

**PW 8:
LGMD (part 1) –
Calpain and dysferlin**

PW8-089	<p><u>THE TRANSCRIPTIONAL ACTIVITY OF MYOD IS REGULATED BY CALPAIN 3 DURING MYOGENESIS.</u> STUELSATZ P¹, VESCHAMBRE P¹, COTTIN P¹ (1) Université Bordeaux 1, INRA USC-2009, Proteolysis and Muscle Development Group (UPCDM), Talence, FRANCE.</p>
To contact the author:: pascalstu@yahoo.fr.	<p>MyoD is part of the myogenic regulatory factors (MRFs) family, which are the essential factors controlling the myogenesis during embryonic development or muscular regeneration in the adulthood. CAPN3 is a calcium-dependent cysteine protease mainly expressed in skeletal muscle. Mutations in capn3 gene, have been identified as responsible for the Limb-girdle muscular dystrophy type 2A (LGMD2A), an autosomal recessive muscular dystrophy. This emphasizes a key role for CAPN3 in maintaining the integrity of muscular fibers, however which intracellular pathway is altered and therefore results in muscular dystrophy still remain controversial.</p> <p>We have shown that CAPN3 inhibits the transcriptional activity of MyoD either in myoblastic cells (C2C12) or in fibroblastic ones (C3H10T1/2). On the contrary, no variation in the transcriptional activity of the other members of the MRFs family (Myf5, Myogenin, or MRF4) is observed. We show that CAPN3 affects the transcriptional activity of MyoD by decreasing the quantity of the endogenous protein MyoD (Western-blots, confocal microscopy experiments), without affecting its mRNA level (Q-PCR). Furthermore, the inhibitory effect of CAPN3 on MyoD is independent of the ubiquitine proteasome proteolytic pathway that is known to play a role during MyoD degradation. Indeed, MyoD mutants resistant to proteolytic degradation by the proteasome are sensitive to CAPN3 activity. Interestingly, in C2C12 differentiating cells, the overexpression of CAPN3 induces a delay in myogenic differentiation with a decrease in the total number of myotubes which correlated to a delay in the apparition of differentiation marker. Experiments are in progress to determine whether CAPN3 acts directly or not on MyoD. This study identifies CAPN3 as a new regulator of the myogenic factor MyoD and could help to understand the mechanisms sustaining the development of the LGMD2A muscular dystrophy.</p>

PW8-090	<p>CAPN3 MUTATIONS IN PATIENTS WITH IDIOPATHIC EOSINOPHILIC MYOSITIS KRAHN M¹, GOICOECHEA M², PÉCHEUX C¹, GARCIA-BRAGADO F², JACQUEMONT S³, LOBRINUS JA³, JEANNET PY³, STROBER J⁴, TESTARD H⁵, PENA-SEGURA JL⁶, ROMERO NB⁷, STREICHENBERGER N⁸, BERNARD R¹, SAENZ A², LETURCQ F⁹, URTIZBEREA JA¹⁰, LÉVY N¹, LOPEZ DE MUNAIN A² (1) Département de Génétique Médicale, Laboratoire de Génétique Moléculaire, Hôpital d'Enfants de la Timone, and INSERM U910, Faculté de Médecine Timone, Université de la Méditerranée,, Marseille, FRANCE. (2) Servicio de Neurologia and Unidad de Genetica and Unidad Experimental, Hospital Donostia, and Servicio de Anatomía Patológica, Hospital Virgen del Camino,, San Sebastian and Pamplona, SPAIN. (3) Service de Génétique médicale, Institut Universitaire de Pathologie, Département médico-chirurgical de pédiatrie, Unité de Neuropédiatrie, CHUV, Lausanne, SWITZERLAND. (4) UCSF, CA, San Francisco, USA. (5) Service de Pédiatrie et Néonatalogie, CHI, Annemasse, FRANCE. (6) Unidad de Neuropediatr&#305;a, Hospital Universitario Miguel Servet,, Zaragoza, SPAIN. (7) INSERM U582 Institut de Myologie, Hôpital de la Pitié- Salpêtrière, Paris, FRANCE. (8) Service de Neuropathologie, Hôpital Neurologique, Lyon, FRANCE. (9) Laboratoire de Biochimie Génétique, Hôpital Cochin, Paris, FRANCE. (10) Hôpital Marin, AP-HP, Paris, FRANCE.</p>
To contact the author:: martin.krahn@ap-hm.fr.	<p>Eosinophilic myositis constitutes a rare pathological entity characterized by eosinophilic infiltration of skeletal muscles, usually associated with parasite infections, systemic disorders, or the intake of drugs or L-tryptophan. The exclusion of such causes defines the spectrum of idiopathic EM. Based on a protein analysis performed in one affected patient, we recently identified <i>CAPN3</i> mutations as a genetic cause of idiopathic eosinophilic myositis in a series of six paediatric patients.</p> <p>Here, we retrospectively included five additional patients diagnosed as being affected with idiopathic eosinophilic myositis. Whereas our previously reported cases of idiopathic eosinophilic myositis with <i>CAPN3</i> mutations were only paediatric cases, we here describe one adult patient. Importantly, as in our previous series, the inclusion was based only on the particular histopathological presentation, in patients for whom no definitive aetiological diagnosis had been established. Disease-causing <i>CAPN3</i> mutations were identified at a homozygous or compound heterozygous state in all patients, thus confirming the diagnosis of primary calpainopathy.</p> <p>Why eosinophilic infiltration can be correlated to defective calpain-3 needs to be further evaluated. Abnormal local interleukin secretion in calpain-3 deficient muscle tissue is one possibility. Also, T lymphocytes, which express the <i>CAPN3</i> transcript, may be implicated in this process, as they are a main component of inflammatory lesions in the vicinity of damaged muscle fiber, and central in the chemotactism of eosinophils by secreting interleukin-5. In this regard, we recently identified one case initially diagnosed as lymphocytic myositis in which primary calpainopathy was diagnosed at the protein and genomic level.</p> <p>Altogether, the presence of marked eosinophilic infiltrates in patients affected with idiopathic eosinophilic myositis caused by <i>CAPN3</i> mutations point to an important role of eosinophils in muscle damage caused by calpain-3 deficiency.</p>

PW8-091	<p><u>TRANSCRIPTIONAL EXPLORATIONS OF CAPN3 IDENTIFY NOVEL SPLICING MUTATIONS, A LARGE-SIZED GENOMIC DELETION AND EVIDENCE FOR MRNA DECAY.</u></p> <p>PÉCHEUX C¹, KRAHN M¹, CHAPON F², BÉROUD C³, DROUIN-GARRAUD V⁴, LAFORET P⁵, ROMERO NB⁶, PENISSON-BESNIER I⁷, BERNARD R¹, URTIZBEREA JA⁸, LETURCQ F⁹, LÉVY N¹</p> <p>(1) Département de Génétique Médicale, Hôpital d'enfants de la Timone, Marseille, FRANCE. (2) Consultation de Pathologies neuromusculaires and Laboratoire de Neuropathologie, CHU Cote de Nacre, Caen, FRANCE. (3) INSERM U827 - Génétique des maladies rares : pathologie moléculaire, études fonctionnelles, banque de données génétiques, Institut Universitaire de Recherche Clinique, Montpellier, FRANCE. (4) Service de Génétique, Hôpital Charles Nicolle, Rouen, FRANCE. (5) Institut de Myologie, Hôpital de la Pitié-Salpêtrière, Paris, FRANCE. (6) INSERM U582 Institut de Myologie, Hôpital de la Pitié-Salpêtrière, Paris, FRANCE. (7) Département de Neurologie, CHU, Angers, FRANCE. (8) APHP, Hôpital Marin d'Hendaye, Hendaye, FRANCE. (9) Laboratoire de Biochimie Génétique, Hôpital Cochin, Paris, FRANCE.</p>
To contact the author:: martin.krahn@ap-hm.fr.	<p>Mutations in the gene encoding calpain-3 (<i>CAPN3</i>) cause autosomal recessive Limb-Girdle Muscular Dystrophy type 2A (LGMD2A) and idiopathic Eosinophilic Myositis. Accurate diagnosis and genetic counselling is based on the identification of disease-causing mutations on both alleles of <i>CAPN3</i> in the patients. Unfortunately, in 20 to 30% of patients, routine genomic analysis fails to identify both disease-causing alleles. These patients should benefit from complementary approaches towards completing mutation's identification.</p> <p>Here, we performed transcriptional analyses in five patients suspected of being affected with LGMD2A, in whom initial DHPLC/genomic mutation screening evidenced no, or a single-allele <i>CAPN3</i> disease-causing mutation thus not sufficient to firmly confirm diagnosis.</p> <p>This allowed to identify and characterise cDNA deletions. Further genomic characterisation allowed to determinate the origin of these deletions, either as splicing defects caused by intronic mutations or an internal multi-exonic deletion. Indeed, we report in the <i>CAPN3</i> gene two novel mutations (c.1745+4_1745+7delAGTG produced from IVS13 and c.2185-16A>G produced from IVS20) and a recurrent large sized-genomic deletion including exons 2 to 8 for which genomic breakpoints have been characterised, in the <i>CAPN3</i> gene. In addition, our results indicate nonsense-mediated mRNA decay as a mechanism for under-expression of <i>CAPN3</i> associated to some specific variations. All abnormalities evidenced at the transcriptional level have been accurately predicted using Splicing Sequences Finder, a novel algorithm for the prediction of deleterious effects on normal splicing. (Note CP and MK contributed equally to this work)</p>

PW8-092	<p><u>TISSUE-SPECIFIC CAPN3 MRNA DECAY IN LIMB-GIRDLE MUSCULAR DYSTROPHY TYPE 2A</u> BLAZQUEZ L¹, AZPITARTE M¹, SÁENZ A¹, GOICOECHEA M¹, OTAEGUI D¹, VILCHEZ J³, LÓPEZ DE MUNAIN A² (1) EXPERIMENTAL UNIT, DONOSTIA HOSPITAL, SAN SEBASTIAN, SPAIN. (2) NEUROLOGY DEPARTMENT, DONOSTIA HOSPITAL, SAN SEBASTIAN, SPAIN. (3) NEUROLOGY DEPARTMENT, HOSPITAL LA FE, VALENCIA, SPAIN.</p>
To contact the author:: lorebg@gmail.com.	<p>Introduction: Limb-girdle muscular dystrophy type 2A (LGMD2A) is an autosomal recessive disorder caused by mutations in the <i>CAPN3</i> gene. This gene is preferentially expressed in muscle tissue, but we have recently described that four different <i>CAPN3</i> transcripts produced by the alternative splicing of exon 6, 15 and 16 are also expressed in White Blood Cells (WBCs) from healthy controls. The sequencing of these transcripts could be applied for LGMD2A diagnosis.</p> <p>Patients and Methods: In the present work, the <i>CAPN3</i> mRNA expression level was measured in muscle and White blood cells from 3 LGMD2A patients and 2 healthy controls by real-time quantitative RT-PCR (RTQ-PCR). Three different Taqman probes designed in the exon junction of different constitutive exons of the <i>CAPN3</i> pre-mRNA were used. TBP (TATA box binding protein) expression level was used to normalize the starting quantity of mRNA. The expression level of healthy controls was taken as the reference value.</p> <p>Results: In WBCs the expression level of the <i>CAPN3</i> mRNA in the three LGMD2A patients was similar to the expression level reported in controls. In muscle, however, LGMD2A patients showed a reduced expression when compared to controls. These reduction was two-fold in the patient who carried a frameshift and a missense mutation, whereas it was up to ten-fold in the two patients who carried a frameshift mutation in each allele.</p> <p>Discussion: These results confirm that in muscle the <i>CAPN3</i> transcripts which carry a mutation which introduces a premature-termination-codon (PTC) are subjected to non-sense mediated mRNA decay (NMD) as previously reported by Stehliková et al. By contrast, in peripheral blood the <i>CAPN3</i> transcripts present a reduced sensitivity to mRNA degradation. These differences might result from the interaction between elements in transcripts that are subjected to NMD and factors in the NMD machinery.</p>

PW8-093	<p><u>MUSCLE LOSS QUANTIFICATION BY CT-SCAN ANALYSIS IN THE CONTEXT OF THE NATURAL HISTORY OF CALPAINOPATHIES PROJECT.</u> LAURENT E¹, BOREL P¹, HOGREL JY², BOUSQUET N¹, FOUGEROUSSE F¹, STOCKHOLM D¹, CALPAIN STUDY GROUP _¹ (1) Généthon, Evry, FRANCE. (2) Institut de Myologie, Paris, FRANCE.</p>
To contact the author:: stockho@genethon.fr.	<p>In the Natural History of Calpainopathies study (F Fougrousse's communication), 37 patients were subjected to Computed Tomography (CT) scans of lower limbs at the beginning of the investigation and 2 years later. The objective was to quantify muscle loss for a better characterization of the muscle damage specificity known in Limb Girdle Muscle Dystrophy and its progression over two years. We applied a methodology based on manual determination of Regions Of Interest (ROI) of individual muscles on 4 cross sections per patient. Cross sectional areas and mean pixel intensity were obtained for each muscle enabling respectively the calculation of the level of atrophy and the degree of adiposity in muscle which is measured using changes in density based on Hounsfield unit. Through this approach, we could recover the proximal pattern of muscle deficiency and were able to correlate it with the muscle strength data (JY Hogrel's communication). Most importantly, we showed evidence of a significant decrease of density in most lower limb muscles in all patients over two years. This study indicates that CT-scans can be a valuable tool for a precise follow-up of muscle disease and could be useful in the context of a clinical assay.</p> <p>Presentation of the Calpain Study Group. Promoter - Généthon : P Borel, N Bousquet, F Fougrousse, E Laurent, I Richard, D Stockholm Centre 1 - Institute of Myology : A Canal, B Eymard, V Doppler, M Fardeau, J Y Hogrel, P Laforêt, G Ollivier, C Payan, A Urtizbera Centre 2 - Hospital San Sebastian : V Bahun, A Lopez de Munain, JJ Pozza, D Salicio Centre 3 - Hospital Saint Pierre : la Reunion. JM Begue, P Boué, C Mignard</p>

PW8-094	<p>NATURAL HISTORY OF CALPAINOPATHIES URTIZBEREA J¹, LOPEZ DE MUNAIN A², MIGNARD C³, BOUÉ P³, DOPPLER V⁴, HOGREL J⁴, STOCKHOLM D⁵, PAYAN C⁴, POZA J², BOUSQUET N⁵, RICHARD I⁵, FOUGEROUSSE F⁵, CALPAIN STUDY GROUP⁵</p> <p>(1) Hôpital Marin de Hendaye, Hendaye, FRANCE. (2) Hôpital Donostia, San Sebastian, SPAIN. (3) Hôpital de Saint-Pierre, Saint-Pierre, FRANCE. (4) Institut de Myologie, Paris, FRANCE. (5) Généthon, Evry, FRANCE.</p>
To contact the author:: fougerou@genethon.fr.	<p>The determination of the natural history is a prerequisite to any future clinical trial in a given myopathy. This is particularly true in calpainopathy where the individual disease progression may markedly vary and where the course itself seems rather slow in the long run. For this purpose, we started to establish the natural history of calpainopathy in a specific group of calpain-deficient patients. The study is multicentric and started in September 2004. We enrolled 21 patients at Paris, 35 at San Sebastian and 30 at the Reunion Island, most with two known mutations in the calpain3 gene. They have been followed up to 2 years and we are likely to expand the observation period up to 5 years or more. After informed consent acceptance of taking part in this study, patients are submitted to exploration every 6 months. In addition to global clinical feature, quality of life questionnaire and evaluation of the pulmonary and cardiac function, the muscular function is explored thoroughly by various means. They include manual and quantitative testing allowing precise investigation of specific muscle groups by QMT, Biodex or MFM. Some of these results will be emphasized in <i>JY. Hogrel's</i> communication. Another complementary exam (CT-scan) will be presented in <i>D. Stockholm's</i> communication.</p>

PW8-095	<p>A TMD/LGMD2J MOUSE MODEL, FINMAJ- TITIN MUTATION KNOCK-IN, EXHIBITS DEFINITE MUSCLE PHENOTYPE CHARTON K¹, DANIELE N¹, SUEL-PETAT L¹, UDD B², RICHARD I¹ (1) GENETHON, EVRY, FRANCE. (2) Folkhalsan Institute of Genetics, Helsinki, FINLAND.</p>
To contact the author:: charton@genethon.fr.	<p>Titin is a giant protein expressed in both skeletal muscles and heart. Titin spans from the Z-disc to the M-line of striated muscle sarcomeres. Several pathogenic mutations were identified in its last two exons causing the phenotype of late onset Tibialis Muscular Dystrophy (TMD). The most common mutation, FINmaj, results in the replacement of 4 amino acids and affects TMD patients in Finland. The disease course is relatively mild with selective atrophy of the <i>tibialis anterior</i> in the early stages. When present on both alleles, the clinical manifestations are completely different and far more severe with progressive atrophy and dystrophy of the girdle muscles leading to wheelchair confinement: this Limb Girdle Muscular Dystrophy phenotype is known as LGMD2J. In addition, the expression of calpain 3, a protease responsible for LGMD2A when mutated, is greatly reduced in LGMD2J muscles, In order to study the physiopathology of these two diseases and to test therapeutic approaches, a mouse model reproducing the FINmaj mutation was created. Although heterozygous and homozygous animals have a normal life span, abnormalities in the transmission ratio suggest a partial embryonic lethality.</p> <p>Whereas the phenotype seems very mild in heterozygous, gait is affected in homozygous animals, possibly reflecting muscle force impairment. In line with this hypothesis, the force of the <i>soleus</i> is reduced. <i>Soleus</i>, <i>tibialis</i> and <i>psaos</i> are indeed severely affected: muscle fibres show signs of inflammation and atrophy, necrosis-regeneration and fibrosis. As seen in LGMD2J, calpain 3 protein level is reduced in heterozygous and homozygous mice, probably related to protein destabilisation because of impaired titin interaction.</p> <p>As a conclusion, this model reproduces symptoms of the human LGMD2J and will therefore be valuable, not only to test therapeutic strategies, but also to document the functional relationship between titin and calpain 3 and other molecular pathways involved in c-terminal titin pathology.</p>

PW8-096	<p><u>DYSFERLINOPATHY IN CHILE, THE TWO FIRST CASES REPORTED SHOW TWO NOVEL MUTATIONS.</u></p> <p>BEVILACQUA JA¹, KRAHN M², PEDRAZA L³, GEJMAN R⁴, GONZALEZ S⁴, LEVY N⁵ (1) Hospital Clínico Universidad de Chile & Instituto de Ciencias Biomédicas, Facultad de Medicina. Universidad de Chile, Santiago, CHILE. (2) Département de Génétique Médicale, Hôpital d'Enfants de la Timone, Marseille, FRANCE. (3) Departamento de Medicina Interna, Clínica Las Condes, Santiago, CHILE. (4) Departamento de Anatomía Patológica, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, CHILE. (5) Département de Génétique Médicale, Hôpital d'Enfants de la Timone & INSERM U491, Faculté de Médecine de Marseille, Marseille, FRANCE.</p>
To contact the author:: JBEVILACQUA@MI.CL.	<p>Dysferlinopathies are autosomal recessive muscular dystrophies caused by mutations in the dysferlin (<i>DYSF</i>) gene that encodes for dysferlin (MIM 603009). Dysferlinopathy manifests as two main clinical phenotypes, distal Miyoshi's myopathy and LGMD2B, however a wide range of clinical phenotypes --- ranging from sub-clinical to severe forms – may also be produced by similar mutations.</p> <p>We are reporting the two first Chilean cases of dysferlinopathy with a molecular genetic analysis. A 26 year old man, from a consanguineous marriage, developed distal myopathy involving the posterior compartment of both legs. Impairment progressed in the in the lapse of three years to tights and the anterior compartment the right arm. CK levels at onset were 21237 UI/dl. Biopsy of the quadriceps showed unspecific dystrophic changes, and absent dysferlin immunostaining. In this patient, a novel one base-pair deletion of the exon 21 was identified (c.1948delC) at a homozygous state. This leads to a shift of the reading-frame resulting in a premature termination codon (p.Leu650TyfsX6). The second case is a 26 year old woman that developed a progressive weakness in her right leg. Examination showed an asymmetrical atrophy of the calves and both anterior recti. Normal walking was impaired, but other muscular groups were less affected. CK levels at onset were 8870 UI/dl. Biopsy of the quadriceps showed minimal dystrophic changes, dysferlin immunostaining was absent. Two disease-causing mutations, at a compound heterozygous state, were detected in her: a frame shifting mutation of the exon 27 (c.2858dupT, p.Phe954ValfsX2) and a novel missense mutation of the exon 13 (c.1276G>A, p.Gly426Arg). The latter, was not retrieved in 200 control chromosomes and affects a highly conserved amino-acid residue located in C2 domain C of dysferlin. We conclude that this novel missense change is pathogenic.</p> <p>Further studies to typify dysferlinopathy in the region will contribute of this dystrophy.</p>

PW8-097	<p>ANALYSIS OF THE DYSF MUTATIONAL SPECTRUM IN A LARGE COHORT OF PATIENTS KRAHN M¹, BÉROUD C², LABELLE V¹, BERNARD R¹, NGUYEN K¹, BASSEZ G³, FIGARELLA-BRANGER D⁴, POUGET J⁵, SALVO E¹, HAMMOUDA EH⁶, EYMARD B³, URTIZBEREA JA⁷, LETURCQ F⁸, LÉVY N¹, AND THE FRENCH NETWORK ON LGMD.⁹</p> <p>(1) Département de Génétique Médicale, Hôpital d'enfants de la Timone et - Inserm U910 :, Marseille, FRANCE. (2) CHU de Montpellier, INSERM, U827, et Université Montpellier 1, Montpellier, FRANCE. (3) Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (4) Laboratoire d'Anatomopathologie, Hôpital Timone, Marseille, FRANCE. (5) Service de Neurologie, Hôpital Timone, Marseille, FRANCE. (6) AFM, Evry, FRANCE. (7) Hopital Marin, APHP, Hendaye, FRANCE. (8) Laboratoire de Biochimie Génétique, Hôpital Cochin, Paris, FRANCE. (9) ., ., FRANCE.</p>
---------	--

<p>To contact the author:: martin.krahn@ap-hm.fr.</p>	<p>Dysferlinopathies belong to the heterogeneous group of autosomal recessive muscular dystrophies. Mutations in the gene encoding dysferlin (<i>DYSF</i>) lead to distinct phenotypes, mainly Limb Girdle Muscular Dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM).</p> <p>Here, we report the results of mutational screening in the largest cohort reported to date. Altogether, 134 patients were included based on clinical suspicion of primary dysferlinopathy and/or dysferlin protein deficiency identified on muscle biopsy samples. Genomic DNA was screened for <i>DYSF</i> mutations using DHPLC analysis, and subsequent sequencing of detected variants, in a routine diagnostic setting.</p> <p>In 89 patients (66%), molecular analysis identified two disease-causing mutations, confirming the diagnosis of primary Dysferlinopathy on a genetic basis. Furthermore, one mutation was identified in 30 patients, without identification of a second deleterious allele. We are currently developing complementary analysis for patients in whom only one or no disease-causing allele could be identified using the genomic screening procedure. Altogether, 64 novel mutations have been identified in this cohort, which corresponds to approximately 25% of all <i>DYSF</i> mutations reported to date. Most of the identified mutations are predicted to produce a truncated protein or one amino-acid substitution, but we also report a high proportion of nonsense mutations as compared to previous series.</p> <p>All mutational and available clinical data from our series, and previous reports in the literature, were included in a novel locus-specific database, the Universal Mutation Database for Dysferlin (UMD-DYSF). This database includes several bioinformatic tools which allow the statistical analysis of mutational data, and the prediction of a pathogenicity score for newly identified intronic, and missense- or isosemantic-exonic sequence variants. The database is therefore a valuable</p>
---	---

<p>PW8-098</p>	<p><u>EXON-SKIPPING OF DYSFERLIN IN CD133+ STEM CELLS ISOLATED FROM NORMAL AND PATIENT AFFECTED WITH DYSFERLINOPATHIES.</u> NAVARRO C¹, FARINI A¹, MEREGALLI M¹, BELICCHI M¹, PAROLINI D¹, RAZINI P¹, KRAHN M², WEIN N², BOURG N³, BARTOLI M³, RICHARD I³, LEVY N², TORRENTE Y¹</p> <p>(1) Stem cell Laboratory, Department of Neurological Science, Fondazione IRCCS Ospedale Maggiore Policlinico, Centro Dino Ferrari, University of Milan, Milan, ITALY. (2) INSERM U910 "Genetique Medicale et Genomique Fonctionnelle" ; Laboratoire de Genetique Moleculaire, Hopital d'enfants de la Timone, Marseille, FRANCE. (3) Généthon CNRS FRE3018, Evry, FRANCE.</p>
----------------	--

<p>To contact the author:: Claire.Navarro@unimi.it.</p>	<p>Mutations in gene encoding Dysferlin are involved in Limb-gird Muscular Dystrophy type 2B (LGMD-2B) and and Miyoshi myopathy (MM), both diseases are characterized by progressive weakness and wasting of skeletal muscles. Dysferlin is abundantly expressed in skeletal and cardiac muscles where its main function reported is membrane repair. So far, no treatment is available for these diseases but new hopes are coming from stem cell therapy. Recently, we found a subpopulation of human muscle or blood derived stem cells expressing the CD133 antigen which are able to efficiently participate in muscle regeneration <i>in vivo</i>. A successful strategy associating exon-skipping strategy and the use of CD133 + stem cell may be tested in the dysferlinopathies. For this reason, we characterized precisely dysferlin expression in normal human CD133+ stem cells obtained from both circulating blood and muscle, either alone or in co-culture experiments with normal or dysferlin null mouse-derived myotubes. In these experiments, we found human dysferlin expression both by RT-PCR and immunostaining experiments. In order to combine exon skipping and stem cell therapy, we designed oligonucleotides anti-sens nucleotides (AONs) targeting consensus regions important to direct splicing machinery, for patients carrying relevant mutations in dysferlin exons. The efficiency of skipping was tested by RT-PCR on CD133 + stem cell isolated from LGMD-2B or MM patients. By this approach we plan to test function of re-expressed dysferlin, after engrafting of human skipped CD133+ cells in a mouse model of dysferlinopathy (<i>Dysf</i> ^{-/-}) crossed with Scid immunodepressed mice. We believe that combined exon skipping and CD133+ stem cell therapy is a very promising approach that has to be deeply explored in the context of dysferlinopathies.</p>
---	--

<p>PW8-099</p>	<p><u>BLOOD MONOCYTES: A VALUABLE MODEL FOR SCREENING ABNORMALITIES OF DYSFERLIN CELL LOCALIZATION AND FUNCTION</u> WEIN N¹, COURRIER S¹, KRAHN M¹, LABELLE V², LETURCQ F³, CAU P¹, LEVY N¹ (1) neuromuscular disorders and laminopathies, Inserm U910, Marseille, FRANCE. (2) Department of medical genetics, Timone hospital, Marseille, FRANCE. (3) Biochemical and molecular genetics, Cassini Laboratory, Paris, FRANCE.</p>
----------------	---

To contact the author::
nicolas.wein@univmed.fr

Dysferlinopathies are a group of autosomal recessive muscular dystrophies, due to defects in the *DYSF* gene. The aim of our project is to set-up functional test in monocytes and to explore therapeutic approaches in dysferlinopathies. Presently, after clinical exploration, initial diagnosis of dysferlinopathies is based on Western blot (WB) from proteins obtained after muscle biopsy or blood monocytes, and our results suggest these latter representing an excellent and easy to obtain cellular model: RT-PCR, WB and IF (with NCL-Hamlet antibody) have demonstrated that monocytes express a *DYSF* mRNA (6.9kb) and produce Dysferlin protein (237kDa). However Dysferlin cannot be detected by WB performed on monocytes after a 48h blood storage, whereas Hamlet antibody still detects a lower yet uncharacterized band (MW = 200 Kda). IF have shown that Dysferlin is present at plasma membrane in resting monocytes while a cytoplasmic staining is shown when monocytes are submitted to mechanical stress or electroporation. Furthermore, we have designed a new diagnosis test using flow cytometry from monocytes. This approach allowed detecting both Dysferlin and monocytes within only 100µl of whole blood from healthy individuals. We are now screening a cohort of patient using flow cytometry and WB analyses in order to valid this method. Finally we are setting up functional tests towards studying monocytes phagocytosis. Our hypothesis is based on the following data: 1/ In monocytes, Dysferlin is associated, as in skeletal muscle cells, with caveolin and annexins, both proteins being involved in monocytes phagocytosis; 2/ phagocytosis requires compensatory plasma membrane supply through exocytosis; 3/ if Dysferlin could play a role in plasma membrane supply after phagocytosis, then phagocytosis could be altered in monocytes from patients affected with dysferlinopathy. In such case, monocyte phagocytosis could be used to evaluate the disease severity and allow the efficiency of potential therapeutic strategies.

PW8-100	<p>OVERLAPPING PROPERTIES OF DYSFERLIN, MYOFERLIN AND FER1L5 VESICLES IN MUSCLE CELLS RAMACHANDRAN U¹, SALEKI K¹, MARLOW G¹, ANDERSON L², BASHIR R¹ (1) School of Biological and Biomedical Sciences, University of Durham, Durham, UNITED-KINGDOM. (2) Institute of Human Genetics, International Centre for Life, Newcastle upon Tyne, UNITED-KINGDOM.</p>
To contact the author:: Rumaisa.Bashir@durham.ac.uk.	<p>The dysferlin gene is mutated in autosomal recessive Miyoshi myopathy, Limb Girdle Muscular Dystrophy type 2B (LGMD2B) and distal anterior compartment myopathy resulting in deficiency of the protein in patient muscle. Dysferlin is a sarcolemmal protein sharing homology with the sperm vesicle fusion protein FER-1, which mediates fusion of intracellular vesicles with the spermatid plasma membrane. Dysferlin has been shown to be involved in sarcolemmal repair through a proposed mechanism that involves fusion of dysferlin containing vesicles to form a “membrane patch” which is added to the membrane disruption site for resealing. Belonging to the ferlin protein family dysferlin shares structural similarities with <i>C. elegans</i> FER-1, a sperm vesicle fusion protein and other mammalian proteins, myoferlin and otoferlin. Through bioinformatic analysis we have identified three novel ferlins, FER1L4, FER1L5 and FER1L6. Homology modeling of all of the ferlin C2 domains and sequence analysis has identified myoferlin and FER1L5 as being the most similar to dysferlin. We have examined the expression and distribution of dysferlin, myoferlin and the novel ferlin FER1L5 in muscle cells with a view to identifying potential compensatory proteins of dysferlin. We demonstrate that in muscle cells dysferlin, myoferlin and FER1L5 are predominantly present in low density vesicles similar to other organelles. Dysferlin and myoferlin vesicles show a dual distribution present in detergent resistant and dissolved fractions in C2C12 cells. FER1L5 is predominantly recovered in dissolved fractions. To examine membrane repair we have adopted siRNA transfection studies. We have successfully generated and characterized myoferlin deficient C2C12 cells. For FER1L5 siRNA knockdown of FER1L5 protein has not been possible but we have performed antibody loading to disrupt protein function. The cellular phenotype of these cells will be discussed.</p>