

**PW 16:**  
**Myofibrillar myopathies  
and distal myopathies**

PW16-193	<p><b><u>RESIDUE R120, MUTATED IN MYOFIBRILLAR MYOPATHIES, IS ESSENTIAL FOR THE QUATERNARY STRUCTURE AND FUNCTIONAL INTEGRITY OF HUMAN ALPHAB-CRYSTALLIN</u></b></p> <p>SIMON S<sup>1</sup>, MICHIEL M<sup>2</sup>, SKOURI-PANET F<sup>3</sup>, LECHAIRE JP<sup>4</sup>, VICART P<sup>5</sup>, TARDIEU A<sup>2</sup></p> <p>(1) CGMC-UMR5534-Stress, Chaperons et Mort Cellulaire, Lyon, FRANCE. (2) Université Paris 6 - FRE2852, Paris, FRANCE. (3) Université Paris 6 - UMR7590, Paris, FRANCE. (4) Université Paris 6 - IFR83, Paris, FRANCE. (5) Université Paris 7 - EA300, Paris, FRANCE.</p>
To contact the author:: stef.labo@gmail.com.	<p>The missense mutation Arg-120 to Gly (R120G) in the human <math>\alpha</math>B-Crystallin sequence is associated with autosomal dominant myopathy, cardiomyopathy, and cataract (Vicart et al., 1998). Previous studies of the mutant showed a significant ability to aggregate in cultured cells and an increased oligomeric size coupled to an important loss of the chaperone-like activity <i>in vitro</i> (for review see Arrigo et al., 2007). In order to further analyze the role of the R120 residue in the structural and functional properties of <math>\alpha</math>B-Crystallin, the following mutants were generated: Arg-120 to Gly (R120G), Cys (R120C), Lys (R120K), and Asp (R120D). The capacity of wild-type and mutant Crystallins to aggregate in two cultured cell lines was evaluated and the protein location was determined. <i>In vitro</i>, the wild-type and mutant Crystallins were expressed in <i>Escherichia coli</i> cells, purified by size exclusion chromatography, and characterized using dynamic light scattering, electron microscopy, and chaperone-like activity assays.</p> <p>The whole of the data showed that the preservation of an Arg residue at position 120 of <math>\alpha</math>B-Crystallin is critical for the structural and functional integrity of the protein. Moreover, each mutation resulted in specific changes in both structural and functional characteristics (Simon et al., 2007).</p> <p>Arrigo, A. P., Simon, S., Gibert, B., Kretz-Remy, C., Nivon, M., Czekalla, A., Guillet, D., Moulin, M., Diaz-Latoud, C., and Vicart, P. (2007). Hsp27 (HspB1) and alphaB-crystallin (HspB5) as therapeutic targets. <i>FEBS Lett.</i></p> <p>Simon, S., Michiel, M., Skouri-Panet, F., Lechaire, J. P., Vicart, P., and Tardieu, A. (2007). Residue R120 Is Essential for the Quaternary Structure and Functional Integrity of Human alphaB-Crystallin. <i>Biochemistry.</i></p> <p>Vicart, P., Caron, A., Guicheney, P., Li, Z., Prevost, M. C., Faure, A., Chateau, D., Chapon, F., Tome, F., Dupret, J. M., et al. (1998). A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. <i>Nat Genet</i> 20, 92-95.</p>

PW16-194	<p><b>PRELIMINARY RESULTS ON APTAMER TECHNOLOGY FOR TREATMENT OF A SUBTYPE OF MYOFIBRILLAR MYOPATHY.</b>  SIMON S<sup>1</sup>, DIMITROVA V<sup>1</sup>, GIBERT B<sup>1</sup>, DIAZ-LATOUD C<sup>1</sup>, ARRIGO AP<sup>1</sup>  (1) CGMC-UMR5534-Stress, Chaperons et Mort Cellulaire, Lyon, FRANCE.</p>
<p>To contact the author::  stef.labo@gmail.com.</p>	<p>To date, 3 mutations (R120G, Q151X and 464ΔCT) in the gene encoding αB-crystallin have been identified as responsible of Myofibrillar Myopathies then called αB-cristallinopathies (Selcen and Engel, 2003; Vicart et al., 1998). In order to develop a pharmacologic treatment for Myofibrillar Myopathies, we realized a large screening of a peptide aptamers library by two-hybrid method. In the preliminary results presented here, we demonstrate that aptamer strategy led us to identify several aptamers able to correct the hyper-sensitivity to oxidative stress of the R120G αB-crystallin mutant in HeLa cells.</p> <p><i>Selcen, D., and Engel, A. G. (2003). Myofibrillar myopathy caused by novel dominant negative alpha B-crystallin mutations. Ann Neurol 54, 804-810.</i>  <i>Vicart, P., Caron, A., Guicheney, P., Li, Z., Prevost, M. C., Faure, A., Chateau, D., Chapon, F., Tome, F., Dupret, J. M., et al. (1998). A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat Genet 20, 92-95.</i></p>

PW16-195	<p><b><u>DEREGULATION OF SMALL CHAPERONE COMPLEXES IN MYOFIBRILLAR MYOPATHIES.</u></b>  SIMON S<sup>1</sup>, FONTAINE JM<sup>2</sup>, MARTIN J<sup>3</sup>, SUN X<sup>2</sup>, HOPPE A<sup>2</sup>, WELSH M<sup>2</sup>, BENNDORF R<sup>4</sup>, VICART P<sup>5</sup>  (1) CGMC-UMR5534-Stress, Chaperons et Mort Cellulaire, Lyon, FRANCE. (2) University of Michigan, Ann Arbor, USA. (3) Loyola University Chicago, Maywood, USA. (4) Center for Clinical and Translational Research, Columbus, USA. (5) Université Paris 7 - EA300, Paris, FRANCE.</p>
To contact the author:: stef.labo@gmail.com.	<p><b>Three mutations (R120G, Q151X, 464ΔCT) in the small heat shock protein (sHsp) αB-crystallin (αBC) have been found to cause inherited myofibrillar myopathy (Selcen and Engel, 2003; Vicart et al., 1998). αBC forms homo-dimers, hetero-dimers with other sHsps, and larger oligomers. In an effort to elucidate the molecular basis for the associated myopathy, we have determined for these mutant αBC proteins i) the formation of aggregates in transfected cells, ii) the partition into different subcellular fractions, iii) the phosphorylation status, and iv) the ability to interact with themselves, with wild-type αBC, and with other sHsps that are abundant in muscles. We found that all 3 αBC mutants have an increased tendency to form cytoplasmic aggregates in transfected cells and significantly increased levels of phosphorylation when compared to the wild-type protein. While wild-type αBC partitioned essentially into the cytosol and membranes/organelles fractions, mutant αBC proteins partitioned additionally into the nuclear and cytoskeletal fractions. Using various protein interaction assays, including quantitative fluorescence resonance energy transfer measurements in live cells, we found abnormal interactions of the various αBC mutants with wild-type αBC, themselves and the other sHsps Hsp20, Hsp22, and possibly with Hsp27. The collected data suggest that each αBC mutant has a unique pattern of abnormal interaction properties (Simon et al., 2007). These distinct properties of the αBC mutants identified are likely to contribute to a better understanding of the gradual manifestation and clinical heterogeneity of the associated myopathy in patients .</b></p> <p><i>Selcen, D. et al (2003). Myofibrillar myopathy caused by novel dominant negative alpha B-crystallin mutations. Ann Neurol</i>  <i>Simon, S. et al (2007). Myopathy-associated alpha B-crystallin mutants: Abnormal phosphorylation, intracellular location, and interactions with other small heat shock proteins. J Biol Chem.</i>  <i>Vicart, P. et al. (1998). A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat Genet.</i></p>

PW16-196	<p><b><u>ASSOCIATION OF SYNEMIN WITH CYTOSKELETAL PROTEINS DURING NORMAL AND PATHOLOGICAL DEVELOPMENT</u></b>  XUE Z<sup>1</sup>, IZMIRYAN A<sup>1</sup>, PAULIN D<sup>1</sup>, LI Z<sup>1</sup>  (1) UPMC, CNRS-UMR 7079, Paris, FRANCE.</p>
To contact the author:: xue@ccr.jussieu.fr.	<p>Desminopathies are subgroups of myofibrillar myopathies caused by mutations in desmin gene. They are characterized by the presence of protein aggregates in muscle cells. Desmin is the main IF protein in mature striated muscle and it is found at the sarcolemma, Z-lines, neuromuscular and myotendinous junctions. Several other IF or IFAP proteins are characterized in muscle: vimentin, nestin, syncollin, and synemin, all containing the typical coiled-coil domains of IF proteins.</p> <p>The screening of many groups of DRM patients shows that synemin associated with muscle protein aggregates. Synemin gene is located on human chromosome 15. It encoded three isoforms (180, 150 and 41 kDa) produced by alternative splicing according to the tissue types: muscles, lens, astrocytes and neurons (Xue et al. 2004).</p> <p>During mouse development, the synemin isoforms show a dynamic and complex distribution. The synemin M was present as early as E5 with vimentin and nestin. Synemin H was found later in nervous and mesoderm derivatives, in E9 embryo when vasculogenesis, somitogenesis and the migration of neural crest cells are under way in the embryo. Later, synemin L was detected in neurons from E13 and in muscle of adult (Izmiryan et al, 2006).</p> <p>None of the three synemin isoforms becomes organized into filaments without an appropriate copolymerization partner. Using a series of constructs by site-directed mutagenesis with substitutions in the 2A and 2B subdomains of synemin and a series of chimeric synemins having the vimentin head, we have showed that the formation of the filamentous homopolymers required at least a vimentin-like head domain and the 2A and 2B regions of the rod domain. The co-localization of synemins with desmin in normal and myopathic muscles, with vimentin in endothelial cells and of synemin with neurofilaments or peripherin in neurons indicates that synemins are key cross-linking proteins connected with different cytoskeletal structures.</p>

PW16-197	<p><b><u>PHYSIOPATHOLOGICAL MECHANISM INVOLVED IN MYOFIBRILLAR MYOPATHIES</u></b>  GOUDEAU B<sup>1</sup>, SIMON S<sup>1</sup>, CASTERAS-SIMON M<sup>1</sup>, VICART P<sup>1</sup>  (1) EA300 Université Paris 7, Paris, FRANCE.</p>
<p>To contact the author::  patrick.vicart@paris7.jus  sieu.fr.</p>	<p>Myofibrillar myopathies (MFM) are rare inherited neuromuscular disorders characterized by adult onset and the accumulation of aggregates containing desmin in cardiac and/or skeletal muscle cells. MFM patients present clinical and genetic heterogeneous patterns. In fact, mutations in several genes, such as desmin, have been involved in this pathology. Desmin belongs to the type III intermediate filament (IF) family and is specifically expressed in all muscles. Its main role is maintaining the functional and structural integrity of myofibrils (Li et al, 1997). Our laboratory has been interested for a long time by understanding molecular mechanisms implied in these diseases and by functional consequences for muscle cells. So, we have identified new mutations in desmin gene : some in the alpha helical central domain, and others in non-helical N- and C-ter extremities. Mutations of the rod domain mainly affect regions necessary to network assembly (Goudeau et al 2006), while mutations in N- and C-ter extremities mostly concern putative phosphorylable sites and/or are included in conserved domains of intermediate filament family (Bar et al, 2007).</p> <p>We demonstrated that in contrast to mutants in rod domain, which have a dominant-negative effect, N- and C-ter mutants can self-assemble and form a proper desmin network when they are expressed in different cell lines. These new mechanisms may involve specific protein-protein interactions modulated in part by phosphorylation that we will further investigate.</p>

PW16-198	<p><b><u>CELLULAR PROTECTION AGAINST DESMIN-RELATED MYOPATHIES MUTATIONS BY PHOSPHORYLATION OF A SMALL HEAT SHOCK PROTEIN, ALPHAB-CRYSTALLIN</u></b>          LAUNAY N<sup>1</sup>, GOUDEAU B<sup>1</sup>, VICART P<sup>1</sup>, LILIEBAUM A<sup>1</sup>          (1) EA300 Université Paris 7, Paris, FRANCE.</p>
To contact the author:: lilienba@ext.jussieu.fr.	<p>In our laboratory, we have analyzed several mutations in the desmin gene found in patients suffering from desmin-related myopathies (DRM) (see related poster from Goudeau B et al.). Several of these mutations lead the formation of aggregates of desmin after 72 hours of expression in cultured myoblasts, while it may take up to sixty years to manifest itself in the patient. To investigate how cells may protect themselves against the apparition of agregates, we undertook the study of the function of one small heat shock proteins (sHSPs), <math>\alpha</math>B-crystallin. In fact, sHSPs act as chaperone, but also in protecting the different cytoskeletal components like actin microfilaments or microtubules networks, and recent results suggest that <math>\alpha</math>B-crystallin also protects the integrity of intermediate filaments (IF) against extracellular stress (Djabali K. et al., J. Cell. Sci. 110 (1997) p2759 – 2769). We demonstrate that chronic perturbations of IF network by expressing mutated desmine molecules result in the activation of the p38/MAPKAP2 kinase pathway and lead to the specific <math>\alpha</math>B-crystallin phosphorylation at serine 59 (Launay N. et al., Exp. Cell Res. 312 (2006) p3570 – 3584). Upstream of p38, we found that RhoK, PKC and PKA are selectively involved in the activation of p38 and phosphorylation of <math>\alpha</math>B-crystallin upon desmin agregation. Finally, we also show that serine 59 phosphorylated <math>\alpha</math>B-crystallin colocalizes with cytoskeletal components, and displays a better protective function than the unphosphorylated <math>\alpha</math>B-crystallin. Thus, disturbance of cytoskeleton leads by converging signaling pathways to the phosphorylation of <math>\alpha</math>B-crystallin, which probably acts as a protective effector of the cytoskeleton.</p>

PW16-199

**ELECTRON MICROSCOPY IN MYOFIBRILLAR MYOPATHIES REVEALS CLUES TO THE MUTATED GENE**

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<p>To contact the author:: k.claeys@institut-myologie.org.</p>	<p>Myofibrillar myopathies (MFMs) are rare inherited or sporadic progressive neuromuscular disorders with considerable clinical and genetic heterogeneity. To date, mutations in five genes are known to cause MFM. These genes encode desmin, <math>\alpha</math>B-crystallin, myotilin, Z-band alternatively spliced PDZ motif containing protein (ZASP), and filamin C. Although clinical observations can provide clues, the overlap in clinical phenotypes associated with the known genes often makes an accurate prediction of the causative gene difficult. Furthermore, the histopathological results and the findings by current immunohistochemical stainings cannot differentiate between the distinct genetic causes. We studied the ultrastructural characteristics in patients with MFM, and differentiated between the MFM subtypes using electron microscopic findings. We analyzed the ultrastructural data in 18 patients with different genetically-proven MFMs, including 8 patients with mutations in desmin, 5 patients with <math>\alpha</math>B-crystallin mutations, 3 patients with ZASP mutations and 2 patients with mutations in myotilin. In one patient with a ZASP mutation, we additionally performed an immunoelectron microscopic (EM) study, using antibodies against desmin, <math>\alpha</math>B-crystallin, ZASP and myotilin. The ultrastructural findings in desminopathies and <math>\alpha</math>B-crystallinopathies were very similar and consisted of electron-dense granulofilamentous accumulations and sandwich formations. They differed in the presence of early apoptotic nuclear changes in <math>\alpha</math>B-crystallinopathies. ZASPopathies were characterized by floccular accumulations of thin filamentous material, and filamentous bundles, which were labeled with the myotilin antibody on immunoelectron microscopy. The presence of tubulofilamentous inclusions with a diameter of 15-18 nm in the sarcoplasm and in some myonuclei in combination with filamentous bundles were characteristic for myotilinopathies. We conclude that MFMs ultrastructural findings can direct diagnostic efforts towards the causal gene mutated, and that electron microscopy should be included in the diagnostic workup of MFMs.</p>
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<p>PW16-200</p>	<p><b><u>CLINICAL AND IMAGING CHARACTERISTICS OF MYOFIBRILLAR MYOPATHIES: THE SALPÊTRIÈRE EXPERIENCE</u></b>          BEHIN A<sup>1</sup>, STOJKOVIC T<sup>1</sup>, CLAEYS K<sup>2</sup>, LAFORÊT P<sup>1</sup>, DUBOURG O<sup>3</sup>, MAISONOBE T<sup>4</sup>, FOURNIER E<sup>4</sup>, FERREIRO A<sup>1</sup>, BÉCANE HM<sup>5</sup>, WAHBI K<sup>5</sup>, DUBOC D<sup>5</sup>, BOUCHE P<sup>4</sup>, STOLTENBURG G<sup>2</sup>, RICHARD P<sup>6</sup>, UDD B<sup>7</sup>, GOUDEAU B<sup>8</sup>, VICART P<sup>8</sup>, EYMARD B<sup>1</sup>          (1) Consultation de Pathologie neuromusculaire, centre de référence des maladies rares neuromusculaires Paris-Est, APHP, GH Pitié-Salpêtrière, Paris, FRANCE. (2) Laboratoire de pathologie musculaire Risler, Institut de Myologie, Paris, FRANCE. (3) Laboratoire de Neuropathologie, APHP, GH Pitié-Salpêtrière, Paris, FRANCE. (4) Fédération de Neurophysiologie, APHP, GH Pitié-Salpêtrière, Paris, FRANCE. (5) Service de cardiologie, APHP, GH Cochin, Paris, FRANCE. (6) Fédération de génétique, UF de cardio et myogénétique, APHP, GH Pitié-Salpêtrière, Paris, FRANCE. (7) Department of Neurology, Vasa Central hospital and University,</p>
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To contact the author:: anthony.behin@psl.aphp.fr.	<p>Myofibrillar myopathies (MMF), defined by an abnormal accumulation of diverse proteins within muscle fibers, correspond to an expanding number of genetic abnormalities.</p> <p><u>Material and methods:</u> In our department, 64 patients from 43 families present with histologically defined MMF. We systematically reviewed the clinical and paraclinical data of genetically characterized patients, including systematic cardiologic and electromyographic assessment, search for ocular abnormalities and muscle imaging.</p> <p><u>Results:</u> A mutation has been found in 17 families (27 cases), including 11 desminopathies (15 cases), 3 zaspopathies (7 cases), 2 myotilinopathies (2 cases) and 1 alpha-B crystallinopathy (3 cases). No filaminopathy has been found yet. Desminopathies were characterized by an early onset (mean age at first symptoms 31 years), lower limb distal weakness predominating on distal muscles in two thirds of cases. Heart involvement was present in all cases, and was the presenting sign of the disease in one half of cases. It varied from conduction abnormalities to dilated, hypertrophic or restrictive cardiomyopathies. A restrictive syndrome was present 65% of cases. Zaspopathies appeared as distal myopathies affecting both anterior and posterior compartments of legs on imaging, with no significant cardiac or respiratory involvement, with a very slow progression. Mean age at onset was 45 years. Myotilinopathies were characterized in both our cases by weakness of foot dorsiflexors occurring after 65 years. Alpha B crystallinopathy presented as a 30 year-old onset, proximal and axial myopathy, associated with cataract.</p> <p><u>Discussion:</u> MMF present clinically as a group of heterogeneous disorders. However, heart involvement, especially when severe and associated with respiratory involvement, should still lead to consider desmin mutations. On the other hand, late-onset, slowly progressive deficit should orientate towards myotilin and ZASP mutations. As noticed in previous series, clinical and imaging characteristics help in distinguishing the different subsets of MMF.</p>

PW16-201	<p><b><u>THE GNE PROTEIN BINDS TO ALPHA-ACTININ 1</u></b></p> <p>AMSILI S<sup>1</sup>, ZER H<sup>2</sup>, HINDERLICH S<sup>3</sup>, BECKER-COHEN M<sup>1</sup>, MACARTHUR DG<sup>4</sup>, NORTH KN<sup>4</sup>, MITRANI-ROSENBAUM S<sup>1</sup></p> <p>(1) Hadassah Hebrew University Medical Center, Jerusalem, ISRAEL. (2) The Hebrew University of Jerusalem, Jerusalem, ISRAEL. (3) Technische Fachhochschule Berlin, Berlin, GERMANY. (4) The Children's Hospital at Westmead, University of Sydney, Sydney, AUSTRALIA.</p>
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Hereditary inclusion body myopathy (HIBM) is a rare neuromuscular disorder caused by mutations in *GNE*, the key enzyme in the biosynthetic pathway of sialic acid. While the mechanism leading from *GNE* mutations to the HIBM phenotype is not yet understood, we searched for proteins potentially interacting with *GNE*, which could give some insights about novel putative biological functions of *GNE* in muscle. We used a Surface Plasmon Resonance (SPR)-Biosensor based assay to search for potential *GNE* interactors in anion exchanged fractions of human skeletal muscle primary culture cell lysate. Analysis of the positive fractions by *in vitro* binding assay revealed  $\alpha$ -actinin 1 as a potential interactor of *GNE*. The direct interaction of the two proteins was assessed *in vitro* by SPR-Biosensor based kinetics analysis and in a cellular environment by a co-immunoprecipitation assay and confocal co-localization in 293T cells. The interaction of *GNE* with  $\alpha$ -actinin 1 might point to its involvement in  $\alpha$ -actinin mediated processes, including cytoskeleton organization and signaling pathways. In addition these studies illustrate for the first time the expression of the non-muscle form of  $\alpha$ -actinin,  $\alpha$ -actinin 1, in mature skeletal muscle tissue, opening novel avenues for its specific function in the sarcomere.

PW16-202	<p><b>LAING EARLY-ONSET DISTAL MYOPATHY: DESCRIPTION OF A FRENCH FAMILY WITH A NOVEL MUTATION IN THE MYH7 GENE</b>  DUBOURG O<sup>1</sup>, MAISONOBE T<sup>2</sup>, SUOMINEN T<sup>3</sup>, EYMARD B<sup>1</sup>, UDD B<sup>3</sup>  (1) Consultation de pathologie neuromusculaire, Groupe Hospitalier Pitie-Salpetriere, Paris, FRANCE. (2) Federation de Neurophysiologie Clinique, Groupe Hospitalier Pitie-Salpetriere, Paris, FRANCE. (3) Neuromuscular Diagnostic Center, University of Tampere, Tampere, FINLAND.</p>
To contact the author:: odile.dubourg@psl.aphp.fr.	<p>Laing early-onset distal myopathy (MPD1) is an autosomal dominant myopathy due to mutations within the slow skeletal muscle fibre myosin heavy chain, MYH7. We report the phenotype of a French family with four affected individuals on two generations. The mode of inheritance was autosomal dominant. Disease onset was in infancy. The first symptoms were pes planus, frequent falls or poor sportive performances. The clinical findings were homogeneous and included distal lower limb weakness in anterior compartment muscles (4/4), distal upper limb weakness in finger extensors (3/4), Achilles tendon retraction (4/4), pes plano-valgus (4/4), lumbar hyperlordosis (3/4) and kyphoscoliosis (3/4). Neck flexors were preserved. The progression was very slow. The functional disability in adulthood was limited to fatigability, calf cramps, ankle instability for lower limbs, and weakness of finger extension for upper limbs. There were no sensory abnormalities. Electromyographic examination showed a myogenic pattern in two patients and a mixed neurogenic and myogenic pattern in the two others. Motor and sensory conduction were normal. Muscle biopsy performed in anterior tibialis muscle in the propositus when he was 8 years old revealed type 1 fibres smallness and fibres with centralized nuclei. Genetic analysis showed a not previously reported heterozygous Glu1508Del mutation in the MYH7 gene.</p>

PW16-203	<p><b>INTERACTION STUDIES ON MYOSPRYN AND M-LINE TITIN</b>  SARPARANTA J<sup>1</sup>, VIHOLA A<sup>1</sup>, HACKMAN P<sup>1</sup>, RICHARD I<sup>2</sup>, UDD B<sup>1</sup>  (1) Folkhälsan Inst. of Genetics and Dept. of Medical Genetics, University of Helsinki, Helsinki, FINLAND. (2) G�n�thon, Evry, FRANCE.</p>
<p>To contact the author::  jaakko.sarparanta@helsinki.fi.</p>	<p>Tibial muscular dystrophy (TMD) is a dominant late onset distal myopathy caused by mutations in M-line titin. In the few homozygotes known, the same mutations lead to the different, more severe limb-girdle muscular dystrophy LGMD2J. Most of the TMD/LGMD2J mutations, including the Finnish FINmaj, are located in M10, the most C-terminal of titin domains.</p> <p>The molecular pathways behind the titinopathies TMD/LGMD2J are unknown, but muscle selectivity and normal sarcomere ultrastructure suggest a signalling or regulatory defect rather than a structural one. Loss of protein interactions of C-terminal titin is likely, caused by direct disruption of the binding or by cleavage of the entire titin C-terminus. Previously, we have shown a secondary defect of calpain 3, a known ligand of M-line titin, in LGMD2J. Since this is not likely to explain the dominant effect in TMD, we have searched for other interactions of C-terminal titin, possibly disrupted by the mutations in TMD/LGMD2J.</p> <p>In a yeast two-hybrid (Y2H) interaction screen, myospryn (CMYA5, cardiomyopathy-associated 5) was identified as a putative ligand of the titin M10 domain. The interaction seems genuine and specific, as it is disrupted in the Y2H system by the FINmaj mutation. The interaction is also supported by coimmunoprecipitation experiments of transfected proteins. According to Y2H studies, the titin binding involves at least the latter of the two FN3 domains and the following SPRY domain of myospryn C-terminus, but a larger region might participate in the interaction. In vivo studies are in progress to obtain further evidence for the putative interaction and to assess its biological function and possible role in TMD/LGMD2J.</p> <p>Myospryn is a large protein belonging to the TRIM superfamily. It has been suggested to function in lysosome-related organelle biogenesis and positioning as well as in regulating the localized activity of protein kinase A in muscle.</p>

PW16-204	<p><b><u>WELANDER DISTAL MYOPATHY: THE MISSING GENE.</u></b>  HACKMAN P<sup>1</sup>, HOLLO S<sup>1</sup>, LUQUE H<sup>1</sup>, TOKOLA M<sup>1</sup>, KERÉ J<sup>2</sup>, EDSTRÖM L<sup>3</sup>,  ÅHLBERG G<sup>3</sup>, UDD B<sup>1</sup></p> <p>(1) Folkhälsan Institute of Genetics and The department of Medical Genetics, University of Helsinki, Helsinki, FINLAND. (2) Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, SWEDEN. (3) Department of Clinical Neuroscience, Karolinska Hospital, Stockholm, SWEDEN.</p>
<p>To contact the author::  peter.hackman@helsinki.fi.</p>	<p>Welander distal myopathy (WDM) is a late onset autosomal dominant disease characterized by slow progression of distal muscle weakness. Usually hands are first affected with weakness of the finger extensor muscles. A few much more severely affected patients proved to be homozygotes. The WDM locus was mapped to chromosome 2p13 by Åhlberg et al already in 1999. Further genotyping during 2004-2007, using additional new microsatellite markers narrowed down the linked region to &lt;980 Kb, with a core region of &gt;530 kb.. All known coding sequences in the linked region, including their putative promotor regions, as well as known hypothetical genes, have been sequenced. Several linked SNP polymorphisms have been identified, but the disease causing mutation is still pending. Large deletions and duplications in the area were excluded by screening with a 250K SNP microarray linkage chip. Analyses of cDNA of all linked genes syntesized from mRNA isolated from muscle biopsies, is being finalized in search of potential splicing mutations. Studies of expression levels with RT-PCR (Taqman) of four linked genes has been performed, and the expression levels of the rest of the genes are being analysed. No abnormal results have so far been obtained and the gene is still unidentified. Total sequencing of the linked genomic area, including all introns and regions between genes, has been started using high throughput segencing in microfabricated high-density picolitre reactors. With all these extended methods we hope to identify the underlying genetic defect causing WDM.</p>

PW16-205	<p><b><u>PROTEOMIC ANALYSIS OF MUSCLE TISSUE FROM HEREDITARY INCLUSION BODY MYOPATHY</u></b>  SALAMA I<sup>1</sup>, MILMAN I<sup>1</sup>, MITRANI-ROSENBAUM S<sup>1</sup>  (1) Hadassah Hebrew University Medical Center, Jerusalem, ISRAEL.</p>
	<p>Hereditary inclusion body myopathy (HIBM) is a unique group of neuromuscular disorders characterized by adult onset and slowly progressive distal and proximal muscle and a typical histology including rimmed vacuoles and filamentous inclusions. The disease is caused by mutations in UDP-N-acetylglucosamine 2-epimerase/ N-acetylmannosamine kinase (GNE), the key enzyme in the biosynthetic pathway of sialic acid. Here we present a proteome comparison between biopsies from 3 healthy individuals and 3 HIBM patients, matched by age, gender and muscle type, carried out in parallel by iTRAQ analysis and by two-dimensional gel electrophoresis. The iTRAQ method resulted in the identification by mass spectrometry of 150 proteins, several of them differentially expressed in HIBM patients versus controls. The 2D analysis detected 35 proteins differentially expressed in HIBM. Results were validated by regular western blots of the same specimens with antibodies of representative proteins.</p> <p>To examine the sialylation profile of the relevant glycoproteins, Western blots were also performed from the very same 2D gels with lectins and specific antibodies against highly sialylated proteins.</p> <p>These studies contribute to a more comprehensive knowledge on the expression and posttranslational modifications of proteins in muscle tissue in general, and to new insights in the understanding of the pathogenesis of HIBM.</p>

