

**PW 9:  
LGMD (part 2) –  
sarcoglycans, caveolin  
and other forms**

PW9-101	<p><b><u>ALTERATIONS OF THE CA<sup>2+</sup> HOMEOSTASIS CORRELATE DYSTROPHIC PHENOTYPE SEVERITY IN NATIVE DELTA-SARCOGLYCAN DEFICIENT SKELETAL MUSCLES</u></b></p> <p>FRAYSSE B<sup>1</sup>, SALMON A<sup>1</sup>, NAGI S<sup>1</sup>, FISZMAN M<sup>1</sup>, FROMES Y<sup>1</sup>  (1) Institut de Myologie-INSERM U582-Université Pierre et Marie Curis, Paris, FRANCE.</p>
To contact the author:: b.fraysse@institut-myologie.org.	<p>Sarcolemmopathies are muscular dystrophies caused by defects of proteins located within or near cell membrane. Underlying Pathological mechanisms remain unclear. Nevertheless, in dystrophin deficient <i>mdx</i> mice it has been shown that skeletal muscle fibers exhibited cytosolic Ca<sup>2+</sup> overload due to an increased sarcolemmal permeability. Ultimately, Ca<sup>2+</sup> dependant processes, such as proteolysis over-activation or mitochondrion dysfunction, have been proposed to trigger muscle fiber death. We challenged herein the calcium hypothesis in the delta-sarcoglycan deficient hamster CHF147 that developed muscular dystrophy mainly affecting proximal skeletal muscles. Using Fura-2, a fluorescent Ca<sup>2+</sup> probe, we determined cytosolic Ca<sup>2+</sup> level ([Ca<sup>2+</sup>]<sub>i</sub>) in mechanically dissected single skeletal muscle fibers (Quadriceps and EDL (<i>extensor digitorum longus</i>)) from control and CHF147 Syrian hamsters. Additionally, we evaluated sarcolemmal permeability to Ca<sup>2+</sup> using the Mn<sup>2+</sup> quenching method. In CHF147 skeletal muscle fibers the [Ca<sup>2+</sup>]<sub>i</sub> was significantly increased (<i>p</i>&lt;0.05). This was more pronounced in Quadriceps fibers (In nM: 100.4 +/- 5.8 vs. 56.3 +/- 4.7 measured in control) than in EDL ones (in nM: 77.4 +/- 4.8 vs. 55.0 +/- 5.6 measured in control). In both muscle types from deficient delta-sarcoglycan hamsters, Mn<sup>2+</sup> quench rate was two fold increased (in % of initial fluorescence vs. control value: Quadriceps: 10.2 +/- 1.3 vs.23.2 +/- 2.2; EDL: 8.9 +/- 0.7 vs.19.7 +/- 2.3; <i>p</i>&lt;0.05). We verified that delta-sarcoglycan deficiency is associated with a cytosolic Ca<sup>2+</sup> overload in striated muscles that appeared to depend on, at least in part, an increase in the sarcolemmal permeability to Ca<sup>2+</sup>. This may be a key determinant pathological step since [Ca<sup>2+</sup>]<sub>i</sub> was more elevated in CHF147 Quadriceps muscle fibers than in EDL ones, in respect to the more pronounced dystrophic phenotype observed in the former muscle. Further analysis are needed to precise the role of alterations in calcium homeostasis in the pathological process affecting delta-deficient muscle.</p>

PW9-102	<p><b>MONITORING OF THE MUSCULAR FUNCTION IN SGCA-NULL MICE</b>  FOUGEROUSSE F<sup>1</sup>, GIANNESINI B<sup>2</sup>, DURAND M<sup>1</sup>, GUERCHET N<sup>1</sup>, BENDAHAN D<sup>2</sup>, COZZONE PJ<sup>2</sup>, RICHARD I<sup>1</sup>  (1) Genethon, RD, Evry, FRANCE. (2) Centre de Résonance Magnétique Biologique et Médicale (CRMBM), UMR 6612 CNRS - Université de la Méditerranée, Faculté de Médecine de la Timone, Marseille, FRANCE.</p>
	<p>We aim at characterizing muscle function in a cohort of different animal models for LGMD2 using mechanical parameters in several isolated muscles. Non invasive techniques will also be applied such as grip, escape or wire tests and measurements of muscular force coupled to magnetic resonance imaging and spectroscopy. The ultimate purpose of the project is to follow the effects of therapies in these models. Alpha-sarcoglycanopathy (LGMD2D) is an autosomal recessively inherited limb-girdle muscular dystrophy caused by mutations in the alpha-sarcoglycan gene, SGCA. Disruption of the SGCA gene in mouse (sgca-null) recapitulates many of the clinical phenotypes observed in patients. These mice developed progressive muscular dystrophy. We first measured the significant decrease of force of the isolated muscles in 2 to 6 month-old sgca-null mice. The most dramatic difference with the wild-type mice was observed after a series of eccentric contractions (the loss of force being more than 40% in 2.5 monthold mice). Considering the severity of the sgca-null mice, we used this model for setting up different assays to get a longitudinal study. Global activity of the animals evaluating the pulling force or the forelimb muscle strength or endurance by different classical means illustrated their weakness. Other experiments of animals of 4 and 6 month-old were able to detect an impressive decrease of force after repeated electrical stimulation coupled to magnetic resonance imaging and spectroscopy. These animals did not show any difference in their energy metabolism compared to the control mice. This approach will help to evaluate the efficiency of pharmaceutical or gene therapies and define parameters that will be worth to study during clinical trial in human.</p>

PW9-103	<p><b>REVISED SPECTRUM OF MUTATIONS IN SARCOGLYCANOPATHIES</b>  TRABELSI M<sup>1</sup>, KAVIAN N<sup>1</sup>, DAOUD F<sup>2</sup>, COMMERE V<sup>3</sup>, DEBURGRAVE N<sup>3</sup>,  BEUGNET C<sup>3</sup>, LLENSE S<sup>3</sup>, BARBOT JC<sup>3</sup>, VASSON A<sup>3</sup>, KAPLAN JC<sup>4</sup>, LETURCQ F<sup>4</sup>,  CHELLY J<sup>4</sup>  (1) Laboratoire de Biochimie et Génétique Moléculaire. Hôpital Cochin et Inserm, U567, paris, FRANCE. (2) Institut Cochin, Université Paris Descartes, CNRS (UMR 8104) et Inserm, U567, paris, FRANCE. (3) Laboratoire de Biochimie et Génétique Moléculaire. Hôpital Cochin, paris, FRANCE. (4) Laboratoire de Biochimie et Génétique Moléculaire. Hôpital Cochin; Institut Cochin, Université Paris Descartes, CNRS (UMR 8104)et Inserm, U567, paris, FRANCE.</p>
To contact the author:: mediha_tr@yahoo.fr.	<p>In order to define the spectrum of mutations in <math>\alpha</math>-, <math>\beta</math>-, <math>\gamma</math>-, <math>\delta</math>-Sarcoglycan (SG) genes, we analyzed these genes in 69 probands with clinical and biological criteria compatible with the diagnosis of autosomal recessive Limb-Girdle-Muscular-Dystrophy. For 48 patients, muscle biopsies were available and Multiplex Western-Blot WB analysis of muscle proteins showed significant abnormalities of <math>\alpha</math>- and <math>\beta</math>-SG. Our diagnostic strategy includes multiplex Western blot, sequencing of SG genes, multiplex quantitative-fluorescent PCR and RT-PCR analyses. Mutations were detected in 57 patients and homozygous or compound heterozygous mutations were identified in 75 % (36/48) of the patients with abnormal WB, and in 52% (11/21) of the patients without muscle biopsy. Involvement of <math>\alpha</math>-SG was demonstrated in 55.3% of cases (26/47), whereas <math>\beta</math>- SG and <math>\gamma</math>- SG were implicated in 25.5 (12/47) and in 17% (8/47) of cases, respectively. Interestingly, we identified 25 novel mutations, and a significant proportion of these mutations correspond to deletions (identified in 14 patients) of complete exon(s) of <math>\alpha</math>- or <math>\beta</math>-SG genes, and partial duplications (identified in 5 patients) of exon 1 of <math>\alpha</math>-SG gene. This study highlights the high frequency of exonic deletions of <math>\alpha</math>- and <math>\beta</math>-SG genes, as well as the presence of a hot spot of duplications affecting exon 1 of the <math>\alpha</math>-SG gene. In addition, protein analysis by multiplex Western blot in combination with mutation screening and genotyping results allowed to propose a comprehensive and efficient diagnostic strategy and strongly suggested the implication of additional genes, yet to be identified, in sarcoglycanopathy-like disorders.</p>

PW9-104	<p><b><u>PULLING MEMBRANE NANOTUBES FOR PROBING THE ROLE OF CAVEOLIN-3 IN THE REGULATION OF MEMBRANE TENSION IN MUSCLE CELLS</u></b>  KÖSTER D<sup>1</sup>, SINHA B<sup>1</sup>, SENS P<sup>2</sup>, NASSOY P<sup>1</sup>, LAMAZE C<sup>3</sup>  (1) Physico Chimie Curie (UMR 168), Institut Curie, Paris, FRANCE. (2) Physico-Chimie Théorique (UMR 7083), ESPCI, Paris, FRANCE. (3) Trafic et Signalisation (UMR 144), Institut Curie, Paris, FRANCE.</p>
To contact the author:: darius.koester@curie.fr.	<p>During stretching, muscle cells are rapidly elongated which causes an increase of membrane area and surface tension. To avoid membrane rupture during this process, reservoirs of rapid available membrane should act as a buffer. We have focused our analysis on the potential role that caveolins and caveolae, which are extremely abundant in all cells that experience intense mechanical stress, can play in this process. Caveolae are small vesicular invaginations of the plasma membrane enriched in cholesterol and glycosphingolipids, and characterized by the presence of oligomerized integral membrane proteins, caveolins. Of the three mammalian caveolin genes, caveolin-1, -2 and -3, caveolin-3 appears essential for normal muscle health and homeostasis.</p> <p>We analyzed by real-time imaging studies the dynamics of the assembly of caveolin oligomers at the sarcolemma of muscle cells in response to global membrane stretching, and measured the forces necessary to pull membrane tubes from the surface of different cell lines including undifferentiated (myoblasts) or differentiated (myotubes) muscle cells.</p> <p>Experiments on mouse lung endothelial cells showed, that the membrane tension of wild type cells is higher than that of Cav-1 deficient ones. By using drugs known to disrupt the actin network, we could show that the cytoskeleton is also an important contributor of the regulation of membrane tension. Finally, the role of caveolae in membrane tension regulation is investigated by the reconstitution of caveolins in a membrane model system.</p> <p>Caveolae and caveolin-3 are implicated in the pathogenesis of genetic myopathies as Duchenne Muscular Dystrophy or Limb Girdle Muscular Dystrophy-1C. Our project will lead not only to a better understanding of the role of caveolae in muscle physiology but also to a furthering of the understanding of skeletal muscle response to tension in muscular dystrophies, and therefore will contribute to elucidate the pathogenesis of these genetic disease.</p>

PW9-105	<p><b><u>FUNCTIONAL CONSEQUENCES OF A LIMB-GIRDLE MUSCULAR DYSTROPHY 1C-ASSOCIATED CAVEOLIN MUTATION ON CA<sup>2+</sup> HOMEOSTASIS AND EVIDENCE OF DIRECT MOLECULAR INTERACTIONS OF CAVEOLIN-3 WITH THE L-TYPE CA<sup>2+</sup> CHANNEL</u></b></p> <p>WEISS N<sup>1</sup>, COUCHOUX H<sup>1</sup>, BICHAQOUI H<sup>2</sup>, LEGRAND C<sup>1</sup>, ALLARD B<sup>1</sup>, RONJAT M<sup>2</sup>, BERTHIER C<sup>1</sup>, JACQUEMOND V<sup>1</sup></p> <p>(1) Université LYON 1, UMR CNRS 5123, Villeurbanne, FRANCE. (2) Institut des Neurosciences de Grenoble GIN, Université Joseph FOURIER, Grenoble, FRANCE.</p>
To contact the author:: christine.berthier@univ-lyon1.fr.	<p>Caveolins constitute a membrane-associated family of proteins believed to regulate various signaling proteins, including ion channels. Mutations in the CAV3 gene which encodes the muscle specific isoform caveolin-3 (Cav-3) lead to muscle diseases such as limb-girdle muscle dystrophy 1C (LGMD-1C). The molecular events responsible for muscle wasting in LGMD-1C remain however largely unknown. The present study aimed at characterizing the functional and molecular links between Cav-3 and the L-type calcium channel. The consequences of a LGMD 1C-associated Cav-3 mutation (P104L) on membrane excitability and intracellular calcium homeostasis was investigated in mouse adult skeletal muscle fibers. YFP-tagged Cav-3<sup>P104L</sup> was expressed in vivo in flexor digitorum brevis muscles and YFP-positive isolated fibers were studied under whole-cell "silicone voltage-clamp" conditions. The L-type Ca<sup>2+</sup> current density was found considerably reduced in Cav-3<sup>P104L</sup> expressing fibers, consistent with our previous data on cultured myotubes (Couchoux et al., 2007, <i>J. Physiol.</i> 580:745). Interestingly intramembrane charge movement was unaltered, suggesting that the total number of voltage-sensing dihydropyridine receptors remained unchanged. Also, there was no detectable alteration of intracellular calcium regulation neither in resting conditions nor subsequently to voltage-activation. These results thus suggest that in differentiating as well as in adult muscle cells, acute expression of Cav-3<sup>P104L</sup> specifically alters the ionic function of the dihydropyridine receptor. We also found that Cav-3 co-fractionnates and co-immunoprecipitates with Cav1.1, the pore forming subunit of the L-type calcium channel, in muscle triadic fractions. Furthermore in-vitro binding assays showed that Cav-3 directly interacts with the I-II interdomain loop of Cav1.1, probably through its alpha interaction domain (AID). Our results thus suggest that the ionic function of the L-type calcium channel is specifically regulated by Cav-3 and that this functional link could be supported by direct molecular interactions. An alteration of these interactions could participate to the physio-pathological mechanisms of skeletal muscle caveolinopathies</p>

PW9-106	<p><b><u>DEVELOPMENT OF NOVEL NON-COVALENT INHIBITORS OF THE 20S PROTEASOME AND APPLICATION TO THE RESCUE OF WILD-TYPE CAVEOLIN-3 IN A CELLULAR MODEL OF LGMD-1C.</u></b></p> <p>BASSE N<sup>1</sup>, COUCHOUX H<sup>3</sup>, GENIN E<sup>2</sup>, ABBACI K<sup>3</sup>, BLAINEAU S<sup>3</sup>, VALA C<sup>2</sup>, BOUVIER-DURAND M<sup>1</sup>, LE RAVALEC V<sup>2</sup>, VIDAL J<sup>2</sup>, REBOUD-RAVAUX M<sup>1</sup>, BERTHIER C<sup>3</sup></p> <p>(1) Enzymologie Moléculaire et Fonctionnelle, FRE 2852, CNRS-Université Paris 6, Institut Jacques Monod, Paris, FRANCE. (2) Chimie et Photonique Moléculaires, UMR 6510, Université Rennes 1, Rennes, FRANCE. (3) Université Lyon 1, UMR CNRS 5123, Villeurbanne, FRANCE.</p>
To contact the author:: christine.berthier@univ-lyon1.fr.	<p>Inhibiting the proteasome appears as a promising therapeutic tool for the treatment of muscle pathologies, including muscle atrophy and several myopathies.</p> <p>We have designed new proteasome inhibitors based on the cyclic natural inhibitor TMC-95A. Several linear mimics that are more readily synthesized than the cyclic parent molecule have been developed that act in a reversible manner without creating a covalent bond with the catalytic Thr1, as do Bortezomib<sup>®</sup>, MG-132 and lactacystin. The first generation of linear mimics was further modified in order to increase the affinity towards the three types of 20S proteasome catalytic sites, the metabolic stability and the cell penetration. The molecules inhibit selectively the proteasome but neither calpain I, nor cathepsin B.</p> <p>Biological activity of several of these inhibitors was evaluated using cultured C2C12 myotubes expressing a mutant form of caveolin-3 (CAV3) harbouring the Limb Girdle Muscular Dystrophy 1C (LGMD-1C)-associated P104L mutation. Previous results have shown that this mutant form of CAV3 acts in a dominant negative fashion and may aggregate with the wild-type form, leading to the ubiquitination and degradation by the proteasome of both molecules. Treatment of CAV3<sup>P104L</sup> expressing myotubes with our inhibitors revealed that they were able to block the dominant negative effect of the LGMD-1C mutant by partly rescuing wild-type caveolin-3 and restoring its membrane localization. This cellular activity was stronger than that of MG 132. Additionally, the cytotoxicity of the proteasome inhibitors was evaluated on muscle and non-muscle cells and our non-covalent inhibitors displayed a lower toxicity than MG132.</p>

PW9-107	<p><b>CHRONIC OPHTHALMOPLÉGIA IN LIMB-GIRDLE MUSCULAR DYSTROPHY 1C</b>          FILOSTO M<sup>1</sup>, TONIN P<sup>2</sup>, VATTEMI G<sup>2</sup>, SCARPELLI M<sup>2</sup>, BARONCHELLI C<sup>3</sup>,          BROGLIO L<sup>1</sup>, TENTORIO M<sup>1</sup>, PADOVANI A<sup>1</sup>, TOMELLERI G<sup>2</sup>          (1) Clinical Neurology, University Hospital "Spedali Civili", Brescia, Brescia, ITALY.          (2) Department of Neurological Sciences and Vision, Section of Clinical Neurology,          University of Verona, Verona, ITALY. (3) Unit of I Pathological Anatomy, University          Hospital "Spedali Civili", Brescia, Brescia, ITALY.</p>
To contact the author:: giuliano.tomelleri@univr.it.	<p>External ophthalmoplegia (EO), although often present in various neuromuscular diseases, is not usually observed in muscular dystrophies except for oculopharyngeal muscular dystrophy. We report on 37-year-old man, healthy until age 25, affected with non fluctuating asymmetric blepharoptosis, bilateral upward and lateral gaze limitation without diplopia, and exophthalmos . He had also difficulty in standing up from the floor, reduced strength of flexors of the thighs and extensors of the feet, bilateral winging of the scapulae, hypertrophy of calf muscles, hypotrophy of quadriceps and pectoralis muscles. Serum CK levels were three to seven times the normal values; anti- acetylcholine receptor antibodies were negative; lactic acid and thyroid function were normal. Electromyography showed myopathic changes and repetitive nerve stimulation was normal. Brain and orbital magnetic resonance imaging was normal. Biopsy of left deltoid muscle showed variability of muscle fibre size with hypertrophic and atrophic fibres, internal nuclei, few necrotic or degenerating fibres, fibre splitting and mild endomysial fibrosis. Immunohistochemistry and Western Blot analysis showed a complete absence of caveolin-3 protein. Sequence analysis of the caveolin-3 gene revealed a heterozygous substitution of C to T at codon 314 of the caveolin-3 gene, changing Pro to Leu at the position 105 of the mature protein. We suggest that LGMD1C should be add to the list of conditions that cause EO..</p> <p>Since limb weakness in LGMD1C may not be evident, caveolin-3 involvement should be considered in the differential diagnosis of myogenic external opthalmoplegia.</p>

PW9-108	<p><b><u>A CAVEOLINE 3 DEFICIT THAT RESEMBLES A DYSTROPHINOPATHY</u></b>  HALBERT C<sup>1</sup>, KRAHN M<sup>1</sup>, FIGARELLA D<sup>1</sup>, CHABROL B<sup>1</sup>  (1) CHU La Timone, Marseille, FRANCE.</p>
To contact the author:: cecilehalbert@yahoo.fr.	<p>Caveolin 3 is present in striated muscle fibers. Mutations in the gene CAV 3 may have 4 different phenotypic expressions : isolated hyperCKemia, distal myopathy, limb girdle muscular dystrophy, or rippling muscle disease. We report the case of a 9-year-old boy, with difficulty in walking and falls for two years. At the clinical examination, there are calf hypertrophy, and an bilateral equine without muscular deficit. He walks on tiptoe. The CK are 10 times higher than normal. The muscle biopsy, performed in front of this presentation, evoking an dystrophinopathy, highlighted normal signal for dystrophin and other membrane complex protein, and heterogeneous signal for caveolin 3. The genetic study has found a new hétérozygous mutation in CAV 3 gene, confirming this diagnosis. Clinical examination of the father of that child reveals rippling muscle at the biceps muscle without any deficit.</p>

PW9-109	<p><b>IDENTIFICATION OF PARTNERS OF THE PROTEINS INVOLVED IN LGMD BY THE YEAST TWO-HYBRID TECHNIQUE</b>          BLANDIN G<sup>1</sup>, MARCHAND S<sup>1</sup>, NOULET F<sup>1</sup>, GICQUEL E<sup>1</sup>, BARRAULT L<sup>1</sup>, BOUCHETEIL J<sup>1</sup>, FORMSTECHE E<sup>2</sup>, MEIL A<sup>2</sup>, COLLURA V<sup>2</sup>, DANIELE N<sup>1</sup>, BARTOLI M<sup>1</sup>, RICHARD I<sup>1</sup>          (1) G�n�thon-CNRS, Evry, FRANCE. (2) Hybrigenics, Paris, FRANCE.</p>
To contact the author:: gaelle.blandin@genetho n.fr.	<p>To improve our understanding of the molecular pathways involved in the pathogenesis of muscular dystrophies and to allow identification of specific processes that may lead to future therapeutic strategies, we chose a domain-based yeast-two hybrid (Y2H) approach to establish a large scale protein-protein interaction (PPI) map of the skeletal muscle cell centred on proteins involved in LGMDs.</p> <p>An initial set of 13 proteins involved in recessive LGMD and/or in muscular atrophic processes were used as baits to perform high-throughput Y2H screenings of a muscular cDNA prey library. The resulting prey interacting proteins were analysed to select a series of new baits for additional screenings. This process was repeated twice and lead to three rounds of screenings. The entire project consisted in 87 high-throughput Y2H screenings and identified more than 1100 proteins that constitute a global network of more than 1600 PPI, with an average of 21 and 1.4 connections per bait and prey, respectively. For each PPI, a statistical score was computed to predict its reliability and coordinates of the prey interacting domains are available. Following the achievement of this muscular interactome map, our Y2H results were compared to public PPI databases and a validation process was initiated to experimentally confirm a subset of interactions by two complementary techniques: <i>in vitro</i> bimolecular assays (Homogeneous Time-Resolved Fluorescence) and co-immunoprecipitation experiments. Finally, comparison of the current list of known neuromuscular disorder genes with our interactome revealed that 30% of them were connected in our map.</p> <p>The availability of the muscular interactome data will undoubtedly expand our knowledge of the function of the proteins involved in neuromuscular disorders and will permit us to go one step further towards the dynamic and systemic comprehension of the muscle system and towards the understanding of the pathophysiology of the diseases.</p>

PW9-110	<p><b><u>CLINICAL CHARACTERIZATION AND MAPPING OF THE LOCUS OF A NEW FORM OF CHILDHOOD-ONSET LIMB-GIRDLE MUSCULAR DYSTROPHY</u></b> BOUCHARD JP<sup>1</sup>, TETREULT M<sup>2</sup>, SROUR M<sup>2</sup>, ALLYSON J<sup>2</sup>, THIFFAULT I<sup>2</sup>, LOISEL L<sup>2</sup>, ROBITAILLE Y<sup>3</sup>, VANASSE M<sup>4</sup>, BRAIS B<sup>2</sup></p> <p>(1) Service de neurologie, Hôpital de l'Enfant-Jésus, Université Laval, Quebec, CANADA. (2) Laboratoire de neurogénétique et motricité, Centre d'Excellence en Neuromique de l'Université de Montréal, Centre de recherche du CHUM, Montreal, CANADA. (3) Département de pathologie, Hôpital Ste-Justine, Montreal, CANADA. (4) Clinique des maladies neuromusculaires, Centre de réadaptation Marie-Enfant, Hôpital Ste-Justine Hospital, Montreal, CANADA.</p>
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Limb girdle muscular dystrophies are a heterogeneous group of pathologies characterized by weakness and wasting of the limb girdle muscles, with typical sparing of the face. To date, seven autosomal dominant forms (LGMD1A-G) and thirteen autosomal recessive forms (LGMD2A-M) have been characterised. All of the LGMD are rare diseases and some of them have only been described in a few families or in ethnic groups. We have recruited a group of five living and reviewed the records of five deceased distantly related French-Canadians of Acadian descent affected by a childhood-onset form of recessive LGMD. All cases originate from the small archipelago of the Magdalen Islands isolated in the Gulf of St-Lawrence. Clinical characterization of the disease was performed on all affected individuals. A SNP genome scan was performed on all affected individuals and one parent to uncover the genetic locus of the disease. All cases present with limb girdle weakness on average at the age of 7 years but they lose walking at a wide range of ages. Children have normal motor milestones and intelligence. With time, they develop macroglossia, decreased pulmonary function, hyperlordosis, large calves and mild to moderate contractures. Creatine kinase levels are elevated (663-10,000 U/L) in the first decades, but are back to normal at later stages. Muscle pathology showed non-specific dystrophic changes without any specific histological findings. Homozygosity mapping was used for analysis based on the likely sharing of the same founder mutation. A chromosomal region of 0.6Mb not previously associated with a muscular dystrophy on chromosome 17q21.31 was uncovered (multipoint LOD score 3.1). The sequencing of the two most promising candidate genes uncovered a rare missense polymorphism in a conserved integrin domain of *ITGA2B*. This study presents the description of a new recessive childhood-onset limb-girdle muscular dystrophy and the mapping of its original chromosomal locus.

PW9-111	<p><b><u>MOLECULAR DIAGNOSIS IN A FRENCH LIMB-GIRDLE MUSCULAR DYSTROPHY COHORT.</u></b>  <b>SALORT-CAMPANA E<sup>1</sup>, FIGARELLA-BRANGER D<sup>2</sup>, PELLISSIER JF<sup>2</sup>, KRAHN M<sup>3</sup>, LEVY N<sup>3</sup>, POUGET J<sup>1</sup></b>  (1) Centre de référence les maladies neuromusculaires et la SLA, Hôpital Universitaire La Timone, MARSEILLE, FRANCE. (2) Laboratoire d'Anatomopathologie, Hôpital Universitaire La Timone, MARSEILLE, FRANCE. (3) Département de Génétique Médicale, Hôpital Universitaire La Timone, MARSEILLE, FRANCE.</p>
To contact the author:: emmanuelle.salort-campana@ap-hm.fr.	<p><b>OBJECTIVE:</b> To determine the distribution of subtypes of Limb-Girdle Muscular Dystrophy (LGMD) among patients in South-East of France. To determine the proportion of patients in whom a molecular diagnosis was available.</p> <p><b>BACKGROUND:</b> LGMD are a heterogeneous group of genetically determined disorders with a primary or predominant involvement of the pelvic or shoulder girdle musculature. Twenty loci have been so far identified, seven autosomal dominant and thirteen autosomal recessive.</p> <p><b>DESIGN / METHODS:</b> We retrospectively analyzed clinical and histopathological data from 154 patients with a progressive LGMD phenotype. Dystrophinopathy, congenital muscular dystrophy, myotonic dystrophy, facio-scapulo-humeral muscular dystrophy, inflammatory, congenital, metabolic myopathies or patients with insufficient data were excluded. We selected 56 patients. Muscle biopsy provided histopathology and immunodiagnostic testing. The protein abnormality along with clinical features directed mutation screening.</p> <p><b>RESULTS :</b> From this evaluation, the distribution of the immunophenotypes was : 12.5% dysferlin deficiency followed by 10.7 % sarcoglycans, 10.7% calpain-3, 8.9% dystroglycans, 1.7% caveolin-3 and 1.7% myotilin deficiencies. In 42.8 % of all patients, a classifying diagnosis was made. The relative frequency of the subtypes was : 17.8% dysferlinopathy, 12.5% FKRP, 7.1% alpha-sarcoglycanopathy, 5.3% calpainopathy, 1.8% caveolinopathy and 57.2% undetermined. Several new mutations in LGMD2A and 2B patients have been found in this population.</p> <p><b>CONCLUSIONS / RELEVANCE</b>  Dysferlinopathy was the most frequent subtype of this population followed by LGMD2I. The proportion of classifying diagnosis is similar to previous studies. Further genetic analysis of this LGMD population is needed. In dystroglycans phenotypes with no FKRP mutations, mutation analysis in various genes, namely LARGE, POMT1, POMT2, fukutin, and POMGnT1 could be performed.</p>

PW9-112	<p><b>CARDIAC ANKYRIN REPEAT PROTEIN IS A TRANSCRIPTIONAL MARKER OF BOTH ATROPHY AND DYSTROPHY IN SKELETAL MUSCLES</b></p> <p>LAURE L<sup>1</sup>, SUEL-PETAT L<sup>2</sup>, ROUDAUT C<sup>3</sup>, OUALI A<sup>4</sup>, BARTOLI M<sup>5</sup>, RICHARD I<sup>6</sup>, DANIELE N<sup>7</sup></p> <p>(1) G�n�thon, CNRS FRE 3087, Evry, FRANCE. (2) G�n�thon, CNRS FRE 3087, Evry, FRANCE. (3) G�n�thon, CNRS FRE 3087, Evry, FRANCE. (4) INRA-Theix, Saint Gen�s Champanelle, FRANCE. (5) G�n�thon, CNRS FRE 3087, Evry, FRANCE. (6) G�n�thon, CNRS FRE 3087, Evry, FRANCE. (7) G�n�thon, CNRS FRE 3087, Evry, FRANCE.</p>
To contact the author:: daniele@genethon.fr.	<p>Muscular dystrophies (MD) are a group of genetic diseases characterized by progressive muscle degeneration and weakness. Muscle atrophy is a very common clinical feature of these pathologies. In an attempt to identify potential therapeutic targets for the correction of muscle wasting in muscular dystrophies, the expression of several pivotal proteins involved in protein metabolism was investigated in 4 MD animal models (deficient for calpain 3, <math>\alpha</math>-sarcoglycan, dystrophin and dysferlin respectively) as well as experimental atrophy induced by transient or definitive denervation. Amongst all the proteins considered: i) FoxO1 expression is increased in every muscle of a LGMD2A murine model and ii) the expression of CARP, a regulator of transcription factors, appears to be the only one systematically rising in all MD models. CARP is also persistently up-regulated in atrophy-induced denervation, whereas MAFbx and MURF1, two E3 ubiquitin ligases known to be involved in muscle wasting, are only transiently over-expressed. CARP over-expression in muscle fibres fails to induce atrophy, indicating that CARP <i>per se</i> cannot initiate the phenomenon. Finally, we propose the down-regulation of these 2 major markers as potential therapeutic strategies: FoxO1 in LGMD2A and CARP in muscular pathologies.</p>

PW9-113	<p><b>CLINICAL FEATURES OF LGMD2H CAUCASIAN PATIENTS</b></p> <p>SCUTIFERO M<sup>1</sup>, SACCONI V<sup>2</sup>, DI GREGORIO MG<sup>1</sup>, VENTRIGLIA VM<sup>2</sup>, PASSAMANO L<sup>1</sup>, CECIO MR<sup>1</sup>, PILUSO G<sup>2</sup>, CANKI-KLAIN N<sup>3</sup>, NIGRO V<sup>2</sup>, POLITANO L<sup>1</sup></p> <p>(1) Department of Experimental Medicine - Cardiomyology and Medical Genetics- Second University of Naples, Naples, ITALY. (2) Department of General Pathology - Second University of Naples, Naples, ITALY. (3) Zagreb University School of Medicine, Croatian Institute for Brain Research, Zagreb, CROATIA.</p>
To contact the author:: luisa.politano@unina2.it.	<p>Limb-girdle muscular dystrophies (LGMD) include a broad group of genetically determined progressive muscle disorders. By definition, patients should present primary or predominant symmetrical atrophy of the pelvic and/or shoulder girdle musculature, elevated serum creatine kinase and a necrotic regeneration pattern. However, the clinical course is characterized by a great variability and the term now includes many different phenotypes ranging from severe forms with onset in the first decade and rapid progression to milder forms with later onset and atypical presentation.</p> <p>LGMD2H – the 10<sup>th</sup> form of autosomal recessive limb girdle muscular dystrophy - was first described in 1976 in Hutterite Brethren population, a genetic and religious isolate from Northern America. The phenotype was reported as extremely variable ranging from asymptomatic individuals, only showing high CK values, to patients with evident muscle involvement, weakness and myalgia of neck and back muscles. In 1996 it was shown that the disease, in Hutterites, is due to mutations in TRIM32 gene, at 9q33.1 chromosome.</p> <p>We investigated 310 LGMD patients without mutation at the other loci to search for mutations of TRIM32. We identified five patients with novel mutated alleles (1,61%), 4 of them coming from Southern Italy and 1 from Croatia. We describe the phenotype associated with these mutations and indicate the clinical features that can help physicians to address the diagnosis.</p>

PW9-114	<p><b><u>LIMB GIRDLE DYSTROPHY: NORTH-WEST TUSCANY EXPERIENCE IN VIEW OF A REGIONAL RARE DISEASE REGISTRY</u></b></p> <p>VOLPI L<sup>1</sup>, CALSOLARO V<sup>1</sup>, FALORNI M<sup>1</sup>, SICILIANO G<sup>1</sup>  (1) Department of Neurosciences, University of Pisa, Pisa, ITALY.</p>
	<p>Limb girdle muscular dystrophies (LGMD) represent a group of muscle diseases characterized by genetic and clinical heterogeneity. At the moment, seven autosomal dominant and 12 autosomal recessive loci have been identified.</p> <p>We characterized the frequency of limb-girdle muscular dystrophy (LGMD) subtypes in a cohort of 82 subjects with increased creatin-kinase (CK) blood levels and skeletal muscle weakness who came to our attention in the last two years.</p> <p>Aims of this study were to evaluate the relative proportion of patients with hyperCKemia with a diagnosis of LGMD, consider the percentage of the different types of LGMD, to describe the clinical pattern of the different forms, as prognostic factors in north-west Tuscany population.</p> <p>Patients underwent a clinical examination, electrophysiological tests, muscle biopsy and molecular analysis.</p> <p>A diagnosis of LGMD was confirmed in 44 patients. Among these, LGMD relative frequency was: dysferlinopathies (LGMD2B) 6.8%, 31.8% Calpainopathies (LGMD2A), 6.8% alpha-sarcoglycan (LGMD2D), 2.3% beta-sarcoglycan (LGMD2E), 2.3% Fukutin-related protein (LGMD2I), undetermined 50%.</p> <p>Clinical disease severity was higher in patients with sarcoglycanopathy, followed by calpainopathy, than dysferlinopathies, than Fukutin-related protein deficiency. The age at the disease onset was 6 years for sarcoglycanopathy, 14 years for calpainopathies and 17 years for dysferlinopathies, 40 years for Fukutin-related protein deficiency.</p>