

P3.30**Normative values for intraepidermal nerve fibers in a Portuguese population**
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Skin biopsy as a complementary in the diagnosis of small fiber neuropathy, has come to be increasingly used by different researchers. There is a need to establish normative standards that allow the comparison of biopsies performed in patients with biopsies performed in healthy individuals in the Portuguese population. Presentation of data collected in the counts of intraepidermal nerve fibers in skin biopsies from healthy volunteers without pathology, with descriptive statistics (mean and standard deviation). We performed two skin biopsies 3 mm in diameter by Punch biopsy of the lower limb. A distal, 10 cm above the external malleolus, and one proximal to 20 cm from the iliac crest. The material is fixed in paraformaldehyde, cryoprotected with 20% glycerol and cryopreserved at -80°C . Are made serial sections of 50μ and is made immunohistochemical staining with antibody to PGP 9.5 counts posterior fibers of the skin. We studied 19 individuals, 6 men and 13 women. For the proximal biopsies, the average is 30 fibers, 3 mm with a standard deviation of 9.55 fibers 3 mm distal to the biopsies, the average is 24.5 fibers 3 mm with a standard deviation of 10, 6 fibers, 3 mm; There is a lower concentration of fibers in biopsies distal compared with proximal biopsies. The results obtained allow to establish a normative database for the Portuguese population, though limited by the availability of volunteers. These data are important in the diagnosis of small fiber neuropathies.

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CONGENITAL MYOPATHIES: POSTER PRESENTATIONS**P3.31****Congenital myopathies – clinical features and frequency of individual subtypes diagnosed in a five-year period: the UK experience**L. Maggi^a, M. Scoto^a, S. Cirak^a, L. Feng^a, S. Lillis^b, T. Cullup^b, S. Robb^a, A. Manzur^a, C.A. Sewry^a, S. Abbs^b, H. Jungbluth^c, F. Muntoni^a^a*The Dubowitz Neuromuscular Centre, UCL Institute of Child Health, London, United Kingdom;* ^b*Diagnostics Genetics Laboratory, GSTS Pathology, Guy's Hospital, London, United Kingdom;* ^c*Clinical Neuroscience Division, IOP, King's College and Evelina Children Hospital, Neuromuscular Service, London, United Kingdom*

Congenital myopathies (CM) are a rare group of neuromuscular disorders, mainly defined on the basis of morphological features. The frequency of single entities of CM is still unsolved. Our aim was to study retrospectively clinical, histological and molecular data of a wide population of patients affected by CM referred to the Dubowitz Neuromuscular Centre in UK. We included 66 patients who were physically assessed at our Centre between 1.1.2005 and 31.12.2009, in whom we arrived to a clinical, histological and/or molecular diagnosis of CM. Muscle biopsy was available in 54 patients and morphological analysis revealed a core myopathy in 31 (57.4%) patients, nemaline myopathy in 9 (16.7%), myotubular/centronuclear myopathy in 7 (13%), congenital fibre-type disproportion in 2 (3.7%), rod-core myopathy in 1 (1.8%); 4 (7.4%) patients had fibre type I predominance, and involvement of one of the genes responsible for CM was confirmed. The following genes were systematically studied: RYR1, SEPN1, ACTA1, MTM1, NEB, TPM3, BIN1, DNM2, MYH7, CFL2. All genes were studied by direct sequencing with the exception of NEB in which we only studied the common exon 55 deletion. To date, a final molecular diagnosis was achieved in 44

(66.7%) patients. RYR1 gene was mutated in 26/44 (59.1%) patients, ACTA1 in 7 (15.9%), SEPN1 in 7 (15.9%), MTM1 in 2 (4.5%), NEB in 1 (2.3%) and TPM3 in 1 (2.3%). The mean age at onset in our patients was 0.8 ± 1.6 years (range 0–7); independent walking was reached in 43 (65.2%) patients. Fifteen (22.7%) patients required non invasive ventilation at mean age of 2.4 ± 3.4 years and 14 (21.2%) had gastrostomy at mean age of 0.8 ± 0.3 years. The clinical course was stable or improved in 56 (84.8%) patients, while 5 (7.6%) patients died. In conclusion, core myopathy was the most frequent CM in our population and RYR1 the gene more frequently involved.

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P3.32**The molecular genetics of monogenic neuromuscular disorders characterised by reduced foetal movement**G. Ravenscroft^a, E. Solis^a, A.K. Charles^b, V.A. Fabian^c, K.N. North^d, G. Baynam^c, N.G. Laing^a^a*The University of Western Australia, The Western Australian Institute for Medical Research, Centre for Medical Research, Perth, Australia;* ^b*School of Pathology and Laboratory Medicine, University of Western Australia, WA, Australia;* ^c*Royal Perth Hospital, Neuropathology, Perth, Australia;* ^d*University of Sydney and The Children's Hospital at Westmead, Faculty of Medicine, Sydney, Australia;* ^e*King Edward Memorial Hospital and The University of Western Australia, Genetic Services of Western Australia, Perth, Australia*

Foetal akinesia refers to a broad spectrum of disorders in which the unifying feature is reduced or absent foetal movement. It is associated with a characteristic set of abnormalities, including: contractures causing arthrogryposis, pterygia, subcutaneous oedema (hydrops), lung hypoplasia, rocker-bottom feet, cranio-facial anomalies (particularly cleft palate, retromicrognathia) and poor muscle bulk. Foetal akinesias can result from primary defects involving any point along the motor system: the central nervous system including the spinal cord, peripheral nerve, the neuromuscular junction and the skeletal musculature; disorders of the connective tissue and environmental factors are also implicated. Mutation detection and genetic counseling is confounded by marked clinical and genetic heterogeneity. Many cases represent the severe end of the spectra of recognized disease entities, such as spinal muscular atrophy, congenital muscular dystrophies and myopathies and in a small percentage of cases mutations are identified in the relevant disease genes. However, the majority of foetal akinesia cases do not have a genetic diagnosis. In order to ascertain novel foetal akinesia disease genes, we are collating a cohort of Australian cases of mostly myopathic origin. To date we have recruited nearly 50 families; seven cases are consanguineous or have multiple affected sibs in the family, suggesting, as in previous studies, a high incidence of recessive diseases. We have excluded the skeletal muscle α -actin gene (*ACTA1*) in all families tested and we are pursuing other disease genes through homozygosity mapping in the consanguineous cases, linkage mapping in families with multiple affected sibs and next generation sequencing.

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P3.33**The expression of amphiphysin-2 during skeletal muscle regeneration**A. Ishii^a, N. Ohkoshi^b, A. Tamaoka^a^a*University of Tsukuba, Neurology, Tsukuba, Japan;* ^b*Tsukuba University of Technology, Neurology, Tsukuba, Japan*

Amphiphysins have been implicated in membrane remodeling in brain and skeletal muscle. Mutations in amphiphysin-2 were recently identified in autosomal recessive centronuclear myopathies. In order to understand the dynamics of amphiphysin-2 during its regeneration, we chronologically evaluate the expression of amphiphysin-2 and caveolin-3 in rat tibial muscles during a cycle of regeneration induced by cardiotoxin injection using immunohistochemistry and Western blot. Tibial muscles of male Wistar rats (7–8 weeks old) were injected with cardiotoxin. The cardiotoxin-injected muscles were removed on 1, 3, 5, 7, 14, and 28 days after the injection. Western blotting was performed as Laemmli's methods. In immunohistochemical studies, amphiphysin-2 and caveolin-3 were weakly stained at T-tubules of some regenerating muscles. In the western blot analysis, amphiphysin-2 was first detected as a visible band on day 5, whereas caveolin-3 was first recognized as a visible band on day 3. During 3–5 days after cardiotoxin injection, satellite cells fuse and differentiate to mature muscle fibers. These results provide evidence that both amphiphysin-2 and caveolin-3 contribute muscle differentiation and membrane deformation.

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P3.34

Correlation of morphological features of skeletal muscle biopsy with the gestational age of newborns with X-linked Myotubular myopathy, and comparison with the muscle pathology of myotubularin1-deficient mice

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The X-linked Myotubular myopathy (XLMTM) due to mutations in the *MTM1*-gene (myotubularin 1) has been clinically well characterised and usually gives rise to a severe phenotype in males presenting at birth with severe congenital myopathy. Although the muscle morphological characteristics are currently well documented (significant number of small muscle fibres with centralised nuclei), the formation and maintenance of this particular structure is not well characterised in human. We aimed to correlate the pathologic features of skeletal muscle biopsy of newborns with *MTM1*-mutations according to the corrected gestational age, and to compare these morphological findings with the pathological characteristics of muscle in myotubularin1-deficient mice. Clinical and muscle biopsies data from 20 XLMTM-newborns were studied. At birth, the age of newborns ranged from 29 to 42 gestational weeks and the age at the time of the muscle biopsy ranged from 0 to 95 days old. Nineteen patients died before the age of 5 months. Indeed, the sequential analysis of morphological features was performed according with the corrected gestational age. Thus, the proportion of myofibers with central nuclei, the myofiber diameters, the ultrastructural abnormalities, the immunocytochemical expression of muscle development markers will be correlated with the corrected age of XLMTM-newborns, as well as with the *MTM1* mutation type (missense, nonsense, splice site, deletion, small insertion or duplication) to try an assessment of the involvement of these different features in the pathological expression of the disease.

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P3.35

Expanding the mutation spectrum of the *MTM1* gene: The first multi-exonic duplication and establishment of a locus-specific database

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Centronuclear myopathies (CNM) are a group of diseases with variable onset and severity sharing as a distinctive histological feature, a high frequency of muscle fibers with centralized nuclei. Myotubular myopathy (MIM#310400) the X-linked form of CNM is characterized by neonatal hypotonia and inability to maintain unassisted respiration. The *MTM1* gene, responsible for this disease, encodes myotubularin, a protein involved in myofiber differentiation and muscle cell architecture. In this work, eight patients were subjected to *MTM1* MLPA analysis, selected according to the following criteria: (i) muscle biopsy compatible with CNM and (ii) exclusion of *MTM1* point mutations by sequencing. We identified the first gross duplication spanning exons 1–5 (c.-76-?_342+?dup) in a 7 year old boy with progressive tetraparesis, ophthalmoparesis, facial diparesis and independent ambulation, the clinical course being milder than the classical myotubular myopathies. Analysis at the mRNA level revealed both normal transcripts and a mutated isoform lacking exon 6 (r.343_444del), suggesting somatic mosaicism. As suspected, this duplication was not detected in the patient's mother. Considering the phenotypic expression in the patient, this mutational event most likely occurred *de novo* during early embryogenesis. We also describe the implementation of a locus-specific database (LSDB) for this gene using the Leiden Open Variation database (LOVD) software. The *MTM1*-LOVD (<http://www.lovd.nl/MTM1>) contains 372 mutation entries identified in 370 patients (last accessed March 2011). A total of 223 unique *MTM1* mutations are listed in this LSDB, including: 207 point mutations, 15 single or multi-exonic deletions and the large duplication described in the present work. Despite the significant advances in this field during the last decade about one third of the CNM cases remain genetically unresolved. Here we show that gross *MTM1* gene duplications may account for a fraction of these cases.

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P3.36

Phenotypic spectrum in myopathies with tubular aggregates

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The sarcoplasmic reticulum of muscle fibers is known to form tubular aggregates (TAs) in various diseases, in some constituting the most striking