

PW 14:
Congenital myopathies –
RYR1, SEPN1 and
myotubular myopathies

PW14-170	<p><u>INTRACELLULAR LOCALISATION OF A MUTATED RYR1 ASSOCIATED WITH SEVERE MMD.</u> FAURÉ J¹, CACHEUX M¹, MONNIER N¹, BROCARD J¹, FOUREST-LIEUVIN A¹, ODDOUX S¹, LUNARDI J¹, MARTY I¹ (1) Grenoble Institut des Neurosciences- Inserm U836- Equipe Muscle et Pathologies, UJF Site Santé, BP 170, 38042 Grenoble, FRANCE.</p>
To contact the author:: julien.faure@ujf-grenoble.fr.	<p>Muscle contraction relies on efficient calcium release from sarcoplasmic reticulum (SR), performed by the ryanodine receptor (RyR1) upon cell stimulation. RyR1 is 5000 amino acids long, anchored in the membrane of SR, and coupled to the voltage-sensitive calcium channel DHPR that initiate muscle contraction after depolarisation of the fibre.</p> <p>Several mutations in the RyR1 gene have been associated to inherited muscle defects, mostly congenital structural myopathies (central core disease, CCD, and multi minicore disease, MmD) and malignant hyperthermia. Although mutations are found along the whole RYR1 gene, those associated with CCD and MmD seem to cluster in the 3' region coding for transmembrane domains of the protein. To date, most of the studies on mutations in these regions have led to the hypothesis that mutated RyR1 is defective in its channel properties.</p> <p>The 14646+2.99 kb A->G mutation has been found in a patient with severe MmD. It generates a cryptic splicing site that leads to an out-of-frame insertion of one exon in the most 3' end of the RyR1 cDNA. The expected protein is shorter than normal RyR1 and lacks its last transmembrane helix (TM6). Moreover, in a biopsy of this patient, the overall level of RyR1 is drastically reduced. To understand this reduction in RyR1 level, we have tested whether removal of TM6 helix could destabilise the mutated RyR1 (mRyR). Surprisingly, when expressed in a cell line, the mRyR construct is mislocalised, compared to normal RyR1 present in the endoplasmic reticulum. We have investigated the precise intracellular targeting of mRyR1 as well as the mechanisms leading to its accumulation. Our work raises the question whether, in addition to channel defaults, intracellular localisation of mutated RyR1 could also explain some of the pathological effects for mutations associated with congenital myopathies.</p>

PW14-171	<p>COMPOUND HETEROZYGOUS MUTATIONS IN RYR1 ASSOCIATED WITH CFTD WITH EXTREME FIBRE SIZE DISPROPORTION</p> <p>CLARKE N¹, MONNIER N², SMITH R³, WADDELL L¹, COOPER S¹, LUNARDI J², NORTH K¹</p> <p>(1) Institute for Neuromuscular Research, Children's Hospital at Westmead, Discipline of Paediatrics and Child Health, University of Sydney, Sydney, AUSTRALIA. (2) Biochimie et Génétique Moléculaire, CHU Grenoble / INSERM, U836, Grenoble, FRANCE. (3) John Hunter Children's Hospital and University Discipline of Paediatrics and Child Health, Newcastle, AUSTRALIA.</p>
<p>To contact the author:: n.clarke@institut-myologie.org.</p>	<p>Background: Congenital fibre type disproportion (CFTD) is a subtype of congenital myopathy in which consistent type 1 fibre hypotrophy, compared to type 2 fibres, is the main histological abnormality. Recessive mutations in <i>RYR1</i> have been associated with several histological patterns, including core myopathies and increased nuclear internalization but not with the pattern associated with CFTD. We present an affected boy, born to healthy non-consanguineous parents, who had slowly progressive severe congenital generalized hypotonia and weakness. He sat unsupported at age 1 year but never walked and he had a myopathic face with ptosis and ophthalmoplegia. Nocturnal non-invasive ventilation and a gastrostomy were required from age 2 years. He died at age 3 years from respiratory failure. Quadriceps muscle biopsies taken at ages 6 months and 3 years showed marked hypotrophy of type 1 fibres and marked type 2 fibre hypertrophy but no other notable abnormalities on standard or electron microscopy. In sequencing the <i>RYR1</i> gene from cDNA (generated from skeletal muscle) there was homozygosity for a novel missense change (c.6104A>T, p.His2035Leu) that was present in genomic DNA in the heterozygous state. Careful analysis revealed low levels of a second transcript in cDNA, that contained a nonsense mutation (c.738T>G, p.Try246Stop). Family studies showed that the Stop mutation was paternally inherited. There was loss of the transcript from this allele in muscle-derived cDNA in the father also, consistent with nonsense-mediated decay. Western blotting showed reduced levels of RYR1 in the proband and his father. Conclusion: Compound heterozygosity for the p.His2035Leu mutation, together with a nonsense (silencing) mutation in <i>RYR1</i> results in a severe congenital myopathy and typical histological changes of CFTD with an extreme difference in the sizes of type 1 and type 2 fibres. <i>RYR1</i> is the fourth gene to be associated with CFTD.</p>

PW14-172	<p><u>CLINICAL, HISTOLOGICAL AND GENETIC HETEROGENEITY OF RYR1-RELATED DISEASES</u> MONNIER N¹, MARTY I², DROUHIN S¹, LAMARIA M¹, TEISSIER N¹, THÉRIER P¹, LABARRE-VILA A³, LUNARDI J¹ (1) Laboratoire de Biochimie et Génétique Moléculaire, CHU Grenoble, Grenoble, FRANCE. (2) INSERM U836, Grenoble Institut des Neurosciences, Grenoble, FRANCE. (3) Centre de Référence des Maladies Neuromusculaires, CHU Grenoble, Grenoble, FRANCE.</p>
To contact the author:: jlunardi@chu-grenoble.fr.	<p>RYR1-related diseases include malignant hyperthermia and structural congenital myopathies among which the most common are core myopathies. Other forms such as centronuclear myopathies and myopathies with predominance of type I fibres or with disproportion of type fibres were also evidenced although less frequently, in patients. However it is not clear whether some of these later forms can represent an early expression of core myopathies.</p> <p>We have analyzed 299 unrelated MH patients. Limited exonic screening has allowed the identification a dominant mutation in 50 % of the families. When performed, histological investigations have revealed the presence of cores in a significant number of MH patients. This caused some confusion as this type of patients were originally referred as MH/CCD patients although they did not present with myopathic symptoms.</p> <p>We have investigated 294 unrelated patients presenting with structural congenital myopathies ranging from congenital onset with severe phenotype to milder non or slowly progressive forms. 191 patients were sporadic cases. Muscle biopsies were characterized by core lesions showing highly variable localisation, size, length and number within the muscle fibres suggesting a continuum from classical unique central cores to multiple small diffuse cores.</p> <p>A dominant mutation was identified in 70 cases while 37 families harboured two recessive mutations. A single mutation was also characterized in 23 other families with unknown AD/AR status. Mapping of the mutations showed a different distribution depending on the nature of diseases and inheritance.</p> <p>Noticeably, more than 40% of the recessive mutations led to silencing of allele expression through various mechanisms. Interestingly, a second mutation has been identified after extensive analysis of the <i>RYR1</i> gene in sporadic cases originally classified as dominant myopathies. This was of importance for the genetic counseling in these families. Myopathic phenotypes will be discussed in relation with the nature of the RYR1 mutations.</p>

PW14-173	<p><u>RYR1 MUTATIONS IN MALIGNANT HYPERTHERMIA : A SURVEY OF 80 FAMILIES INVESTIGATED IN MARSEILLES</u> BENDAHAN D¹, MONNIER N², FOUTRIER-MORELLO C¹, KOZAK G¹, LUNARDI J², COZZONE PJ¹ (1) CRMBM UMR CNRS 6612 Faculté de Médecine de la Timone, 27, bd. J. Moulin, 13005, Marseille, FRANCE. (2) Département de Génétique, CHU la Tronche, Grenoble, FRANCE.</p>
To contact the author:: david.bendahan@univmed.fr.	<p>Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disease triggered by commonly used volatile anaesthetics and/or succinylcholine. In vitro muscle contracture testing (IVCT) is the gold standard test to establish an individual's risk of MH susceptibility (MHS) (2) while investigations of muscle energetics (1) and genetic analyses are commonly performed in order to better understand the genetic and metabolic bases of this infraclinical myopathy. MHS is characterised by a genetic heterogeneity and two genes, RYR1 and CACNA1S, have been associated with the disease. 29 causative mutations have been reported so far in the RYR1 gene. We report in the present study the results of 80 MHS families investigated in Marseilles.</p> <p>Among these 80 families, 44 have been genetically investigated. One causative mutation has been found in 17 families i.e. the porcine mutation (G341R) in 6 families and the R2458H mutation in two other families. A double mutation (R2676W/T2787S) has been reported in a single family (3) and so far the causative aspect of each of them is discussed. In four families, a single mutation has been found but the causative feature has not been clearly established. Among them, the T2787S mutation is present. Finally, none of the 20 common mutations has been found in the 22 remaining families. For these families, further investigations should have to be performed on mRNA from muscle samples.</p> <p>Overall, mutations in the RYR1 gene have been found in half of the investigated families. For the 50% remaining, additional investigations should be performed either in the RYR1 gene or in other candidate genes such as the gene coding for the DHPR receptor.</p> <ol style="list-style-type: none"> Bendahan D, Kozak-Ribbens G, Rodet L, Confort-Gouny S, and Cozzone PJ. ³¹Phosphorus magnetic resonance spectroscopy characterization of muscular metabolic anomalies in patients with malignant hyperthermia: application to diagnosis. <i>Anesthesiology</i> 88: 96-107, 1998. Bendahan D, Guis S, Monnier N, Kozak-Ribbens G, Lunardi J, Ghattas B, Mattei JP, and Cozzone PJ. Comparative analysis of in vitro contracture tests with ryanodine and a combination of ryanodine with either halothane or caffeine: a comparative investigation in malignant hyperthermia. <i>Acta Anaesthesiol Scand</i> 48: 1019-1027, 2004. Guis S, Figarella-Branger D, Monnier N, Bendahan D, Kozak-Ribbens G, Mattei JP, Lunardi J, Cozzone PJ, and Pellissier JF. Multiminicore disease in a family susceptible to malignant hyperthermia: histology, in vitro contracture tests, and genetic characterization. <i>Arch Neurol</i> 61: 106-113, 2004.

PW14-174	<p>IMPLICATION OF OXIDATIVE STRESS IN SEPN1-RELATED MYOPATHY ARBOGAST S¹, MUNTONI F², FERREIRO A¹ (1) Inserm U582, Université Pierre et Marie Curie Paris 6, Institut de Myologie, IFR 14, Paris, FRANCE. (2) Dubowitz Neuromuscular Centre, Imperial College, Hammersmith Hospital, London, UNITED-KINGDOM.</p>
<p>To contact the author:: s.arbogast@myologie.ch ups.jussieu.fr.</p>	<p>Mutations of the selenoprotein N gene (<i>SEPN1</i>) have been identified as responsible for <i>SEPN</i>-related myopathy (<i>SEPN</i>-RM), an early-onset muscle disorder. Selenoprotein N is the only selenoprotein implicated in a human genetic disorder but its function remained unknown. SelN has structural similarities with other selenoproteins which are involved in redox homeostasis. Therefore, we hypothesized that 1/ SelN depletion increases intracellular oxidant activity in cells, 2/ SelN plays a role in defence against oxidative stress.</p> <p>To verify this, we performed ex-vivo experiments using the dichlorofluorescein assay, comparing intracellular oxidant activity in human myoblast and fibroblast primary cultures from 4 controls and from 4 patients carrying homozygous or compound heterozygous nonsense <i>SEPN1</i> mutations. Then, we assessed protein oxidation by measuring protein carbonyls that are sensitive indices of oxidative injury. Furthermore, we examined the susceptibilities of the cells to different concentrations of hydrogen peroxide (H₂O₂).</p> <p>Myoblasts devoid of SelN show a significant increase in intracellular oxidant activity compared to controls whereas fibroblasts do not. Furthermore, we demonstrated that the average content of protein carbonyls both in fibroblasts and in myotubes expressing <i>SEPN1</i> mutations was significantly increased compared to control cells. Fibroblasts devoid of SelN are more sensitive to H₂O₂ -induced oxidative stress than normal fibroblasts, as evidenced by a significantly greater cell death. Pre-treatment with the antioxidant N-acetyl cysteine restored cell survival against H₂O₂ to a level comparable to controls.</p> <p>This study establishes for the first time that oxidative stress is implicated in <i>SEPN</i>-RM, and that SelN protects human cells against oxidative stress.</p>

PW14-175	<p>DEFECTS IN EXCITATION-CONTRACTION COUPLING IN X-LINKED MYOTUBULAR MYOPATHY</p> <p>AL-QUSAIRI L¹, WEISS N², SANOUDOU D³, BERBEY C², MESSADDEQ N¹, KRETZ C¹, ALLARD B², BEGGS AH³, MANDEL JL¹, JACQUEMOND V², LAPORTE J¹, BUJ BELLO A¹</p> <p>(1) IGBMC, INSERM U596, CNRS UMR 7104, ULP, Collège de France, Illkirch, FRANCE. (2) Université Claude Bernard-Lyon 1, UMR CNRS 5123, Villeurbanne, FRANCE. (3) Children's Hospital, Harvard Medical School, Boston, USA.</p>
To contact the author:: abb@igbmc.u-strasbg.fr.	<p>X-linked myotubular myopathy (XLMTM) is a severe congenital disease that affects the skeletal musculature leading to early postnatal death of most patients. The gene responsible for the disorder, <i>MTM1</i>, encodes a lipid phosphatase named myotubularin. <i>Mtm1</i> knockout mice develop a progressive generalized myopathy with reduced life expectancy and present a muscle pathology that resembles that of XLMTM patients.</p> <p>The aim of our study is to identify the molecular mechanisms leading to XLMTM pathogenesis. For this, we have analyzed the transcriptome of <i>Mtm1</i> knockout (KO) skeletal muscle before (2 week-old) and after (5 weeks) the appearance of muscle weakness by using Affymetrix arrays and found a highly active transcriptional response in mice at symptomatic stages of the disease. Since we have observed that overexpressed myotubularin is associated to the sarcolemma and triads and muscle weakness is very severe in KO mice, we focused on genes involved in calcium homeostasis. We found that genes linked to the excitation-contraction (E-C) coupling machinery are deregulated at the transcriptional (Q-PCR) and/or protein level in muscles of 5 week-old <i>Mtm1</i> KO mice, with some of them being already altered at early stages of the disease.</p> <p>We thus measured intracellular Ca²⁺ transients elicited by voltage-clamp depolarisations in muscle fibres from 4-5 week-old control and <i>Mtm1</i> KO mice. The peak Ca²⁺ transient was strongly reduced in myotubularin deficient fibres whereas resting Ca²⁺ and time course of Ca²⁺ removal from the cytoplasm remained essentially unaffected. These results strongly suggest that failure of excitation-contraction coupling due to alteration of calcium release from the sarcoplasmic reticulum accounts for muscle weakness in <i>Mtm1</i> deficient mice. We also found ultrastructural alterations of the triads in <i>Mtm1</i>-deficient muscle that may contribute to the E-C uncoupling. These results provide novel insights into the pathomechanism of XLMTM and may open novel therapeutic strategies for patients.</p>

PW14-176	<p><u>A NOVEL MTM1 MUTATION IN A CASE OF X-LINKED MYOTUBULAR MYOPATHY</u> PEDEMONTE M¹, D'APICE MR², OTTONELLO C¹, CONTE G², MATTIOLI G¹, SCAPOLAN S¹, NOVELLI G², MINETTI C¹, BRUNO C¹ (1) Istituto Giannina Gaslini, Genova, ITALY. (2) Policlinico Universitario Tor Vergata, Roma, ITALY.</p>
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X-linked myotubular myopathy is a rare disease, characterised by neonatal hypotonia, muscle weakness and respiratory distress in affected males, leading often to early death. It is caused by mutations in the *MTM1* gene, localised on the long arm of the X chromosome (Xq28), which encodes a phosphatase called myotubularin. To date, around 200 different mutations have been reported in the *MTM1* gene, and most of them are private mutations.

We report a novel *MTM1* gene mutation in a neonate with X-linked myotubular myopathy. The boy was the first child of healthy non-consanguineous parents. There was no family history of neurological diseases. Pregnancy was characterized by reduction of fetal movements in the last months. At birth, he presented generalized muscle hypotonia, lack of spontaneous movements, respiratory insufficiency and he was intubated. Muscle biopsy revealed the typical features of myotubular myopathy. Molecular analysis of *MTM1* gene revealed a novel *MTM1* mutation in exon 14 (c.1481-1482delG, p.R494RfsX7). This mutation is predicted to result in the truncation of myotubularin, introducing a stop codon due to a frame shift, and were not detected in 100 healthy chromosomes. The mother did not carry the mutation supporting a *de novo* origin of this mutation. He was placed on ventilator since two months of age, and underwent a percutaneous endoscopic gastrostomy (PEG) at age of 5 months. He is currently 10 months-old with good respiratory monitor, progressive improvement of his nutritional indices and no complications to date. Since germline mosaicism has been described in several cases, prenatal diagnosis has been proposed to the family.

PW14-177

“DARK NECKLACE” FIBERS MYOPATHY, A PECULIAR MORPHOLOGICAL PATTERN OF CONGENITAL MYOPATHY

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<p>To contact the author:: nb.romero@institut-myologie.org.</p>	<p>Congenital myopathies are characterized by peculiar structural changes of muscular fibers. Recently, working on a series of cases close by their clinical and pathological features from the centronuclear myopathies, we identified a group of patients which presented a curious morphological pattern characterized by the presence of a variable amount of “dark necklace” fibers (DNF).</p> <p>Necklace fibers were found in both type1 and type2 fibers. They showed a basophilic subsarcolemmal ring deposit, few micrometers apart from the plasma membrane. The ring stained intensively with HE, GT, NADH-TR, SDH and COX, but was not detected on the myofibrillar ATPase. Occasional nuclei align with the necklace.</p> <p>EM analysis showed that the necklace was situated about 3µm from the sarcolemma. It consists of normally oriented and disorganized myofibrils with remarkably small diameter, surrounded by increased, normally structured mitochondria and numerous sarcotubular profiles.</p> <p>Immunohistochemical studies showed an intense labeling using anti-SERCA1 and SERCA2 antibodies, but not with other proteins of the sarcoplasmic reticulum (calsequestrin, ryanodine receptor, triadin), and the T-tubule (dihydropyridine receptor-alpha1subunit). In addition, there was a marked reaction with anti-desmin and aB-crystallin antibodies, but not for myotilin antibodies.</p> <p>Clinically, the four cases identified were sporadic. Symptoms began in the first decade, as a slowly progressive proximal pattern of weakness predominantly in lower limbs. No facial or extraocular muscle involvement was observed. Serum CK were slightly elevated in two cases and normal in the two others. Electromyography showed unspecific myopathic changes in all of them, in one case nerve conduction studies showed distal slowing of latencies.</p> <p>The peculiar structural alterations were not present in any other cases of centronuclear myopathies. Preliminary molecular genetics analysis allowed to exclude <i>DNM2</i> and <i>BIN</i> genes. <i>MTM</i> gene study is in progress. The mechanism of this structural defect in myofibrillar organization and organelle positioning remains to be elucidated.</p>

PW14-178	<p>IMPLICATION OF AMPHIPHYSIN 2 (BIN1) IN AUTOSOMAL RECESSIVE CENTRONUCLEAR MYOPATHIES AND T-TUBULES ORGANIZATION TOUSSAINT A¹, NICOT AS¹, TOSCH V¹, KRETZ C¹, BÖHM J¹, OLDFORS A², LAPORTE J¹, MANDEL JL¹ (1) IGBMC, Illkirch, FRANCE. (2) Sahlgrenska University Hospital, Göteborg, SWEDEN.</p>
To contact the author:: anne.toussaint@igbmc.u-strasbg.fr.	<p>Centronuclear myopathies (CNM) are characterized by muscle weakness and abnormal centralization of nuclei in muscle fibres without excessive regeneration. The severe neonatal X-linked form (myotubular myopathy, XLMTM) is due to mutations in the gene encoding the phosphoinositides phosphatase myotubularin (MTM1), while mutations in the membrane tubulating GTPase dynamin 2 (DNM2) have been found in some autosomal dominant cases. We identified homozygous mutations in the amphiphysin 2 gene (also called BIN1) in four families with autosomal recessive CNM. Two different missense mutations in the BAR (Bin1/Amphiphysin/RVS167) domain disrupt its membrane tubulation properties in transfected cells, while a partial truncation of the C-terminal SH3 domain abrogates the interaction with dynamin 2 and its recruitment to the membrane tubules. Although amphiphysin 2 is ubiquitously expressed, mutations in the gene induce a muscle phenotype. Amphiphysin 2 was shown to be located to T-tubules, and in the muscle of patients mutated in the amphiphysin 2 gene, we observed anomalies in the localization of markers of T-tubules biogenesis and organization. Thus we are investigating the role of amphiphysin 2 in the biogenesis and organization of these structures in skeletal muscle. Initial results indicate that it co-localizes with markers of the sarcotubular system in murine skeletal muscle. In parallel we are analyzing the impact of amphiphysin 2 mutations and T-tubule organization in several forms of myopathies with nuclei centralization.</p>

PW14-179	<p><u>MORPHOLOGICAL REAPPRAISAL OF CENTRONUCLEAR MYOPATHY (CNM) AFTER THE IDENTIFICATION OF DNM2 MUTATIONS</u> BEVILACQUA J², BITOUN M¹, TARATUTO A³, MONNIER N⁴, GUICHENEY P¹, FARDEAU M¹, ROMERO N¹ (1) Inserm, U582, Institut de Myologie ; AP-HP, Groupe Hospitalier Pitié-Salpêtrière ; UPMC Université Pierre et Marie Curie-Paris 6, UMR S582, IFR14, Paris, FRANCE. (2) Departamento de Neurología y Neurocirugía, Hospital Clínico Universidad de Chile and Instituto de Ciencias Biomédicas Universidad de Chile, Santiago, CHILE. (3) FLENI, Department of Pathology, Buenos Aires, ARGENTINA. (4) Laboratoire de Biochimie et Génétique Moléculaire, CHU Grenoble, Grenoble, FRANCE.</p>
To contact the author:: nb.romero@institut-myologie.org.	<p>Muscular biopsies of congenital myopathy patients showing a high rate of central nuclei as the most important finding were usually classified as having CNM. With the identification of DNM2 mutations as the cause of autosomal centronuclear myopathy (Bitoun et al. 2005), two main groups arose from CNM patients, those mutated on DNM2 and those excluded for DNM2 mutations (about 50% of patients in each group). Interestingly, myotubular myopathy and some recessive forms of CNM myopathy are caused by mutations in proteins functionally linked to DNM2 (i.e. myotubularin, amphiphysin-2).</p> <p>We reviewed a large series of biopsies with diagnosis of CNM before <i>DNM2</i> mutation identification. According to our observations, proper observation of morphological criteria is important to orientate molecular analysis of the different CNM subgroups. Nuclear “centralization” is very typical of <i>DNM2</i> related cases, and should be distinguished from random nuclear “internalization”. Additional structural alterations within muscular fibers are another criterion useful to suggest or discard <i>DNM2</i> related CNM. Typical aspects of radiating sarcoplasmic strands “spoke of wheel” pattern is seen with mutations in <i>DNM2</i> almost exclusively. On the other hand, the association of nuclear internalization (but no centralization) with pseudo-core lesions would lead to mutations in <i>RYR1</i> as the first possibility.</p> <p>Amongst the CNM diagnosed cases, we identified at least two other subgroups. One showing internalized nuclei associated with a peculiar subsarcolemmal alteration that resembled a “dark lace”. In these cases, nuclei aligned with the “lace” of distorted material. In another group, nuclear internalization was higher than normal but the hallmark of the biopsy was the presence of rounded fibers with a strong subsarcolemmal positive staining with oxidative techniques.</p> <p>Percentages of fibers with centralized or internalized nuclei, with associated histopathological features (i.e. presence or not of radiating sarcoplasmic strands) are good indicators for underlying genotypes.</p>

PW14-180	<p><u>DYNAMIN 2: EXPRESSION IN HUMAN SKELETAL MUSCLE AND LOCALISATION IN AN IN VIVO MOUSE MODEL AFTER TRANSFECTION OF WILD TYPE OR MUTANTS</u></p> <p>DURIEUX AC¹, BITOUN M¹, PRUDHON B¹, GUICHENEY P¹ (1) INSERM, U582, Institut de Myologie; Université Pierre et Marie Curie-Paris 6, Paris, FRANCE.</p>
<p>To contact the author:: ac.durieux@institut-myologie.org.</p>	<p>Autosomal dominant centronuclear myopathy (AD-CNM) is a rare congenital myopathy, clinically characterized by delayed motor milestones and muscle weakness and often associated with ptosis and ophthalmoplegia. The gene responsible for AD-CNM has been identified as <i>DNM2</i>, which encodes dynamin 2 (DNM2). This protein is a member of the large GTPase family that includes three dynamin-encoding genes (<i>DNM1</i>, 2 and 3), with distinct tissue expression patterns and several isoforms. RNA was extracted from human brain, skeletal muscles and cultured primary muscle cells. RT-PCR was performed to determine the dynamin isoforms expressed in these tissues. The 3 genes are expressed in brain, whereas only <i>DNM2</i> is expressed in skeletal muscle, human primary myoblasts and myotubes. In <i>DNM2</i>, alternative forms of exons 10 and 13 can be differentially spliced leading potentially to 4 different transcripts. By RT-PCR and sequencing we found that only 2 of these transcripts were detected in skeletal muscle: transcripts 1 (containing exons 10a and 13b), and 2 (containing 10b and 13b), corresponding to the longest sequences i.e. 870 amino acids. However, in brain and in primary cultured myoblasts and myotubes only transcript 1 is expressed. The cellular localisation of <i>DNM2</i> in skeletal muscle was unclear from standard immunohistochemical staining of muscle sections. To investigate this further, a construct encoding wild type (WT) <i>DNM2</i> transcript 1 tagged with GFP was electro-transferred into mouse <i>tibialis anterior</i> muscle. In transfected muscles GFP-tagged WT <i>DNM2</i> was distributed homogenously in the cytoplasm and localized to the sarcolemma. In contrast, when GFP-tagged mutant R465W <i>DNM2</i> was transfected, staining was restricted to the sarcolemma. The R465W mutation is the most common AD-CNM mutation reported. Consequences of this alteration on the function of <i>DNM2</i> into skeletal muscle remain to be determined.</p>

PW14-181	<p><u>SPECTRUM OF DYNAMIN 2 MUTATIONS IN AUTOSOMAL CENTRONUCLEAR MYOPATHY AND AXONAL CHARCOT-MARIE-TOOTH DISEASE.</u></p> <p>BITOUN M¹, PRUDHON B¹, DURIEUX AC¹, BEVILACQUA J¹, STOJKOVIC T², OLDFORS A³, MAURAGE CA⁴, EYMARD B², FARDEAU M¹, ROMERO N¹, GUICHENEY P¹</p> <p>(1) INSERM U582, Institut de Myologie, UPMC Paris6, AP-HP, Paris, FRANCE. (2) Centre de référence de Pathologie Neuromusculaire Paris-Est, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (3) Department of Pathology, Sahlgrenska University Hospital, Göteborg, SWEDEN. (4) Service de Neuropathologie, CHU de Lille, Lille, FRANCE.</p>
To contact the author:: m.bitoun@institut-myologie.org.	<p>Dynamin 2 (DNM2) mutations have been associated with two distinct clinical presentations: autosomal dominant centronuclear myopathy (CNM), a congenital myopathy, and dominant intermediate and axonal Charcot-Marie-Tooth disease (CMT), a peripheral neuropathy. To date, the sequencing of <i>DNM2</i> gene has lead us to identify 13 heterozygous mutations in 39 CNM families and one mutation in an axonal CMT patient. Five DNM2-CNM mutations are located in the middle domain (MD), seven in the Pleckstrin Homology (PH) domain and one in the GTPase effector domain (GED) of the protein. The CMT-DNM2 mutation (p.K559del) is located in the PH domain in which five DNM2 mutations have been previously identified. Therefore, mutations in the PH domain cause both CNM and CMT. PH domains are classically involved in targeting proteins to the plasma membrane. Structural studies of this domain from other proteins suggest that the α-sheets in the N-terminal part of the domain are involved in the interaction with membrane phosphoinositides and that the C-terminal part includes an α-helix involved in protein-protein interactions. Four out of the seven CNM-mutations in this domain, affecting residues 618, 619 and 625 in the α-helix, cause severe neonatal CNM. The 6 CMT-DNM2 mutations are restricted to a region from position 537 to 570 that includes the α-sheet region. Only one CNM-DNM2 mutation (p.E560K) was located in this 'CMT region' and is associated with an atypical presentation of CNM. This CNM mutation p.E560K is associated with the muscle morphological features of CNM, which are absent in the CMT patient with the p.K559del mutation. These data enlarge the spectrum of DNM2 mutations in CNM and CMT and highlight the clinical and morphological heterogeneity associated with DNM2 mutations. The severity of the clinical phenotype associated with α-helix PH domain CNM mutations suggests that this region is particularly important for DNM2 function in muscle.</p>