

**PW 37:  
Myotonias and  
miscellaneous**

PW37-457	<p><b>SEROLOGICAL ASSAY FOR THE DIAGNOSIS OF AUTOIMMUNE K+ CHANNELOPATHIES</b>  MARTIN-MOUTOT N<sup>1</sup>, BERTHOMIEU M<sup>1</sup>, SEAGAR M<sup>1</sup>  (1) INSERM U641, Universite de la Mediterranee-Aix Marseille 2, Marseille, FRANCE.</p>
<p>To contact the author::  nicole.martin-moutot@univmed.fr.</p>	<p><i>Autoimmune responses against voltage-gated ion channels are suspected in several diseases, but evidence is compelling in the case of two: Lambert-Eaton myasthenic syndrome (voltage-gated Ca<sup>2+</sup> channels, Cav) (O'Neill et al. 1988) and neuromyotonia (voltage-gated K<sup>+</sup> channels, Kv) (Benetar, 2000). These disorders affect the PNS and principally the axonal terminals of motoneurons.</i></p> <p>Acquired neuromyotonia (NM) or Isaac's syndrome is a disease in which peripheral nerve hyperactivity leads to myokymia (muscle twitching, cramps and impaired relaxation resulting from spontaneous motoneurone discharge). In some patients NM is associated with CNS symptoms (hallucinations, mood changes, insomnia) and this has been designated as Morvan's syndrome. Patients with NM respond to plasmapheresis and passive transfer has been demonstrated. Mice injected with IgG from an NM patient displayed increased quantal content, probably resulting from an increment in Ca<sup>2+</sup> influx owing to increased action potential duration (Sinha et al. 1991). Thus the effects of NM IgG resemble those of certain K<sup>+</sup> channel blockers, suggesting that NM patients produce anti-Kv autoantibodies.</p> <p>Based on published studies and our experience in serological assay of Cav autoantibodies (Martin-Moutot et al. 2006), our laboratory has set-up serological assay for circulating antibodies against Kv as an aid to diagnosis. Routine assays for autoantibodies rely on immunoprecipitation of channel / <sup>125</sup>I-ligand complexes from solubilized mammalian brain membranes. Potassium channels in detergent extracts of rat brain membranes were labeled with a radioligand (<sup>125</sup>I-dendrotoxine) specific for these channels. Patient autoantibodies that immunoprecipitate the ligand/channel complex can thus be titrated.</p> <p>Benetar M, (2000) Q J Med 93: 787-797.  Martin-Moutot N, de Haro L, Santos RG, Mori Y, Seagar M (2006) Neurobiol Dis. 22 57-63.  O'Neill J. H., Murray N.M.F., Newsom-Davis J. (1988) Brain 111, 577-596.  Sinha S, Newsom-Davis J, Mills K, Byrne N, Lang B, Vincent A. (1991) Lancet 338, 75-77.</p>

PW37-458	<p><b><u>SPECIFICITY OF ELECTROMYOGRAPHIC EXERCISE TESTS FOR DIFFERENTIATION BETWEEN THE INVOLVED ION CHANNELS AND TYPES OF MUTATIONS IN INHERITED MUSCLE CHANNELOPATHIES AND IN MYOTONIC DYSTROPHY TYPE 2</u></b></p> <p>LEONARDIS L<sup>1</sup>, ZIDAR J<sup>1</sup>  (1) Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Ljubljana, SLOVENIA.</p>
To contact the author:: lea.leonardis@kclj.si.	<p>The role of electromyographic short and long exercise tests in diagnosing inherited muscle channelopathies is well established. It has been reported that such tests can point to the affected genes or specific mutations. Our aim was to re-examine this hypothesis and, in addition, to test patients with myotonic dystrophy type 2 (DM2). We examined 24 patients: 11 with potassium aggravated myotonia (PAM) (<i>SCN4A</i>: 9 Val445Leu mutation, 2 Ala699Thr), 6 with myotonia congenita (MC) (different <i>CLCN1</i> mutations), two with hypokalaemic periodic paralysis (HypoK-PP) (<i>CACNA1S</i> and <i>KCNJ2</i> mutations), and 5 with DM2, where aberrantly spliced RNA produces altered chloride channel protein.</p> <p>Results of the long and short exercise tests were normal or near to normal in 9 PAM (all Val445Leu) patients. A decrease of the compound muscle action potential (CMAP) late after completion of the long exercise and also after short exercise was noted in one of the two Ala699Thr patients but was normal in the other. Five of the 6 MC patients had autosomal dominant and one recessive form of the disease. In none of them, the results of tests differed significantly from normality. The HypoK-PP1 patient, tested when taking acetazolamide, had no abnormalities. In patient with Andersen-Tawil's syndrome, a progressive decline in CMAP amplitudes after long exercise was noted. In DM2 patients, we found minor deviations from normality with no specific pattern.</p> <p>Number of the examined patients is rather small, especially in the subgroups. The results were largely normal in PAM Val445Leu patients while one Ala699Thr patient a decline of muscle response after long exercise was noted. All MC patients, those with dominantly and recessively inherited disease forms, were normal on these tests. The Hypo-PP patient's CMAPs declined on long exercise. Specificity of the electromyographic exercise tests seems to be limited, but they may help in decisions for genetic analyses.</p>

PW37-459	<p><b><u>THE PRESYNAPTIC CALCIUM CHANNEL P/Q TYPE AS A POTENTIAL PARTNER FOR THE BASEMENT MEMBRANE PROTEIN PERLECAN.</u></b>  OERTEL J<sup>1</sup>, WATSCHINGER K<sup>2</sup>, FONTAINE B<sup>1</sup>, NICOLE S<sup>1</sup>  (1) Inserm U546, Paris, FRANCE. (2) Universität Innsbruck, Institut für Pharmazie, Abteilung für Pharmakologie und Toxikologie, Innsbruck, AUSTRIA.</p>
To contact the author:: oertel@chups.jussieu.fr.	<p>Hereditary skeletal muscle channelopathies are characterized by abnormal muscle excitability. They result from mutations in genes coding for skeletal muscle voltage-gated ion channels involved in the propagation of action potential and excitation-contraction coupling. Related diseases that clinically resemble these channelopathies include Schwartz-Jampel syndrome (SJS, <i>MIM</i> 255800), which is a rare autosomal recessive human disorder. It is characterized by muscle hyperactivity clinically related to <i>myotonia</i>. SJS is due to a lack of perlecan, the major heparan sulfate proteoglycan of basement membranes (BMs). The synaptic BM is rich in laminin isoforms that play a crucial role in the organization of neuromuscular junctions (NMJs). The C-terminus of the laminin beta2 subunit binds directly to the pore forming alpha1 subunit of the presynaptic P/Q type voltage-gated calcium channel (CaV2.1) by a leucine-arginine-glutamate (LRE)-motif. Two LRE-motifs are present in the human perlecan (aa 4149-4151 and 4299-4301), which is highly enriched at the BM of NMJs. This observation led us to study a possible interaction between the BM protein perlecan and CaV2.1 <i>in vivo</i> and <i>in vitro</i>. First, the SJS mouse mutant line was used to analyze the correlation between the CaV2.1 expression and the absence of perlecan. Immunostaining and confocal microscopy of dissected longitudinal and transversal muscle slices revealed an up-regulation of CaV2.1 at the NMJ in the SJS line compared to wildtype mice. Secondly, immunocytochemical experiments using the recombinant HEK293 expression system showed a selective colocalization at the outer membrane of endogenously expressed perlecan and the transfected alpha1 subunits of CaV2.1. These preliminary results suggest an interaction, direct or indirect, between the presynaptic calcium channel and perlecan. We currently investigate this issue further by biochemical analyses.</p>

PW37-460	<p><b>AUTOSOMAL RECESSIVE MYOTONIA CONGENITA : CLINICAL, ELECTROPHYSIOLOGICAL AND GENETIC STUDY IN 6 FAMILIES</b>  BIROUK N<sup>1</sup>, BELAÏDI H<sup>1</sup>, REGRAGUI W<sup>1</sup>, KABLY B<sup>1</sup>, URTIZBEREA A<sup>2</sup>, OUAZZANI R<sup>1</sup>  (1) Service de Neurophysiologie clinique, Hôpital des Spécialités, Rabat, MOROCCO.  (2) Institut de Myologie, Hôpital de la Salpêtrière, Paris, FRANCE.</p>
To contact the author:: birna@menara.ma.	<p>Myotonia congenita (MC) can be Autosomal recessive (Becker) or Autosomal dominant (Thomsen). These 2 forms are due to mutations in CLCN1 gene coding for chloride channel of muscle membrane. This is a study of the clinical and electrophysiological phenotypic features and genetic data in 6 Moroccan families. Twelve patients with Becker MC had clinical evaluation for age at onset, myotonia distribution, muscle hypertrophy and weakness and electrophysiological examination for the pattern of chloride channelopathy. Family investigation consisted of clinical and electrophysiological examination of at risk relatives. Molecular analysis was performed in one family. Age at onset ranged from 2 and 10 years. Myotonia was the predominant symptom that improved by exercise in all cases and worsened by cold in 5 patients. It was noticed in orbicularis oculi muscles in 5 cases. Eight patients had moderate weakness in limb girdle muscles. Muscle hypertrophy was a constant sign. All patients had myotonic discharges at needle EMG examination. The effort test and repetitive stimulation showed typical patterns in 4 cases. Autosomal recessive inheritance was confirmed by established consanguinity and normal clinical and electrophysiologic examination of the parents in all families. An homozygous mutation in the CLCN1 gene was demonstrated in one patient. Functional disability was moderate in all cases due mostly to myotonia which responded partially to symptomatic treatment. Becker MC has homogeneous phenotype. The clinical signs are explained by loss of function in the chloride channel responsible of muscle membrane hyperpolarisation.</p>

PW37-461	<p><b>INTRANUCLEAR LOCALIZATION OF PHOSPHO-B-DYSTROGLYCAN (PY892) AT RAT BRAIN</b></p> <p>RODRIGUEZ-MUÑOZ R<sup>1</sup>, MORNET D<sup>2</sup>, MARTÍNEZ-ROJAS D<sup>1</sup>  (1) CINVESTAV-IPN, Physiology, Biophysic and Neurosciences Department, México D. F., MEXICO. (2) Institut National de la Sante´ et de la Recherche Me´dicale, Equipe ERI 25, Muscle et Pathologies, Universite´ de Montpellier1, Unite´ de Formation et de Recherche de Me´decine, Montpellier, France, FRANCE.</p>
<p>To contact the author::  damartin@fisio.cinvestav.mx.</p>	<p>□-Dystroglycan (□-DG) is a transmembrane protein that links the extracellular matrix with the cytoskeleton. This protein is a Dystrophin-associated protein (DAP) that has an important role in cell signaling, and cytoplasm and nuclear organization (Fuentes-Mera, 2006). Recently, it has been reported that □-DG undergoes tyrosine phosphorylation in a cell adhesion-dependent manner. However, phosphorylated □-DG has not been detected in nuclei.</p> <p>In the present work, we studied the sub-nuclear localization of □-DG and its phosphorylated status at nuclei from cerebral cortex of rat. We found that the nuclear pattern of □-DG was different that in rat brain extract. The canonical □-DG band of 47kDa was detected in rat brain extract with anti-□-DG antibodies (NCL-43DG, G5 and JAF), but was undetectable with anti-phospho Y892 antibody that recognizes the □-DG C-terminal phosphorylated tyrosine 892 residue, indicating that it is not phosphorylated. The □-DG band was almost undetected in nuclei, nuclear matrix and nuclear membrane fractions. However, some protein bands with upward or downward mobility shifts (250, 190, 115, 65, 44, 41, 38 and 35 kDa) were detected in nuclear extract and nuclear matrix fraction. These bands were more immunoreactive in nuclear that in rat brain extracts. We hipothethized, that these nuclear proteins are post-transductional modified □-DG. With the anti-phospho Y892 antibody, we confirmed that □-DG bands expressed in nuclei are phosphorylated. The phospho-□-DG bands of upward mobility shifts may be poly-glycosilated isoforms.</p> <p>For the first time, we have demonstrated the enriched expression of phospho-□-DG isoforms at nuclei from cerebral cortex and nuclear matrix fraction. These results give new insights about the possible functions of phospho-□-DGs in the nuclear architecture and the anchorage of signaling proteins at nuclei.</p>

PW37-462	<p><b>EFFECTS OF CTGF OVEREXPRESSION ON MYOGENIC CELLS IN VITRO.</b>  AMBROSI I<sup>1</sup>, NOIREZ P<sup>1</sup>, MOULY V<sup>2</sup>, FISZMAN M<sup>1</sup>, KELLER A<sup>3</sup>, DUBOIS C<sup>4</sup>, ALAMEDDINE H<sup>1</sup>  (1) Inserm U582, Université Paris 6, Institut de Myologie, Paris, FRANCE. (2) Inserm U787, Université Paris 6, Institut de Myologie, Paris, FRANCE. (3) CNRS UMR 7149 – CRRET, Université Paris 12 – Val de Marne, Paris, FRANCE. (4) Inserm U515, Université Paris 6, Paris, FRANCE.</p>
<p>To contact the author::  h.alameddine@institut-myologie.org.</p>	<p>Fibrosis, characterized by excessive accumulation of extracellular matrix (ECM), is a hallmark of muscle biopsies in several muscular dystrophies such as Congenital (CMD) or Duchenne Muscular Dystrophies (DMD). Cell therapy trials have shown that increased connective tissue hindered the dispersion and incorporation of grafted cells into regenerating myofibers. Consequently, the success of this therapeutic strategy depends on the comprehension of molecular mechanisms leading to dysregulation of the balance between production and/or hydrolysis of ECM components.</p> <p>The involvement of Transforming Growth Factor beta (TGFβ) in fibrotic processes is well established. Other factors, among which Connective Tissue Growth Factor (CTGF), have equally been implicated. This protein, member of the CCN family (Cyr61/Ctgf/Nov), appears to be involved in the development of fibrosis in various pathological situations. It was shown to act as a downstream mediator of TGFβ but could also act through a TGFβ independent mechanism. In dystrophic muscles, the role played by CTGF in the fibrotic process is emerging and remains to be fully assessed.</p> <p>In this study, we have examined the consequences of exposing myogenic cells to CTGF. At first, we have investigated the production of endogenous CTGF by myogenic cells and its regulation during myogenesis <i>in vitro</i>. Then, we have overexpressed human-CTGF in myoblasts to explore its effect on cell proliferation and differentiation and compared them to control cells. Stably transfected clones were screened for h-CTGF expression by western blotting and quantitative real time Q-PCR has confirmed CTGF overexpression. The effect of CTGF on myoblast proliferation was quantified using the <i>Neutral Red</i> assay. Myogenic differentiation was evaluated by comparing the fusion capacity of clones overexpressing the protein versus their control clones. Data on CTGF expression, regulation and the functional consequences resulting from myoblasts exposure to CTGF will be presented.</p>

PW37-463	<p><b>NEUROMUSCULAR DISORDERS IN AVIATION MEDICINE</b>  MARTINEZ PEREA MDC<sup>1</sup>, LISTE H<sup>2</sup>, RUGGIERO M<sup>3</sup>, ANDRADA L<sup>4</sup>, CANAVERIS G<sup>5</sup>  (1) HOSPITAL AERONAUTICO CENTRAL.JEFE SERVICIO NEUROLOGIA INFANTIL, BUENOS AIRES, ARGENTINA. (2) HOSPITAL AERONAUTICO CENTRAL.NEUROLOGIA, BUENOS AIRES, ARGENTINA. (3) INSTITUTO DE MEDICINA AERONAUTICA Y ESPACIAL, BUENOS AIRES, ARGENTINA. (4) JEFE SERV. CLINICA MEDICA.INSTITUTO DE REHABILITACION PSICOFISICA, BUENOS AIRES, ARGENTINA. (5) INSTITUTO DE MEDICINA AERONAUTICA Y ESPACIAL, BUENOS AIRES, ARGENTINA.</p>
To contact the author:: mdeposadas@intramed.net.	<p><b>NEUROMUSCULAR DISORDERS in AVIATION MEDICINE</b>  Dres. *Martínez de Posadas, M. ;*Liste H, *** Ruggiero ,# Andrade L. Hospital Aeronáutico Central, * Neuropediatric, ** Neurology, *** Instituto de Medicina Aeronáutica y Espacial, #Instituto de Rehabilitación Psicofísica. Buenos Aires. Rca ARGENTINA.</p> <p><b>INTRODUCTION:</b> The physicians need to be aware of the NMD and physiological flight conditions. The primary difference between the aircraft and ground is the barometric pressure reduced. Although most healthy travelers can compensate for this hypoxemia, this may not be true for Neuromuscular Disorders (NMD).</p> <p><b>OBJECTIVES:</b> To present NMD in aviation, special jobs dispositions, and airline travel passenger's medical condition.</p> <p><b>Methods:</b> This paper reports three cases: 2 with DM (Myotonic Dystrophy), and 1 patient with DMD (Duchenne Muscular Dystrophy). Methodology was used, including the historical information, neurophysiologic studies, and molecular test.</p> <p><b>Case 1:</b> Male Patient, 19 years that enters in specific tasks for Security Force presenting myotonic stiffness of the hand with generalized myotonia in right arm, while presses trigger of gun. The molecular study confirms DM (more than 99 of CTG triplets' repeats).</p> <p><b>Case 2:</b> Male patient 36 years, private aircrew, in a routine annual flight examination show impaired in muscular relaxation.</p> <p><b>Case 3:</b> Male child, 10 years, presents DMD. His molecular test presents deletion Xp21 exon 48. He needs fly to Hospital for medical controls. His pulmonary function was normal. At 15 years he becomes wheelchair bound, cardiac muscle and pulmonary function are affected. He request permission to travel by air.</p> <p><b>Results</b> Some forms of NMD inevitably preclude military /security jobs aeromedical dispositions. <b>Patient 1:</b> had been transferred to other Department. <b>Patient 2:</b> After wide consultation wasn't returned to fly. <b>Patient 3,</b> at the first time he could airline travel; at 15 years, couldn't travel.</p> <p><b>Conclusions:</b> These cases offer some more generally applicable lessons in occupational, aviation medicine and Neuromuscular Disorders. Recognition of this disease during the early stages is the primary hurdle for the physician in screening to selective occupational jobs. The diagnosis has ramification for the patients and their offspring's futures and will necessitate genetic counseling.</p>

PW37-464	<p><b>PRACTICAL EDUCATION IN BIOLOGY FOR GENETIC DISEASE ASSOCIATIONS : A FRENCH ACTION UNIQUE IN EUROPE !</b>  MATHIEU M<sup>1</sup>, KARLIN D<sup>1</sup>, THIMONIER J<sup>1</sup>, LANGLET C<sup>1</sup>, HAMMOND C<sup>1</sup>  (1) Association Tous Chercheurs, Marseille, FRANCE.</p>
To contact the author:: mathieu@inmed.univ-mrs.fr.	<p>Rare diseases associations are increasingly interested and involved in research, from which they hope to gain better knowledge and prevention of their disease. Because the time scales of research and patients waiting for treatments intrinsically differ, it is essential to train members of disease associations with the various aspects of research, in order to contribute to a closer relationship between the scientific community and these associations.</p> <p>Accordingly, since 2004, the association <i>DNA school in Marseilles</i> (renamed <i>Tous Chercheurs</i> in 2007) has developed practical training in molecular biology and genetics for rare disease associations. These 3-day sessions, unique in Europe, take place in a laboratory where the trainees work "as" researchers, together with trainers who are experienced research fellows. They learn to observe, formulate hypotheses and carry out experiments. In addition to the practical work, every session includes discussions with researchers specialized in the pathology under the scope of training. The trainees thus understand concretely how researchers work and their constraints.</p> <p>Between 2004 and 2007, <i>Tous Chercheurs</i> formed more than 150 patients during 17 sessions, highlighting that such an approach is meeting a crucial need and desire from patient associations. At the same time, more and more associations want to benefit from these training sessions. To achieve this, and to make access easier for associations from all over France, it was essential to expand our action to a national scale. This was possible in 2007 thanks to the French-speaking Federation of <i>DNA Schools</i>, built around 6 members distributed throughout France, to whom we transferred our skills. Acting as initiator and catalyser of this type of associations training, we look forward to extend this successful story to a Europe-wide level.</p> <p>The development of this innovative action was made possible thanks to the support of the AFM, but also the Inserm and CNRS.</p>

