portugal

jpinto@med.up.pt

Autism spectrum disorders (ASD) are neurodevelopmental conditions showing extreme genetic heterogeneity. Array CGH offers superior sensitivity for identification of submicroscopic copy number variants (CNV) and it is advocated to be the first tier genetic testing for patients with autism spectrum disorder (ASD). The aim of this study was to correlate pathogenic CNVs or potencially pathogenic CNVs with clinical features in syndromic and non-syndromic ASD patients. Additionally, we would like to identify which variants of uncertain significance (VOUS) taking into account their gene contents or localization should be revised from time to time.

Agilent 4x180K microarrays and cytogenomics 4.0.2.21 software were applied for the study of ASD patients. From 173 patients two main groups were established: ASD isolated patients (non-syndromic) (95) and ASD patients with additional features (syndromic) (78) and organised in two main groups: ASD patients and ASD patients with others features.

We identified a total of 31 pathogenic or potentially pathogenic CNVs plus 33 VOUS. Within these, 16 pathogenic CNVs plus 11 VOUS were found in non-syndromic ASD patients and 15 pathogenic CNVs plus 22 VOUS in syndromic ASD patients. In 137 patients (79 and 58 with non-syndromic and syndromic, respectively), we found no pathogenic CNVs but we identified 215 VOUS. The pathogenic or potentially pathogenic CNVs sizes in ASD patients ranged between 18 Kb and 3Mb in different genomic regions from different chromosomes. A 22q11.21 duplication was present in at least 3 patients with syndromic ASD.

In our study, we are able to identify 19% of ASD patients related with some pathogenic or potentially pathogenic CNVs. The majority were isolated CNVs but we also identified 3 patients with a similar duplication on 22q11.21 presenting ASD and macrocephaly. We would like to highlight the clinical usefulness of aCGH as a first-tier test in evaluation of syndromic and non-syndromic ASD patients. It is highly recommended to perform the study of all pathogenic/potentially CNVs and also for some VOUS in the progenitors using aCGH or other techniques such as Q-PCR or MLPA. DNA from patients with no pathogenic/potentially CNVs with the aCGH test should be submitted to an ASD genes panel.

CO_04
CHL1 TETRASOMY IN A PATIENT WITH AUTISM SPECTRUM DISORDER AND DEVELOPMENTAL DELAY: A CASE REPORT

Teresa Saraiva[1], Maria João Sá[1,2], Gabriela Soares[1], Ana Maria Fortuna[1,2]

[1] *Serviço de Genética Médica, Centro Materno Infantil do Norte, Centro Hospitalar do Porto, Porto, Portugal*

[2] *Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto - UMIB/ICBAS/UP*

teresaosaraiva@gmail.com

The *CHL1* gene is located at the chromosomal sub-band 3p26.3 and encodes a protein that is part of the L1 family of neural cell adhesion molecules. It is highly expressed in the central and peripheral nervous system and plays an important role in synaptic plasticity and nervous system development. Few patients have been previously described in the literature with mutations in the *CHL1* gene. Mice functional studies showed that *CHL1* haploinsuficiency is associated with mental impairment. In humans, 3p26.3 microdeletions including only this gene have been described in the literature associated with a spectrum of neurodevelopmental disorders. To date, only two microduplications encompassing only *CHL1* gene have been reported in patients with autism spectrum disorder, developmental delay and minor dysmorphic facial features.

The authors report a 4 years old boy with autism spectrum disorder and developmental delay. Chromosomal microarray analysis showed an approximately 346 Kb 3p26.3 tetrasomy that only involved the *CHL1* gene. This alteration was transmitted from his apparently unaffected mother. She had epilepsy in childhood.

This is the first report of *CHL1* tetrasomy in a patient with autism spectrum disorder and developmental delay. The phenotypic variability observed between mother and son can be suggestive of incomplete penetrance, variable clinical expression, effect of undetected copy number variants, differences in genetic background and epigenetic phenomena. Because the number of reports is limited, the identification of new cases can be helpful to characterize the clinical features associated with this copy number variant and to understand the potential effect of *CHL1* overexpression in cognitive development.

CO_05
EXPANDING THE *MEF2C* HAPPLO- INSUFFICIENCY SYNDROME: REPORT OF A NEW *MEF2C* MUTATION

Joana Rosmaninho-Salgado[1], Lina Ramos[1,2], Jorge M Saraiva[1,3], Sérgio B Sousa[1,2]

[1] *Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal*

[2] *Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal*

[3] *University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra, Portugal*

jrosmaninho.salgado@chuc.min-saude.pt

MEF2C haploinsufficiency syndrome was recently recognized as a neuro-developmental disorder, characterized by severe intellectual disability with absent speech and limited walking abilities, hypotonia, seizures, a variety of minor brain anomalies and minor dysmorphisms. We report a case of a patient with a *de novo MEF2C* mutation, not previously described in the literature.

We describe a 10-years old patient born at term, with non-consanguineous parents, that was referred with severe milestones delayed with absent speech and walk and a postnatal microcephaly. He was first observed at eleven months of age for generalized hypotonia and poor eye contact. At age of 14-16 months of age he experienced the first episodes of epileptic seizures. At four years of age, a brain MRI was performed with a description of areas compatible with hypoxic-ischemia lesions. Nerve conduction studies were suggestive of alterations at dorsal column-medial lemniscus pathway level. Extensive etiological study was performed with inconclusive results: karyotype, *FRAXA*, *DGUOK* and *UBE3A* molecular analyses, biochemical metabolic screening (lactic acid, pyruvate acid, ammonia, plasmatic and urinary aminoacids, urine organic acids, blood and urinary creatine), CSF analysis, biochemical mitochondrial studies and an array-based comparative genomic hybridization (aCGH) assay. Subsequently, clinical exome was performed and a heterozygosity for pathogenic variant in *MEF2C* gene, c.70A>G (p.Arg24Gly) was found. This variant was considered likely pathogenic as it was not previously reported in literature or any database. The amino acid residue affect is highly conserved and it is predicted to be disease causing by *in silico* prediction tools.

MEF2C encodes myocyte-specific enhancer factor 2C protein, expressed in neurons and it regulates the activity of many genes involved in brain development, because it is expressed in excitatory and inhibitory neurons. Thereby alterations in *MEF2C* might prompt to neurodevelopmental disorders by inhibiting the number of excitatory synapse and thus regulating synaptic transmission. The presented report constitutes one of the 9 described cases, with a new mutation, not previously described. This case will help in the delineation of the clinical phenotype associated with this gene and the previous brain MRI report should be revised. This diagnosis allows a more personalized management and accurate genetic counselling.

CO_06

JOUBERT SYNDROME AND RELATED DISORDERS: OUR EXPERIENCE

André M Travessa[1], Patrícia Dias[1], Isabel Cordeiro[1], Ana Berta Sousa[1]

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

at1@campus.ul.pt

Classical Joubert syndrome (JS) is a neurodevelopmental disorder characterized by the typical "molar tooth sign" on head MRI, hypotonia, developmental delay (DD), ataxia, irregular breathing pattern and abnormal eye movements. The designation JS and related disorders (JSRDs) describes individuals with additional findings, observed in other ciliopathies, including retinal dystrophy, renal disease, ocular colobomas, hepatic fibrosis and polydactyly. JSRDs are highly heterogeneous genetic pathologies. We report our experience with four cases of JSRDs diagnosed in our Department.

Between 2003 and 2016, four patients were referred to our department because of a suspected diagnosis of JS. Medical charts were retrospectively reviewed to assess patients' clinical and genetic data.

All patients presented to our consultation with "molar tooth sign" on head MRI, neurological symptoms including hypotonia and ataxia, DD, nystagmus and visual loss. In all cases, family history was unremarkable with no consanguinity.

The first patient was an 8-year-old boy with retinal dystrophy and cystic kidney disease with mild renal failure. The diagnosis of JS with oculorenal disease was clinically made. Sanger sequencing of *MKS1*, *MKS3*, *INPP5E*, *NPHP1*, *RPGRIP1L* and *CC2D2A* genes was normal. The patient died at 9 years of age.

A 3-year-old girl also presented with retinal dystrophy. A NGS panel of genes responsible for JS was performed and the variants c.394C>T (p.Gln132*) and c.599G>A (p.Arg200His) were found in compound heterozygosity in the *ARL13B* gene. Parental segregation analysis concluded that each variant was inherited from a different parent.

In a 6-month-old boy with typical signs and symptoms of JS, the variants c.1219_1220delAT (p.Met407Glufs*14) and c.1666delA (p.Ile556Phefs*17) were found in compound heterozygosity in the pleomorphic gene *CEP290* in a multi-gene NGS panel; they were inherited from his mother and father, respectively.

More recently, a 3-year-old girl presented with prenatal hydrocephaly that required ventriculoperitoneal shunt placement at 7 months of age. A NGS panel of genes causative for ciliopathies revealed the variant c.2364_2365del (p.Lys789Argfs*9) in homozygosity in *CSPP1*, a gene recently associated with JS. JSRDs are a complex group of pathologies both in its clinical manifestations and genetic etiology.

NGS multi-gene panels are now a first line analysis and allowed molecular diagnosis in three of our cases. Only the older case remained without a genetic diagnosis possibly because of the limited number of genes analyzed.

Establishing genotype/phenotype correla-tions in JSRDs is important for a more accurate prognosis and counselling, but the significant clinical overlap between the ciliopathies makes this a difficult task. Long term follow-up of patients with JSRDs might help in this endeavor.

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Poster abstracts
Resumos de posters

P_01

NEW INSIGHTS INTO XLOS PHENOTYPE: THE CLINICAL, NEUROCOGNITIVE AND BEHAVIOURAL FEATURES OF 4 PATIENTS FOLLOWED AT CGM/CHP

Carla Carmona[1,2], Nuno Maia[1,2], Paula Jorge[1,2], Gabriela Soares[1], M João Nabais[1,2], Nataliya Tkachenko[1], Ana Rita Soares[1], Rosário Santos[1,2], Ana Maria Fortuna[1,2]

[1] *Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal*

[2] *Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto - UMIB/ICBAS/UP*

carla.carmona@chporto.min-saude.pt

Opitz G/BBB syndrome (OS) has two inheritance subtypes: autosomal dominant (OMIM #145410) and X-linked (XLOS, OMIM #300000). Although XLOS has been defined as a developmental/intellectual disability syndrome (DD/ID) several patients with a normal intelligence have been described. This is suggestive that a yet unidentified genetic modifier factor has a protector effect on the ID phenotype development. The goal of this study was to characterize our XLOS patients not only on their clinical features but also on the neurocognitive and adaptive functioning aiming a better understanding of this genetic condition and a more appropriate intervention and disease management.

We evaluated the clinical features of 4 XLOS patients, with a mutation on *MID1* gene aged 6, 10, 17 and 22 years diagnosed between 0 and 5 years old. Patient's outcome was evaluated based on neurocognitive profile using the Griffiths Mental development Scales, the Wechsler Scales (WISC-III and WAIS-III), the educational level and school curriculum, their professional career and general autonomy. Neurocognitive profile and general autonomy will be presented. Results: We found global developmental and intellectual (DQ/IQ) values in the normal range in three patients. One of them, albeit a normal verbal IQ, had a mild cognitive delay conditioned by deficits in specific cognitive domains. Three had difficulties in tasks evaluating fine motor skills and language. These impairments justified the implementation of an adapted curriculum and speech therapy. All of them had a normal level of adaptive functioning. Co-morbidities were observed in one patient reporting depressive mood and low self-esteem.

Although XLOS has been frequently defined as a developmental/intellectual disorder, it is important to evaluate the individual cognitive and adaptive potential of patients with XLOS in order to provide an adequate disease management and avoid social stigmatization.

P_02

NEURONAL CEROID-LIPOFUSCINOSES IN PORTUGAL: THE REFERENCE CENTER CASUISTRY AND INTERESTING CASES

Francisco Laranjeira[1], Isaura Ribeiro[1,2], Carla Caseiro[1], Eugénia Pinto[1], Sónia Rocha[1], Lúcia Lacerda[1,2]

[1] *Unidade de Bioquímica Genética, Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal*

[2] *Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto - UMIB/ICBAS/UP*

ferlaranjeira@gmail.com

Neuronal Ceroid-Lipofuscinoses (NCLs) is a group of lysosomal storage disorders, one of the subsets of inherited metabolic disorders. The overall prevalence of NCL makes it the most common neurogenetic storage diseases and a must-think-of suspicion when dealing with pediatric age neurodegenerative conditions, despite the onset may occur at later ages. NCLs are genetically heterogeneous as, presently, thirteen genes are known to be involved - *PPT1, TPP1, CLN3, CLN5, CLN6, MFSD8, CLN8, CTSD, DNAJC5, CTSF, ATP13A2, GRN* and *KCTD7* - usually with a certain degree of correlation with age of onset, some clinical features and course, although the outcome is always fatal as there is no treatment available yet. Symptoms present in a progressive way, and usually include intellectual and motor deterioration, seizures and visual loss.

Definitive classification of NCL type is based mostly on gene defect because protein/enzymatic assays are only possible for a few ones and lipofuscin pigment accumulation – a hallmark of these diseases – patterns are not fully type-specific.

NCL3 is the most prevalent type and has a very frequent disease causing allele carrying a "1kb deletion". There might be a therapeutic hope for NCL2 affected patients, currently in clinical trials.

This cohort includes patients with clinical suspicion of NCL that were laboratory confirmed in our center over the last decades. For NCL1 and NCL2 testing enzyme activity assays were used and PPT1 or TPP1 gene analysis, respectively, was performed to search for causative genetic variants, accordingly. For the diagnosis of the other types of NCL, variant specific PCR amplification and sequencing were the standard procedures. Recently, next generation sequencing (NGS) analysis was used to solve some unusual situations.

Thirty-nine patients, belonging to 32 families, were diagnosed so far. Thirteen families were affected with NCL3, 8 with NCL2,

7 with NCL6 and one family with the cause laying in each NCL1, NCL5, NCL7 and NCL8. Data available regarding patient's clinical and laboratory findings is presented. Some cases are depicted in more detail because either the complex diagnostic quests required special strategies or are illustrative of benefits and limitations of NGS.

The most prevalent type of NCL in Portugal is NCL3 and the "1kb deletion" allele is present in all families, except two. If approved, therapeutic for NCL2 will have a great impact in NCL treatment as this is the second most prevalent type.

NGS approach unravelled a concomitant condition in a patient affected also with NCL6. This is an example of what might be underlying some unusual clinical presentations that don't fit a specific disease and often delay the diagnosis.

Family study gives precious information, even more necessary in the NGS approaches.

P_03

LYSOSOMAL LEUKODYSTROPHIES: KRABBE DISEASE AND METACHROMATIC LEUKODYSTROPHY

Isaura Ribeiro[1,2], Carla Caseiro[1], Eugénia Pinto[1], Elisabete Silva[1], Sónia Rocha[1], Helena Ribeiro[1], Célia Ferreira[1], Francisco Laranjeira[1], Dulce Quelhas[1,2], Lúcia Lacerda[1,2]

[1] Unidade de Bioquímica Genética, Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal

[2] Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto - UMIB/ICBAS/UP

isaura.ribeiro@chporto.min-saude.pt

Leukodystrophies (LD) are genetic disorders that lead to white matter abnormalities in the central nervous system, with or without peripheral involvement. These disorders have a large spectrum of clinical manifestations, from neonatal to adult forms. There are over thirty conditions categorised as LD. This work will focus on two lysosomal LD, Krabbe disease (KD) and Metachromatic leukodystrophy (MLD). KD and MLD are neurodegenerative disorders of autossomic recessive nature, characterized by demyelination of the central nervous system. KD is caused by mutations in the galactosylceramidase gene (GALC).This gene encodes the galactocerebrosidase (GALC) enzyme that is involved in the catabolism of galactosylceramide and galactosylsphingo-sine.

In MLD, mutations on arylsulfatase A gene (ARSA) lead to arylsulfatase A (ARSA) enzyme deficiency or lack of cofactor due to mutations on prosaposin gene, causing accumulation of sulphatides in various tissues.

To report casuistic, biochemical and genetic data as well as clinical phenotypes of KD and MLD Portuguese patients, diagnosed since 1982 until 2016. Molecular data of this group of patients will also be presented and discussed. Biochemical diagnosis of KD and MLD is achieved by measurement of GALC and ARSA activity, respectively, either in leukocytes or cultured skin fibroblasts and urinary sulphatides excretion in MLD. Diagnosis is completed by genotyping analysis of GALC and ARSA genes. Screening of pseudodeficiency alleles in both LD is important due to high frequency of these alleles in the general population. 64 patients were diagnosed with KD and MLD. MLD account for 63% of the total lysosomal LD, in which 56% are infantile forms. Regarding KD, 87% of these patients were infantile forms. GALC gene molecular analysis revealed that the 30 kb deletion was particularly common (22% of KD patients) and in homozygosity is associated with infantile forms. In MLD patients, mutations c.459+1G>A (allele I) and c.1428T>G (p.I179S) account for about 80% of the mutated alleles. Allele I in homozygosity is associated with an infantile onset.

Current data demonstrates that MLD is the most common lysosomal LD in Portuguese population.

Molecular characterization may allow to establish genotype-phenotype correlations, as well as to offer genetic counseling and prenatal diagnosis to affected families.

With this work we also intend to highlight the importance of a multidisciplinary approach, combining clinical, neuroimaging and laboratory tests to perform a reliable and early diagnosis of these entities.

P_04

THE IMPACT OF CHROMOSOMAL MICROARRAYS IN THE DIAGNOSTIC YIELD OF A NEURODEVELOPMENT CLINIC - 3 YEARS OF EXPERIENCE

Ana R Soares[1], Diana Gonzaga[2], Inês V Matos[2], Inês Maio[2], Nataliya Tkachenko[1], Gabriela Soares[1], Raquel Lemos[3], Joaquim Sá[4], Paula Rendeiro[3], Ana M Fortuna[1,5], Catarina Prior[2]

[1] *Medical Genetics Department, Centro Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar Porto, Porto, Portugal*

[2] *Neurodevelopment Unit, Centro Materno-Infantil do Norte, Centro Hospitalar do Porto, Porto, Portugal*

[3] *Laboratório de Citogenética, CGC Genetics*

[4] *Departamento Clínico, CGC Genetics*

[5] *Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto - UMIB/ICBAS/UP*

anaritamsoares@gmail.com

Chromosomal Microarray (CMA) is being increasingly used by clinical geneticists and neurodevelopment paediatricians as a first-line test in the postnatal evaluation of patients with multiple anomalies not attributable to a well-defined genetic syndrome, non-syndromic developmental delay/intellectual disability (DD/ID) and/or autism spectrum disorder (ASD). CMA analysis allowed the identification of new syndromes caused by submicroscopic chromosomal imbalances.

The aim of this work was to report the experience of a Neurodevelopment Paediatrics clinic in a Central Hospital since the introduction of CMA analysis in clinical practice.

Our study included the patients in whom CMA analysis was requested and which results were retrieved from January 2013 to December 2016. Clinical characterization of patients, type of the detected variation and specific diagnosis were described.

Seventy-five patients (43 males and 32 females) were analysed, with a medium age at first consultation of 7.95 years old. The detection rate was about 15%. From 19 positive CMAs, 11 provided a specific diagnosis of a submicroscopic syndrome. Furthermore, variants of unknown significance (VOUS) were found in 8 cases.

With this work the authors intended to highlight CMA's role in a Neurodevelopment clinic of a Central Hospital from a small European country. Accordantly to the literature, it proves to be an useful test in the clinical practice allowing new specific diagnosis.

P_05

GLUCOSE TRANSPORTER TYPE 1 DEFICIENCY SYNDROME - A CASE REPORT

Ana M Amaral[1], Juliette Dupont[1], João P Monteiro[2], Ana B Sousa[1]

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

[2] *Centro de Desenvolvimento da Criança Torrado da Silva - Serviço de Pediatria - Hospital Garcia de Orta, Almada, Portugal*

anamiguelamaral@gmail.com

Glucose transporter type 1 deficiency syndrome (MIM 606777) was described by Dr. De Vivo in 1991. It is a rare genetic metabolic disorder classified as an epileptic encephalopathy with a variable phenotypic spectrum. It is caused by mutations in the *SLC2A1* gene and is inherited predominantly as an autosomal dominant trait.

The classic phenotype is characterized by infantile-onset seizures, developmental delay, acquired microcephaly and complex movement disorders, including ataxia, dystonia and chorea. Seizures, in general refractory to treatment, begin before two years of age in 90% of the cases. Affected individuals present varying degrees of cognitive impairment, ranging from learning difficulties to severe intellectual disability.

Neurocognitive outcome often improves substantially when a ketogenic diet is started before 6 months of age. The epilepsy and the movement disorder respond well to treatment independently of when it is started.

We describe a 3 year-old girl who was referred to the Genetics Department at 2 years of age due to global developmental delay, post-natal microcephaly and epilepsy, which started when she was 14 months and responded well to a low dose of valproate. At the time she was the only child of a young non-consanguineous couple with an unremarkable family history.

On physical examination she had microcephaly (<5th centile), an inability to coordinate voluntary movements and sialorrhea. She presented with moderate developmental delay, especially in language and motor skills. Array-CGH was normal.

Overtime her developmental delay worsened progressively, and it was decided to perform whole exome sequencing (WES), trio approach. This analysis detected a *de novo*, heterozygous variant in the *SLC2A1* gene, c.968_972+3del (p.Val232Alafs*53).

The clinical manifestations in our patient are consistent with the diagnosis of Glucose Transporter Type 1 Deficiency Syndrome. The *SLC2A1* variant identified by WES was not present in either parent. Also, it is a frameshift variant, which was classified as likely pathogenic (class 2). Thus, we conclude it is highly likely this variant is the cause of our patient's phenotype.

Molecular diagnosis was determinant: although she was no longer in the therapeutic window of opportunity (first 6 months of life) to avoid neurocognitive damage, a ketogenic diet will be beneficial for both seizure and movement disorder control.

This case clearly illustrates the clinical usefulness of performing WES. Reaching an etiological diagnosis led to a change in treatment which will likely improve the patient's quality of life, and enabled proper genetic counselling concerning recurrence risk in future pregnancies.

P_06

DELETION OF 18Q12.3: A CASE REPORT

Marta Souto[1], Pedro Botelho[1], Márcia Martins[2], Fernanda Pereira[3], Osvaldo Moutinho[4], Rosário Pinto Leite[1]

[1] Laboratório de Citogenética, Centro Hospitalar de Trás-os-Montes e Alto Douro

[2]Consulta de Genética, Centro Hospitalar de Trás-os-Montes e Alto Douro

[3] Serviço de Pediatria, Centro Hospitalar do Nordeste

[4] Serviço de Ginecologia/Obstetrícia, Centro Hospitalar de Trás-os-Montes e Alto Douro

marta_dantas_souto@hotmail.com

The 18q deletion syndrome has an incidence of 1 in 40 000 live births. The clinical phenotype varies depending on the segment deleted and is characterized by mental retardation, developmental delay, seizures, obesity, abnormal behavior, short stature and craniofacial dysmorphisms.

We report the case of a patient with mental retardation, dysmorphic features, hypotonia, growth retardation, severe expressive speech delay and Duane syndrome, with a deletion in 18q resulting from an insertion of 15 and 18 chromosome.

Cytogenetic and array analysis showed a female karyotype presenting a *de novo* rare chromosome rearrangement: an insertion of the 18q21q23 on the 15q22 region, with deletion 18q12.3, involving only the *MIR4319* and *SETBP1* genes.

In literature there are few cases described with 18q12.3 deletion with mild dysmorphic features, mental retardation and impairment of expressive language. Recent studies associated the *SETBP1* haploinsufficiency with expressive speech delay. The authors present a literature review of 18q12.3 deletion.

P_07

AN INVERTED DUPLICATION 8P: A CASE REPORT

Rosário Pinto Leite[1], Marta Souto[1], Pedro Botelho[1], Osvaldo Moutinho[2], Márcia Martins[3]

[1] Laboratório de Citogenética, Centro Hospitalar de Trás-os-Montes e Alto Douro

[2] Serviço de Ginecologia/Obstetrícia, Centro Hospitalar de Trás-os-Montes e Alto Douro

[3] Consulta de Genética, Centro Hospitalar de Trás-os-Montes e Alto Douro

mlleite@chtmad.min-saude.pt

The partial duplication (dup) of the short (p) arm of chromosome 8 is a rare syndrome. To date, no more than 50 cases with direct or inverted duplication 8p has been published. The prevalence of both types is estimated to be 1/22000-30000 of the population. Clinical manifestations vary from healthy to several degrees of mental retardation, multiple congenital anomalies – like hypotonia, heart defects, brain malformations (Dandy-Walker syndrome, dilation of the third ventricle and agenesis of the corpus callosum) and facial dysmorfism. The phenotypic outcome depends on the amount of duplicated genetic material. The authors present a case of inverted duplication and deletion of 8p in 6 years old boy with severe mental retardation, macrocephaly and minor facial dysmorfism. Cytogenetic analysis revealed extra material on the short arm of chromosome 8. Parents karyotypes were normal. Fluorescence in situ hybridization technique identified the extra material as chromosome 8. Array Comparative Genomic Hybridization technique revealed a 910Kb segment deletion on 8p23.3 and a 19.3Mb duplication on 8p23.3p21.3. Despite being a large duplication, this case presented mental retardation as the main characteristic of dup 8p syndrome. All new cases detected should be reported in order to obtain a more precise correlation between genotype/ phenotype to be used for genetic counselling.

P_08

POLYMICROGYRIA - AN ATYPICAL DIAGNOSIS OF 22Q11.2 MICRODELETION

Teresa Carminho Rodrigues[1], Fabiana Ramos[1], Jorge Saraiva[1,2], Sérgio Sousa[1,3]

[1] *Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra (CHUC)*

[2] *University Clinic of Pediatrics, Faculty of Medicine, University of Coimbra*

[3] *Faculty of Health Sciences, University of Beira Interior, Covilhã*

mtcarminho@gmail.com

Polymicrogyria, a type of neuronal migration disorder, is characterized by stable neurologic deficits; Symptoms vary according to the extension, but often include hypotonia, seizures, developmental delays/impaired cognitive development and microcephaly. It can result from both genetic and environmental etiologies (including intrauterine viral infections) and can occur as an isolated finding or as part of a syndrome. To date the only gene known to be associated with isolated bilateral frontoparietal polymicrogyria is *GPR56* but it has also been described in 22q11.2 microdeletion, Aicardi syndrome and in few inborn errors of metabolism.

A 17 months-old boy, no relevant family history, with left side spastic hemiparesis, initially diagnosed as a lesion of brachial plexus at 6 months of age. Frequent episodes of chocking with nasal regurgitation were noted at 2 months of age. Magnetic resonance showed widespread polymicrogyria involving frontoparietal, insular and temporal posterior areas; he also has a prenatal history of atrial septal defect and polycystic left kidney. Currently, he has recurrent respiratory infections, mild developmental delay and some facial dysmorphisms. The suspicion 22q11.2 microdeletion syndrome was confirmed by targeted deletion analysis (MLPA). Familiar studies showed it was a *de novo* deletion.

This atypical case with exuberant neurological involvement shows the remarkable variability in clinical phenotype of 22q11.2 microdeletion. There are practical guidelines published for the management of this syndrome with multisystemic approach including cardiac, endocrine, renal, skeletal, dental, ophthalmology, otorhinolaryngology and immunological surveillance. Prenatal diagnosis should be considered in presence of heart and some type of renal defects. 22q11.2 microdeletion arises *de novo* in ~90% of the cases and in this group recurrence risk in less than 1%. In about 10% of cases one parent can be affected, with 50% recurrence risk. In both situations molecular prenatal diagnosis can be offered.

P_09

MLPA® AS AN APPROACH IN THE DIAGNOSIS OF X-LINKED INTELLECTUAL DISABILITY

Luís Carneiro[1], Nuno Maia[1,2], Paula Jorge[1,2], Raquel Chaves[3,4], Rosário Santos[1,2], Isabel Marques[1,2]

[1] *Unidade de Genética Molecular, Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal*

[2] *Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto - UMIB/ICBAS/UP*

[3] *Laboratory of Cytogenomics and Animal Genomics, Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*

[4] *Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisboa, Portugal*

miguelcarneiro99@gmail.com

Intellectual disability (ID) is an etiologically heterogeneous group of disorders that affects about 2-3% of the general population. In up to 50% of the cases, a genetic etiology has been implicated, but despite extensive evaluation, the cause for ID remains unknown in a significant percentage of cases. Etiological identification is of extreme importance not only for appropriate patient management but also for genetic counselling and prenatal diagnosis in family members.

Copy number variations (CNVs), have been implicated in the etiology of ID, but are frequently too small to be detected by conventional cytogenetic methodologies. As such, in recent years these have been gradually replaced by novel molecular methodologies that have improved the ID diagnostic yield and helped unravel various genetic syndromes. Submicroscopic chromosomal duplications and/or deletions involving the subtelomeric regions are responsible for up to 5 to 7% of all ID cases, and are considered an important genetic cause for X-linked intellectual disability (XLID). These imbalances can be detected by different techniques such as chromosomal microarray (CMA) and Multiplex ligation-dependent probe amplification (MLPA®). MLPA® offers a low-cost and a rapid means of scanning a great variety of genes and is now widely used in molecular investigation of genetic diseases. However, most of the MLPA® probes are present for only some genes and a limited number of target sequences, constituting a limitation of the technique.

Herein, MLPA®-P106 MRX that covers 46 exons of 16 different genes will be tested for the evaluation of non-syndromic XLID (NS-XLID). Our goal is to assess the clinical utility of MLPA®-P106 MRX in the evaluation of NS-XLID, with or without malformations, in 100 male patients with normal karyotype and negative for molecular screening of fragile-X syndrome. No deletion/duplication has been detected, leading to no effective diagnostic yield and consequent requirement of additional methodologies. Our current results lead us to conclude that MLPA®-P106 MRX has limited use for screening NS-XLID.

Testing more cases and comparing results with CMA results will allow further conclusions. However, without detailed

clinical phenotyping, most tests can result in a low diagnostic yield. Evidence shows that MLPA®, CMA and newer tests like whole genome sequencing (WGS) or whole exome sequencing (WES) shall be combined for broader diagnosis. Determining the first-tier test in the routine evaluation of ID will also depend on accuracy, reliability, efficiency, cost and availability of various techniques.

P_10

MICRODELETION SYNDROME: STUDY OF A PATIENT COHORT USING ONE MLPA® PANEL

Marta Rosas[1,2], Cristina Candeias[2,3], Raquel Chaves[4,5], Manuela Mota Freitas[2,3], Natália Oliva Teles[2,3], Maria da Luz Fonseca e Silva[2]

[1] Aluna do Mestrado de Biologia Clínica Laboratorial, Universidade de Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal

[2] Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal

[3] Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto - UMIB/ ICBAS/UP

[4] Universidade de Trás-os-Montes e Alto Douro (UTAD), Departamento de Genética e Biotecnologia (DGB), CAG - Laboratório de Citogenómica e Genómica Animal, Vila Real, Portugal

[5] Universidade de Lisboa, Faculdade de Ciências, BioISI – Biosystems & Integrative Sciences Institute, Lisboa, Portugal

ana.marta.25@hotmail.com

Chromosomal microdeletions are usually associated with genetic syndromes and are typically one to two Mb long. They have been proven to cause multisystem pathologies frequently associated with intellectual disability, developmental delay, multiple congenital anomalies, autistic spectrum disorders and diverse phenotypic findings. Multiplex Ligation-dependent Probe Amplification (MLPA®), MRC-Holland, is a good screening method for chromosomal microdeletions and several of these syndromes have been described in the past years. Recently a new MLPA® microdeletion panel for laboratory use, aiming to test patients for several new microdeletion syndromes, SALSA® MLPA® probemix P297 Microdeletion syndromes-2, was commercially available.

Eighty five samples were selected for possible inclusion in this study, since they had been analysed both for chromosomal studies and SALSA® MLPA® Probemix P245 and their respective results were normal. Forty seven patients were excluded because a diagnosis had meanwhile been established and the remaining undiagnosed 38 patients were selected for this work. These 38 patients were screened for microdeletions using the new panel (SALSA® MLPA® probemix P297-C1 Microdeletion syndromes-2).

From the total of 38 samples, 37 were normal and one revealed a microdeletion in the long arm of chromosome 3, band 3q29, involving the gene *PAK2*. In this sample, the *DLG1* gene, also located at 3q29 and present in the SALSA® MLPA® probemix P297, showed a normal number of copies. This result is in agreement with the previous analysis with SALSA® MLPA® Probemix P245 that includes two probes for 3q29, both targeting the same gene (*DLG1*), but no probes targeting *PAK2* gene. Parents blood samples were requested in order to establish if this alteration is inherited or *de novo*.

With this work the authors demonstrate the importance of the combined use of panels P297 and P245 for a more complete investigation of Microdeletion syndromes.

P_11

Y-CHROMOSOME MICRODELETIONS SCREENING: PROSPECTIVE STUDY OF 40 IDIOPATHIC CASES OF AZOOSPERMIA AND SEVERE OLIGOZOOSPERMIA

Natália Oliva-Teles[*1,2], *Cristina Candeias*[*1,2], *Manuela Mota Freitas*[1,2], *Elisa Lopes*[1], *Fernanda Paula Oliveira*[1], *Maria do Céu Ribeiro*[1], *Sílvia Pires*[1], *Isac Braga*[3], *Nuno Rossano Louro*[3]

[1] *Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal*

[2] *Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto - UMIB/ICBAS/UP*

[3] *Departamento de Cirurgia, Serviço de Urologia, Centro Hospitalar do Porto, Porto, Portugal*

[*] *These authors contributed equally to this work*

natalia.teles@chporto.min-saude.pt

In recent decades many publications have been issued in relation with infertility, the inability to achieve conception or take a pregnancy to live birth and Y-chromosome microdeletions. Infertility affects about one in six couples and may have many different causes. About half of the infertility identified cases may be attributed to male causes, particularly in males presenting with azoospermia and severe oligozoospermia (with sperm concentrations <5 x 106/mL). Abnormalities of the sex chromosomes (X and Y) explain about 6% of male infertility; the most common aneuploidy is Klinefelter Syndrome (47,XXY), followed by a XXY/XY constitution (mosaic Klinefelter Syndrome) and, more rarely, XX or 45,X/46,XY constitutions. Y-chromosome microdeletions are detected in approximately in 10%-15% of men with nonobstructive azoospermia or severe oligospermia; an increasing number or reports have been published lately associating total or partial Y-chromosome microdeletions with azoospermia factor regions (AZFa, AZFb and AZFc). The main objective of this study was to investigate the presence of structural chromosomal abnormalities by classical cytogenetics methods and AZF microdeletions by Multiplex Ligation-dependent Probe Amplification (MLPA®), MRC-Holland.

The authors have analyzed blood samples collected in litium heparin for classical cytogenetic studies (karyotype) and EDTA for molecular cytogenetics, from a total of 40 individuals (26 severe oligozoospermia and 14 azoospermia), between 01.01.2015 and 31.05.2016. All DNA samples were analyzed for AZF region chromosome Y-microdeletions by MLPA (SALSA® MLPA® kit probemix P360-A1 Y-chromosome microdeletions).

One case showed a 46,XX chromosomal constitution, the other 39 were normal (46,XY). Two cases (5%) showed large amplifications of two probes in the AZFa region, six samples (15%) revealed partial deletions in the AZFb-c regions and two samples (5%) revealed a large amplification of two probes in 12q14.2 (OMIM #613993-Spermatogenic failure).

The current work shows the conclusions of "Projecto REF 2014.200 (141-DEFI/170-CES): Investigação de Azoospermia e Oligozoospermia Masculinas através de Estudos Citogenéticos – Cariótipo, aplicação de Técnicas de FISH e de MLPA". Thus it was demonstrated that the Multiplex Ligation-dependent Probe Amplification (MLPA®) is adequate for the identification of Y-chromosome microdeletions in the AZF region. The results that were obtained show both the usefulness of routine cytogenetic tests and the use of MLPA for Y-chromosome microdeletions screening in infertile couples. The analysis of partial AZFa, AZFb or AZFc microdeletions is important for the study of infertility in other male members of their families and the provision of appropriate genetic counselling.

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FMR1 BEYOND INTELLECTUAL DISABILITY: IMPLICATIONS IN REPRODUCTIVE FUNCTION

Bárbara Rodrigues[1], Isabel Marques[2,3], Nuno Maia[2,3], Maria J Ortigão[4], Alexandra Marques[5], Isabel S Pereira[5], Rosário Santos[2,3], Paula Jorge[2,3]

[1] *Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal*
[2] *Unidade de Genética Molecular, Centro de Genética Médica Doutor Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal*
[3] *Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto - UMIB/ICBAS/UP*
[4] *Serviço Sistemas de Informação, Centro Hospitalar do Porto, Porto, Portugal*
[5] *Banco de Gâmetas, Centro de Procriação Medicamente Assistida, Centro Hospitalar do Porto, Porto, Portugal*

be_rodrigues_@hotmail.com

Fragile X Syndrome (FXS; OMIM #300624), the most common inherited cause of intellectual disability, is caused by an expansion of more than 200 CGG triplet repeats (full mutation) located at the 5 'UTR end of the *FMR1* gene. Other disorders associated with this gene include Premature Ovarian Insufficiency (FXPOI), which occurs in ~20% of women with a premutated *FMR1* allele (55 to 200 CGG repeats). Yet the mechanism underlying the ovarian insufficiency and the implication of *FMR1* remains unknown.

The severity of FXS as well as the frequency of premutated alleles in the general population, justifies the screening of expanded *FMR1* alleles in potential oocyte donors. Premutation carriers are excluded as donators, since their oocytes may have the full mutation.

Several recent studies suggest a higher predisposition to premature ovarian dysfunction in carriers of "normal" *FMR1* alleles, sized under 26 or over 34 CGG triplet repeats (26>CGG>34). However, this phenomenon is difficult to explain and is still controversial.

Taking advantage of the detailed and systematic hormonal and genetic studies carried out on potential oocyte donors for the public gamete bank at the Centro Hospitalar do Porto, this study aims to contribute towards understanding the implications of *FMR1* in the reproductive function. Descriptive and statistical analyses were performed using results obtained from 50 potential oocyte donors (100 *FMR1* alleles), including *FMR1* CGG repeat number, reproductive (number of antral follicles) and hormonal functions (FSH, LH and Estradiol concentrations). Alleles with 17 (p=2,71×10-2), 38 (p=4,92×10-4) and 48 (p=2,54×10-2) CGG repeats seem to be more frequent in the oocyte donors than in the control population. Interestingly, donors with an estradiol concentration higher than 80 pg/ml, a predictor of poor ovarian response, have one allele with CGG repeat number below 26 (p=8×10-8), designated as "low-normal". To better understand the correlation between "low-normal" *FMR1* alleles and reproductive function, stability of these alleles will be determined by analyzing the AGG interspersion pattern within the CGG stretches. Overall, our results corroborate previous investigations suggesting that the *FMR1* gene plays an important role in ovarian function.

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PATTREC: AN EASY-TO-USE CNV DETECTION TOOL OPTIMIZED FOR TARGETED NGS PANELS

Iria Roca[1], Ana Fernández-Marmiesse[1], L González-Castro[2], Sofia Gouveia [1], P Palacios[3], J Maynou[3], H Fernández[2], María Luz Couce[1]

[1] *Unidad de Diagnóstico y Tratamiento de Enfermedades Metabólicas Congénitas, Hospital Universitario de Santiago de Compostela, Spain*
[2] *Galician Research and Development Center in Advanced Telecomunications, Vigo, Spain*
[3] *Progenika Biopharma, Derio, Spain*

iria.roca.otero@gmail.com

Genetic laboratories use custom-commercial targeted next-generation sequencing (tg-NGS) gene panels to identify disease-causing variants. Although the high coverage offered by these tests enable the detection of copy number variation (CNVs), which account for a large proportion of the genetic burden in rare diseases, a robust, easy-to-use tool for automatic CNV detection is still lacking. We describe PattRec, a novel CNV detection tool optimized for targeted NGS data. We also present a novel method for simulating tg-NGS data.

PattRec was developed using comprehensive statistical analyses of a broad real targeted NGS dataset (300 DNA samples analyzed using three different targeted NGS panels). In-solution hybridization capture (SureSelect XT; Agilent Technologies) was used as the enrichment method and captured fragments were sequenced as paired-end 100-base reads in the MiSeq platform (Illumina).

The key features of PattRec are 1) a pre-analytical parameter to identify the most suitable controls for a specific test; 2) a pre-analytical filter to discard polymorphic or false-positive CNVs; 3) absence of need for same-sex controls, thereby increasing the number of potential controls and decreasing the risk of false positives; 4) SNP filtering to reduce false-positive deletions; 5) creation of in-house CNV MySQL database; 6) easy-to-use, intuitive interface (unlike other methods where the user needs to manage multiple packages in R or Python in PattRec the user simply has to introduce the bam files for the test and controls, the bedfile containing the regions of interest, the fasta file containing the genome sequence, and the relevant search parameters); 7) output in xlsx format with a colour code in addition to p values for certain parameters to prioritize CNVs that are more likely to be pathogenic.

Pattrec is a powerful tool for detecting deletions (100% sensitivity). With the exception of exomeDepth, PattRec has significantly higher sensitivity for both simulated and real data. Its performance is comparable to that of exomeDepth for simulated data, but in addition, it offers several novel features, such as sex independence, SNP filtering, filtering of common CNVs, an in-house CNV population database, and an output file that helps user to prioritize potentially real CNVs.

PattRec is implemented in Java and R languages, and is packaged as a desktop application with an easy-to-use interface for Ubuntu. A limited-time full trial version of PattRec is freely available at https://goo.gl/forms/uFmrGvFsSQ9MMPIz1.

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PORTUGUESE NEWBORN SCREENING PROGRAM: 36 YEARS AT THE SERVICE OF PUBLIC HEALTH

Lurdes Lopes, Carmen Sousa, Ivone Carvalho, Helena Fonseca, Ana Marcão, Hugo Rocha, Laura Vilarinho

Newborn Screening, Genetics and Metabolism Unit, Human Genetics Department National, National Institute of Health Doutor Ricardo Jorge, Porto

lurdes.lopes@insa.min-saude.pt

Newborn screening programs have a key role in national public health strategies, and have as main goal the detection of potentially fatal or disabling conditions in newborns, providing a window of opportunity for treatment, often before the infant displays any signs or symptoms of a disease or condition. Early detection and treatment can have a positive impact on the severity of the condition in the newborn.

Portuguese Newborn Screening Program (PNSP) was established in late 70's with the screening for phenylketonuria and congenital hypothyroidism, being available for all newborns in our Country and is performed in a single laboratory, which processes over 400 samples per day. Since the beginning, PNSP development is guided in respect to Wilson and Jungner criteria (WHO recommendations) with the purpose of maximizing the benefits/costs ratio. Throughout the last 35 years several pilot studies were undertaken, that in some cases resulted in the expansion of the number of screened disorders and others that don't. Nowadays a total of 25 disorders are screened as part of PNSP (congenital hypothyroidism and a group of 24 metabolic disorders) being a pilot study for the newborn screening of cystic fibrosis in its final stage, with extremely promising results. Since its beginning, more that 3.5 million Portuguese newborns were screened (from 1993 the coverage rate is over 99%) and a total of 1.885 individuals were identified as being affected by one of the screened conditions. Birth prevalence's are 1: 2.968 for congenital hypothyroidism; and 1: 2.283 for the group of metabolic disorders.

Following the continuous improvement strategy implemented in the PNSP, future developments will depend on a permanent evaluation of new technical possibilities that could allow the detection of more treatable disorders as well as of the progresses in therapy effectiveness that may justify the screening of others. The positive impact of the PNSP in the newborn population and its continuous search for improvement makes it a key and leading hedge strategy in public health policies.

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GENETIC VARIANTS OF *CYP2D6* AND *IL*-6 IN CHILDREN INFECTED WITH SCHISTOSOMIASIS HAEMATOBIA

Mónica C Botelho[1,2], Rita Cordeiro[1], Pedro C Lacerda[3], Paulo Pinho e Costa[3,4], Adriano Bordalo[5], Joachim Richter[6], Helena Alves[1,7]

[1] INSA, National Institute of Health Dr. Ricardo Jorge, Department of Health Promotion and Chronic Diseases, Porto, Portugal

[2] I3S, Instituto de Investigação e Inovação da Universidade do Porto, Portugal

[3] INSA, National Institute of Health Dr. Ricardo Jorge, Department of Human Genetics, Porto, Portugal

[4] UMIB-ICBAS, University of Porto, Portugal

[5] ICBAS, Institute of Biomedical Sciences, Laboratory of Hydrobiology and Ecology, University of Porto, Portugal

[6] Institute of Tropical Medicine and International Health, Charité–Universitätsmedizin Berlin, Germany

[7] Fundação Professor Ernesto Morais, Porto, Portugal

monicabotelho@hotmail.com

Urogenital schistosomiasis is a chronic infection caused by the human blood fluke Schistosoma haematobium. Schistosomiasis haematobia is a known risk factor for cancer leading to squamous cell carcinoma of the urinary bladder (SCC). This is a neglected tropical disease endemic in many countries of Africa and the Middle East. Schistosome eggs produce catechol-estrogens. These estrogenic molecules are metabolized by cytochrome P450 oxygenases to active quinones that cause alterations in DNA, known to promote breast or thyroid cancer. Our group has shown that schistosome egg associated catechol estrogens induce tumor-like phenotypes in urothelial cells, possibly due to the formation of parasite estrogen-host cell chromosomal DNA adducts. These estrogen metabolites might also contribute to schistosomiasis associated infertility. Interleukin-6 (IL-6) is a pleiotropic proinflammatory cytokine, highly expressed in the female urogenital tract and reproductive organs. It has been implicated in estrogen metabolism imbalance. In the present study we investigated polymorphic variants in *CYP2D6* and the -174 G/C (rs1800795) promoter polymorphism of the IL-6 gene on a cohort of 18 S. haematobium infected children from Guiné-Bissau. We found that 25% of the infected children are carriers of the CYP2D6*5 allele, which is characterized by deletion of the entire *CYP2D6* gene, and that 6.25% have the IL6 -174C variant, that is known to be associated with lower IL6 secretion. We recently demonstrated that *S. haematobium*-infected children are more frequently stunted and wasted than non-infected children, and these polymorphisms may represent potential biomarkers for growth development and metabolism disorders in these patients. On the other hand, they may have prognostic significance, namely regarding the development of cancer, something which will need to be addressed in further studies.

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