

**PW 18:  
Congenital myasthenias,  
Andersen syndrome and  
myasthenia gravis**

PW18-214	<p><b>CLINICAL FEATURES OF THE CONGENITAL MYASTHENIC SYNDROME ASSOCIATED WITH DOK7 MUTATIONS</b></p> <p>PETIT F<sup>1</sup>, BEN AMMAR A<sup>2</sup>, ALEXANDRI N<sup>1</sup>, GAUDON K<sup>3</sup>, RICHARD P<sup>3</sup>, ROUCHE A<sup>4</sup>, FOURNIER E<sup>5</sup>, BAUCHÉ S<sup>4</sup>, KOENIG J<sup>4</sup>, STOJKOVIC T<sup>1</sup>, ZAGNOLI F<sup>6</sup>, VIOLLET L<sup>7</sup>, PELLEGRINI N<sup>8</sup>, ORLIKOWSKI D<sup>8</sup>, PETIOT P<sup>9</sup>, STOLTENBURG G<sup>10</sup>, PATURNEAU-JOUAS M<sup>4</sup>, FARDEAU M<sup>10</sup>, HANTAÏ D<sup>4</sup>, EYMARD B<sup>1</sup></p> <p>(1) APHP, Centre de Référence en Pathologie Neuromusculaire Paris-Est, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (2) Institut National de Neurologie, Université Tunis El Manar, Tunis, TUNISIA. (3) APHP, UF Cardiogénétique et Myogénétique, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (4) Inserm U582, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (5) APHP, Service de Neurophysiologie, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (6) Service de Neurologie, Hôpital des Armées Clermont-Tonnerre, Brest, FRANCE. (7) Service de Pédiatrie, Réanimation Infantile, Hôpital Raymond Poincaré, Garches, FRANCE. (8) Service de Réanimation Médicale, Hôpital Raymond Poincaré, Garches, FRANCE. (9) Service de Neurologie, Hôpital de la Croix Rousse, Lyon, FRANCE. (10) Unité de Morphologie Neuromusculaire, Institut de Myologie, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE.</p>
To contact the author:: d.hantai@institut-myologie.org.	<p>Congenital myasthenic syndromes (CMS) differ from autoimmune myasthenia by an early onset of symptoms, a possible familial history, and the absence of autoimmunity. Among CMS, there is a large genetic heterogeneity. Ten genes have been described so far, coding for proteins implicated in the neuromuscular transmission. Mutations of <i>DOK7</i> have been recently described in recessive forms of CMS. This gene, localized to 4p16.2, is coding for a post-synaptic protein responsible for activating phosphorylation of MuSK.</p> <p>We report clinical and molecular data of 13 patients with CMS caused by <i>DOK7</i> mutations. The onset of symptoms varies from birth to early childhood. All patients display a characteristic “limb-girdle” pattern of weakness, with frequent ocular, facial, bulbar or respiratory involvement. The disease course is variable but often progressive, with fluctuations of symptoms, and may lead to loss of ambulation in adulthood. In electromyography, decrement without repetitive response is determinant for the diagnosis. Muscle biopsy shows type I fiber predominance and type II fiber atrophy. This pattern can be suggestive of CMS, although it is non-specific. In addition, we observed an increase of the lipid content of the fibers in 5/12 biopsies. Cholinesterase inhibitors are not efficient and may even worsen CMS caused by <i>DOK7</i> mutations. We identified several novel mutations in our patients. The frameshift mutation c.1124_1127dupTGCC is present in half of the alleles. <i>DOK7</i> is associated with a distinct phenotype of CMS, and appears to be among the most frequent genes mutated, with <i>CHRNE</i> and <i>RAPSN</i>.</p> <p><i>Supported by APHP, Inserm, ANR Maladies Rares, CMCU and AFM.</i></p>

PW18-215	<p><b><u>CONGENITAL MYASTHENIC SYNDROME WITH COLQ MUTATIONS: A LONG WAY TO DIAGNOSIS</u></b></p> <p>LARUE S<sup>1</sup>, BEHIN A<sup>1</sup>, LAFORET P<sup>1</sup>, STERNBERG D<sup>2</sup>, RICHARD P<sup>2</sup>, BEILLEVAIRE T<sup>3</sup>, HEZODE M<sup>3</sup>, RIGAL O<sup>4</sup>, GAUDON K<sup>2</sup>, CLAEYS K<sup>5</sup>, STOLTENBURG G<sup>5</sup>, HANTAI D<sup>6</sup>, EYMARD B<sup>1</sup></p> <p>(1) APHP, Centre de Reference en pathologie neuromusculaire, Institut de Myologie, GH Pitié-Salpêtrière, Paris, FRANCE. (2) APHP, UF cardiogénétique et myogénétique, GH Pitié-Salpêtrière, Paris, FRANCE. (3) APHP, Fédération de neurophysiologie, GH Pitié-Salpêtrière, Paris, FRANCE. (4) APHP, service de biochimie, Hôpital Robert Debré, Paris, FRANCE. (5) Unité de morphologie neuromusculaire, Institut de Myologie, GH Pitié-Salpêtrière, Paris, FRANCE. (6) Inserm U582, UPMC, Institut de Myologie, GH Pitié-Salpêtrière, Paris, FRANCE.</p>
To contact the author:: anthony.behin@psl.aphp.fr.	<p>Congenital myasthenic syndromes (CMS) are a group of inherited disorders in which neuromuscular transmission is impaired, with several possible clinical presentations. The diagnosis of CMS may be particularly difficult in patients with a myopathic pattern in which fluctuations may be overlooked or even absent.</p> <p>Herein, we report two sibs with AChE deficiency with a 16 years follow-up before CMS was suspected. A 30 year-old female achieved normal motor skills, but proximal muscle weakness and progressive postural scoliosis were noted after the second year of life. Facial strength and eye movements were spared. Muscle biopsy was performed when she was aged 12, showing a moderate lipidosis, leading to consider the diagnosis of metabolic myopathy. A thorough workup demonstrated no abnormalities, but L-carnitin and coenzyme Q10 prescription led to dramatic clinical improvement. However, the persistence of muscular weakness led to perform a second muscle biopsy displaying no lipidosis. EMG was thus performed, and special attention was paid to double CMAP in response to single stimulation of median nerve. Neuromuscular transmission study revealed a decremental response of 70%.</p> <p>Although less impaired, her brother aged 32, had presented the same clinical profile from childhood on. His muscular biopsy at age 23 had demonstrated a “non specific dystrophic pattern”. Whereas initial EMGs had shown myogenic patterns, a study of nerve-muscle transmission disclosed the same abnormalities as in her sister. Molecular genetic testing of COLQ gene revealed a missense R340H mutation and a splicing IVS2+1:G-&gt;C mutation.</p> <p>This report highlights the importance of a critical and repeated assessment in patients presenting with atypical metabolic or dystrophic features, and the utmost importance of performing a nerve-muscle conduction study in difficult or unsolved cases.</p>

PW18-216	<p><b>CHARACTERIZATION OF CONGENITAL MYASTHENIC SYNDROMES (CMS): THE EXPERIENCE OF THE FRENCH CMS NETWORK</b>  HANTAÏ D<sup>1</sup>, RICHARD P<sup>2</sup>, KOENIG J<sup>1</sup>, EYMARD B<sup>3</sup>  (1) Inserm U582, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (2) APHP, UF de Cardiogénétique et Myogénétique, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (3) APHP, Centre de référence en Pathologie Neuromusculaire Paris-Est, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE.</p>
To contact the author:: d.hantai@institut-myologie.org.	<p>and all members of the French CMS network.</p> <p>Congenital myasthenic syndromes (CMS) represent a heterogeneous group of diseases caused by genetic defects affecting neuromuscular transmission. CMS can present at any time from birth to adulthood, though usually within the first 2yr of life, and result in a spectrum of diseases ranging from wild weakness to severe disability with life-threatening episodes.</p> <p>The characterization of CMS comprises two complementary steps: establishing the diagnosis and identifying the pathophysiological type of CMS. A study oriented by the clinics, the EMG and the study of the neuromuscular junctions in muscle biopsies has identified several genes in which mutations cause the disease. New variants, especially in novel genes, have to be characterized by expressing them in cell culture systems or in rodent muscle to establish that they are indeed causing the CMS.</p> <p>Among the 210 index patients followed by the clinicians of the French CMS network, 98 have been traced to mutations in the genes coding for the collagenic tail of acetylcholinesterase <i>COLQ</i> (14); the acetylcholine receptor subunits <i>CHRNA1</i> (2), <i>CHRNB1</i> (1), <i>CHRND</i> (1) and <i>CHRNE</i> (45); rapsyn, <i>RAPSN</i> (19), the muscle-specific receptor tyrosine kinase MuSK, <i>MUSK</i> (3) and, recently identified, the MuSK-interacting protein dok-7, <i>DOK7</i> (13). In our experience mutations in the choline acetyltransferase <i>CHAT</i> or in the muscle sodium channel <i>SCN4A</i> were never identified.</p> <p>Some mutations in given CMS genes appear to be more frequent in some areas and this is the case for North Africa. Among the 25 Maghrebian index patients followed by our network, 12 were mutated in the founder <i>CHRNE</i>1293insG mutation. The knowledge of the ethnic background can be instrumental in diagnosing CMS patients rapidly.</p> <p>Despite comprehensive characterization, the phenotypic expression of one given gene involved is variable, and the aetiology of many CMS remains to be discovered.</p> <p><i>Supported by APHP, Inserm, ANR Maladies Rares, CMCU and AFM.</i></p>

PW18-217	<p><b><u>CONGENITAL MYASTHENIC SYNDROME DUE TO MUTATIONS IN THE MUSK GENE: CHARACTERIZATION OF A FACTOR FAVOURING AXONAL GROWTH</u></b></p> <p>BAUCHÉ S<sup>1</sup>, LIPECKA J<sup>1</sup>, VINH J<sup>2</sup>, DEMAY-THOMAS E<sup>2</sup>, BEN AMMAR A<sup>3</sup>, CHEVESSIER F<sup>4</sup>, FARAUT B<sup>1</sup>, WITZEMANN V<sup>4</sup>, ROSSIER J<sup>2</sup>, EYMARD B<sup>5</sup>, KOENIG J<sup>1</sup>, HANTAÍ D<sup>1</sup></p> <p>(1) Inserm U582, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (2) CNRS UMR 7637, ESPCI, Paris, FRANCE. (3) Institut National de Neurologie, Université Tunis El Manar, Tunis, TUNISIA. (4) Max Planck Institut, Heidelberg, GERMANY. (5) APHP, Centre de Référence en Pathologie Neuromusculaire, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE.</p>
To contact the author:: d.hantai@institut-myologie.org.	<p>Congenital myasthenic syndromes (CMS) are rare hereditary diseases characterized by a dysfunction of the neuromuscular transmission. Our group has identified <i>MUSK</i> mutations (one nonsense and one missense) in a CMS patient. Study of the patient's muscle biopsy shows <i>inter alia</i> a sprouting of the axonal endings. The over-expression of the missense mutation in mouse skeletal muscle causes an axonal outgrowth at the neuromuscular junction, similar to that observed in the patient.</p> <p>We established that the MuSK mutation expressed by cultured muscle cells was responsible for the release in their medium of a soluble growth factor increasing the axonal growth of cultured motoneurons. We used 2D gel electrophoresis followed by mass spectrometry for characterizing this factor. We identified, among thirty differentially expressed proteins, a fragment of a heparan sulfate proteoglycan, the perlecan. We have controlled the protein potency on axonal growth by carrying out blocking experiments using anti-perlecan antibodies.</p> <p>We proposed that this axonal growth might be favourable to the patient condition and had to be accompanied by the formation of new neuromuscular junctions. Indeed, we noticed an increased number of neuromuscular contacts both in nerve-muscle cocultures and in the KI mouse bearing the MuSK missense mutation. It is conceivable that the motoneurone, previously conditioned by perlecan or another muscle factor, could in turn release a factor, likely agrin, able to aggregate or increase the transcription of acetylcholine receptors.</p> <p>In summary, a mutation of a synaptic muscle protein leads to the discovery of a growth factor, perlecan or one of its fragments. This factor by inducing an axonal sprouting would induce the formation of new neuromuscular junctions in a CMS patient and compensate for the defective synaptic transmission.</p> <p><i>Supported by APHP, Inserm, ANR Maladies Rares, CMCU and AFM.</i></p>

PW18-218	<p><b><u>ACTIN CYTOSKELETON TRANSIENTLY ASSOCIATES WITH LIPID RAFT DURING AGRIN-ELICITED ACETYLCHOLINE RECEPTOR CLUSTERING</u></b>  PATO C<sup>1</sup>, DUTRAIT M<sup>1</sup>, RECOUVREUR M<sup>1</sup>, CARTAUD A<sup>1</sup>, STETZKOWSKI-MARDEN F<sup>1</sup>, CARTAUD J<sup>1</sup>  (1) Institut Jacques Monod, Paris, FRANCE.</p>
To contact the author:: cartaud@ijm.jussieu.fr.	<p>Liquid-ordered membrane microdomains or lipid rafts are involved in the clustering of nicotinic acetylcholine receptors at the neuromuscular junction (Stetzkowski-Marden et al., <i>J. Lipid Res.</i>, 2006). Upon activation of the Muscle-specific tyrosine kinase receptor MuSK by the neurotropic factor agrin, a complex cascade of signalling events involving protein tyrosine phosphorylation and Rho family GTPases reorganize the actin-based cytoskeleton, eventually leading to AChR clustering. Among strategies developed by cells to initiate and sustain response to extracellular signals, membrane-raft dynamics controlled by proteins that are linked to the actin cytoskeleton has recently been recognized. In this work, we have explored the possibility that AChR clustering results from an actin-based coalescence of rafts containing critical postsynaptic components. We show that upon agrin treatment of C2C12 myotubes, actin and several actin assembly factors Cdc42, N-WASP, ARP 2/3 and cortactin were transiently recruited to raft fractions after Ca<sup>++</sup>/Mg<sup>++</sup> isotonic buffer extraction. The recruitment of these proteins followed a complex mechanism: in the first few minutes upon agrin engagement, a dissociation of these assembly factors from rafts occurred whereas, their robust and transient recruitment to the raft fractions was later observed. Our data point out a model of raft-actin cytoskeleton dynamics in which uncoupling of rafts from the skeleton allows raft coalescence to proceed through actin repolymerization. New strategies to investigate <i>in situ</i> raft-actin dynamics during agrin signalling are currently developed.</p> <p>This work was supported by the CNRS, Universités Paris 6 &amp; 7 and by grants from ANR and AFM. C.P. was a recipient of a Post-doctoral ANR fellowship.</p>

PW18-219	<p><b><u>COLQ CONTROLS ACETYLCHOLINE RECEPTOR EXPRESSION AND MUSCLE DIFFERENTIATION</u></b>  SIGOILLOT S<sup>1</sup>, LAMBERGEON M<sup>1</sup>, BOURGEOIS F<sup>1</sup>, LEGER J<sup>2</sup>, LEGAY C<sup>1</sup>  (1) Inserm U686 Laboratoire de biologie des jonctions neuromusculaires normales et pathologiques, Paris, FRANCE. (2) Plateforme Ouest Genopole, Nantes, FRANCE.</p>
To contact the author:: severine.sigoillot@univ-paris5.fr.	<p>Congenital Myasthenic Syndromes (CMS) are inherited muscular disorders affecting neuromuscular transmission. Mutations in the acetylcholinesterase (AChE) collagen-like tail subunit gene (ColQ) underlie synaptic basal lamina associated congenital myasthenia with end-plate AChE deficiency (CMS-1C). Until now, most of the defects observed in patients with CMS due to mutations in ColQ have been interpreted in the light of the absence of AChE. However, considering currently known ColQ interactions in the basal lamina of the neuromuscular junction (NMJ) with MuSK (Muscle Specific Kinase) and Perlecan, we made the hypothesis that ColQ could control <i>per se</i> postsynaptic development and organization independently of AChE. MuSK is a signalling platform implicated in the transcription and accumulation of acetylcholine receptors (AChR). The heparan sulfate proteoglycan Perlecan binds to Dystroglycan (DG) known to be crucial for synapse differentiation and muscle integrity.</p> <p>To study ColQ effects on these two signalling pathways, a wt and a ColQ deficient (ColQ<sup>-/-</sup>) cell lines were compared using various techniques such as microarrays, classic RT-PCR and TaqMan Low Density Arrays (TLDA), western blotting and immunofluorescence. We show that the absence of ColQ leads to a disorganization of the extracellular matrix that could lead to defects in muscle cell differentiation as well as in synaptic protein expression. In particular, the absence of ColQ upregulates AChR expression and proteins involved in AChR aggregation but downregulates proteins involved in AChR cluster stabilization. Since ColQ controls AChR expression, it suggests that there is an adaptive mechanism linking AChE levels to those of AChR. In addition, pathological effects could be the results of DG low expression and low stimulation by Perlecan and Laminin, both of which are downregulated in ColQ<sup>-/-</sup> cells.</p> <p>Thus, CMS-1C would not be only due to the absence of AChE in basal lamina, but probably also linked to developmental disorders with maturation defects.</p>

PW18-220	<p><b><u>SOLEUS MUSCLE CHARACTERISTICS IN A MURINE MODEL OF CONGENITAL MYASTHENIC SYNDROME WITH END PLATE AChE DEFICIENCY</u></b></p> <p>VIGNAUD A<sup>1</sup>, MOUISEL E<sup>1</sup>, HOURDÉ C<sup>1</sup>, FOUGEROUSSE F<sup>3</sup>, BUTLER-BROWNE G<sup>1</sup>, BACOU F<sup>2</sup>, CHATONNET A<sup>2</sup>, FERRY A<sup>1</sup></p> <p>(1) Inserm U787, UPMC, Institut Myologie, paris, FRANCE. (2) inra, umr 866, montpellier, FRANCE. (3) genethon, RD, evry, FRANCE.</p>
To contact the author:: ferry@chups.jussieu.fr.	<p>Acetylcholinesterase (AChE) plays an essential role in neuromuscular transmission. Not surprisingly, neuromuscular transmission during repetitive nerve stimulation is severely depressed in the AChE knockout mice (KO). However, it was not known whether the deficit in AChE leads to changes in the skeletal muscle fibers. The in vitro contractile properties of postural and locomotor soleus muscles of adult KO and normal (wild type, WT) mice were studied and completed by histological and biochemical analyses. Our results show that muscle weight, cross-sectional area (CSA) of muscle fibres and absolute maximal isometric force were reduced in KO mice as compared to WT mice (<math>p &lt; 0.05</math>). Interestingly, the relative amount of slow myosin heavy chain (MHC-1) in muscle homogenates and the percentage of muscle fibres expressing MHC-1 were both decreased by AChE deficiency (<math>p &lt; 0.05</math>). Surprisingly, AChE ablation did not result in modifications in twitch kinetic, absolute maximal power, fatigue resistance and citrate synthase activity (<math>p &gt; 0.05</math>), despite the reduced number of slow muscle fibres. In conclusion a deficit in AChE leads to alterations in the structure and function of muscles, a substantial part of which are not simply related to the reduced body weight of the KO mice. Therefore, our results suggest that this murine model of congenital myasthenic syndrome with end plate AChE deficiency combines alterations in both neurotransmission and intrinsic muscle properties.</p>

PW18-221	<p><b><u>LOSS OF IK1 CURRENT IN MYOTUBES FROM ANDERSEN'S SYNDROME PATIENTS</u></b>  BENDAHOU S<sup>1</sup>, SACCONI S<sup>2</sup>, ARRIGHI N<sup>2</sup>, LARROQUE MM<sup>1</sup>, CHAPON F<sup>3</sup>, VICART S<sup>4</sup>, STERNBERG D<sup>4</sup>, FONTAINE B<sup>4</sup>, BARHANIN J<sup>1</sup>, DESNUELLE C<sup>2</sup>  (1) University of Nice Sophia Antipolis, IPMC, UMR 6097 CNRS, Valbonne, FRANCE. (2) CHU Pasteur of Nice - Centre de Référence Maladies Neuromusculaires et de Sclérose Latérale Amyotrophique - INSERM U 638 / IFR 50, Nice, FRANCE. (3) Service de Neurologie et Laboratoire de Neuropathologie, CHU de Caen, Caen, FRANCE. (4) Fédération de Neurologie, Groupe Hospitalier Pitié-Salpêtrière, and INSERM U546, Faculté de Médecine Pitié-Salpêtrière, Paris, FRANCE.</p>
To contact the author:: bendahhou@ipmc.cnrs.fr .	<p>Andersen's syndrome (AS) is a rare disorder that manifests with a triad: periodic paralysis, cardiac arrhythmia, and development anomalies. Muscle weakness is a feature of this disease that has been reported in two-thirds of the patients. The <i>KCNJ2</i> gene remains the only gene linked to AS, and encodes for the alpha subunit of the Kir2.1 potassium channel. Several studies have shown that AS mutations lead to a loss of function of the potassium channel activity <i>in vitro</i>. However, <i>in vivo</i> studies on isolated patient myotubes have not been reported.</p> <p>We have performed myotube cultures from muscle biopsies of controls and patients presenting with clinically and genetically defined AS disorder, and our cultures reached 95% of myoblast cells. No morphological difference was observed between AS and control myoblasts at each passage of the cell culture. Cellular proliferation and viability were quantified in parallel with direct cell counts and showed no difference between control and AS patients. It has also been suggested that the alteration in Kir2.1 function would result in impaired myoblast fusion and inhibition of myotube formation (J. Cell Biol. 153:F9-F12). Our data show no significant difference in myoblast fusion index among AS and control patients.</p> <p>Current recordings carried out on differentiated myoblasts from healthy individuals and from AS patients revealed the absence in AS myoblasts of the inwardly rectifying Ba<sup>2+</sup>-sensitive current. One consequence of the Ik1 loss in the AS myoblasts is a shift of the rest membrane potential towards depolarizing potentials. Our data strongly suggest a loss-of-function of the Kir2.1 channel <i>in ex vivo</i> in AS myoblasts carrying known AS mutations. Our findings describe for the first time the functional consequences of AS mutations <i>in ex vivo</i> differentiated myoblasts and provide the first clues to the periodic paralysis manifestations observed in AS patients.</p>

PW18-222	<p><b><u>MYASTHENIA GRAVIS: REASONS FOR UNSATISFACTORY OUTCOME REVEALED BY A PROSPECTIVE STUDY</u></b>  DUNAND M<sup>1</sup>, BORRUAT FX<sup>2</sup>, BOTEZ S<sup>1</sup>, ROUX-LOMBARD P<sup>3</sup>, KUNTZER T<sup>1</sup>  (1) Neurology Service, Unité Nerf-Muscle, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, SWITZERLAND. (2) Hôpital Ophtalmique Jules-Gonin, Unité de Neuro-Ophtalmologie, Lausanne, SWITZERLAND. (3) Immunology Service, Unité Immunologie Clinique, HU Genève, Genève, SWITZERLAND.</p>
To contact the author:: Murielle.Dunand@chuv.ch.	<p><b>Introduction:</b> in myasthenia gravis (MG), thanks to therapeutic advances, one should aim at a complete or near complete remission within a few months, allowing patients to return to habitual professional and social life. This stage has to be maintained and exacerbations prevented. In daily practice this can be hard to achieve. This difficulty to treat with success some myasthenic patients triggered this study.</p> <p><b>Objective:.</b> To determine the causes leading to unsatisfactory outcome in a group of recent MG patients.</p> <p><b>Methods:</b> We randomly included 41 MG patients registered in our neuromuscular database at the Muscle-Nerve Unit, University Hospital, Lausanne, Switzerland. Follow-up was performed either by the myologist which assessed diagnosis or by other neurologists which referred lately the patients to our Nerve-Muscle Unit. Outcome was rated at each consultation according to Myasthenia Gravis Foundation of America Postintervention Status. Complete Stable remission, Pharmacologic Remission, Minimal Manifestations and Improved Status were considered satisfactory outcome. Patients scoring Unchanged (U), Worse (W) or Exacerbated (E) during follow-up were taken into account. Reasons leading to unsatisfactory responsiveness were analysed by Fisher's exact tests.</p> <p><b>Results:</b> During follow-up, 54% of patients scored unsatisfactory, related to insufficient medication (36%), infectious diseases (23%) and no compliance (28%). Unsatisfactory outcome rate at the last visit was 19.5%. Unsatisfactory outcome during follow-up was significantly (P=0.004) associated to unsatisfactory outcome at the last visit. Care from the beginning by myologists lead to better outcome, with only 6.7 % of unsatisfactory outcome at the last visit versus 27% in the group followed-up initially by general neurologists.</p> <p><b>Conclusion:</b> This study points out that 54% of our MG patients had an unsatisfactory outcome during follow-up. Part (60%) of this unsatisfactory outcome could be prevented by tailoring adequate treatments during regular appointments by interested specialists in myology.</p>

PW18-223	<p><b>MAJOR ROLE OF THE CHEMOKINE CCL21 IN THYMIC HYPERPLASIA IN MYASTHENIA GRAVIS</b>  LE PANSE R<sup>1</sup>, CIZERON-CLAIRAC G<sup>1</sup>, RUHLMANN N<sup>1</sup>, BISMUTH J<sup>1</sup>, TRUFFAULT F<sup>1</sup>, BERRIH-AKNIN S<sup>1</sup>  (1) CNRS UMR 8162, Le Plessis-Robinson, FRANCE.</p>
To contact the author:: rozen.lepanse@u-psud.fr.	<p>Early-onset Myasthenia Gravis (MG) with anti-AChR antibodies is commonly associated with thymic hyperplasia, characterized by the presence of germinal centers (GC) containing B cells producing pathogenic antibodies. We demonstrate a specific and strikingly increased expression of the chemokine CCL21 in the hyperplastic thymus of MG patients. CCL21 is commonly considered as a chemokine dedicated to the recruitment of naïve T cells and sensitized dendritic cells necessary for lymphoid follicle development. By immunocytochemistry, we show that CCL21 over-expression was localized to specific endothelial vessels. We also observed, surrounding ectopic GC, the presence of numerous high endothelial venules (HEV) known to participate in T and B cell recruitment in secondary lymphoid organs. However, we showed that in hyperplastic thymuses, HEV were not responsible for CCL21 over-expression and the phenotype of the CCL21-positive endothelial cells is currently under investigation. The analysis of the chemotactic properties of CCL21 revealed that, at increasing concentrations, CCL21 was very powerful in recruiting B cells compared to the well known B-cell chemokine, CXCL13, whose expression is also increased in MG thymuses, stressing a novel property of CCL21. Altogether, these results point to a crucial role played by CCL21 in triggering thymic hyperplasia by recruiting both peripheral T and B cells.</p>

PW 18-224	<p><b>REGULATION OF CHEMOKINE PRODUCTION BY ESTROGENS. IMPLICATION FOR MYASTHENIA GRAVIS</b>  NANCY P<sup>1</sup>, KERLERO DE ROSBO N<sup>1</sup>, BERRIH-AKNIN S<sup>1</sup>  (1) CNRS UMR 8162, Hopital Marie Lannelongue, Le Plessis Robinson, FRANCE.</p>
To contact the author:: sonia.berrih-aknin@u-psud.fr.	<p>In myasthenia gravis (MG), there is a clear relationship between thymic pathology and gender, with thymic hyperplasia affecting essentially young female patients (9:1). We have demonstrated that the expression of alpha and beta estrogen receptors (ER) is significantly elevated in thymocyte populations of hyperplastic MG thymuses as compared to control thymuses, an observation which may be related to upregulation of ER expression by proinflammatory cytokines. To further investigate the contribution of estrogens in the formation of thymic GC, we analyzed their effect on the expression of molecules involved in the anti-acetylcholine receptor (AChR) response (MHC class II, AChR) and of chemokines implicated in germinal center (GC) development (CXCL13, CCL21, and CCL19). Stimulation of thymic epithelial cells (TEC), which express AChR and MHC proteins, in the presence of beta-17 estradiol resulted in profound decrease in HLA-DR at both mRNA and protein levels; decreased expression of AChR subunits was also observed, albeit not to the same extent. Real-time PCR analysis of CXCL13, CCL21, and CCL19 showed that beta-17 estradiol downregulates the expression of these three chemokines in TEC; however, little or no difference was observed at the protein level. Interestingly, the expression of certain cytokines, upregulated in a proinflammatory environment, was downregulated in the presence of beta-17 estradiol. Our results suggest that the balance between estrogens and proinflammatory cytokines could be of importance in thymus homeostasis and influence the progression of the autoimmune response in MG patients.</p>

PW18-225

**FUNCTIONAL AND PHENOTYPIC ANALYSIS OF REGULATORY T CELLS IN  
MYASTHENIA GRAVIS PATIENTS**

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Myasthenia Gravis (MG) is an autoimmune disease characterized by antibody-mediated dysfunction of the neuromuscular junction. Regulatory CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> T cells (Treg) are responsible for maintenance of the peripheral self-tolerance. Our previous study showed a severe functional defect of thymic Treg of MG patients, while their number, as evaluated by the CD25 marker, was normal.

The aim of the study was to investigate the causes of Treg functional defect.

- 1) We first investigated whether this defect was thymic-specific or could also be found in the periphery. Functional studies using peripheral Tregs confirmed that they display a functional defect in suppressive activity, similar to Tregs isolated from the thymus. These results emphasize that the defect is not only related to the activation status of MG thymuses, but is probably intrinsic.
- 2) Since the CD4<sup>+</sup>CD25<sup>+</sup> population includes both Treg (FoxP3-positive, IL-7alpha receptor-negative) and effector cells (FoxP3-negative, IL-7alpha receptor-positive), we wondered whether the functional defect in MG Tregs could be explained by the presence of effector cells in the CD4<sup>+</sup>CD25<sup>+</sup> population studied. Analysis of FoxP3 and of IL-7a receptor showed that the numbers of effector T cells were not increased in MG patients, either in the thymus or at the periphery.
- 3) FoxP3 is normally inducible by activation of CD4<sup>+</sup>CD25<sup>-</sup> cells. We observed that inducibility of FoxP3 in CD4<sup>+</sup>CD25<sup>-</sup> T cells is less efficient in MG patients than in control individuals, suggesting that the ability of CD4<sup>+</sup>CD25<sup>-</sup> to become CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg with suppressive activity may be affected. The low inducibility of FoxP3 in MG cells suggests a defect of FoxP3 regulation that could be related to the functional defect.

PW18-226	<p><b><u>ROLE OF MYOSIN VA IN THE MAINTENANCE OF THE VERTEBRATE NEUROMUSCULAR JUNCTION</u></b>  RODER IV<sup>1</sup>, PETERSEN Y<sup>1</sup>, RUDOLF R<sup>1</sup>  (1) Institute of Toxicology and Genetics, Forschungszentrum Karlsruhe, Karlsruhe, GERMANY.</p>
To contact the author:: ruediger.rudolf@itg.fzk.de.	<p>Myosin Va is known as molecular motor involved in vesicular transport and its functional absence leads to lethal conditions in humans (Griscelli and Elejade syndromes) and rodents (<i>dilute</i> phenotype). We thus examined the role of myosin Va in the maintenance of the neuromuscular junction (NMJ) by a series of <i>in vitro</i> and <i>in vivo</i> studies. First, co-expression of the NMJ marker, rapsyn-GFP, and a dominant negative myosin Va (dnMyoVa) in differentiating C2C12 cells led to massive clustering of rapsyn-GFP which was not observed in control cells. Second, in <i>dilute</i> mice we found a direct correlation between increasing postnatal propensity for seizures (leading to death at around P21) and NMJ fragmentation as well as reduction in NMJ size. Third, NMJs showed a similar amount of fragmentation and size reduction in live wildtype mice in which myosin Va function was locally deactivated in tibialis anterior muscle. Fourth, <i>in vivo</i> overexpression of rapsyn-GFP (intracellular) and labeling with <math>\alpha</math>-bungarotoxin (a marker for acetylcholine receptor exposed to the cell surface) showed partial co-localisation of both markers under control conditions. This pattern was altered in the absence of functional myosin Va, i.e. <math>\alpha</math>-bungarotoxin positive vesicular structures were strongly increased. Since, furthermore, most of these double positive vesicular structures were also labeled with a truncated myosin Va-YFP construct, this data suggests that myosin Va is important for the endocytosis or recycling of acetylcholine receptors in the NMJ. Finally, we show evidence that absence of functional myosin Va also leads to impaired muscle contractility. In summary, we found <i>in vitro</i> and <i>in vivo</i> evidence for an important role of myosin Va in the functional maintenance of the vertebrate NMJ.</p>

PW18-227	<p><b><u>ROLE OF COMPARTMENTATION OF PKA SIGNALLING FOR THE MAINTENANCE OF THE NEUROMUSCULAR JUNCTION</u></b>  RODER I<sup>1</sup>, LISSANDRON V<sup>2</sup>, MARTIN J<sup>1</sup>, CHOI K<sup>1</sup>, ZACCOLO M<sup>2</sup>, RUDOLF R<sup>1</sup>  (1) Institute of Toxicology and Genetics, Forschungszentrum Karlsruhe, Karlsruhe, GERMANY. (2) Venetian Institute of Molecular Medicine, Padova, ITALY.</p>
<p>To contact the author::  ruediger.rudolf@itg.fzk.de.</p>	<p>Fragmentation of the neuromuscular junction (NMJ) and a dramatically shortened halflife of the acetylcholine receptor are hallmarks of dystrophic mdx muscle. Since cyclic AMP (cAMP) mediated signalling is thought to be important for aspects of NMJ maintenance we investigated the role of protein kinase A (PKA) in the muscle postsynapse. In particular, we studied the function of A kinase anchoring protein (AKAP)-mediated PKA microdomain organisation for the integrity of the NMJ. Using GFP-based cAMP sensors targeted to the microdomains of the different PKA regulatory subunit isoforms we found the RI<math>\alpha</math>-sensor to be localised in an AKAP-specific manner in the NMJ of wildtype mice. Conversely, in dystrophic mdx mice, the RI<math>\alpha</math>-sensor was mostly displaced from the NMJ. Measurement of cAMP-levels in the different regulatory subunit microdomains <i>in vivo</i> revealed AKAP-dependent microdomain specific responses to different agonists and a strongly altered cAMP handling in dystrophic versus wildtype muscles. Expression of disruptor peptides in wildtype muscles completely abolished the AKAP-dependent localisation of the RI<math>\alpha</math>-sensor in the NMJ and led to a fragmentation of the NMJs similar to that in dystrophic mdx muscles. In summary, our results suggest an important role of AKAP-mediated PKA microdomain organisation for the maintenance of structural integrity of the NMJ.</p>