

**PW 10:
FSHD and OPMD**

PW10-115	<p><u>STUDIES ON THE EXPRESSION AND FUNCTION OF DUX4C, A GENE LOCATED CLOSE TO THE FSHD LOCUS.</u></p> <p>TASSIN A¹, VANDERPLANCK C¹, ANSSEAU E¹, MARCOWYCZ A¹, MAHEMUTI L¹, BARRO M², LAOUDJ-CHENIVESSE D², BELAYEW A¹, COPPÉE F¹</p> <p>(1) Laboratory of Molecular Biology, University of Mons-Hainaut, Mons, BELGIUM. (2) INSERM ERI 25 "Muscle et Pathologies", CHU A. de Villeneuve, University of Montpellier I, Montpellier, FRANCE.</p>
To contact the author:: frederique.coppee@umh.ac.be.	<p>Facioscapulohumeral muscular dystrophy (FSHD) is a dominant disease linked to contractions of a repeat array in the 4q35 subtelomeric region. In non-affected individuals the array comprises 11-100 tandem copies of a 3.3-kb element named D4Z4 in which we have identified the DUX4 double homeobox gene. The FSHD deletions reduce the D4Z4/DUX4 copy numbers to between 1 and 10, and activate genes in the vicinity by chromatin loop alterations. We have shown that DUX4 was activated and played a major role in FSHD.</p> <p>We now focus on a homologous gene (DUX4c) mapped 42 kb centromeric of the repeat array. The DUX4c mRNA and protein were expressed in control human primary myoblasts. The protein was observed in nuclei by immunofluorescence with a specific antiserum, and it was induced upon differentiation to myotubes. The protein level detected by Western blot increased 1.5-2-fold in FSHD versus control or DMD (Duchenne Muscular dystrophy) myotubes. It was also induced in FSHD (2-10-fold) and in DMD (3-4-fold) muscle biopsies as compared to controls. The increase in DMD samples might result from the multiple muscle degeneration/regeneration cycles characteristic of the disease.</p> <p>In a functional study we transfected human TE671 rhabdomyosarcoma cells with <i>pC/Neo</i> expression vectors for either DUX4, DUX4c, DUX1 or without insert. We observed a strong up-regulation of PCNA (proliferating cell nuclear antigen) 24h post-transfection in DUX4c expressing cells only. These cells continued to proliferate in differentiation medium instead of aligning to fuse into myotubes like the other transfected cells. Since DUX4c specifically induced the MYF5 transcription factor, these findings suggested a role in muscle regeneration not restricted to FSHD.</p> <p>We propose that as well an excess (in FSHD patients) as a reduced amount (in families where the D4Z4 deletion removes the DUX4c gene) of DUX4c expression could affect muscle regeneration and contribute to the FSHD pathology.</p>

PW10-116	<p><u>FRG1 OVER-EXPRESSION INHIBITS MUSCLE DIFFERENTIATION.</u> PICOZZI P¹, CABIANCA D¹, GABELLINI D¹ (1) Stem Cell Research Institute, DIBIT-HSR, Milano, ITALY.</p>
To contact the author:: gabellini.davide@hsr.it.	<p>Facioscapulohumeral muscular dystrophy (FSHD) is the third most important myopathy. Currently, no therapeutic treatment is available for FSHD. Unlike the majority of genetic diseases, FSHD is not caused by mutation in a protein-coding gene. Instead, FSHD is the result of a complex epigenetic cascade activated by deletion of a 3.3 kb subtelomeric repeat (D4Z4) located on chromosome 4q35. D4Z4 appears to regulate chromatin structure at 4q35 and its partial deletion causes de-repression of several 4q35 genes. Notably, we found that FSHD is caused by over-expression of the 4q35 gene FRG1 and we generated the first animal model of the disease.</p> <p>Several evidences suggest a differentiation defect in FSHD. First, global gene expression profiling of muscle biopsies obtained from FSHD, normal as well as other muscular dystrophies showed that among the genes altered in an FSHD-specific and highly significant manner, many are involved in myogenesis, cellular differentiation, and cell-cycle control, suggesting that a partial block in the normal differentiation program occurs in FSHD muscle. Second, primary myoblasts derived from FSHD patients were reported to manifest increased susceptibility to oxidative stress, morphological aberrations and early cell cycle arrest suggestive of a defect in early myogenesis. Third, mesoangioblasts (a new class of adult stem cells of mesodermal origin) derived from FSHD patients have been shown to display impaired differentiation capabilities.</p> <p>To determine if these phenotypes are mediated by FRG1, we compared the differentiation capability of naïve C2C12 muscle cells, control stable cell lines containing the empty vector and stable C2C12 cells over-expressing FRG1. Interestingly, very few and very small myotubes were present in FRG1 over-expressing cells. Moreover, several myogenic markers were down-regulated in FRG1 over-expressing cells by real-time RT-PCR.</p> <p>Collectively, our preliminary results reveal that FRG1 over-expression inhibits muscle differentiation.</p> <p>The molecular mechanism responsible for FRG1 inhibition of muscle differentiation is under investigation.</p>

PW10-117	<p><u>FUNCTIONAL AND BIOCHEMICAL CHARACTERIZATION OF SKELETAL MUSCLES OF FRG1 OVER-EXPRESSING TRANSGENIC MICE, MODEL OF FSHD</u> GERMINARIO E¹, ESPOSITO A¹, PERON S¹, TUPLER R², DANIELI-BETTO D¹ (1) Dept Human Anatomy and Physiology, University of Padova, Padova, ITALY. (2) Dept Biomedical Sciences, University of Modena and Reggio Emilia, Modena, ITALY.</p>
To contact the author:: daniela.danieli@unipd.it.	<p>Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary neuromuscular disorder characterized by progressive weakness and atrophy of the facial, shoulder, abdominal and pelvic girdle muscles. Evidence indicates the over-expression of genes mapped at distal long arm of chromosome 4 (4q35) as responsible for FSHD. Transgenic mice over-expressing FRG1 (FSHD Region Gene 1) at low, medium and high level were utilized to confirm this hypothesis. Indeed FRG1 transgenics develop a progressive muscular dystrophy whose degree of severity correlates with the expression level of the transgene. Contractile properties of soleus, extensor digitorum longus and biceps muscles were studied in vitro at 30 °C. Calcium-sensitivity and specific tension of single fibres from soleus, vastus lateralis (VL) and biceps muscles were also analyzed. All muscles showed a progressive loss of twitch and tetanic tensions, confirmed by the lower specific tension recorded in single fibres. The pCa-tension relationship of VL and biceps fibres was significantly shifted to the right, particularly in mice expressing FRG1 at the highest level. Functional properties were correlated to the expression of myosin heavy chains (MyHC), troponin C (TnC) and troponin T (TnT) isoforms. SDS-PAGE and WB analyses of dystrophic muscles show a general shift toward a slow phenotype of both MyHC and TnC isoforms and an altered expression of fast TnT isoform. Interestingly, two-dimensional gel electrophoresis of VL proteins showed different TnT isoforms that correlated to mRNA alternative splicing products previously observed. Taken together these results indicate that <i>FRG1</i> over-expression exerts its effects on a subset of genes that might induce a transition from fast to slow fibre-type composition and determine changes that influence the physiology of the contractile apparatus. AFM-France (grant 12055) is gratefully acknowledged.</p>

PW10-118	<p>MYOBLASTS FROM AFFECTED AND NON AFFECTED FSHD MUSCLES EXHIBIT MORPHOLOGICAL DIFFERENTIATION DEFECTS</p> <p>BARRO M¹, CARNAC G¹, FLAVIER S¹, MERCIER J³, VASSETZKY Y², LAOUDJ-CHENIVESSE D¹</p> <p>(1) INSERM, ERI25; Université Montpellier1, UFR Médecine, EA 4202, MONTPELLIER, FRANCE. (2) CNRS-UMR8126 Université Paris-Sud 11, Institut de Cancérologie Gustave-Roussy, VILLEJUIF, FRANCE. (3) INSERM, ERI25; Université Montpellier1, UFR Médecine, EA 4202; CHU de Montpellier, Service de Physiologie Clinique, Hôpital A. de Villeneuve, MONTPELLIER, FRANCE.</p>
To contact the author:: barro@montp.inserm.fr.	<p>Facioscapulohumeral dystrophy (FSHD) is a muscular hereditary disease with a prevalence of 1 in 20 000 caused by a partial deletion of a subtelomeric repeat array on chromosome 4q. However, very little is known about the pathogenesis as well as the molecular and biochemical changes linked to the progressive muscle degeneration observed in these patients.</p> <p>Therefore, we decided to evaluate vulnerability to an induced oxidative stress, differentiation capacity and morphological abnormalities in primary myoblasts derived from clinically affected muscles of FSHD patients and compare them to cells from muscles of healthy individuals. We wanted to ascertain whether myoblasts derived from clinically unaffected muscles present the same abnormalities than those from affected muscles.</p> <p>We have created a panel of primary myoblast cell cultures from the FSHD patients and matched control individuals. Our results show that primary myoblasts derived from both clinically unaffected and affected muscles are more susceptible to an induced oxidative stress than control myoblasts. Moreover, we demonstrate that both types of FSHD primary myoblasts differentiate into multinucleated myotubes which present morphological abnormalities. Whereas control myoblasts fuse to form branched myotubes with aligned nuclei, FSHD myoblasts fuse to form either thin and branched myotubes with aligned nuclei or large myotubes with random nuclei distribution.</p> <p>In conclusion, we postulate that these abnormalities could be responsible for muscle weakness in FSHD patients and provides an important marker for FSHD myoblasts.</p>

PW10-119	<p><u>FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY (FSHD) MYOBLASTS EXHIBIT AN ALTERATION OF THE CELL CYCLE AND A PREMATURE SENESCENT STATE.</u></p> <p>DODILLE-PLAISANT M¹, ALLEGRA M¹, LOUBAT A¹, SACCONI S¹, SACCONI S², PONZIO G³, DELPLACE S¹, CORBEL L¹, DESNUELLE C¹, DESNUELLE C², ROSSI B¹</p> <p>(1) INSERM U638, IFR50, Nice, FRANCE. (2) CHU Nice, Centre de référence pour les maladies neuromusculaires, Hôpital de l'Archet, Nice, FRANCE. (3) INSERM U634, IFR 50, Nice, FRANCE.</p>
To contact the author:: allegra@unice.fr.	<p>Facioscapulohumeral muscular dystrophy (FSHD) is an inherited neuromuscular disorder affecting a characteristic pattern of muscles. The physiopathological mechanism has not been elucidated yet, but relies on the deletion of the D4Z4 repeat in the 4q subtelomeric region thus affecting expression of a panel of genes in FSHD patients. Indeed most studies focus on determining which genes are involved in FSHD disease and suggest implication of genes controlling proliferation, differentiation and cell cycle progression. Besides, FSHD myoblasts present a proliferation defect in vitro.</p> <p>Aiming at better characterize this proliferation defect, we investigated in the present study the progression of FSHD myoblasts through the cell cycle. After cell synchronization, these myoblasts abnormally accumulated in the G0/G1 phase compared to control myoblasts. We revealed that this G0/G1 block in FSHD myoblasts results from the decreased CDK4 and CDK2 activities and, consequently, altered Rb phosphorylation state. In addition, expression of the cyclin-dependent kinase (CDK) inhibitors p21 and p57 are affected in FSHD myoblasts in comparison to control myoblasts: p21 is up-regulated during all the cell cycle phases and in contrast p57 is drastically diminished. Moreover, concerning the expression of p53 and MyoD, key proteins controlling cell cycle progression, we found up-regulation of p53 and stabilized level of MyoD during the S phase in patient myoblasts compared to control myoblasts.</p> <p>Finally, focusing on the consequences of the p53 overexpression on FSHD myoblasts fate, we demonstrated that patient myoblasts are not apoptotic or necrotic, but enter an early senescent state.</p> <p>Altogether our data provide further insights in the physiopathology of FSHD disease, showing that dysregulation of several regulators involved in cell cycle control are responsible, after onset of engagement in the pathogenic mechanism of the disease, for withdrawal of FSHD myoblasts from the cell cycle and that these myoblasts are prematurely senescent.</p>

PW10-120	<p><u>ALTERATION OF EXPRESSION AND LOCALIZATION OF MUSCLE-SPECIFIC ISOFORMS OF FRAGILE X RELATED PROTEIN 1 IN MYOBLASTS AND MYOTUBES OF FSHD PATIENTS.</u></p> <p>DAVIDOVIC L¹, SACCONI S², DELPLACE S², BECHARA E¹, DESNUELLE C², BARDONI B¹</p> <p>(1) Institut de Pharmacologie Moléculaire et Cellulaire – CNRS UMR6097, Valbonne, FRANCE. (2) INSERM U638, CHU de Nice, Centre de Référence pour les Maladies Neuromusculaires, Nice, FRANCE.</p>
To contact the author:: bardoni@ipmc.cnrs.fr desnuelle.c@chu-nice.fr.	<p>Facioscapulothumeral muscular dystrophy (FSHD) is the third most common hereditary disease of muscle. Several lines of evidence suggest that FSHD is due to alteration of the expression of FRG1. Transgenic mice overexpressing FRG1P in skeletal muscle were described to develop muscular dystrophy. Even if its precise function is unknown, the FRG1 protein has been reported to be localized to nucleoli, Cajal bodies and speckles. This localization suggests that FRG1 is a component of the spliceosome. Starting from this observation, we hypothesized that the correct generation of the splicing isoforms of FXR1P (Fragile X Related Protein 1) could be altered in FSH patients. FXR1P is one of the homologous proteins of Fragile X Mental Retardation Protein (FMRP), whose absence causes Fragile X Syndrome. Like FMRP, FXR1P is an RNA binding protein involved in several steps of mRNA metabolism, in particular translational control and intracellular localization of a subset of mRNAs (most still unknown). FXR1P plays a vital role during Xenopus and mouse embryogenesis since its complete or partial inactivation has dramatic and muscle-specific effect. Seven distinct FXR1P splicing isoforms have been identified. Four of them are widely expressed in all cell lines and mouse tissues (with the exception of skeletal muscle and heart), whereas the 3 long isoforms are only expressed at high level in skeletal muscle and heart. In agreement with our hypothesis, we observed a reduction of the expression of FXR1P muscle-specific isoforms (mRNA and protein) in cultured myoblasts and myotubes obtained from muscle of FSHD patients. In FSHD myoblasts FXR1P is only localized in the cytoplasm, while in normal cells it appears to be present both in nucleus and in cytoplasm. These results suggest that altered expression/localization of FXR1P may be implicated in the pathogenesis of FSHD and influence the physiopathology of this disease.</p>

PW10-121	<p><u>SELECTIVE MUSCLE INVOLVEMENT IN FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY (FSHD): THE ROLE OF MYOBLASTS AND 4Q35 GENE OVEREXPRESSION</u></p> <p>SACCONI S¹, VILQUIN JT², SALVIATI L³, FABBRI G³, TUPLER R⁴, TEUSAN R⁵, LECUNFF M⁵, LEGER J⁵, DESNUELLE C¹</p> <p>(1) Centre de référence des maladies neuromusculaires, Nice, FRANCE. (2) INSERM U523, Paris, FRANCE. (3) Università di Padova, dipartimento di Genetica, Padova, ITALY. (4) Univesità di Modena e Reggio Emilia, Dipartimento di scienze Biomediche, Modena, ITALY. (5) INSERM U533- Genopole, Nantes, FRANCE.</p>
To contact the author:: sacconi@unice.fr.	<p><u>Background</u></p> <p>FSHD is an autosomal dominant myopathy characterized by selective muscle involvement. We previously demonstrated that, in contrast with myoblasts issued from non affected territories, FSHD myoblasts from affected territories display defect in myogenic differentiation.</p> <p>This study is aimed to correlate the extent of muscle involvement to the alteration in differentiation observed in FSHD myoblasts issued from differentially affected territories and gain more inside in FSHD physiopathological process.</p> <p><u>Methods</u></p> <p>We analyzed 4 myoblasts cultures issued from non dystrophic FSHD vastus lateralis (VL) muscle, 4 from subscapularis (SS) FSHD affected muscle with different level of dystrophy and 4 matched controls. Dynamic gene expression profile was performed on a specific chip containing 6682 genes. 5 timepoints were analyzed: the cell cycle withdrawal (D0), and 24, 48, 72 and 144 hours after inducing myogenic differentiation. The results were analyzed using SAM multiclass analysis and expressed in term of variant or invariant gene during differentiation correlated or not correlated with variation in control myoblasts gene expression. Pathway connectivity analysis and visualisation of different pathways was done using a specific metasearch tool. The expression of 4q35 gene FRG1, ANT1 was analyzed both by microarray and Taqman real time PCR.</p> <p><u>Results</u></p> <p>Controls myoblasts issued from VL and SS muscles display clear differences in gene expression at D0 and during differentiation. The dynamic gene expression profile in FSHD myoblasts during differentiation confirm the defect in both cell cycle withdrawal and differentiation mostly in FSHD cell culture from highly dystrophic muscles. 4q35 genes overexpression were seen only in dystrophic SS derived muscle cultures after differentiation suggesting a role of these gene in differentiation defect.</p> <p><u>Conclusions</u></p> <p>Our results suggest that selective muscle involvement in FSHD patients can be due to different alteration in the ability of derived myoblasts to regenerate muscle. The role of 4q35 gene overexpression will be discussed as well as the implications for autologous cell therapy.</p>

PW10-122	<p><u>MYOFIBRIL FORMATION DEPENDS FROM TC10 ACTIVATION THROUGH THE RHO-GEF OBSCURIN IN HUMAN SKELETAL MUSCLE</u> COISY-QUIVY M¹, BOURRET A¹, FORT P², MERCIER J¹, PHILIPS A¹ (1) INSERM ERI25 / EA 4202 UM1, Montpellier, FRANCE. (2) CRBM UMR 5237 CNRS, Montpellier, FRANCE.</p>
To contact the author:: philips@montp.inserm.fr.	<p>Identifying and characterizing key elements of sarcomeric signal transduction and their roles in the control of myofibrillogenesis are essential to elucidate basic mechanisms of the cell biology of muscle, leading to a molecular understanding of associated diseases.</p> <p>Using an <i>in vivo</i> approach in ascidians, we have recently shown that the Rho GTPase TC10 is required for myofibrillogenesis. This result is now confirmed in human primary myotubes, in which we demonstrate that the inhibition of TC10 activity and of its expression block myofibrillar organization. To characterize the TC10-dependent pathway implicated in this process, we have searched an activator (RhoGEF) of TC10 and we have identified Obscurin. This protein is the third member of the titin superfamily, it localizes to myofibrils at the M-bands in later stages of development and in adult animals. And it has been proposed that Obscurin is indispensable for myofibrillogenesis. We show that TC10 co-immunoprecipitates with Obscurin in human myotubes and we suggest that this interaction is direct because it is confirmed <i>in vitro</i> with the two purified proteins. Moreover, we display that this interaction leads to the activation of TC10 in human primary myotubes.</p> <p>The implication of Obscurin in pathology still remains to be demonstrated but aberrant RhoGEF functions have been associated with human diseases, such as cancer. Thus, their constitutive activation or inactivation can be the cause of myopathies of unknown etiology. In muscular biopsies from FSHD patients, we have observed a weak expression of TC10. As our results involve TC10 in myofibrillogenesis, we will determine if its deregulation observed in FSHD is associated to the physiopathological process. A reduction in the TC10 activity may indeed participate in the muscular loss. The more accurate our views of the sarcomere and myofibrillogenesis become, the more likely we are to identify and treat muscle diseases successfully.</p>

PW10-123	<p><u>CLINICAL, CT AND MRI PECULIARITIES IN 4Q35 LINKED FACIOSCAPULOPERONEAL MUSCULAR DYSTROPHY. REPORT OF TWO RUSSIAN FAMILIES CLINICAL, CT AND MRI PECULIARITIES IN 4Q35 LINKED FACIOSCAPULOPERONEAL</u></p> <p>KAZAKOV V¹, RUDENKO D², KOLYNIN V³</p> <p>(1) Pavlov State Medical University and City Hospital 2, St-Petersburg, RUSSIA. (2) Pavlov State Medical University and City Hospital 2, St-Petersburg, RUSSIA. (3) Pavlov State Medical University and City Hospital 2, St-Petersburg, RUSSIA.</p>
To contact the author:: valerykazakov@mail.ru.	<p>We give the description of two autosomal dominant with 4q35 linked facioscapuloperoneal muscular dystrophy families in which the patients were re-examined by V.K. after 28 -37 years and the typical changes of the pattern of muscle affections on the different stages of the disease were established and confirmed by muscle CT or MRI.</p> <p><u>The disease began with initial involvement of the isolated facial muscles or their parts and shoulder girdle muscles and some time later of the anterior tibial muscles. The developed scapulooperoneal phenotype with slight affection of the isolated facial muscles or their parts existed in the clinical picture a very long period. The muscles of the thigh (posterior group, namely), pelvic girdle (gluteus maximus, namely) and biceps brachii (slight weakened) are involved in two probands much later and comparatively less degree then the muscles of the scapulo-peroneal region.</u></p> <p>The muscle CT and MRI in observed patients showed more often and severe involvement of the tibialis anterior, extensor digitorum longus and extensor hallucis longus, posterior thigh muscles (semimembranosus, long head of biceps femoris and semitendinosus), rectus femoris and some time later of adductors of thighs and gastrocnemius (medial heads) and soleus with sparing of peroneus longus and deep posterior compartment of lower leg, quadriceps, gracilis and sartorius muscles. The radiological muscle pattern does not fully correlate with clinical pattern of muscle weakness. The posterior thigh muscles and quadriceps clinically had a normal strength although the total/severe involvement of some hamstrings and rectus femoris was revealed on MRI and CT study. Thus the clinical, CT and MRI study shows the inaccuracy of the term "facioscapuloperoneal" muscular dystrophy becomes evident. The name "facioscapulolimb muscular dystrophy, type 2 (FSLD2), a descending with a "jump" with initial FSP phenotype" instead the name facio-scapulo-peroneal muscular dystrophy would be more correct.</p>

PW10-124	<p><u>ANALYSIS OF GROWTH FACTORS EXPRESSION IN AFFECTED AND SPARED MUSCLES OF OPMD PATIENTS</u> BOUAZZA B¹, KRATASSIOUK G², PERIE S³, ST GUILY JL³, BUTLER-BROWNE GS¹, SVINARTCHOUK F⁴ (1) UMR-S 787 Inserm, Université Pierre et Marie Curie Paris VI, Institut de Myologie, Paris, FRANCE. (2) CNRS FRE 2944, Institut Andre Lwoff, Villejuif, FRANCE. (3) Service d'O-R-L et de Chirurgie Cervico-faciale, Hôpital Tenon, Paris, FRANCE. (4) Genethon, CNRS FRE 3087, Evry, FRANCE.</p>
To contact the author:: svinart@genethon.fr.	<p>In order to determine the role of growth factors, cytokines and chemokines in the physiopathology of muscle disease we have compared the level of expression of 174 factors in both the affected and non affected muscles of Oculo-Pharyngeal Muscular Dystrophy (OPMD) patients by means of antibodies arrays. OPMD is characterized by progressive eyelid drooping (ptosis) and swallowing difficulties (dysphagia). The pharyngeal and cricopharyngeal muscles (CP) are specific targets in OPMD. In the present study expression of 174 factors in CP (affected) muscle was compared versus Sternocleidomastoid (SCM) (spared) muscles in 8 OMPD patients.</p> <p>We found an important individual variability in the expression of some factors in affected and spared muscles. Nevertheless, the expression of sixty factors was persistently different between these two groups. Many of the differentially expressed factors in our study are known to be involved in the formation of fibrosis in both the liver and the lung, thus indicating the possibility that the treatment used in hepatic fibrosis may have a beneficial effect in OPMD.</p>

PW10-125

PROTEOMIC INVESTIGATION OF THE MOLECULAR PATHOPHYSIOLOGY OF OCULO-PHARYNGEAL MUSCULAR DYSTROPHY (OPMD)

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<p>To contact the author:: bouazza@ext.jussieu.fr.</p>	<p>Oculo-pharyngeal muscular dystrophy (OPMD) is an adult onset disorder (40-50 years) characterised by progressive eyelid drooping (ptosis) and swallowing difficulties (dysphagia). This autosomal dominant disease is caused by an amplification of a GCG repeat in the PABPN1 gene from 8 to 13 repeats which results in the expansion of a polyalanine stretch in the N-terminus of the PABPN1 protein. The molecular mechanisms which cause only a few muscle groups to be specifically affected in OPMD patients are not yet understood. Previously we have shown that satellite cells isolated from Cricopharyngeal muscle (CP) of OPMD patients have a lower myogenicity and reduced proliferative life span compare to the cells isolated from healthy donors or from the unaffected Vastus lateralis (VL) of OPMD patients. In the present study, in order to find out consequences of mutated PABPN1 expression we performed two types of proteomic comparisons. In the first we compared the proteomes of myoblasts isolated of spared muscles (VL) from OPMD patients versus the same muscles from healthy donors. In the second analysis myoblasts from affected (CP) muscles were compared to myoblasts of healthy donors.</p> <p>Cytoplasmic and nuclear protein extracts from the myoblasts were separated by 2D gel electrophoresis and differentially expressed proteins were identified by MALDI-ToF mass spectrometry. We found that the number of differentially expressed proteins is substantially higher in the second type of analysis (83 versus 51) thus indicating a more important perturbation of the proteomie in the affected muscles. These differentially expressed proteins are involved in detoxification, energy metabolism, protein folding and also implicated in histone acetylation/deacetylation process. Our results indicate that histone deacetylases could be a possible therapeutic target for OPMD treatment.</p>
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<p>PW10-126</p>	<p><u>PABPN1 POLYALANINE TRACT DELETION AND LONG EXPANSIONS MODIFY ITS AGGREGATION PATTERN AND EXPRESSION</u> KLEIN AF¹, EBIHARA M², ALEXANDER C², DICAIRE MJ², ROULEAU GA³, BRAIS B² (1) UMRS787 – Groupe Myologie; Inserm / UPMC-ParisVI; Institut de Myologie, Paris, FRANCE. (2) Laboratoire de neurogénétique du mouvement, Centre d'excellence en neuromique de l'Université de Montréal, CRCHUM, Université de Montréal, Montréal, CANADA. (3) Centre d'excellence en neuromique de l'Université de Montréal, CRCHUM, Université de Montréal, Montréal, CANADA.</p>
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Oculopharyngeal muscular dystrophy (OPMD) is a late-onset autosomal dominant disease caused by expansions of a (GCN)₁₀/polyalanine tract in the gene coding for the Poly(A) Binding Protein Nuclear 1 (PABPN1). The pathological hallmark of the disease is the presence of filamentous intranuclear inclusions (INI) in the nucleus of skeletal muscle fibers. In cellular and animal models of the disease, INIs can be reproduced by overexpression of *PABPN1*. Studies on other diseases caused by similar polyalanine expansions have shown that expression of mutated proteins does not cause INIs formation but induces their abnormal accumulation in the cytoplasm. This relocalization appears to be correlated to the length of the polyalanine tract. This study presents the impact on the subcellular localization of PABPN1 produced by large expansions of its polyalanine tract or by its deletion. Large tracts of more than 24 alanines result in the nuclear accumulation of PABPN1 in SFRS2-positive functional speckles and in a significant decline in cell survival. These large expansions do not cause INIs formation nor do they lead to cytoplasmic accumulation. Deletion of the polyalanine tract induces the formation of aggregates that are located at the nuclear membrane, highlighting the possible role of the N-terminal polyalanine tract in PABPN1 nucleo-cytoplasmic transport. We also show that even though five other proteins with polyalanine tracts tend to aggregate when over-expressed they do not co-aggregate with PABPN1 INIs. This study presents the first experimental evidence that there may be a relative loss of function in OPMD by decreasing the availability of PABPN1 through an INI-independent mechanism.

PW10-127	<p><u>A DROSOPHILA MODEL FOR THE OCULOPHARYNGEAL MUSCULAR DYSTROPHY (OPMD): STUDY AND DEVELOPMENT OF THERAPEUTIC STRATEGIES</u> CHARTIER A¹, VAN DER MAAREL SM², VERRIPS T³, SIMONELIG M¹ (1) Dept Genetics and Development, Institute of Human Genetic, CNRS UPR, Montpellier, FRANCE. (2) Department of Human Genetics, Leiden University Medical Center, Leiden, THE NETHERLANDS. (3) Cellular Architecture and Dynamics (CAD), Utrecht University, Utrecht, THE NETHERLANDS.</p>
	<p>Oculopharyngeal muscular dystrophy (OPMD) is a late onset disease characterized by eyelid dropping, swallowing difficulties and limb weakness. Alanine expansions in the coding region of poly(A) binding protein N1 (PABPN1) resulting from GCG trinucleotide repeat extensions lead to the dominant autosomal inheritance of OPMD. In skeletal muscle fibers of OPMD patients, mutant PABPN1 protein aggregates as fibrillar nuclear inclusions, which are the hallmark of the disease.</p> <p>We have generated a <i>Drosophila</i> model that recapitulates muscular phenotypes with similarities to those of OPMD: i) muscle degeneration ii) fibrillar nuclear inclusions containing PABPN1 in muscle nuclei (Chartier et al., <i>EMBO J.</i> 2006). Molecular analysis of this model showed that muscle disorder in <i>Drosophila</i> is induced by an intrinsic toxicity of PABPN1 brought by the RNA binding domain and enhanced by the alanine expansions. We investigated the potential of single-chain intracellular antibody (intrabody) as therapeutic agent for OPMD. We could induce muscle expression of several intrabodies against PABPN1 (Verhensen et al., <i>HMG</i> 2006), in the <i>Drosophila</i> OPMD model and we found that several of them can prevent PABPN1 toxicity and muscular phenotypes.</p>