

**PW 13:**  
**Nucleopathies and other  
dominant dystrophies**

PW13-155	<p><b><u>GERMINAL MOSAICISM FOR LMNA MASQUERADING AS AUTOSOMAL RECESSIVE CONGENITAL MUSCULAR DYSTROPHY (CMD)</u></b></p> <p>MAKRI S<sup>1</sup>, CLARKE NF<sup>2</sup>, RICHARD P<sup>3</sup>, MAUGENRE S<sup>2</sup>, DEMAY L<sup>3</sup>, TERKI N<sup>4</sup>, BONNE G<sup>2</sup>, GUICHENEY P<sup>2</sup></p> <p>(1) Service de neurologie. Etablissement Hospitalier Spécialisé Ali Ait Idir, Alger, ALGERIA. (2) Inserm, U582. Institut de Myologie, Paris, FRANCE. (3) AP-HP, Groupe Hospitalier Pitié-Salpêtrière. UF Cardiogénétique et Myogénétique. Service de Biochimie Métabolique, Paris, FRANCE. (4) Service d'anatomopathologie. CPMC, Alger, ALGERIA.</p>
To contact the author:: makrisamira@yahoo.fr.	<p>Background: Mutation p.Arg527Pro in the lamin A/C, encoded by <i>LMNA</i> gene, was identified several times in dominant Emery-Dreifuss muscular dystrophy and cases with muscular dystrophy, lipodystrophy, and cardiac rhythm disturbances. We found this p.Arg527Pro mutation in two sisters, from consanguineous unaffected parents, with a congenital muscular dystrophy (CMD) phenotype. Patients: Reduced fetal movements and neonatal hypotonia were reported in both affected children. The patients walked at age 4 and 2 years respectively and now ambulate with difficulty. Neurological examination at ages 13 and 7 years respectively showed generalised muscle weakness with scapular winging, no facial weakness, marked contractures, hyperlordosis and scoliosis. Electrocardiography and echocardiography were normal. Respiratory function tests and CK levels were normal. IQ and brain MRI were also normal. On muscle biopsy there was marked fibrosis in younger child.</p> <p>Immunohistochemical analysis showed normal dystrophin, sarcoglycans and merosin.</p> <p>Results: A Genome-wide 10 cM microsatellite screen and extra genotyping of specific loci excluded all know CMD loci. This included the <i>LMNA</i> locus for both classical autosomal recessive or autosomal dominant inheritance patterns as normal siblings shared the same haplotypes as the affected children. No new locus was seen on the 10 cM microsatellite screen. <i>LMNA</i> sequencing, requested based on the clinical phenotype, found both affected children are heterozygous for a missense mutation in the gene <i>LMNA</i> encoding Lamin A/C, p.Arg527Pro, which was not present in either parent in DNA samples derived from lymphocytes. This pattern is best explained by the presence of gonadal mosaicism in a parent. Conclusion: <i>De novo</i> mutations are common in <i>LMNA</i> and typically mimic the pattern of autosomal recessive inheritance. Linkage analysis is non-informative in this situation, therefore patients are best selected for <i>LMNA</i> sequencing on clinical features, bearing in mind the broad range of clinical phenotypes that exists (severe congenital CMD to LGMD).</p>

PW13-156	<p><b>DESIGN OF A CLINICAL TRIAL FOR RISK STRATIFICATION FOR PRIMARY PREVENTION OF SUDDEN DEATH IN LAMIN A/C MUTATED PATIENTS</b>  BENEDETTI S<sup>1</sup>, SACCO F<sup>2</sup>, ZERBINI G<sup>3</sup>, MORANDI L<sup>4</sup>, PEGORARO E<sup>5</sup>, TREVISAN C<sup>5</sup>, COMI G<sup>6</sup>, FERRARI M<sup>7</sup>, PREVITALI SC<sup>6</sup>, PAPPONE C<sup>2</sup>  (1) Diagnostica e Ricerca San Raffaele, Milano, ITALY. (2) Dept. Arrhythmology, IRCCS San Raffaele, Milano, ITALY. (3) Dept. Endocrinology, IRCCS San Raffaele, Milano, ITALY. (4) Istituto Nazionale Neurologico C Besta, Milano, ITALY. (5) Dept. Neurology, University of Padova, Padova, ITALY. (6) Dept. Neurology, IRCCS San Raffaele, Milano, ITALY. (7) Università Vita-Salute San Raffaele, Milano, ITALY.</p>
To contact the author:: benedetti.sara@hsr.it.	<p>Mutations in <i>LMNA</i> gene, encoding lamin A/C, have been associated with a high risk of sudden death. The implant of a cardioverter defibrillator (ICD) is to date the only effective intervention, but no specific guidelines are available. To define a risk stratification protocol for ICD implant, we designed a clinical trial that may in the future involve other italian centers. Patients bearing <i>LMNA</i> alterations are subjected to extensive cardiological examination, including ECG, Holter monitoring, electrophysiological study (EPS), echocardiogram and heart MRI. In addition, neurological evaluation and cutaneous biopsy are performed and family history collected. Patients are advised to undergo ICD or Reveal implant according to proposed indications and are re-evaluated every six months for at least 5 years. Major arrhythmic events (MAE: arrhythmic syncope, cardiac arrest, appropriate ICD shock) occurring during follow-up will be compared in the two groups to evaluate the effectiveness of risk stratification performed. Moreover, the efficacy of different clinical variables as predictors of MAE and the benefits/ adverse events ratio among ICD patients will be evaluated. To date, 9 patients were enrolled (age 39±13), six displaying cardiac symptoms. Four patients were given the indication to implant ICD. Interestingly, exams performed in these patients highlighted some common features, revealing underlying defects in initial stages of cardiac disease. Indeed, while none of our patients developed sustained ventricular tachycardia during EPS, two showed a prolongation of the AH interval after injection with Verapamil, a calcium antagonist able to reveal intranodal conduction defects. In one case this result was associated with alterations at heart MRI, revealing initial features of DCM with areoles of fibrotic substitution in the conduction tissue. Notably, this patient was asymptomatic except for bradycardia. Follow-up will allow to correlate these results with increased risk of MAE, therefore better addressing the use of ICD in <i>LMNA</i> mutated patients.</p>

PW13-157	<p><b><u>NUCLEAR DEFECTS IN SYNDROMIC LAMINOPATHIES WITH MUSCLE INVOLVEMENT.</u></b>  LATTANZI G<sup>1</sup>, CAMOZZI D<sup>2</sup>, SCHENA E<sup>2</sup>, COLUMBARO M<sup>1</sup>, BONNE G<sup>3</sup>, WEHNERT M<sup>4</sup>, SQUARZONI S<sup>1</sup>  (1) IGM-CNR Unit of Bologna, Bologna, ITALY. (2) Cell biology lab. Istituto Ortopedico Rizzoli, Bologna, ITALY. (3) Inserm U582 - Institut de Myologie G.H. Pitie-Salpetriere, Paris, FRANCE. (4) Institute of Human Genetics, University of Greifswald, Greifswald, GERMANY.</p>
To contact the author:: lattanzi@jolly.bo.cnr.it.	<p>Laminopathies are a heterogeneous group of human disorders linked to mutations in the <i>LMNA</i> gene encoding A type lamins or in genes encoding lamin A-associated proteins. Numerous mutations of the <i>LMNA</i> gene cause overlapping clinical phenotypes, such as the S143F lamin A/C mutation which causes a progeroid syndrome with muscle involvement. Here we report a comparative study of two different cell lines, one bearing the S143F <i>LMNA</i> mutation, the other affected by double R298C/R298C <i>LMNA</i> and delK37 emerin mutation, obtained from patients affected by muscle wasting and systemic involvement. The first patient was affected by Werner syndrome with muscle involvement, the second patient was affected by a severe form of Charcot-Marie Tooth neuropathy (CMT2). Our results show that nuclear defects consisting of impressive invaginations of the nuclear envelope and accumulation of unprocessed prelamin A characterize both cell lines. Prelamin A accumulation had not been detected previously in laminopathies with muscle involvement and could explain the nuclear envelope disorganization as well as the severity of the clinical phenotype.</p>

PW13-158	<p><b>ADULT ONSET CARDIOMYOPATHY DUE TO A NEW MUTATION IN LAMIN A/C GENE</b></p> <p>POZA JJ<sup>1</sup>, INDAKOETXEA B<sup>1</sup>, GARCÍA-BRAGADO F<sup>2</sup>, OLIVÉ M<sup>3</sup>, FERRER I<sup>3</sup>, MOLANO J<sup>4</sup>, MARTÍN-GARCÍA A<sup>4</sup>, QUESADA JF<sup>4</sup>, COBO AM<sup>5</sup></p> <p>(1) Servicio de Neurología. Hospital Donostia., San Sebastián, SPAIN. (2) Anatomía Patológica. Hospital Virgen del Camino, Pamplona, SPAIN. (3) Instituto de Neuropatología. Servicio de Anatomía Patológica. Hospital de Bellvitge, Barcelona, SPAIN. (4) Genética Molecular. Hospital Universitario La Paz, Madrid, SPAIN. (5) Hôpital Marin, Hendaye, FRANCE.</p>
To contact the author:: juanjose.pozaaldea@osakidetza.net.	<p>Introduction: Mutations in the LMNA gene have been associated with a heterogeneous series of human diseases. The neuromuscular phenotypes may involve the skeletal muscle, heart or both. Muscle morphology usually shows only mild myopathic or non-specific changes but exceptionally, myofibrillar changes have been described.</p> <p>Objectives: To describe a patient with adult-onset cardiomyopathy due to a novel unreported lamin A/C gene mutation</p> <p>Patient: A 53-year-old woman was referred because of sudden dysarthria and right hemiparesia which spontaneously recovered in a few days. She had no vascular risk factors. Brain MRI confirmed the existence of two recent ischemic lesions one in cerebellum and another one in the pons. Angio-MRI was normal. The G20210A mutation in prothrombin (factor II) was detected. Holter-ECG showed frequent extrasystoles. Echocardiogram was normal. Several members of her father's family, including her father, experienced cardiac arrhythmias or stroke and sudden death between the ages of 40 and 60. She reported slight paravertebral and pelvic muscle weakness from her forties Clinical examination showed a 4/5 in MRC scale weakness in paravertebral and pelvic muscles without contractures. Adipose tissue distribution was normal. ENG-EMG study was normal. The serum CK level was 154U/L (n.v. &lt;190U/L). Muscle MRI demonstrated striking atrophy of paravertebral muscles, glutei and posterior group of thighs and less prominent changes in quadriceps, sparing the rectus femoris. At mild-calf level, atrophy of the soleus and the medial head of gastrocnemius were evident. Muscle biopsy of the vastus lateralis showed myopathic changes with variation in the fiber size, and scarce fibers containing basophilic, desmin-positive subsarcolemmal deposits. Analysis of the lamin A/C gene detected a novel heterozygous mutation Asp357Asn in a highly conserved region of the gene.</p> <p>Conclusion: The novel Asp357Asn mutation in lamin A/C gene manifests as an adult-onset cardiomyopathy with frequent sudden death and mild proximal myopathy.</p>

PW13-159	<p><b><u>HEART-HAND SYNDROME OF SLOVENIAN TYPE: A NEW KIND OF LAMINOPATHY</u></b></p> <p>RENOU L<sup>1</sup>, STORA S<sup>1</sup>, BEN YAOU R<sup>2</sup>, SINKOVEC M<sup>3</sup>, DEMAY L<sup>4</sup>, RICHARD P<sup>4</sup>, PETERLIN B<sup>3</sup>, BONNE G<sup>1</sup></p> <p>(1) Institut National de la Santé et de la Recherche Médicale, U582, IFR14, Institut de Myologie, Université Pierre et Marie Curie-Paris VI, Faculté de médecine, Paris, FRANCE. (2) Institut National de la Santé et de la Recherche Médicale, U582, IFR14, Institut de Myologie, Université Pierre et Marie Curie-Paris VI, Faculté de médecine, Association Institut de myologie (AIM), Groupe hospitalier Pitié-Salpêtrière, Paris, FRANCE. (3) Division of Medical Genetics, Dpt. Obstetrics and Gynecology, UMC Ljubljana, Ljubljana, SLOVENIA. (4) AP-HP, Groupe Hospitalier Pitié-Salpêtrière, U.F. Cardiogénétique et Myogénétique Moleculaire, Service de Biochimie Métabolique, paris, FRANCE.</p>
To contact the author:: r.benyaou@institut-myologie.org.	<p>Heart-hand syndrome (HHS) is a heterogeneous group of genetic disorders characterized by congenital cardiac and limb deformities. Five subtypes are currently reported, among them the Slovenian type (HHS-S) associating bracydactyly and cardiac conduction defects. Due to cardiac involvement similarities between laminopathies and HHS-S, we clinically reassessed 12 members and then analyzed <i>LMNA</i> gene in the original HHS-S family. We then used Universal mutation Database (UMD®) software to perform in silico predictions. We finally studied fibroblasts from 2 affected members and control subject by immunofluorescence, western blot and RT-PCR techniques to study the consequences of the identified <i>LMNA</i> variant at molecular, protein and cellular levels.</p> <p>In addition to the HHS, one patient showed proximal myopathy. At the molecular level, we identified a new <i>LMNA</i> intronic variant (IVS9-12 T&gt;G) heterozygous in all affected subjects. UMD® software predicted creation of a new cryptic acceptor splice site 11 nucleotides upstream of the wild type site. mRNA study revealed the presence of a frameshift in the <i>LMNA</i> mRNA leading to a premature stop codon and the production of a truncated protein of 550 amino acids observed on fibroblast western. Fibroblasts showed nuclear abnormalities similar to what is classically observed in laminopathies.</p> <p>Finally, we identified a new intronic <i>LMNA</i> variant that lead to the production of a truncated lamin A/C that is expressed in patient fibroblasts. HHS-S might be considered as a new lamins A/C related disorder thus widening the clinical spectrum of laminopathies.</p>

PW13-160	<p><b><u>LAMIN A/C MEDIATED NEUROMUSCULAR JUNCTION DEFECTS IN EMERY-DREIFUSS MUSCULAR DYSTROPHY</u></b>  MEJAT A<sup>1</sup>, DECOSTRE V<sup>2</sup>, RENO L<sup>2</sup>, KESARI A<sup>3</sup>, STEWART C L<sup>4</sup>, BONNE G<sup>2</sup>, HOFFMAN E<sup>3</sup>, MISTELI T<sup>1</sup>  (1) National Cancer Institute, NIH, BETHESDA, USA. (2) Inserm U582 - Institut de Myologie, PARIS, FRANCE. (3) Research Center for Genetic Medicine, Children's National Medical Center, WASHINGTON DC, USA. (4) Institute of Medical Biology, SINGAPORE, SINGAPORE.</p>
To contact the author:: mejata@mail.nih.gov.	<p>The <i>LMNA</i> gene encodes lamins A and C, two intermediate filament type proteins that are important determinants of interphase nuclear architecture as they play essential roles in maintaining the integrity of the nuclear envelope and chromatin structure. Mutations in the human lamin A/C gene (<i>LMNA</i>) lead to a wide spectrum of human diseases including autosomal dominant Emery-Dreifuss muscular dystrophy (AD-EDMD) which affects skeletal and cardiac muscle. The cellular mechanism by which mutations in genes encoding nuclear envelope proteins cause striated muscle abnormalities in EDMD has been elusive. <i>LMNA</i><sup>H222P/H222P</sup> and <i>LMNA</i><sup>-/-</sup> mice develop severe muscle wasting, highly reminiscent of human EDMD and have been used as EDMD animal models. Here we show that these mice fail to innervate muscle and exhibit aberrant neuromuscular junctions (NMJ). In both mice models, several nuclear envelope components crucial for proper recruitment and positioning of synaptic nuclei are mislocalized, leading to a reduction in their number and their mispositioning at the NMJ. Consequently, <i>LMNA</i><sup>H222P/H222P</sup> and <i>LMNA</i><sup>-/-</sup> muscles show several signs of functional denervation including mis-expression of neurotransmission-defect marker genes and altered epigenetic chromatin modifications. These defects are due to loss of interaction between lamin A and inner nuclear membrane proteins since muscle defects are recapitulated upon transient knockdown of <i>LMNA</i> or expression of a dominant negative form of a lamin A interacting protein. Failed innervation is also a hallmark of biopsies from EDMD patients suggesting these defects are relevant to human disease. These results strongly suggest that lamin A/C mediated neuromuscular junction defects are largely responsible for the disease phenotype in EDMD and they provide the first insights into the cellular and molecular mechanisms for the muscle-specific phenotype of EDMD.</p>

PW13-161	<p><b><u>DELETION OF LAMIN A/C LYSINE 32 IS RESPONSIBLE FOR ABNORMAL MUSCLE MATURATION ASSOCIATED WITH DIFFERENTIATION DEFECTS IN MICE</u></b></p> <p>BERTRAND A<sup>1</sup>, RENOU L<sup>1</sup>, GUENEAU L<sup>1</sup>, DECOSTRE V<sup>1</sup>, LACÈNE E<sup>1</sup>, ARIMURA T<sup>1</sup>, MALISSEN M<sup>2</sup>, BONNE G<sup>1</sup></p> <p>(1) INSERM U582 - Institut de Myologie, Paris, FRANCE. (2) Centre d'immunologie INSERM-CNRS de Marseille-Luminy, Marseille, FRANCE.</p>
To contact the author:: a.bertrand@institut-myologie.org.	<p>Lamin A and C, encoded by <i>LMNA</i> gene, localize at the inner face of the nuclear membrane and interact with multiple proteins and DNA. Mutations reported all along the <i>LMNA</i> gene are responsible for multiple diseases including Emery-Dreifuss muscular dystrophy (EDMD). Among them, the deletion of the lysine 32 leads to a severe phenotype with the first clinical signs appearing before the age of 2 and a loss of ambulation before the age of 10.</p> <p>Homozygous knock-in mice reproducing this mutation show a pronounced reduction of lamin A/C protein levels. At birth, they are undistinguishable from their wild type littermates but they rapidly develop an overall growth retardation in weight and size and die around 15 days of life. Histological analysis of mutant muscles shows a reduction of fibre cross section area, an increased proportion of fibres with central nuclei and embryonic myosin heavy chains expression compared to wild type littermates resembling a maturation defect. Culture of mutant myoblasts shows no reduction of myoblasts proliferation but a delayed differentiation with major nuclear abnormalities: several nuclear proteins interacting with lamin A/C are mislocalized, the chromatin is abnormally distributed and the nuclear shape is severely altered.</p> <p>In conclusion, these preliminary data suggest that KI-<i>Lmna</i><sup>delK32</sup> mice rapidly develop severe and progressive signs of muscular dystrophy probably due to improper post-natal muscular differentiation.</p>

PW13-162	<p><b><u>N-ACETYLCYSTEINE TREATMENT OF EMERY-DREIFUSS MUSCULAR DYSTROPHY LMNA H222P/H222P MOUSE MODEL: BLUNTING OF HARMFUL PROCESSES AND PRESERVATION OF PROTECTIVE RESPONSES</u></b></p> <p>DECOSTRE V<sup>1</sup>, KHOUZAMI L<sup>2</sup>, VARNOUS S<sup>1</sup>, CARMELLE P<sup>2</sup>, PERIER M<sup>2</sup>, ADAMY C<sup>2</sup>, ARIMURA T<sup>1</sup>, SALMON A<sup>1</sup>, ENOND C<sup>4</sup>, MEUNE C<sup>3</sup>, PECKER F<sup>2</sup>, BONNE G<sup>1</sup></p> <p>(1) Inserm U582, IFR14, Institut de Myologie, Paris, FRANCE. (2) Inserm U841, IMRB, Creteil, FRANCE. (3) AP-HP, Hôpital Cochin, Service de Cardiologie, Paris, FRANCE. (4) Université Pierre et Marie Curie-Paris VI, Faculté de médecine, Paris, FRANCE.</p>
To contact the author:: v.decostre@institut-myologie.org.	<p>Emery-Dreifuss muscular dystrophy (EDMD) is characterized by cardiac dysfunction and dilation associated with skeletal muscle defects. It is caused by mutations in the <i>LMNA</i> gene encoding the nuclear proteins lamin A/C that form a meshwork under the nuclear membrane. The <i>Lmna</i><sup>H222P/H222P</sup> mouse model reproduces one of the mutations identified in EDMD patients and presents cardiac and skeletal muscle defects reminiscent of the disease.</p> <p>Left ventricle dysfunction and dilation in 7-month old female <i>Lmna</i><sup>H222P/H222P</sup> mouse was associated with systemic and cardiac depletion in glutathione, elevation in oxidative stress marker (Malondealdehyde: MDA contents), secreted proinflammatory cytokine tumor necrosis factor (sTNF) and fibrosis. One-month oral NAC treatment prevented cardiac function and morphologic worsening assessed by echocardiography, normalized systemic glutathione, MDA, and sTNF contents.</p> <p>We explored the possible molecular mechanisms underlying the cardiac beneficial effects of NAC by focusing on ROS and sTNF-related signalling. Oxidative stress induced upregulation of the cardiac histone deacetylase Sirt1 mRNA in <i>Lmna</i><sup>H222P/H222P</sup> mouse. This corroborated with decreased acetylation of histone-3 and downstream caspase-dependant death, illustrated by the reduced processing of proapoptotic protein BID and caspase-3. MAPKinase pathways were activated in 7-month old <i>Lmna</i><sup>H222P/H222P</sup> mouse : 1) ERK1/2 phosphorylation was associated with TNFR2 protein upregulation, both participating to survival pathway scheduling ; 2) Increased JNK phosphorylation correlated with TNFR1 protein upregulation, both illustrating deleterious response.</p> <p>NAC treatment decreased the severity of the cardiac phenotype induced by the <i>Lmna</i><sup>H222P</sup> mutation by both blunting adverse TNFR1/JNK pathway and preserving survival Sirt1/TNFR2/ERK1/2 pathway.</p>

PW13-163

**PHENOTYPICAL FEATURES OF AUTOSOMAL RECESSIVE CHARCOT-MARIE-TOOTH DISEASE ASSOCIATED WITH R289C MUTATION IN THE LAMIN A/C GENE**  
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The most frequent form of axonal autosomal recessive Charcot-Marie-Tooth neuropathy is a laminopathy. R289C is the only mutation described so far in all reported families. This is a phenotype study of patients with R289C mutation in Lamin A/C gene.

Fifteen patients belonging to 5 Moroccan consanguineous families were examined clinically and electrophysiologically. In one patient, a peroneal nerve biopsy was performed. Linkage to 1q21.2 was then demonstrated and a mutation in the coding region of the lamin A/C gene was identified by direct sequencing.

Mean age at onset was  $14.6 \pm 3.8$  years (range: 8 to 20 years).

Most of the cases had the common CMT clinical phenotype. Four patients, with the longest disease duration, had pronounced atrophy and weakness of scapular muscles. The severity was variable even in the same family. The electrophysiological findings were consistent with axonal form of CMT. The mean MNCV was  $53.5 \pm 7.6$  m/s (range: 36 to 66) for median nerve. The CMAP amplitudes in upper limbs were either reduced (in 5 patients) or normal (in 9 patients). Sensory responses were abolished in most of patients for both upper and lower limbs. Needle electromyography showed neurogenic pattern in both distal and proximal muscles predominantly in lower limbs.

Morphological finding showed axonal neuropathy. R289C mutation in the lamin A/C gene was identified in all families

The main phenotype characteristics of CMT with Lamin A/C mutations are: onset during the last teens or the second decade, impairment of proximal muscles, the variability of functional disability even within the same family and the axonal disorder process. This form is described mostly in North Africa and is due to the same mutation suggesting a probable founder effect.

PW13-164	<p><b><u>X-LINKED EMERY-DREIFUSS MUSCULAR DYSTROPHY. A 14 YEARS RETROSPECTIVE OF ROUTINE DIAGNOSIS</u></b>  <b>BEN YAOU R<sup>1</sup>, DEBURGRAVE N<sup>2</sup>, GUENEAU L<sup>3</sup>, BEUGNET C<sup>2</sup>, BONNE G<sup>3</sup>, CHELLY J<sup>2</sup>, LETURCQ F<sup>2</sup>, RÉSEAU FRANÇAIS EMERY-DREIFUSS ET AUTRES NUCLÉOPATHIES R<sup>4</sup></b></p> <p>(1) Institut National de la Santé et de la Recherche Médicale, U58IFR14, IFR14, Institut de Myologie, Université Pierre et Marie Curie-Paris VI, Faculté de médecine, AP-HP, Groupe hospitalier Cochin, Laboratoire de Génétique Moléculaire, Association Institut de myologie (AIM), Groupe hospitalier Pitié-Salpêtrière, paris, FRANCE. (2) AP-HP, Groupe hospitalier Cochin, Laboratoire de Génétique Moléculaire, Pavillon Cassini, Paris, France, paris, FRANCE. (3) Institut National de la Santé et de la Recherche Médicale, U582, IFR14, Institut de Myologie, paris, FRANCE. (4) Laboratoire de Génétique Moléculaire (Groupe hospitalier Cochin), Institut de Myologie (Groupe hospitalier Pitié-Salpêtrière), France, FRANCE.</p>
To contact the author:: r.benyaou@institut-myologie.fr.	<p>Emery-Dreifuss Muscular Dystrophy (EDMD) is a rare autosomal or X-linked recessive condition, associating muscular dystrophy, joint contractures and cardiac disease. X-linked forms are caused by <i>EMD</i> gene mutations (Emerin). Since the identification of the causative gene in 1994, routine molecular diagnosis of emerinopathies in France has been exclusively performed in the « laboratoire de biochimie et génétique moléculaire » (LBGM) at Cochin hospital (Paris). We aimed to perform a retrospective assessment of the LBGM's 14 years experience in this field.</p> <p>During the 1994-2008 period, 379 patients (239 families, 13 countries) have been referred to LBGM for emerin analysis. Where Emerin protein expression was not studied by immunofluorescence (IF), western blot (WB) was performed on muscle or lymphoblastoid cell lines when available. <i>EMD</i> gene was secondarily studied by sequencing method.</p> <p>An <i>EMD</i> mutation was identified in 139 subjects (83M / 56F) corresponding to 53 families. Previous Emerin analysis in 83 of them had showed an abnormal IF and/or Emerin expression (absent, reduced, mosaic) in 78 patients while it was normal for the remaining 5 (female carriers). <i>EMD</i> mutations were out-of frame insertions/deletions (23 families), in-frame deletions/insertions (5 families), point mutations (non sense in 17 families, missense in 1 family) and intronic mutations (7 families)</p> <p>Clinically, the 76 male subjects had EDMD while the others had isolated cardiac disease (5) or were still asymptomatic (2). 45 female carriers were asymptomatic while 11 (19%) showed clinical symptoms of disease (6 EDMD, 4 isolated cardiac disease and 1 limb girdle myopathy) due to variable degree of X chromosomes reciprocal inactivation.</p> <p>Finally, this retrospective analysis revealed a large cohort of mutated cases (53 families, 21% of the referred cohort) and that Emerin IF / WB analysis remain a good pre-screening tool before molecular analysis of the <i>EMD</i> gene.</p>

PW13-165	<p><b><u>RELOCALISATION OF CENTROSOMAL PROTEINS AT THE NUCLEAR ENVELOPE DURING MYOGENESIS: ROLE OF THE NESPRINS</u></b>  <b>ESPIGAT-GEORGER A<sup>1</sup>, MERDES A<sup>1</sup></b>  (1) ISTMT UMR2587 CNRS - Pierre Fabre, Toulouse, FRANCE.</p>
<p>To contact the author::  aude.espigat@istmt.cnrs  .fr.</p>	<p>During the differentiation process, a drastic rearrangement of the microtubular cytoskeleton occurs in muscular cells. While in myoblasts, the microtubules radiate from the centrosome, they adopt a linear array parallel to the axis of the cell in myotubes. This reorganization is accompanied by the relocalisation of centrosomal proteins to the nuclear envelope (NE). The mechanisms leading to this large reorganisation are until now totally unknown. Preliminary work has suggested that this relocalisation depends on the activation of proteins localised at the NE at the onset of differentiation. Among the few NE proteins characterized to date, nesprins present a remarkable profile. Although they seem to be ubiquitously expressed, specific nesprin isoforms are produced in muscle cells. These isoforms are targeted to the NE through the C-terminal domain and present a long spectrin-repeat rod domain promoting various protein-protein interactions. In particular, nesprins interact with proteins of the SUN family, forming a physical link between the nucleoskeleton and the cytoskeleton. Interestingly, such a link involving SUN proteins and the protein ZYG-12, has been described between the nucleus and the centrosome in <i>C. elegans</i>. Supported by these data, we investigated the potential role of the nesprins in the relocalisation of the centrosomal protein to the NE in C2C12. Using a siRNA approach and overexpression of GFP-tagged nesprins, we demonstrated the implication of nesprin1 in the relocalisation of PCM-1 and pericentrin, two centrosomal proteins at the NE.</p>

PW13-166	<p><b>SPECIFIC CT SCANNER MUSCLE PATTERN HELPS TO DIFFERENTIATE RETRACTILE LAMIN A/C AND COLLAGEN VI RELATED MYOPATHIES.</b>  DECONINCK N<sup>1</sup>, DION E<sup>2</sup>, FERREIRO A<sup>3</sup>, EYMARD B<sup>3</sup>, RICHARD P<sup>6</sup>, ALLAMAND V<sup>4</sup>, BENYAOU R<sup>4</sup>, BONNE G<sup>5</sup>, STOJKOVIC T<sup>3</sup></p> <p>(1) Clinique de Neurologie, Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles (ULB), Bruxelles, BELGIUM. (2) Imagerie médicale, AP-HP Hôpital Louis Mourier, Colombes, FRANCE. (3) Centre de référence neuromusculaire, Paris Est, APHP Pitié - Hôpital Salpêtrière INSERM U582, Paris, FRANCE. (4) Inserm, U582, IFR14, Institut de Myologie, Paris, FRANCE. (5) Université Pierre et Marie Curie-Paris VI, Faculté de médecine, Paris, FRANCE. (6) AP-HP, Groupe Hospitalier Pitié-Salpêtrière, U.F. Myogénétique et Cardiogénétique, service de Biochimie Métabolique, Paris, FRANCE.</p>
To contact the author:: nicolas.deconinck@hude rf.be.	<p>Autosomal dominant Emery-Dreifuss dystrophy (EDMD) is caused by <i>LMNA</i> gene mutation while mutations in the collagen VI genes (<i>COL6A1</i>, <i>COL6A2</i>, and <i>COL6A3</i>) cause Bethlem myopathy (BM) and Ullrich congenital muscular dystrophy (UCMD). The presence of contractures, with a muscular dystrophy pattern is found in both conditions, making the differential diagnosis sometimes complex. In this context, the recognition of a specific muscle pattern involvement using straightforward imaging techniques may orient clinicians to appropriate genetic analysis.</p> <p>In a retrospective study we assessed upper and lower limb muscle CT scanner in two patient cohorts suffering from either <i>LMNA</i> mutated EDMD or <i>COL6</i> mutated BM or UCMD. We systematically assessed fatty infiltration level and distribution, muscle volume, and fascia appearance of selected muscles in all patients. Assessment was performed by two independent blinded investigators.</p> <p>In both groups, important fatty infiltration was found in the thighs. However, rectus femoris infiltration was selectively present in BM/UCMD patients; posterior thigh muscles fatty infiltration was significantly found in EDMD patients. In upper arms, severe infiltration was found in triceps and biceps muscles in BM/UCMD patients. A detailed analysis will be presented.</p> <p>From this study, we can conclude that EDMD and BM/UCMD contractile myopathies are characterized by a selective pattern of muscle involvement especially in thigh muscles. Assessing fatty infiltration level with a standard CT scanner technique in selected muscles may help differentiating both conditions and orientating towards appropriate genetic analysis.</p>

PW13-167	<p><b><u>CLINICAL, PATHOLOGICAL, AND GENETIC CORRELATIONS IN IBMPDF PATIENTS WITH A MUTATION IN VALOSIN CONTAINING PROTEIN (VCP GENE)</u></b>  STOJKOVIC T<sup>1</sup>, HAMMOUDA H<sup>2</sup>, RICHARD P<sup>3</sup>, LAFORËT P<sup>1</sup>, LOPEZ DE MUNAIN A<sup>4</sup>, BESNIER-PENISSON I<sup>5</sup>, FERRER X<sup>6</sup>, EYMARD B<sup>1</sup>  (1) AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Myology Institute, Pitié-Salpêtrière Hospital, Paris, FRANCE. (2) Association Francaise contre les Myopathies, Evry, FRANCE. (3) AP-HP, Groupe Hospitalier Pitié-Salpêtrière, UF Cardiogénétique et Myogénétique, Service de Biochimie Métabolique, Paris, FRANCE. (4) Department of Neurogenetics, Donostia Hospital,, San Sebastian Donostia, SPAIN. (5) Department of Neurology, Angers, FRANCE. (6) Department of Neurology, Bordeaux, FRANCE.</p>
To contact the author:: pascale.richard@psl.aph p.fr.	<p><b>Background :</b> Hereditary inclusion body myopathy with Paget disease of bone (PDB) and frontotemporal dementia (FTD), or IBMPDF disease, is an autosomal dominant disorder related to mutations in Valosin-containing protein (VCP).</p> <p><b>Objective:</b> The aim of the study was to describe the clinical, pulmonary, cardiac and histopathological features of 19 patients presenting an IBMPDF disease associated with mutations in the VCP gene.</p> <p><b>Material and methods:</b> PDB was investigated on standard X-rays, bone scintigraphy and alkaline phosphatase dosage. Cognitive impairment was assayed on clinical data and neuropsychological tests. Immunohistochemical studies were performed in biopsies using antibodies directed against dystrophin, sarcoglycans, merosin, dysferlin, alpha-dystroglycan, and desmin. Genetic diagnosis was obtained by direct sequencing of the 17 coding exons of VCP gene.</p> <p><b>Results:</b> The age at onset ranges from 20 to 62 years old. The clinical pattern was characterized by an early involvement of proximal upper limb with scapular winging. Axial and distal muscles of lower limbs were often and early affected, whereas facial, oculobulbar muscles were spared. Ten patients were wheelchair-bound and 4 patients required aid for walking. Two patients required mechanical assisted ventilation and 7 patients had reduced vital capacity. There was no cardiac involvement. Paget disease was observed in 8 patients. Cognitive dysfunction was observed in 9 cases. Seven patients died as the consequence of weakness and respiratory distress. Muscle biopsy revealed rimmed vacuolar myopathy and genetic analysis revealed heterozygous missense mutations in VCP gene in all patients.</p> <p><b>Conclusion:</b> In our series of 19 cases with IBMPDF disease, we observed an intra-familial variability in terms of severity, distribution of weakness and presence or absence of Paget disease or cognitive impairment.</p>

PW13-168	<p><b><u>AUTOSOMAL DOMINANT VACUOLAR MYOPATHY LINKED TO 19P13.3. REPORT OF THE FIRST SPANISH FAMILY</u></b>  <b>TEIJEIRA S<sup>1</sup>, SAN MILLÁN B<sup>1</sup>, FERNÁNDEZ JM<sup>2</sup>, GUTIÉRREZ E<sup>3</sup>, CABELLO A<sup>4</sup>, VIÉITEZ I<sup>1</sup>, ASTUDILLO A<sup>5</sup>, RIBAS T<sup>6</sup>, NAVARRO C<sup>1</sup></b>  (1) Department of Neuropathology, Hospital Universitario de Vigo (Meixoeiro), Vigo, SPAIN. (2) Department of Clinical Neurophysiology, Hospital Universitario de Vigo (Xeral-Cíes), Vigo, SPAIN. (3) Department of Neurology, Hospital "12 de Octubre", Madrid, SPAIN. (4) Department of Pathology, Hospital "12 de Octubre", Madrid, SPAIN. (5) Department of Pathology, Hospital Central de Asturias, Oviedo, SPAIN. (6) Department of Pathology, Hospital de León, León, SPAIN.</p>
<p>To contact the author::  carmen.navarro.fernande  z.balbuena@sergas.es.</p>	<p><i>Introduction.</i> An autosomal dominant vacuolar myopathy (ADVM) linked to 19p13.3 (OMIM 601846) has been recently described in two large Italian families. It is characterized by adult onset, variable muscle weakness with distal to proximal progression, dysphonia and dysphagia, arreflexia, mixed neuropathic and myopathic EMG changes and numerous "rimmed vacuoles" in muscle biopsies.</p> <p><i>Patients and methods.</i> We present a Spanish family with five affected members in two consecutive generations. Age at onset was variable, with distal weakness and amyotrophy, and further extension to upper limbs. In four patients a muscle biopsy was performed under informed consent. Techniques included acetylcholinesterase, acid phosphatase, alizarin red, and immunohistochemistry for Dystrophin, DRP<sub>2</sub>, LAMP<sub>2</sub>, Tau, B-amyloid, ubiquitin and C5b9. Ultrastructural examination was performed.</p> <p><b>DNA from 6 members of the family (4 clinically affected and 2 asymptomatic) was extracted from peripheral lymphocytes and linkage analysis was performed to 19p13 using microsatellite markers.</b></p> <p><i>Results.</i> Muscle biopsy findings were similar in the four biopsied individuals. There was a dystrophic pattern of variable intensity with striking and numerous autophagic vacuoles located at the periphery of muscle fibers or centrally. Acid phosphatase activity was increased, abnormal calcium deposits were abundant and acetylcholinesterase stain disclosed an abnormal pattern with fine deposits all over the vacuoles. Ubiquitin was positive within the vacuoles. Tau, B-amyloid and PrP were negative. LAMP2 was positive. Electron microscopy showed numerous autophagic vacuoles with membranous and multilamellar bodies and myelin figures, some of which were membrane-bound. Linkage analysis showed co-segregation in the critical region 19p13.3 previously described.</p> <p><u>Summary and conclusions</u>  This is the first Spanish family with ADVM linked to 19p13.3. Muscle pathology is somehow distinctive among the large group of vacuolar myopathies, with particular immunohistochemical findings.</p>

PW13-169	<p><b>NOVEL MYOSIN HEAVY CHAIN IMMUNOHISTOCHEMICAL DOUBLE STAINING FOR THE DIAGNOSTIC ROUTINE ASSESSMENT OF FIBER TYPES</b>  RAHEEM O<sup>1</sup>, HUOVINEN S<sup>2</sup>, SUOMINEN T<sup>3</sup>, HAAPASALO H<sup>2</sup>, UDD B<sup>4</sup>  (1) Department of Medicine, Neuromuscular pathology, University of Tampere, Tampere, FINLAND. (2) Department of Pathology, Pirkanmaa Hospital District Center for Laboratory Medicine, Tampere, FINLAND. (3) Neurogenetics, University of Tampere, Tampere, FINLAND. (4) Department of Neurology, Tampere University Hospital, Tampere, FINLAND.</p>
To contact the author:: oiraheem@cc.jyu.fi.	<p><b>Introduction</b>  The ATPase properties of different muscle fiber types is widely used for histochemical diagnostic purposes. The ATPase staining method has a central position in most routine muscle biopsy diagnostic laboratories. However, the method is very laborious and there are disadvantages such as weakening of staining over time and the non-specific staining of capillaries making a distinction of highly atrophic muscle fibers difficult. Since fiber types by ATPase are paralleled by differences in myosin heavy chain isoforms the full assessment of fiber type distribution can also be achieved by immunohistochemical staining.</p> <p><b>Objectives</b>  To develop a reliable and advanced immunohistochemical method for the detection and separation of different fiber types and their subtypes on one slide only.</p> <p><b>Methods</b>  Frozen sections from muscle biopsies were used for myosin double stainings made with the BondMax (Vision biosystems) and/or BenchMark (Ventana medicals) immuno-stainers, using antibodies against myosin heavy chains: slow myosin and myosin A4.74. Slow myosin was stained using a DAB based detection kit with hematoxylin counterstaining and myosin A4.74 with an AP red based detection system.  Histochemical ATPase stainings were also performed on serial sections of the same normal controls and myotonic dystrophy type2 samples to compare the fiber type distribution.</p> <p><b>Results</b>  All ATPase based fiber types were easily separated by the double immunostaining technique. Slow type1 fibers stained brown, fast type 2A fibers stained with a deep shade of pink and type 2C fibers being myosin isoform hybrid fibers stained reddish-brown. Moreover, this technique was able to also separate type IIX fibers with a light shade of pink not accessible by conventional ATPase method. Capillaries or other structures were not immunostained.</p> <p><b>Discussion</b>  The immunohistochemical double staining of myosin heavy chains seems to be a reliable method for the detection and separation of different fiber types and subtypes. Immunohistochemical double staining of myosins can be used as an alternative for the more laborious ATPase staining method in routine histopathology, and proved to provide even more detailed information of fast subtypes and highly atrophic fibers. immunostaining technique.</p>

