mdx, dilated cardiomyopathy, cation influx, calcium regulation, TRP channels, TRPV2

P05- Congenital muscular dystrophies / Dystroglycanopathies- N° 66 to N° 75

Congenital muscular dystrophies (other than dystroglycano- #2421

P05- 66- Investigation of calcium current properties and leak conductance in mouse muscle fibers overexpressing a type 1 Hypokalemic Periodic Paralysis mutant L-type calcium channel suggests a role of acidification in attacks of paralysis

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Missense mutations in the gene encoding the alpha1 subunit of the L-type calcium channel CaV1.1 induce type 1 Hypokalemic Periodic Paralysis (HypoPP1). These mutations mainly occur at arginine residues in the fourth transmembrane segment of voltage-sensor domains. Very few studies have investigated the acute effects of these mutations on channel function and muscle membrane electrical properties because of the difficulty to express Cav1.1 in heterologous systems. In the present study we successfully transferred by electroporation the genes encoding the turboGFP-tagged human wildtype (WT) and R1239H HypoPP1 mutant Cav1.1 into hind limb mouse muscles. The expression profile of the two channels showed a regular striated pattern indicative of the localization of the channels in the t-tubule membrane. Measurement of the L-type current using the silicone-clamp technique showed that the maximal conductance and the voltage-dependence of the Cav1.1 channel were significantly reduced and shifted towards negative potentials respectively in fibers expressing R1239H Cav1.1 as compared to fibers expressing WT Cav1.1. Applying voltage ramps from a holding potential of 0, -20, -40 or -60 mV to -120 mV in the presence of an external low-chloride, sodium-free and potassium-free solution revealed a significant higher leak conductance measured between -80 and -120 mV in fibers expressing R1239H Cav1.1. Acidification of the external solution significantly increased the leak inward current, leak conductance and the fluorescence of an internally loaded pH indicator in fibers expressing R1239H. These data suggest that an elevated leak inward current, likely carrying protons, flows at resting membrane potentials in fibers expressing R1239H Cav1.1 and that muscle acidification could contribute to favor the onset of muscle paralysis in HypoPP1.

calcium channel, periodic paralysis, cell electrophysiology

Congenital muscular dystrophies (other than dystroglycano- #2589

P05- 67- YAP mediated mechanosensing defects of LMNA mutant myoblasts also affect cell-cell contacts

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The mechanisms underlying the cellular response to mechanical forces are critical for muscle development and functionality. The LINC (LInker of the Nucleoskeleton and Cytoskeleton) complex, enables transmission of forces between the nucleus and the extracellular matrix via the actin cytoskeleton. Mutations in LINC-complex associated proteins, including lamins cause human muscular dystrophies but the molecular mechanisms still remain to be elucidated. Recently, mechanosensing defects have been reported in patient-derived immortalized myoblasts carrying mutations in A-type Lamins (Bertrand et al., 2014). These mutant myoblasts were shown not to adapt their cytoskeletal organisation to the stiffness of their environment. Also MKL-1 and Yes-Associated Protein (YAP) signaling, two important mechanotransducers, were shown to be misregulated on low stiffness substrates in mutant myoblasts. In this study, we hypothesized that mechanosensing defects in LMNA mutant myoblasts also affect cell-cell contact through deregulation of YAp. Cell-cell contact induced the cytoplasmic relocalization of YAP in WT but not in LMNA myoblasts. Further investigations revealed no defects in the HIPPO pathway signaling upstream of YAp. YAP expression and activity but also the LATS mediated phosphorylation of YAP at serine 127 were increased in LMNA mutant myoblasts. In conclusion, our results indicate that the mechanical regulation of YAP as sensor and mediator of mechanical inputs from cell-cell interaction is impaired in LMNA mutant myoblasts, presumably through a disturbed actin-dependent regulation of YAp.

mechanotransduction, yap, hippo, LaminA/C

Congenital muscular dystrophies (other than dystroglycano- #2594

P05- 68- Inactivation of Myostatin: a potential therapeutic tool against Autosomal Dominant Centronuclear Myopathy.

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Context: The unique mouse model for autosomal dominant centronuclear myopathy (KI-Dnm2R465W/+), associated to mutations of dynamin 2 gene (Dnm2) reproduce some of the clinical features reported in human, notably muscle atrophy and weakness. Myostatin (Mstn), a member of TGF? family, is a master negative regulator of skeletal muscle mass. We hypothesized that inactivation of Mstn could limit muscle atrophy and weakness reported in the KI mouse model. To test this hypothesis, we intercrossed KI micewith mice inactivated for Mstn (KO-Mstn) to generate a double mutated lineage (KIKO mice).

Results: Animals were followed over a 12 months period. Muscle force (grip strength test) and motricity (rotator test) were significantly reduced in 1-month old KI mice. A significant loss of muscle mass and volume (microRMI) were observed in KI from 2 months of age. The analysis of tibialis anterior muscle mass was correlated with the decrease of muscle volume determined by microMRI (r=0.9). From 2 to 12 months, all these parameters remained below control values.

When compared to KI mice, KIKO mice presented a 30% and 50% increase of muscle grip strength at 1 and 12 months. Furthermore, the time spent on rotarod was increased by 20% and 50% at 1 and 12 months. In agreement with these data, muscle mass was increased by 45% at 1 and 12 months and the volume by 32% to 42% at 1 and 12 months.

Molecular analyzes showed that inactivation of Mstn allowed for an increase of total and phosphorylated forms of several proteins involved in the IGF1/Akt/mTOR pathway, together with a down regulation of several factors involved in ubiquitin-proteasome and autophagy-lysosome proteolytic pathways. Overall, we demonstrated that inactivation of Mstn improves muscle mass and function of KI mice. An activation of muscle protein synthesis and a reduction in muscle proteolysis probably account for this beneficial effect.

Conclusion:These results are very promising since genetic inactivation of Mstn showed a real benefit for AD-CNM mice by restoring the strength, the motricity and by blocking the loss of muscle mass. The perspective to this work is to evaluate the efficiency of an anti-Mstn based pharmacological approach to restore muscle function after the establishment of the disease.

Centronuclear myopathy , Dynamin , Myostatin , Animals models , Therapy

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Congenital muscular dystrophies (other than dystroglycano- #2939

P05- 69- Increased muscle water T2 in congenital myopathy: the example of KI-Dnm2R465W, a murine model for autosomal dominant centronuclear myopathy

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Centronuclear myopathies are non-dystrophic congenital myopathies characterized by the presence of a high proportion of muscle fibers with internalized or centralized nuclei, without extensive muscle degeneration, regeneration, inflammation or fibrosis. The knock-in KI-Dnm2R465W mouse (Dnm2) models the autosomal dominant contronuclear myopathy with the most frequent mutation in the DNM2 gene in patients. Differently from human, this model presents mild strength reduction and slight atrophy, with very few histological alterations. The objective of this study was to non-invasively characterize Dnm2 mice with Nuclear Magnetic Resonance imaging (NMR), in comparison to histological findings. For NMR, 16 Dnm2 and13 wild-type (WT) mice were evaluated (3-6 months of age), while 4 Dnm2 and 2 WT mice were evaluated histologically. The NMR study included anatomical evaluation and muscle T1 and T2 measurements, performed in a 4T magnet under isoflurane anesthesia. Despite similar body weight, atrophy could be detected in Dnm2 mice by comparing the cross-section area (CSA) of the caudal limb (Weight: Dnm2 = 30.5±4.31 g, WT = 31.6±2.35 g, p=0.66; CSA: Dnm2 = 47.7±3.6 mm2, WT = 54.8±3.5 mm2, p>0.001). No differences were observed for muscle T1 (T1: Dnm2 = 1.37±0.06 s, WT = 1.37±0.05 s, p=0.72), but muscle water T2 was increased in Dnm2 mice (T2: Dnm2 = 31.7±1.2 ms, WT = 30.3±1.5 ms, p>0.001). Even if heterozygous Dnm2 mice have a mild phenotype, this non-invasive NMR study could identify muscle atrophy and increased muscle T2. Increased muscle T2 is considered an index of disease activity in different muscle pathologies, such as muscular dystrophies and inflammatory myopathies. Nevertheless, both mice and patients with dynamin-2 related centronuclear myopathy present a stable or slowly progressive disease manifestation. We hypothesize that the increased muscle T2 in Dnm2 mice can be related to altered intracellular organization, since the histological analysis didn't show pathological alterations usually related to increased muscle T2, such as necrosis and inflammation. These results indicate that NMR, especially T2 relaxometry, is sensitive enough to identify alterations in a pre-clinical stage in a murine model for autosomal dominant centronuclear myopathy, the heterozygous KI-Dnm2R465Wmouse. Nevertheless, these results also indicate that increased muscle T2 can be observed in a stable pathological process.

NMR, T2, congenital myopathy, dynamin-2, murine model

Congenital muscular dystrophies (other than dystroglycano- #3001

P05- 70- Whole Exome Sequencing identified mutations in the MSTO1 gene in a family with congenital muscular dystrophy and mitochondrial dysfunction.

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We studied a family with two sisters presenting a clinical phenotype of congenital muscular dystrophy with neurodevelopmental delay, hyperCKemia, failure to thrive and cerebellar ataxia. Muscle biopsy in the older sister showed both myopathic and neurogenic changes, and respiratory chain complexes activity was normal. In order to find the genetic basis of this condition, we performed Whole Exome Sequencing (WES) on genomic DNA from the entire family-of-four as part of the European Union funded project Neuromics.
Among 6,451 rare variants (MAF ? 0.1% or not reported) found in both affected sisters, we found 943 nonsense, missense, frameshift or splice site variants. Filtering assuming a recessive inheritance model, identified compound heterozygous variants (c.1033C>T p.R345C and c.1128C>A p.F376L) in the MSTO1 gene. MSTO1 is located in the outer membrane of the mitochondrion, and is involved in the regulation of mitochondrial distribution and morphology. While MSTO1 mutations have never been reported before, we considered this a strong functional candidate for the observed phenotype. Additional studies

showed that the mitochondrial network is clearly fragmented in the older patient's fibroblasts, supporting the view of mitochondrial dysfunction in this disease.
The RT-PCR analysis on patient's showed a strong reduction of MSTO1 transcript expression, and Western blotting analysis revealed protein absence in patient's fibroblasts, confirming the pathogenicity of the identified mutations. These results suggest that MSTO1 might be the causative gene in our patients. Identification of further patients with MSTO1 mutations will provide additional information on the role of this new gene in neuromuscular disorders.
The EU Neuromics grant (2012-305121, www.neuromics.eu) is acknolwedged

MSTO1, WES, congenital muscular dystrophy, mitochondrial dysfunction

Congenital muscular dystrophies (other than dystroglycano- #3031

P05- 71- Laminin-alpha2 congenital muscular dystrophy: lama2-deficiency leads to decreased muscle mass and activation of fetal muscle progenitors during development

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Laminin-alpha2 congenital muscular dystrophy(LAMA2-CMD), also called Merosin deficient congenital muscular dystrophy type 1A (MDC1A), is a fatal disease caused by mutations in LAMA2, the gene that encodes laminin alpha2 chain present in laminins 211 and 221, major constituents of the basement membrane around skeletal muscle fibers. Efforts to treat this disease have focused on alleviating symptoms post-natally. However, crippling muscle weakness is evident already from birth, so studies about the development of the disease in utero are essential. Skeletal muscle development, or myogenesis, is a gradual and complex process which starts early in embryogenesis and goes on until after birth. Here, we evaluated the role of Lama2-deficiency during muscle development in the mouse embryo to pinpoint disease onset in utero.

We first undertook a detailed analysis of laminin isoform distribution during epaxial myogenesis in normal mouse embryos. We found that laminin alpha2 deposition correlates with myotome development and the onset of secondary myogenesis, while most of primary myogenesis occurs in the absence of assembled laminins. In LAMA2dyW-/- embryos, a model for LAMA2-CMD, skeletal muscle development is already impaired at E10.5, resulting in smaller myotomes than in controls. This results in the formation of smaller muscle masses during primary and secondary myogenesis. Strikingly, we found that during secondary myogenesis, LAMA2dyW-/- fetuses also have a reduced number of Pax7-positive muscle progenitor cells, while the number of Myogenin-positive cells is increased, indicating an overactivation of myogenic differentiation. While TUNEL assay shows no significant apoptosis and phospho-histone 3 staining reveals no difference in proliferation levels, RT-qPCR analysis revealed that JAK STAT signaling is activated in LAMA2dyW-/- fetuses. Moreover, Sure Fire analysis revealed that activation of JAK STAT signaling in LAMA2dyW-/- fetuses occurs through STAT3 phosphorylation. These results suggest this depletion correlates with the activation of the JAK STAT signaling pathway.

We propose that the lack of laminins 211/221 leads to reduced muscle mass, visible already in the myotome, and to increased differentiation of muscle progenitors at fetal stages, and that these defects constitute the first signs of the disease.

Laminin-alpha2 congenital muscular dystrophy, Embryonic development, Muscle progenitors, JAK STAT signaling pathway

Dystroglycanopathies- #2473

P05- 72- Fkrp rescue in blood-derived cd133+ cells isolated from patients affected by congenital muscular dystrophies

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Dystroglycanopathies are a group of heterogeneous muscular dystrophies caused by mutations occurring in 6 genes (POMT1, POMT2, POMGnT1, FKRP, Fukutin and LARGE) involved in the glycosylation of ?-dystroglycan (?-DG). Actually there are no effective therapies for these disorders. One proposed approach could be the development of a combined gene and cell therapy. For this purpose, we selected patients affected by mutations in the coding sequence of fukutin related protein gene (FKRP). We firstly isolated blood-derived CD133+ cells from healthy and dystrophic patients and demonstrated their self-renewal capacity. HEK cells were transduced with a lentiviral vector coding for the FKRP gene (pLenti-CAG(FKRP)-Rsv(GFP-puro). A pLenti-CAG-Rsv(GFP-puro) was used as control. To evaluate the lentiviral infectious efficiency, we transduced HEK cells with three different increasing MOI: 1, 5, 10; HEK cells showed a high viability after lentiviral infection (more than 80%). The efficiency of transduction increased from 55% (MOI 1), to 88% (MOI 5) until a maximum of 92% (MOI 10). We thus selected MOI 10 for all future experiments performed on blood-derived CD133+ cells. Green fluorescent protein (GFP) expression was used to define the infectious efficacy by cytofluorimetric analysis. Transduced cells maintained proliferation capacity and > 90% vitality. q-RT-PCR and WB analysis confirmed the expression of FKRP in engineered CD133+ cells isolated from dystrophic patients. Engineered human blood-derived CD133+ cells were induced to differentiate in myotubes when co-cultured in the presence of a feeder layer of mouse myogenic cells. Q-RT-PCR and WB analysis confirmed the expression of early myogenic markers (PAX7, MYF5, M-cadherin and MRF4).
Data demonstrate that blood-derived CD133+ cells can be easily isolated with no invasive procedure from FKRP mutated patients and manipulated in vitro. Therefore genetically FKRP engineered cells can be considered as a tool for future investigations in dystroglycanopathies

Dystroglycanopathies, Fkrp, cd133+ stem cells, gene therapy

Dystroglycanopathies- #2546

P05- 73- Mild Phenotype of congenital muscular dystrophy secondary to GTDC2 mutation

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Abnormal alpha-dystroglycan (alpha-DG) glycosilation is associated with congenital muscle disorders (CMD), characterized by early onset muscle weakness, variably severe central nervous system and eye abnormalities, and dystrophic changes in muscle biopsy.

We present the case of a patient with a mild phenotype of CMD secondary to a mutation in GTDC2: the proband is a 35-year-old female, first child of healthy non-consanguineous french parents. After a mildly delayed motor development (the patient was able to walk by the age of 18 months, but she was never able to run), she developed a gait disorder at the age of 3 years. The patient showed progressive muscle weakness and she was wheelchair bound by the age of 14 years. Intellectual functions were normal; she was able to finish university studies. At the age of 25 years, she was diagnosed of partial complex epilepsy and treated with levotiracetam and oxcarbazepine. EEG showed generalized intercritical epileptic activity.

Neurological examination showed normal intelligence; proximal weakness, scapular winging; rigid spine; scoliosis; elbow and ankle contractures. CPK was elevated (2500 UI). Muscle biopsy showed dystrophic changes. Immuno-histochemistry studies were normal for the usual membrane proteins, but alpha-DG was slightly reduced. Alpha-DG was absent in Western blot. Cerebral MRI was normal.

Mutation screening was performed in FKRP, POMGnT1, POMT1, POMT2, LARGE and FUKUTIN, ISPD, SGK196 and TMEM5 genes without detecting any pathogenic alterations. Finally, two heterozygous variants (c.1471C>T and c.1334G>A) were detected in glycosyltransferase-like domain-containing 2 gene (GTDC2 gene).

GTDC2 gene mutations have been recently involved in Walker Warburg syndrome (WWS). As for the other genes involved in WWS, GTDC2 gene is implicated in the glycosilation of the transmembrane protein dystroglycan. Interaction with extracellular matrix components such as laminin, requires alpha-DG to be glycosylated. This case report expands the phenotype of alpha-dystroglycanopathies: mutations in the GTDC2 gene may be associated with milder phenotypes, without cerebral or ocular malformations

CMD, GTDC2

Dystroglycanopathies- #2556

P05- 74- NGS: a new strategy for molecular diagnosis of alpha-dystroglycanopathies.

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Alpha-Dystroglycanopathies (a-DGpathies) are a group of rare inherited neuromuscular disorders characterized by modified glycosylation of alpha-dystroglycan. Up to now mutations in 16 genes mostly with direct link to glycosylation pathway have been identified in patients with a-DGpathies. Due to an extremely broad clinical spectrum (from type II lissencephaly-LIS II, to Congenital Muscular Dystrophy-CMD and Limb Girdle Muscular Dystrophy-LGMD) and relatively poor phenotype-genotype correlation, molecular diagnosis of a-DGpathies is difficult and requires searching for mutations gene by gene. Therefore, a-DGpathy is a good candidate for NGS strategy based on a glycosylation gene panel that we applied designing a panel comprising the 16 known genes in a-DGpathies and 29 other genes involved in glycosylation and Congenital Disorders of Glycosylation (CDG).
We first validated our panel design by assessing DNA from patients with known mutations. Then, DNA from 23 new cases were tested, leading to the identification of mutations in 8 different genes. All mutations were confirmed by Sanger sequencing.

Out of the 23 cases, a molecular diagnosis was established for 8/11 LIS II fetuses (72%) and 4/12 patients with DMC or LGMD (33%) with identified mutations in one of the 16 a-DGpathies known genes. In another patient presented with LGMD, a heterozygous ins-del mutation was identified in SLC35A2 (Chr X) encoding for UDP-galactose transporter which is usually associated with another type of glycosylation linked to CDG. Complementary studies to confirm link between mutation and pathology are in progress.

Considering the possibility of an inadequate coverage of a few regions in the remaining undiagnosed cases, the 16 a-DGpathies genes were analyzed by Sanger sequencing without identifying any mutations. Therefore, the efficacy of mutation identification was comparable between NGS and Sanger sequencing. One limit of our NGS panel is the low coverage of the FKRP mutation:

c.826C>A, p.Leu276Ile. So, for LGMD patients without identified FKRP mutation, Sanger sequencing of this region should be systematically performed.

Thanks to this new technology, assessing in one single step all the genes linked to a-DGpathies leads to a gain of several months in the establishment of molecular diagnosis which benefits the families waiting for prenatal diagnosis. In addition, the design of our panel allows screening in parallel other glycosylation genes potentially involved in a-DGpathies.

alpha-DGpathy Dystroglycanopathies LIS II POMT1 Glycosylation NGS

Dystroglycanopathies- #3273

P05- 75- The pathophysiological consequences of different levels of dystrophin following antisense based exon-skipping in two muscles of the mdx mouse

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We examined the effects on muscle physiology and pathology of restoring different levels of dystrophin acutely and chronically in mdx mice with established dystrophic pathophysiology (12 weeks and 24 weeks old). Dystrophin expression was induced efficiently using cell penetrating peptides linked to an antisense sequence targeting exon 23 which contains a premature stop mutation in the mdx mouse. We assessed muscle physiology in the tibialis anterior (TA) muscle of the mouse using an in situ protocol under terminal anaesthesia. To assess muscle physiology in the diaphragm we used strips of diaphragm in an in-vitro system. In both cases we examined the force-frequency relationship and established maximum specific tetanic force. We then subjected the muscles to a 10% stretch while stimulating them to contract for 10 cycles. This eccentric exercise was highly damaging to the TA muscle but not the diaphragm. We present data showing that 15% of normal levels of dystrophin was sufficient to prevent eccentric exercise induced damage to the TA following various dosing regimens. Unlike the TA, both acute and chronic administration of PPMO significantly increased specific force in the diaphragm while a similar effect was only seen in the TA when treated chronically with the higher dose of PPMO from 12 weeks old. We present a pathological analysis to explain the differences between the muscles and further show differences in the fibre-type response to treatment. While caution must be applied when extrapolating these results to DMD patients, the results suggest that moderate levels of dystrophin may be sufficient to slow-down or possibly prevent disease progression whereas higher levels of dystrophin will also improve muscle force production but this will depend on the age of treatment and the specific muscle.

dystrophin, mdx, antisense, DMD, dystrophic, diaphragm, tibialis anterior, physiology, muscle

P06- Congenital myopathies- N° 76 to N° 93

Congenital myopathies- #2355

P06- 76- Mechanosensing defects in muscular dystrophies related to nuclear envelope mutations

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Recent data indicate that mutations in nuclear envelope genes cause both defects in mechanotransduction signaling and force transmission across the nuclear envelop. A central role in this mechanosensory process has been attributed to A-type lamins, which together with the LInker of the Nucleoskeleton and Cytoskeleton (LINC)-complex enables force transmission across the nuclear envelop. Whereas a basic picture is that extracellular mechanical forces are transmitted from outside the cell to the nucleus, we hypothesize that mutations in A-type lamin (LMNA) or in nesprin can affect the mechanical transmission from the nuclear envelop to the extracellular matrix. To test this hypothesis, human myoblasts with either LMNA or nesprin-1 mutations were cultured on soft substrates (12 kPa) and compared to control (WT) myoblasts. On soft surface, LMNA and nesprin-1 mutated myoblasts exhibited enlarged and increased number of focal adhesions, increased stress fibers and enlarged cell spreading area compared with WT. Further, nesprin-1 mutant exerted significantly higher traction forces on the substrate compared with WT myoblasts. These abnormalities were greatly reduced after treatment with Y27632, or SU6656, which inhibit Rho-associated protein kinase or the Src family kinase respectively, suggesting an abnormal activation of Rho-dependent Src pathway in mutant cells. Treatment with the MLCK inhibitor ML7 also significantly reduced the spreading area in mutant cells but without modifying the number and distribution of perinuclear actin stress fibers. We concluded that the integrity of the LINC complex and the lamina is required for proper regulation of the cytoplasmic actin contractility in soft substrates, through an apparent Src-dependent ROCK pathway.

Congenital muscular dystrophy, Mechanotransduction, LINC complex

Congenital myopathies- #2441

P06- 77- X-linked centronuclear myopathy: insights into myotubularin MTM1 function from yeast.

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X-linked centronuclear myopathy (XLCNM) is a muscle disorder characterized by neonatal hypotonia and abnormal organelle positioning in skeletal muscle. This myopathy is due to different mutations in the MTM1 gene encoding the phosphoinositide