

c.826C>A, p.Leu276Ile. So, for LGMD patients without identified FKRP mutation, Sanger sequencing of this region should be systematically performed.

Thanks to this new technology, assessing in one single step all the genes linked to a-DGpathies leads to a gain of several months in the establishment of molecular diagnosis which benefits the families waiting for prenatal diagnosis. In addition, the design of our panel allows screening in parallel other glycosylation genes potentially involved in a-DGpathies.

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#### *alpha-DGpathy Dystroglycanopathies LIS II POMT1 Glycosylation NGS*

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Dystroglycanopathies- #3273

#### **P05- 75- The pathophysiological consequences of different levels of dystrophin following antisense based exon-skipping in two muscles of the mdx mouse**

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We examined the effects on muscle physiology and pathology of restoring different levels of dystrophin acutely and chronically in mdx mice with established dystrophic pathophysiology (12 weeks and 24 weeks old). Dystrophin expression was induced efficiently using cell penetrating peptides linked to an antisense sequence targeting exon 23 which contains a premature stop mutation in the mdx mouse. We assessed muscle physiology in the tibialis anterior (TA) muscle of the mouse using an in situ protocol under terminal anaesthesia. To assess muscle physiology in the diaphragm we used strips of diaphragm in an in-vitro system. In both cases we examined the force-frequency relationship and established maximum specific tetanic force. We then subjected the muscles to a 10% stretch while stimulating them to contract for 10 cycles. This eccentric exercise was highly damaging to the TA muscle but not the diaphragm. We present data showing that 15% of normal levels of dystrophin was sufficient to prevent eccentric exercise induced damage to the TA following various dosing regimens. Unlike the TA, both acute and chronic administration of PPMO significantly increased specific force in the diaphragm while a similar effect was only seen in the TA when treated chronically with the higher dose of PPMO from 12 weeks old. We present a pathological analysis to explain the differences between the muscles and further show differences in the fibre-type response to treatment. While caution must be applied when extrapolating these results to DMD patients, the results suggest that moderate levels of dystrophin may be sufficient to slow-down or possibly prevent disease progression whereas higher levels of dystrophin will also improve muscle force production but this will depend on the age of treatment and the specific muscle.

*dystrophin, mdx, antisense, DMD, dystrophic, diaphragm, tibialis anterior, physiology, muscle*

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<b>P06- Congenital myopathies- N° 76 to N° 93</b>
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Congenital myopathies- #2355

#### **P06- 76- Mechanosensing defects in muscular dystrophies related to nuclear envelope mutations**

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Recent data indicate that mutations in nuclear envelope genes cause both defects in mechanotransduction signaling and force transmission across the nuclear envelop. A central role in this mechanosensory process has been attributed to A-type lamins, which together with the Linker of the Nucleoskeleton and Cytoskeleton (LINC)-complex enables force transmission across the nuclear envelop. Whereas a basic picture is that extracellular mechanical forces are transmitted from outside the cell to the nucleus, we hypothesize that mutations in A-type lamin (LMNA) or in nesprin can affect the mechanical transmission from the nuclear envelop to the extracellular matrix. To test this hypothesis, human myoblasts with either LMNA or nesprin-1 mutations were cultured on soft substrates (12 kPa) and compared to control (WT) myoblasts. On soft surface, LMNA and nesprin-1 mutated myoblasts exhibited enlarged and increased number of focal adhesions, increased stress fibers and enlarged cell spreading area compared with WT. Further, nesprin-1 mutant exerted significantly higher traction forces on the substrate compared with WT myoblasts. These abnormalities were greatly reduced after treatment with Y27632, or SU6656, which inhibit Rho-associated protein kinase or the Src family kinase respectively, suggesting an abnormal activation of Rho-dependent Src pathway in mutant cells. Treatment with the MLCK inhibitor ML7 also significantly reduced the spreading area in mutant cells but without modifying the number and distribution of perinuclear actin stress fibers. We concluded that the integrity of the LINC complex and the lamina is required for proper regulation of the cytoplasmic actin contractility in soft substrates, through an apparent Src-dependent ROCK pathway.

*Congenital muscular dystrophy, Mechanotransduction, LINC complex*

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Congenital myopathies- #2441

#### **P06- 77- X-linked centronuclear myopathy: insights into myotubularin MTM1 function from yeast.**

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X-linked centronuclear myopathy (XLCNM) is a muscle disorder characterized by neonatal hypotonia and abnormal organelle positioning in skeletal muscle. This myopathy is due to different mutations in the MTM1 gene encoding the phosphoinositide

phosphatase myotubularin. Disease-causing mutations are found all along the protein sequence and not only in the phosphatase catalytic domain. We used the yeast *Saccharomyces cerevisiae* as a eukaryotic model to analyze the in vivo phosphatase activity of different disease mutants. We investigated the cellular effects resulting from expression or overexpression of different MTM1 disease-causing mutants in the yeast *Saccharomyces cerevisiae*. The expression of human MTM1 resulted in the enlargement of the vacuole/lysosome, a consequence of its phosphatase activity. We used the vacuolar size as a marker for the MTM1 in vivo activity. Our results show that some mutations responsible for severe forms of myopathy are active phosphatases. Therefore, these mutations would lead to XLCNM disease through a mechanism not directly linked to their phosphatase activity. To further question this finding, we used the myotubularin Mtm1 mouse knock-out (KO) model that reproduces faithfully the histopathological findings in human patients. Expression of phosphatase-dead mutants improved most phenotypes of Mtm1 KO mice. This shows that the maintenance of normal skeletal muscles is largely independent from myotubularin phosphatase activity, while defects in the activity may participate in the onset of the disease. These results show that yeast *S. cerevisiae* is a good model to determine the in vivo activity of human myotubularin and can be used to further define the molecular impact of patient mutations.

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*centronuclear myopathy, yeast model, myotubularin, phosphoinositide*

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Congenital myopathies- #2444

**P06- 78- The MTMR2 myotubularin-related protein, a new target to suppress MTM1 myopathy.**

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The X-linked centronuclear myopathy (XLCNM) and the Charcot-Marie-Tooth neuropathy type 4B (CMT4B) are neuromuscular diseases caused by mutations in lipid phosphatases: the myotubularin MTM1 for the XLCNM and the myotubularin-related protein MTMR2 for CMT4B. Although they are involved in two distinct pathologies, MTM1 and MTMR2 are ubiquitously expressed in human tissues and very similar in sequence, catalytic function and domain organization.

Our recent results show that in yeast cells, MTM1 and MTMR2 lead to different vacuolar morphologies linked to different subcellular localizations: MTM1 is associated with membranes and its in vivo phosphatase activity induces a vacuolar enlargement, whereas MTMR2 is not associated with membranes and thus cannot dephosphorylate its lipid substrates and the yeast vacuoles remain fragmented. Interestingly, truncation of MTMR2 restores the MTM1-like phenotype in yeast cells. Thus, specific sequences seem to be involved in functional differences between MTM1 and MTMR2, which opens a complete new field of investigation.

Based on this, the aim of my PhD project is to determine if the expression of modified forms of MTMR2 ameliorates the XLCNM-like phenotypes of the Mtm1 KO mice.

Elaborate a therapeutic approach targeting the specific sequences of the endogenous MTMR2 and rendering it more similar to MTM1 in XLCNM patients' muscles. It would compensate for non-functional MTM1 and thus restore a normal muscle organization and contractility.

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*Centronuclear myopathy, myotubularin, MTM1, MTMR2, AAV*

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Congenital myopathies- #2445

**P06- 79- X-linked centronuclear myopathy: using the yeast *Saccharomyces cerevisiae* to better understand the cellular function of the human Myotubularin MTM1**

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Myotubularin MTM1 is a lipid phosphatase with specificity towards the phosphoinositides PtdIns(3)P and PtdIns(3,5)P2. Different mutations in the MTM1 gene were isolated from patients with a muscle disease, X-linked centronuclear myopathy (XLCNM). We investigated the cellular effects resulting from expression or overexpression of different MTM1 disease-causing mutants in the yeast *Saccharomyces cerevisiae*. The expression of human MTM1 resulted in the enlargement of the vacuole/lysosome, a consequence of its phosphatase activity. We used the vacuolar size as a marker for the MTM1 in vivo activity. Microscopic observation allowed the classification from the most to the least active MTM1 forms in vivo: MTM1, MTM1R69C, MTM1V49F, MTM1N180K, MTM1R421Q and the inactive control MTM1C375S. These results were confirmed by in vitro phosphatase assays and in vivo lipid dosage. Yeast cells producing MTM1V49F and MTM1R69C proteins affected in the PH-GRAM domain displayed a clear defect in membrane trafficking at the endosomal level, contrary to what was observed with MTM1 and MTM1N180K. Therefore, these mutations could lead to XLCNM disease through a mechanism not directly linked to their phosphatase activity but rather to their membrane trafficking regulation. These results show that yeast *S. cerevisiae* is a good model to determine the in vivo activity of human myotubularin and might be used to further define the molecular impact of patient mutations.

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*MTM1, Yeast, myopathy*

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Congenital myopathies- #2446

**P06- 80- Study of Myotubularin, a phosphatase responsible for centronuclear myopathy by using as a model the yeast *Saccharomyces cerevisiae*.**

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Centronuclear myopathy (CNM) is a genetic muscular disease characterized at the histological level by nuclei at the center of the myofibers instead of the periphery. Mutations in three genes (MTM1, DNM2 and BIN1) are associated with this pathology. MTM1 on the X chromosome affects males and codes for an enzyme called myotubularin. Myotubularin is a phosphoinositide lipid phosphatase. The cellular processes controlled by MTM1 at the muscular level are still unknown. MTM1 study in human cells is complicated due to the presence of 14 homologues. Thus, we used the unicellular eukaryotic yeast model *Saccharomyces cerevisiae* to study MTM1. Yeast has a similar organellar organization as human cells and encodes only 1 myotubularin, termed Ymr1 (yeast myotubularin related 1). To better understand the cellular function of MTM1, we are studying the two main domains of this protein: the PH-GRAM N-terminal lipid binding domain and the C-terminal catalytic phosphatase domain. The two domains were tagged with different fluorescent reporters (GFP and mCherry) and either expressed or co-expressed into yeast cells. The yeast cells were observed by fluorescence microscopy to analyze the cellular localization and function of these MTM1 domains. We also tested two CNM patient mutants affected into the PH-GRAM domain. The results show that intermolecular interactions between the N-terminal PH-GRAM and the C-terminal catalytic domain are required to restore a catalytically active MTM1 phosphatase.

*Myopathy, myotubularins, MTM1, phosphoinositides, PH-GRAM domain, yeast*

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Congenital myopathies- #2454

#### **P06- 81- A role for endocytic proteins in muscle mechanotransduction**

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Costameres represent specialized focal adhesion sites of muscle fibres, located between the plasma membrane and sarcomeres, the contractile units of muscle. When disrupted, they directly contribute to the development of several distinct myopathies.

We have shown that the ubiquitous clathrin heavy chain (CHC), well characterized for its role in intracellular membrane traffic and endocytosis from the plasma membrane (PM), is a component of costameres in muscle cells and forms large plaques connected to  $\gamma$ -actinin and actin filaments. Depletion of CHC leads to defective costamere formation and maintenance both in vitro and in vivo and induces sarcomere disorganization and a loss of contractile force due to the detachment of sarcomeres from the PM.

At costameres, CHC is co-expressed with dynamin 2 (DNM2), another key protein of the intracellular membrane trafficking machinery which is mutated in autosomal dominant centronuclear myopathy (CNM). Similarly to clathrin, DNM2 is required for proper  $\gamma$ -actinin organization in vitro. With this project, we aimed at pursuing the characterization of this unconventional role of the endocytic machinery in costamere formation and maintenance.

We analyzed the role of DNM2 and several actin binding proteins on clathrin plaque function at costameres by siRNA depletion combined to high resolution electron microscopy. Dynamics of clathrin plaques were determined for the first time in vivo by intravital microscopy of muscle transduced with an rAAV construct expressing the mu2 subunit of the AP2 clathrin adaptor.

Finally, we focused on the possible link between costamere and CNM pathophysiology. Using muscles from a knock-in mouse model of DNM2-related myopathy, we analyzed the structure and defects of costameres by immunocytochemical approaches.

Our results suggest that CHC, DNM2 and the actin cytoskeleton contribute to the formation and maintenance of the contractile apparatus through interactions with costameric proteins and highlight an unconventional role for clathrin flat lattices in skeletal muscle which may be relevant to pathophysiology of several neuromuscular disorders.

*endocytosis, clathrin, dynamin 2, centronuclear myopathy, cytoskeleton, actin*

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Congenital myopathies- #2535

#### **P06- 82- Pathophysiology of tubular aggregate myopathies**

*Georges Arielle PECHE (1), Catherine KOCH (1), Johann BOHM (1), Jocelyn LAPORTE (1)*

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Tubular aggregate myopathies (TAM) are progressive muscle diseases characterized by abnormal accumulations of membrane tubules in muscle fibers. Our team identified dominant mutations in STIM1 in TAM. STIM1 is the main calcium sensor of the endo/sarcoplasmic reticulum (ER/SR). Following a stimulus, calcium ( $\text{Ca}^{2+}$ ) is released from the ER/SR into the cytoplasm where it triggers muscle contraction. Upon store depletion, STIM1 unfolds, oligomerizes and activates the ORAI1 channel to allow  $\text{Ca}^{2+}$  entry. This mechanism is known as store-operated calcium entry (SOCE). We demonstrated that STIM1 mutations lead to an impairment of  $\text{Ca}^{2+}$  homeostasis in TAM myoblasts. However, the physiopathology of tubular aggregates and the link between STIM1 mutations and muscle dysfunction need to be unraveled.

We aim to decipher the sequence of physiological events that leads from STIM1 mutations to the formation of tubular aggregates and to muscle dysfunction in cellular and animal models.

In order to study the impact of STIM1 mutations on clustering and ORAI1 recruitment and opening, C2C12 murine myoblasts or HeLa cells were transfected with mutant or wild-type (WT) STIM1 constructs. Unlike WT STIM1, mutant STIM1 constitutively clusters and recruits ORAI1. Calcium level measurements showed a higher basal  $\text{Ca}^{2+}$  level and a higher cytoplasmic  $\text{Ca}^{2+}$

increase after addition of exogenous Ca<sup>2+</sup> in patient's myoblasts and in cells transfected with mutant STIM1. Together it strongly suggests STIM1 mutations lead to a constitutive activation of SOCE.

There is currently no mammalian model for TAM. We therefore generated AAVs harboring WT or mutant STIM1, and injected them into the tibialis anterior of WT mice. We are currently assessing the impact of STIM1 mutations on muscle structure and function by histological and ultra-structural analyses at different time-points post transduction. To establish a correlation between the molecular and cellular alterations, the muscle function and the development of the disease, a second cohort of transduced mice will undergo specific muscle force measurements.

In conclusion, we aim to shed light on the physiological mechanisms leading to TAM and identify actionable cellular defects that could be rescued at the cellular level and in the animal model.

*tubular aggregates, STIM1, ORAI1, clustering, calcium*

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Congenital myopathies- #2547

**P06- 83- Reducing dynamin 2 rescues a severe congenital myopathy in mice**

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Centronuclear myopathies (CNM) are congenital disorders associated with skeletal muscle weakness and abnormally located myonuclei. An autosomal dominant form of CNM results from mutations in the gene encoding dynamin 2 (DNM2), and loss-of-function mutations in the gene encoding myotubularin (MTM1) result in X-linked centronuclear myopathy (XLCNM), which promotes severe neonatal hypotonia and early death. Currently, no effective treatments exist for XLCNM.

The main goal of this study was to validate a novel rescue approach for CNM. Recent data suggested some CNM-causing DNM2 mutations increase the dynamin oligomer stability and GTPase activity. Also overexpression of wildtype DNM2 in skeletal muscle cause a CNM-like phenotype. We hypothesize myotubularin and dynamin 2 function in a common pathway, where either MTM1 loss-of-function or DNM2 gain-of-function lead to CNM. To test this we reduced DNM2 expression in XLCNM mice (Mtm1-/-) that exhibit a progressive myopathy leading to death by 12 weeks. Mtm1-/-Dnm2+/- mice survived up to 2 years. Classical CNM histological features including fiber atrophy and nuclei mispositioning were strongly reduced, and muscle strength was increased. Downregulation of Dnm2 after disease onset could stop and potentially revert the progression of the phenotype. We are now developing a translated approach to downregulate Dnm2 in XLCNM mice. This step is essential in the preclinical development of this novel therapeutic target.

Based on the above results we used the same strategy to test the therapeutic potential of DNM2 in a different disorder. Our results identify a second muscle disease that may be treated by reducing DNM2 as a novel therapeutic target. This exciting unpublished data will be presented here.

While DNM2 is a key mechanoenzyme for important cellular processes, its reduction is beneficial for several muscle diseases and represents a novel potential therapeutic approach.

*congenital myopathy, myotubular myopathy, preclinical research, dynamin 2, myotubularin, therapy*

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Congenital myopathies- #2588

**P06- 84- Compound null/missense RYR1 mutations in a cohort of centronuclear myopathy patients**

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Mutations in the RYR1 gene give rise to diverse skeletal muscle phenotypes, such as classical central core disease and susceptibility to malignant hyperthermia. Next-generation sequencing has recently shown that RYR1 is implicated in a wide variety of additional myopathies, in particular both nonspecific and distinct congenital myopathies, including centronuclear myopathies. In this work, we screened a cohort of 23 families with centronuclear myopathy or nonspecific congenital myopathies in which centralized nuclei were present as a prominent feature in the muscle biopsy, and found seven families, totaling 10 patients, having RYR1 as the implicated gene after whole exome sequencing. Common clinical features in the cohort were an early onset in infancy with motor developmental delay, stable course with preservation of ambulation, proximal muscle weakness and normal or mildly elevated serum CK levels. Ptosis and/or ophthalmoparesis were found in 7/10 patients, facial weakness in 7/10, thoracic deformities (pectus excavatum, depression) in 5/10, and spinal involvement in 6/10. Muscle MRI imaging was done in 5 patients. The most consistently severely affected muscles were the gluteus maximus (5/5 patients) and



adductor magnus (4/5). The rectus femoris was less affected than either of the vastus in 4/5, and the biceps brachii was more affected than the triceps in 4/5. All patients had moderate signal change in paraspinal muscles and finger flexors. Muscle biopsy was performed in 9 patients and showed, in addition to a variable prominence of nuclear centralization, chosen as the inclusion criteria, a predominance of type 1 fibers in 9/9, intermyofibrillary architecture abnormalities (moth-eaten, mini-core like areas) in 7/9, and moderate increase in fatty/connective tissue in 3/9. All seven families showed an autosomal recessive inheritance pattern, and six of them harbored compound heterozygous mutations in the RYR1 gene, in which one of the mutations was null (nonsense, primary splice site disrupting, or new acceptor site creating) and the other was a missense predicted as pathogenic through variant effect evaluation software. Twelve out of the 14 mutations were novel, and were evenly distributed throughout the length of the gene, from exons 8 to 101, without a particular hot spot. This work confirms imaging, biopsy and clinical features of RYR1-related myopathies and illustrates how such findings can help guide a molecular diagnosis.

*RYR1 gene, centronuclear myopathy, congenital myopathy*

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Congenital myopathies- #2798

**P06- 85- MYOCAPTURE: 1000 exomes for gene identification and diagnosis in myopathies**

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More than 200 different myopathies have been described but about half of patients are still devoid of a genetic diagnosis, suggesting the implication of yet unlinked genes and unsuspected clinical and genetic heterogeneity. To tackle these rare genetic diseases, we launched a national consortium (?Myocapture?) bringing together research teams expert in genetics and myology, several diagnosis labs, DNA, cells and tissues banks, the national sequencing center (Centre National de Génotypage), and associated clinicians from the FILNEMUS network. Homogeneous patient cohorts were defined based on detailed clinical, histological and ultrastructural analyses. Exome sequencing was performed on 1000 patients and relatives to identify the causative mutations. Bioinformatic pipelines and specific analytic tools were developed in-house to proceed to variants filtering and ranking. The data obtained highlighted several cases: 1) some patients were linked to mutations in known myopathy genes, mainly in very large genes that were not routinely tested in diagnostic laboratories; 2) some cases were due to mutations in genes previously implicated in other muscle diseases, thus enlarging the phenotypic spectrum; 3) several novel myopathy genes were identified. Examples of genes newly linked to congenital myopathies will be presented. The overall analysis of the Myocapture data has defined ?rules? to increase the success of the exome approach. Also, a significant proportion of patients were not resolved and are still being scrutinized. We are also building an integrated knowledge base dedicated to myopathies, in order to provide analytical tools for sequence analysis and better gene prioritization, and to provide an integrated view of pathways implicated in muscle homeostasis and function under normal and pathological conditions. Taken together, the unbiased exome approach of Myocapture aims to refine the classification of myopathies and for the identification of novel pathways implicated in different myopathies and representing new drug targets.

*exome, NGS, mutations, congenital myopathy, genetic*

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Congenital myopathies- #2862

**P06- 86- Intramuscular delivery of a Dnm2 antisense in vivo-morpholino oligonucleotide rescues the muscle pathology in a murine model of myotubular myopathy**

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Centronuclear myopathies (CNM) are a group of rare genetic muscle diseases characterized by the presence of nuclei in central position of hypotrophic myofibers and the predominance of type I fibers. No curative treatment is available. The most severe form, X-linked myotubular myopathy (XLMTM), results in neonatal muscle hypotonia and weakness, respiratory insufficiency and premature death. It is caused by mutations in the MTM1 gene, which encodes a 3-phosphoinositide phosphatase named myotubularin. The two other forms of centronuclear myopathy have an autosomal inheritance and are caused by mutations in the DNM2 and BIN1 genes, encoding dynamin 2 and amphiphysin 2, respectively. Some cellular and molecular alterations are similar amongst the various forms of CNM, suggesting functional relationships between these three proteins. Interestingly, it was recently reported that Mtm1 deficient mice have increased levels of dynamin 2 in muscle and genetic downregulation of Dnm2 restored lifespan and improved muscle strength and pathology of mutant mice (Mtm1y<sup>-/-</sup>; Dnm2<sup>+/-</sup>).

We therefore developed a therapeutic strategy aimed at reducing dynamin 2 level during the postnatal period by using specific antisense morpholino oligonucleotides (PMO). We screened various Dnm2 PMOs ex vivo and selected one for in vivo studies. Intramuscular administration of this PMO in the tibialis anterior and quadriceps muscles of Mtm1 KO mice normalized

pathological dynamin 2 overexpression and ameliorated the pathological hallmarks of the disease, such as muscle hypotrophy and abnormal positioning of mitochondria. Most importantly, the contractile force of myotubularin-deficient muscles improved significantly. Altogether, these results indicate that DNM2 antisense PMOs might represent a novel strategy for the treatment of myotubular myopathy.

*Myotubular myopathy, in vivo-PMO, dynamin 2, myotubularin, muscle*

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Congenital myopathies- #2911

**P06- 87- Molecular diagnosis of COLVI-related myopathies and Ehlers-Danlos syndromes: a new targeted custom panel analyzed by High Throughput sequencing**

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Ullrich Congenital Muscular Dystrophy (UCMD) and Bethlem myopathy represent the two opposite end points of a phenotypic spectrum caused by collagen VI abnormalities and characterized by muscle weakness, contractures, joint hypermobility, and frequently cutaneous signs (follicular hyperkeratosis, soft velvety skin, keloid). Ehlers-Danlos syndrome (EDS) is a group of connective tissue diseases which in some types associate joint hypermobility, abnormal bruising, cutaneous symptoms (as skin hyperextensibility, atrophic scarring) and constitute a differential diagnosis of collagen VI myopathies. Our laboratory has a long experience in collagen VI molecular diagnosis. Recent evolutions in sequencing allowed us to enlarge our diagnostic tools by generating a custom panel of 13 genes associated to muscular weakness and joint hyperlaxity then sequenced by high throughput sequencing (NGS).

We designed a panel of 13 genes (530 targets, about 90kb) including the COL6 genes (A1, A2 and A3), COL12A1, and genes associated with EDS type I, II, III, VI, VII and kyphoscoliotic type. After DNA fragmentation, coding exons of the selected genes were captured and sequenced on a MiSeq sequencer (Illumina). Bioinformatic analysis was performed by Genodiag (ICM, Pitié Salpêtrière), and the variant files were then analysed in our laboratory. Mutations and potential pathogenic variants were confirmed by Sanger sequencing.

DNA of 20 patients was analysed. Median coverage was satisfactory and from the 530 regions sequenced, one or several nucleotides were missed in less than 1% of the targets. This technique allowed the detection of a number of genetic variations, and knowledge of phenotypic data was essential to the interpretation of their pathogenicity in order to deliver a better genetic counselling.

*hyperlaxity, COLVI, Ehlers-Danlos, NGS*

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Congenital myopathies- #2937

**P06- 88- Preclinical approaches to downregulate dynamin 2 in centronuclear myopathies**

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Centronuclear myopathies (CNM) are a group of rare severe muscle diseases characterized by muscle weakness. Muscle biopsies from CNM patients show hypotrophic fibers with centralized nuclei. Three forms have been described: X-linked, Autosomal-recessive (AR) and Autosomal-dominant forms caused by mutations in Myotubularin (MTM1), Amphiphysin 2 (BIN1) and Dynamin 2 (DNM2) respectively. However, the physiopathological mechanisms are barely understood and no specific therapy is available.

Using a novel "cross therapy" approach, our group has shown that genetic reduction of Dnm2 in the Mtm1 Knock-out (KO) mice restores a normal lifespan (from 1-3 months to 2 years) with improved muscle structure and function. Therefore, we aim to translate this proof-of-principal experiment by reducing Dnm2 expression using deliverable agents. Several shRNA sequences that target specifically mRNA were selected and screened on human embryonic Kidney (HEK) and mouse C2C12 myoblasts. The best shRNA candidates for downregulating Dnm2 were then selected for adeno-associated virus (AAV) production, and used to inject in vivo into mice to confirm the reduction of Dnm2 expression detected in cells.

Our overall goal is to develop a translated approach to downregulate Dnm2 in vivo, and to show an improvement in lifespan and disease pathology in Mtm1 KO mice. This would confirm that reducing DNM2 is a novel therapeutic target for X-linked CNM.

*centronuclear myopathies, dynamin 2, myotubularin 1, BIN1, gene therapy*

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Congenital myopathies- #2978

**P06- 89- Deep RNA profiling identified CLOCK and molecular clock genes as pathophysiological signatures in collagen VI myopathy.**

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Collagen VI myopathies are genetic disorders due to mutations in collagen 6 A1, 2, and 3 genes, ranging from the severe Ullrich congenital muscular dystrophy to the milder Bethlem Myopathy, which is recapitulated by collagen VI null (Col6a1-/-) mice. Abnormalities in mitochondria and autophagic pathway have been proposed as pathogenic causes of collagen VI myopathies, but the link between collagen VI defects and these metabolic circuits remains unknown.

To unravel the expression profiling perturbation in muscles with collagen VI myopathies we performed a deep RNA profiling in both Col6a1-/- mice and COL6 patients. An interactome map that highlights the connection between the altered pathways, including circadian rhythms, and collagen VI pathology was built up.

Intriguingly, Bmal1-/- mice, a well-characterized model displaying arrhythmic circadian rhythms, showed profound deregulation of the collagen VI pathway and autophagy-related genes. The involvement of circadian rhythms in collagen VI myopathies is new and links autophagy and mitochondrial abnormalities. It also opens new avenues for therapies of hereditary myopathies to modulate the molecular clock or potential gene-environment interactions that may modify muscle damage pathogenesis.

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*Collagen VI, congenital myopathy, circadian rhythm, CLOCK gene, RNAseq*

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Congenital myopathies- #2982

**P06- 90- A new X-linked congenital myopathy with atypical cores associated with SRPK3 deficiency: description of a French family**

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Congenital myopathies are a heterogeneous group of inherited muscle disorders characterised by the presence of distinctive morphological features on skeletal muscle biopsy. Cores myopathies are characterised by the presence of cores in the muscle fibers, which correspond to broad areas of myofibrillar disorganization, Z-line streaming, and lack of mitochondria. In addition to the histologically well-defined core myopathies, areas of myofibrillar disorganization (e.g. minicores, core-like areas, atypical cores etc.), have been described in clinically heterogeneous congenital myopathies.

Here we describe the clinical and histopathological features of two affected male individuals mutated in the X-linked muscle specific kinase SRPK3 belonging to a non-consanguineous French family.

The phenotype consisted of delayed gait acquisition with the early development of marked axial and milder proximal muscle weakness, more progressive in adult age.

Whole body MRI showed bilateral fatty infiltration of thigh muscles.

Muscle morphological lesions consisted of: 1) multiple and poorly defined areas of uneven oxidative staining; 2) type 1 predominance or uniformity; 3) prominent nuclear internalizations; 4) presence of frequent muscle fibers splitting.

With our detailed clinico-histopathological analysis of SRPK3 X-linked congenital myopathy with atypical cores we alert clinicians and pathologists on the recognition of this novel entity.

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*Congenital myopathies, Core myopathies, SRPK3 genes*

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Congenital myopathies- #3007

**P06- 91- Mild clinical presentation in KLHL40-related Nemaline Myopathy (NEM 8)**

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To date, eleven genes have been identified for nemaline myopathy (NM), a common congenital myopathy. Mutations in the KLHL40 (kelch-like family member 40) gene (NEM 8) are common cause of severe/lethal NM. Clinically, these patients present foetal akinesia, hyperkinesia and contractures, respiratory failure and swallowing difficulties at birth. The survival varies between the neonatal period to adolescence.

We present the case of an 8-year-old girl from first degree cousins Moroccan parents, with no medical history. The pregnancy was uneventful and she was born eutrophic at 39 gestational weeks. She was quickly hospitalised in the neonatal unit for axial hypotonia, difficult eye contact and poor sucking.

Her evolution slowly improved. Hypomimia, deficit of the orbicularis oculi and drooling were noticed. Had control was achieved at 6 months and walking at 20. At 3 years, she was able to run and ride a bike but had difficulties in climbing stairs. She had a waddling gait and predominant axial weakness. All the activities were performed very slowly. The patient complained a lot about tiredness and used a manual wheel chair for long distances. The neck flexors and extensors were weak. Her coughing was rather inefficient and the sniff nasal inspiratory pressure was low.

Last seen at 8 years, her height was on the 5th percentile for a height on the 50th, and had normal intelligence. She walked 599 meters at the 6-minute walking test and was able to climb some steps without the rail. The Gowers sign was negative. Axial tonus was stable and she had respiratory and digestive autonomy.

At first, an extensive metabolism work-up was carried on and no specific abnormality was detected. The karyotype was normal and molecular diagnosis for Prader Willi and Steinert myotonic dystrophy was negative.

A muscle biopsy performed at the age of 3 years showed the presence of nemaline bodies.

The molecular analysis for ACTA1, TPM2, TPM3, KBTBD13, LMOD3, TNNT1, CFL2 and KLHL41 by exon sequencing did not find any abnormalities. The linkage analysis for the NEB locus was not contributory. A mutation search in KLHL40 found a homozygous variation of the KLHL40 gene, c.1498C>T (p.Arg500Cys), never described before and predictive to be pathogenic. Western blot analysis revealed absence of KLHL40 protein expression supporting the pathogenicity of c.1498C>T.

To our knowledge, this is the first case KLHL40-related nemaline myopathy presenting with a mild clinical phenotype.

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#### *nemaline myopathy, KLHL40*

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Congenital myopathies- #3050

#### **P06- 92- Further evidence that loss of function mutations of TNNT1 cause Nemaline Myopathy (NM) and are not restricted to specific ethnic groups.**

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Introduction: ANM was initially reported only in the old order Amish community (Johnston et al, 2000). The homozygous p.Glu180\* none sense founder mutation in the skeletal muscle slow troponin encoding gene TNNT1 causes a lethal type of nemaline myopathy (NM) with neonatal tremors, progressive contractures especially of the hips and knees, severe pectus carinatum and kyphosis with progressive chronic respiratory distress, axial, limb and facial muscle weakness and muscle atrophy, and nemaline bodies on muscle histology. Recently, three Dutch (Van Der Pol et al, 2013) and one Hispanic patient (Marra et al, 2015) were identified having TNNT1 mutations and NM. Clinical features were very similar to those previously described in the ANM. Dutch patients were respectively homozygous for the splicing mutation TNNT1 c.309+1G>A leading to the skipping of exon 8 and compound heterozygous for this mutation and a heterozygous exon 14 deletion. Homozygosity for TNNT1 p.Ser108\* was found in the patient with Hispanic ancestry. Complete loss of slow troponin in ANM muscle was associated with the homozygous p.Glu180\* mutation (Jin et al, 2003). The progressive deterioration of muscle function in ANM leading finally to death was suggested to be due to the physiological down-regulation of embryonic fast and cardiac troponin T during the first months of life in ANM skeletal muscle.

Case report: We report on a male patient from consanguineous North African parents presenting with axial, limb and facial hypotonia from birth. He died from respiratory distress at the age of 6y. Muscle biopsy showed type 1 hypotrophy and nemaline bodies. Molecular analysis and homozygosity mapping excluded currently known genes in NM, but found a homozygous c.16G>T/p.Glu6\* mutation in TNNT1. Western blot analysis of the muscle showed complete absence of slow skeletal troponin T. No cardiac isoform of troponin T was detected in our patient's muscle biopsy on western-blot analysis.

Discussion: No clear correlation between the mild phenotypic variability observed in patients with ANM and their TNNT1 mutations could be established until now. Our findings suggest that the absence of cardiac troponin T in post-natal skeletal muscle in our patient triggered a more severe form of TNNT1 associated NM, although the reason for the lack of cardiac troponin T remains to be elucidated. Our findings further show that TNNT1 mutations with NM are not restricted to the Amish community or European ancestry.

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#### *Nemaline Myopathy, TNNT1, Amish Nemaline Myopathy*

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Congenital myopathies- #3237



**P06- 93- Case report of ?double trouble? patient- combination of Ullrich congenital muscular dystrophy and spastic ataxia Charlevoix-Saguenay type.**

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We present a case report of a five year old boy of a Russian origin with symptoms of Ullrich CMD. He was born with floppy infant signs and had motor delay at first year of life. Our patient started to walk independently since 23 months, had gait disturbances and muscular wasting and could not get up if fallen. He also had drop head syndrome and have been able to control his head only at 3-3,5 y.o. He developed funnel chest deformity, ankles, knees, hips, shoulders and elbows contractures in combination with hyper elasticity in phalangeal joints. Skin abnormalities included follicular hyperkeratosis on the hips, ankles, shoulders, arms, and face; a rough keloid scar appeared after muscle biopsy on the right hip. Muscle tone was diffusely reduced, DTR were not presented. CK level was within a normal range (164, 176 U/l). Muscle MRI showed diffuse fat replacement in hips and ankles with a ?tiger? pattern, very specific to collagen VI. The only test not confirming Ullrich CMD was EMG study. EMG registered MUAP's with median amplitude 2696 mV, and maximum amplitude 4683 mV, and signs of progressive loss of motor units. It was unexpected as we did not expect neurogenic disorder in our patient. All types of SMA related to SMN gene were excluded. NGS was performed and we found 3 mutations in COL6A2 gene (mutations related to Ullrich CMD) and 3 mutations in SACS gene (mutations related to spastic ataxia Charlevoix-Saguenay type). All motor development features, clinical features, CK level and MRI findings could be explained by collagenopathy- mutations in COL6A2 gene, but not EMG results. Neurogenic changes in our patient's EMG may be explained by SACS mutations that could be a cause of the specific pathway of this slow current neurogenic disorder. Vermis atrophy and degeneration of cerebellar hemispheres leads to pyramidal tract suffering and subsequent degeneration of the lower motor neuron, which damage we observed on the needle EMG. Usually patients with spastic ataxia Charlevoix-Saguenay type are characterized with cerebellar ataxia, nystagmus, lesions of pyramidal tracts, spasticity, high DTR and peripheral neuropathy. Cognitive functions are not involved. Sometimes it can be an atypically late onset or peripheral neuropathy only. We think that our patient has both disorders, with clinical features of Ullrich CMD dominating at present, co-existing with spastic ataxia Charlevoix-Saguenay type, which may become more active later and complicate Ullrich CMD.

*Ullrich congenital muscular dystrophy, spastic ataxia Charlevoix-Saguenay type, COL6A2 gene, SACS gene, neurogenic changes, EMG, MUAP's, muscle MRI,*

**P07- Distal myopathies / Myofibrillar myopathies- N° 94 to N° 103**

Distal myopathies- #4466

**P07- 94- Decline in Upper and Lower Extremity Muscle Strength Observed Over 1 Year in a Prospective, Observational GNE-Myopathy Natural History Study**

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GNE Myopathy (GNEM) is a rare, severely debilitating adult-onset myopathy with progressive muscle weakness caused by a mutation in the GNE gene that encodes an enzyme critical to the biosynthesis of sialic acid (SA). To gain a better understanding of the clinical presentation and progression of the disease, a natural history study was initiated in 2014. Up to 100 subjects are planned to be enrolled and followed for a minimum of 3 y under a research protocol.

This is an international, prospective, observational study in subjects with genetically confirmed GNEM. Five study sites in North America and Europe are participating. Enrolled subjects may continue to take substances they feel provide benefit and may participate in clinical trials of investigational products for GNEM treatment. Muscle strength was measured by hand held dynamometry (HHD) at baseline, 6, and 12 months during the first year and then yearly thereafter. Individual muscle group strength in the upper extremities (grip, shoulder abductors, elbow flexors and extensors) and lower extremities (hip flexors, extensors, abductors, adductors, and knee flexors) were combined to generate composite scores (UEC and LEC). Strength was compared with age- and gender-matched normative values.

A total of 48 subjects who had completed 12 months of follow up were evaluated. The mean age of subjects was 41 y, 56% were men, and most were white (94%). Eighteen subjects (37.5%) could walk >200m in the 6-minute walk test (6MWT) at baseline; 30 subjects (62.5%) could walk

In this broad cohort of subjects with GNEM, substantial muscle strength impairment was observed at baseline and declined measurably over 1 y in both the upper and lower extremities, regardless of walking ability. These findings are consistent with other observational studies of GNEM showing the progressive decline of muscle strength in a period as little as 1 y.