Stem cells therapies- #3246

**P25- 386- Induction of mouse embryonic stem cells differentiation using Mimic miRNAs- functional analysis in co-culture with the C2C12 myoblasts**

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Proper functioning of skeletal muscles requires precise spatiotemporal control of growth, development, and tissue regeneration. All of these processes are controlled by miRNAs (miRNAs), i.e. small, single-stranded RNA molecules either initiating degradation of mRNAs or inhibiting target gene expression. Recent research demonstrated which miRNAs, both muscle-specific (myo-miRNA) or expressed in other tissues, take part in the regulation of myogenic differentiation and muscle development. In vitro experiments conducted by us aimed to determine whether the increase in the levels of selected miRNAs impacts myogenic differentiation of pluripotent mouse embryonic stem cells (ESCs). First, we determined fusion index of C2C12 myoblasts co-cultured with ESCs that were transfected with miRNA Mimics. Next, we estimated the ability of such ESCs to create hybrid myotubes in co-culture with the C2C12 myoblasts. Finally, we analysed the expression level of transcription factors and miRNAs that secure pluripotency and those associated with myogenic differentiation. Over-expression of miRNA, such as miR-1, miR-133a/b, and miR-145 enhanced the ESCs ability to fuse with myoblasts. miR-1 increased the proportion of fusion with C2C12 myoblasts and hybrid myotubes. Transfection of ESCs with miR-145 increased the levels of miR-133a as well as miR-133b and miR-1 increased the level of let-7. It did not impact, however, at the expression of mRNAs encoding myogenic markers. On the other hand, transfection of ESCs with miR-133b or miR-145 induced the expression of genes involved in the mesoderm formation and myogenic differentiation, such as Pax3 and Kdr. Thus, by using transient expression of selected miRNA one can induce myogenic differentiation of mouse ESCs in vitro.

ES cells, skeletal muscles, myoblasts, cell differentiation, miRNA

Stem cells therapies- #3249

**P25- 387- Directed Embryonic stem cells differentiation- the use of mesodermal and myogenic miRNAs.**

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miRNAs are known regulators of embryonic stem cells (ESCs) pluripotency and differentiation. Lentiviral miRNAs overexpression have been reported to induce ESCs differentiation towards cardiomyocytes and other cell types. We tested if transient miRNA driven by mimic miRNAs overexpression could affect mouse ESCs differentiation. ESCs transfected with exogenous miRNAs were differentiated in embryoid bodies, i.e. ESCs aggregates which serve as a model for ectoderm, endoderm, and mesoderm formation, cultured under conditions supporting myogenic differentiation. Several miRNAs were tested, among them molecules specific for mesodermal lineages or muscles. Tulesent miRNAs overexpression lead to the decreased expression of miRNAs encoding pluripotency factors and increased expression of those encoding myogenic markers as Pax3, Pax7, MyHC, suggesting differentiation into myogenic precursor cells or skeletal myoblasts. Meanwhile introduction of other miRNAs inhibited differentiation of skeletal myoblasts, as it decreases expression of mRNAs encoding MyoD, Myf5, MyoG. Importantly, transient miRNA overexpression turned out to be relatively stable suggesting that this technique could be used for directed stem cells differentiation into other lineages.

embryonic stem cells, miRNAs,
Recombinant adeno-associated virus (rAAV)-based vectors are promising tools for the gene therapy of Duchenne muscular dystrophy (DMD). Several studies in murine and canine models of DMD reported significant phenotype improvements without any notable toxicity following the injection of substantial amounts of vector. While this has raised hope for future translations in DMD patients, long term maintenance of the therapeutic benefit is an important, and yet unresolved issue. In previous studies conducted in DMD mice, we and others have demonstrated that rAAV-mediated transgene expression is progressively reduced, even after injection of clinically relevant vector doses. Our first study highlighted several restriction factors with a negative impact on rAAV transduction, notably the loss of vector genomes resulting from muscle cell necrosis, but also the oxidative damage affecting transgene mRNA molecules. This study supported the fact that the tissue context in which rAAV vectors are delivered is of critical importance and can significantly affect their efficiency. In addition, it opened new avenues for improvement since we can now consider counteracting these restriction phenotypes prior to rAAV injection. In the case of DMD, oxidative stress seems to occupy a central position in both muscle cell pathophysiology and rAAV transgene mRNA degradation. Therefore, we designed an innovative strategy using a relevant antioxidant agent routinely used in human medicine. DMD mice, pre-treated or not with this compound, were subsequently injected with an rAAV vector carrying a reporter transgene. The transduction efficiency, together with the expression and activity of the transgene, were monitored and compared two months later. The outcome of this innovative approach will certainly pave the way for future combinatorial protocols using pharmacological agents and rAAV vectors in DMD muscles.

Duchenne muscular dystrophy, gene therapy, pharmacology, oxidative stress

Young Investigator Poster- #2456
P26- 390- Nuclear distribution in muscle from a mouse model of the Dynamin 2-linked autosomal dominant centronuclear myopathy
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Dynamin 2 (DNM2) is a key protein of endocytosis and intracellular membrane trafficking through its function in vesicle release from membrane compartments. In addition, DNM2 regulates architecture and dynamics of actin and microtubule cytoskeletons. Mutations in the DNM2 gene cause autosomal dominant centronuclear myopathy (CNM) and rare forms of Charcot-Marie-Tooth neuropathy. We constructed a knock-in mouse model expressing the most frequent human DNM2 mutation in CNM (Knock In-Dnm2R465W). Heterozygous (HTZ) mice developed a myopathy sharing similarities with human disease including muscle atrophy, impairment of contractile properties and morphological abnormalities. However, no noticeable nuclear centralization is present in muscle sections from HTZ mice. In order to determine if peripheral nuclear positioning is altered in HTZ muscles, number and distribution of myonuclei as well as volume of myonuclear domain were compared in isolated fibres from HTZ and wild-type (WT) Tibialis anterior muscle from 3 weeks to 7 months of age. The size of HTZ fibres is lower at 3 weeks of age compared to WT littermate. At two months, HTZ fibres reach size comparable to WT and, afterward, progressive atrophy occurs up to 7 months of age. The number of myonuclei/100μm remains constant from 3 weeks to 5 months but is reduced at 7 months. Consequently, number of nuclei/volume is increased in HTZ fibres at 3 weeks and thereafter is similar to WT up to 7 months of age. The nearest neighbour distance for each nucleus was used as an index of nuclear distribution. The mean value is normal but the range of values for individual fibres is modified from 2 to 7 months of age. As a consequence of the described changes, the volume of myonuclear domain appears altered only at 3 weeks of age. These preliminary data suggest that peripheral nuclear distribution is modified during muscle remodelling occurring in the CNM-mouse model. A better characterization is required to determine the potential impairment of mechanisms controlling nuclear positioning or nuclear anchorage and the consequences on muscle function.

dominant centronuclear myopathy, dynamin 2, mouse model, nuclear distribution

Young Investigator Poster- #2461
P26- 391- Sarcoplasmic reticulum Ca2+ release in MTM1-deficient muscle fibers exhibits reduced amplitude, slow onset and spatial heterogeneities over the full range of voltage activation: defects are partially rescued by phosphatidylinositol 3-kinase inhibition
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Mutations in the gene encoding the phosphoinositide (PtdInsP) phosphatase MTM1 are responsible for myotubular myopathy. The associated fatal muscle weakness is believed to result from deficiency of excitation-contraction coupling but why MTM1 is necessary for proper function of this process is unclear. Using confocal line-scan detection of voltage-clamp-activated rhod-2 Ca2+ transients we found that, in muscle fibers from MTM1-KO mice, the spatially averaged sarcoplasmic reticulum (SR) Ca2+ release flux routinely yields a secondary rising phase at mid-activation voltages and exhibits decreased peak amplitude and increased time to peak over the full range of voltage activation. For instance, in response to a 500 ms-long pulse from -80
to +10 mV the mean peak amplitude of Ca2+ release was, on average, depressed by a factor of 2.5 and delayed by 16 ms in MTM1-KO fibers (n=13) as compared to wild type fibers (WT, n=20). MTM1-KO muscle fibers exposed for 1 hour to the PtdIns 3-kinase inhibitors Wortmannin and LY294002 exhibited a 60 to 80% enhancement in peak Ca2+ release amplitude while no effect of this treatment was observed in WT fibers. A more prolonged pre-exposure of MTM1-KO fibers to the two inhibitors did not further enhance Ca2+ release, suggesting that, in absence of MTM1, PtdIns 3-kinase activity acutely contributes to depress Ca2+ release. The rescuing effect of Wortmannin and LY294002 on Ca2+ release was not reproduced by 3-methyladenine, a specific inhibitor of class III PtdIns 3-kinase. In MTM1-KO fibers, confocal Ca2+ images revealed strong heterogeneity of Ca2+ release along the scanned line, with spatially segregated regions exhibiting alterations recapitulating the ones observed at the global level. Concurrent measurements of rhod-2 and di-8-anepps fluorescence revealed that at least some of the local Ca2+ release defects are associated with disrupted t-tubule network. Overall results demonstrate that MTM1 deficiency generates complex, spatially heterogeneous Ca2+ release defects characterized by reduced and slowed or delayed ryanodine receptor Ca2+ channel activation. Co-localized defects in t-tubule network and Ca2+ release is consistent with a causal link between the two while results from PtdIns 3-kinase inhibition suggest that accumulation of phosphoinositide products of PtdIns 3-kinase class I and/or II, play an acute role in the depression of Ca2+ release. This work is supported by AFM-Téléthon.

**Congenital myopathy, skeletal muscle, excitation-contraction coupling, calcium signaling, myotubular myopathy**

Young Investigator Poster- #2467

P26-392- Hacd1-knockout mice are protected against high-fat-diet-induced obesity and insulin resistance

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Mutation in HACD1/PTPLA gene causes a myopathy characterized by muscle weakness and exercise intolerance in Labrador retrievers. HACD1 participates to the elongation of very long chain fatty acids (C?18) within the endoplasmic reticulum and we recently proved that HACD1 is dynamically regulated in differentiating myoblasts, where it regulates cell membrane composition and fluidity. As a consequence, HACD1 deficiency in mouse and dog impairs myoblast fusion during development, leading to the observed reduction in muscle mass in adulthood. We decided to explore the metabolic capacities of Hacd1-knockout (KO) mice as we hypothesized that their reduced muscle mass and spontaneous locomotion would lead to an altered sensitivity to insulin, given that skeletal muscles constitute the main glucose sink. Counterintuitively, Hacd1-KO mice exhibited higher glucose tolerance and insulin sensitivity, both in normal and high fat diet. Moreover, during high fat diet, despite eating the same quantity of food, Hacd1-KO mice exhibited a resistance to obesity, with reduced weight gain and fat accumulation. Analysis of skinned muscle fibers revealed that mitochondrial beta oxidation and uncoupled respiration were markedly elevated in Hacd1-KO mice, suggesting that this increased catabolic activity consumed increased levels of lipids and glucose and partially compensated the over ingested fat during high fat diet. Lipidomic analyses revealed altered levels of mitochondria-specific lipid species that could impair inner membrane organization and thus respiratory complex function. Taken together, our results demonstrate that HACD1 plays a major role in muscle metabolism and suggest that HACD1 loss-of-function could be linked to the nosological group of mitochondrial myopathies in which energy deficit contributes to exercise intolerance. In parallel, the protective effect of HACD1 deficiency towards diet-induced obesity and insulin resistance may provide grounds for the management of metabolic syndrome.

Young Investigator Poster- #2471

P26-393- Polyamine metabolism is a novel disease modifier underlying heterogenous muscle involvements in merosin-deficient congenital muscular dystrophy type 1A

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Merosin-deficient congenital muscular dystrophy type 1A (MDC1A) is caused by mutations in Lama2 gene, which compromises the structural stability of skeletal muscle and nerves. The MDC1A mouse model, dy2/Jdy2J, has reduced laminin-?-2 expression and displays progressive muscle weakness and paralysis. Strikingly, the hind limb muscles, e.g. tibialis anterior (TA), exhibit more severe paralysis, atrophy and fibrosis compared to the forelimb muscles, e.g. triceps. Our initial microarray study showed downregulation of Adenosyl-methionine decarboxylase (Amd1) and Spermine oxidase (Smox), two enzymes that maintain cellular level of polyamines, in the TA muscles, but not in the triceps. Polyamines are naturally occurring polycations involved in cell proliferation, differentiation and apoptosis, yet their involvements in muscle growth and disease remain to be investigated.

We sought to characterize the contribution of altered polyamine metabolism in MDC1A pathology and assess whether modulation of polyamine level can protect against laminin deficiency, leading to improved dy2/Jdy2J phenotypes. We analyzed the TA and triceps from 1, 3, 5, 12, and 20-weeks old dy2/Jdy2J mice for polyamine levels using LC/MS-MS and generated lentiviral vector to stably overexpress Amd1 and Smox in vivo.

We observed significantly lower Amd1 and Smox expression in dy2/Jdy2J TA compared to triceps starting at 1 week of age, which corresponds to the levels of three polyamines, namely putrescine, spermidine and spermine in the muscles. Furthermore, early intervention of dy2/Jdy2J pups with lentiviral vector overexpressing Amd1 and Smox showed significant improvement in muscle histology and open field activity.

Our findings showed that muscle-specific decrease of polyamine level and alteration of its metabolism correlate with the difference in phenotypic severity observed in different dy2/Jdy2J mice. Remarkably, modulation of polyamine levels can ameliorate disease phenotypes in dy2J/dy2J mice, indicating its treatment potential for MDC1A.
During skeletal muscle regeneration, muscle stem cells (MuSCs) recapitulate the myogenic program to repair the damaged myofibers. A subset of MuSCs does not enter into the myogenic program but self-renews to return into quiescence. The control of this process is crucial to maintain skeletal muscle homeostasis. Recent studies highlight the importance of a metabolic switch in the regulation of stem cell fate and particularly the exit from and the entry into quiescence. AMPK being a master regulator of cellular metabolism, it seems to be an excellent candidate in the control of MuSC fate choice. AMPK activation mainly depends on the increase of the AMP/ATP ratio and induces an increase of the anabolic/catabolic ratio.

We deciphered the role of AMPK\(^1\), in the control of MuSC self-renewal versus differentiation (AMPK\(^1\) being the only catalytic subunit expressed in these cells). In ex vivo (isolated fibers) and in vitro (β7Int+ sorted MuSCs) experiments, we showed that MuSCs deficient for AMPK\(^1\) differentiated in a lower extend and self-renewed much more (+269\%) than WT MuSCs. In vivo, we used specific conditional mouse strain in which AMPK\(^1\) is deleted in MuSCs (Pax7CRE-ERT2/+:AMPK\(^1\)fl/fl) and the model of cardiotoxin-induced muscle regeneration. 28 days after injury, we observed i) a strong decrease in the size of regenerating myofibers (-48\%); ii) an increase in the total number of fibers per muscle (+56\%); and iii) an increase in the total number of satellite cell per muscle (+28\%). This phenotype is not observed in mice deleted for AMPK\(^1\) in the myofibers (HSACRE/+;AMPK\(^1\)fl/fl). The metabolism of MuSCs was explored in vitro by analyses of mitochondrial respiration and measurement of the activity of key enzymes of glycolysis. Our results suggest that in absence of AMPK\(^1\), MuSCs are not able to provide enough mitochondria-driven energy for differentiation and thus are prone to self-renewing while they maintain their metabolism toward glycolysis. The identification of the metabolic targets of AMPK in MuSCs is under investigation.

To conclude, our work establishes a new and important role of AMPK\(^1\) in MuSC fate choice by switching their metabolism at the time of differentiation/self-renewal during skeletal regeneration, linking for the first time self-renewal and metabolism in this context.

*stem cells fate, skeletal muscle regeneration, metabolism switch*
**INTRODUCTION.** The adult-onset form of myotonic dystrophy (DM1), has a wide phenotypic spectrum and potentially may affect any organ including CNS. METHODS. We enrolled 63 patients with established clinical-genetic diagnosis of DM1, that underwent neurological assessment, psychological and neuropsychological evaluation and quality of life interview. A subgroup of 20 patients underwent 3T-MRI protocol including morphologic and functional investigation. Brain atrophy was measured with Voxel-based morphometry (VBM) and calculating Parenchymal Brain Fraction (PBF). fMRI examination investigated cortical BOLD response during a self-awareness task.

RESULTS. Neurological examination showed mild to severe muscle involvement (MIRS mean 2.98±0.92). Most of our patients presented with a heterogeneous cognitive profile, characterized by prominent impairment in executive and mnesic domains with visuo-spatial involvement (WCST-Cat 36.7%, WCST-Pe 40%, ROCF-copy 46.9%, CBT 40%); impaired illness awareness was found in 52.1% and was smoothly associated to executive impairment (p=0.075).

VBM revealed several clusters of reduced cortical GM in DM1 patients compared to healthy controls (TFCE p>0.001 corrected for multiple comparisons). Atrophy was diffuse in both cerebral hemisphere and particularly in peririodic area, orbitofrontal, dorsolateral frontal insular, temporoparietal, anterior and posterior cingulated.

We found a negative relationship between verbal memory performance and the grade of atrophy respectively in left postcentral, left middle and inferior temporal gyri and left supramarginal gyrus,(p>0.05). PBF correlates with impaired cognitive performance for visuo-spatial and executive functions. Within-group activation maps showed in patients higher activation in frontal and midline regions during self-awareness task. fMRI revealed frontal and midline brain hypoactive regions in patients with reduced illness-awareness.

**CONCLUSIONS**

Our data indicate the existence of a correlation between brain atrophy, expressed with PBF, and impairment in typical cognitive domains for adult DM1 patients; further studies ongoing could better explore the relationship between behavioural parameters and VBM data. Moreover the association between illness-unawareness and specific brain regions hypoactivation may be a persistent and distinctive feature of this multisystemic disease.
correct folding. Analysis of autophagic activity in DMD myoblasts revealed a modulated level of autophagy adaptors and of Atg proteins but also an increased level of LC3-II protein, which is associated with autophagosome membranes. Moreover, we observed that autophagosome maturation is not altered in DMD myoblasts, suggesting that autophagic activity may be increased in myoblasts from DMD patients.

Further in-depth characterization of PQC actors will provide the first comprehensive description of shared proteostasis alterations occurring in DMD myoblasts. Furthermore, it will give hints to which manipulation of PQC could delay or stop muscle degeneration and thus unravel therapeutic strategies toward muscle dystrophies.

Protein Quality Control, Duchenne Muscular Dystrophy

Young Investigator Poster- #2553
P26- 398- Calpain 3 rAAV-mediated gene transfer- Pilot study of biodistribution and toxicity in Non-Human Primates
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Genetic defects in capn3 (CAPN3) lead to limb-girdle muscular dystrophy type 2A, a disease of the skeletal muscle that affects predominantly the proximal limb muscles. This protease was proposed to play a key role in plasticity of the skeletal muscle fiber through the cleavage of different substrates. We previously demonstrated the potential of adeno-associated virus-mediated transfer of the CAPN3 gene to correct the pathological signs in a murine model for limb-girdle muscular dystrophy type 2A after intramuscular and locoregional administrations. Nevertheless, systemic injection was associated to a cardiotoxicity that was circumvented by the incorporation of a cardiac specific miRNA (mir208a) target sequence (Roudaut, 2013).

To further evaluate the safety of the new generation of the cassette, we designed two rAAV vectors including the macaca fascicularis CAPN3 CDS under control of hDesmin promoter and in presence or not of a mir208a target sequence. Five 2/3-year-old male primates were included; 3 with rAAV with miRT and 2 with rAAV without miRT. Both vectors were injected to evaluate the biodistribution and toxicity of our vectors after systemic delivery of a 3e13vg/kg dose of rAAV. According to the previous experiments performed on mice and the early onset of cardiotoxicity due to the ectopic expression of the transgene, necropsy and samples collection were performed after one month of expression.

Evaluation of the cardiac status was thoroughly examined at different levels, including biomarkers, echocardiogram and histology. Detailed analyses were performed on 6 different sections of the heart. Muscles of upper (triceps brachis, vastus lateralis) and lower (soleus, tibialis anterior, biceps femoris, and gastrocnemius) limbs were collected as well as temporalis in head, and diaphragm and psoas in trunk part. Most of organs were biopsied including lung, liver, spleen, lymphoid nodes, kidney, testis, brain and spinal cord. The results of this pilot study in non human primates will be presented.

CAPN3, gene transfer, primate

Young Investigator Poster- #2554
P26- 399- In the pursuit of a mouse model for the sarcoglycanopathies
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The sarcoglycanopathies are a group of rare type of muscular dystrophies characterized by early onset of muscle weakness and early loss of ambulation. The disease is caused by mutations in the genes coding for the sarcoglycans (SGC), which participate in the dystrophin-associated glycoprotein complex (DGC) at the membrane of the muscle fiber. The absence of at least one of these proteins leads to the absence of the whole SGC complex, disrupting the DGC structure and leading to progressive damage upon muscle contraction. Unfortunately, no effective treatment has been developed thus far. We showed that some missense mutations in the sarcoglycan genes can still yield functional proteins if correctly addressed at the membrane and that pharmacological rescue from their early degradation by the ER-associated degradation (ERAD) pathway leads to the restoration of the complex integrity. In the process of developing new therapeutic drug based on this principle, the candidates have to be tested in an in vivo model of the disease. Previous work in our lab described an ?-sarcoglycan (SGCA) mouse model carrying the most frequent missense mutation. However, this mouse model did not present any manifestation of the disease, possibly due to a deficiency in the recognition of this protein as being misfolded by the ERAD pathway. Therefore, we initiated the development of a new mouse model. A missense mutation in the beta-sarcoglycan (SGCB) gene was chosen based on its ability to be rescued in vitro by several drug candidates. Using the CRISPR/Cas9 system and a homologous donor, the mouse variant of the human mutation was introduced in ES cells that were injected into blastocyst. Chimeric mice have been generated and evaluation of the germ line transmission is on-going. The mutant mice will be characterized at phenotypic level for the presence of a dystrophic presentation. In the meantime, we are testing in vivo the addressing to the membrane of several rescueable missense mutations by AAV transfer in a KO background. We are currently screening for the mutations that fail to be present at the membrane due to their early degradation and that are rescued by pharmacological treatment. These models will be used to test candidate drugs.

sarcoglycan, AAV, mutation, in vivo

Young Investigator Poster- #2562
P26- 400- Implication of CD8+ T cells in the pathophysiology of amyotrophic lateral sclerosis
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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder caused by the loss of motoneurons. ALS leads to the atrophy and paralysis of striated muscles, which cause death within 3 to 5 years. Approximately 20% of familial ALS are caused by mutations in SOD1 gene. Mice overexpressing human SOD1 mutations develop a motor syndrome with features of human disease.

A chronic inflammatory response, associated with the accumulation of blood-derived immune cells in the central nervous system, is a pathological feature of ALS. In the early phase of the disease, CD4+ lymphocytes invade the CNS and seem to negatively regulate the inflammatory response. However, the early symptomatic phase is characterized by a substantial increase of CD8+ T cells in the CNS. Those CD8+ T cells are effectors of adaptive and innate immunity and can promote cytotoxic effects.

The contribution of this effector cell population in the neurodegenerative process has been poorly investigated. Here, we propose to explore the impact of the infiltration of CD8+ T cells on the development of the disease. Our results show that mutant SOD1G93A CD8+ lymphocytes, but not wildtype, trigger motoneurons death, whereas cortical, hippocampal and striatal neurons are not sensitive to this neurotoxicity. Moreover, our co-culture experiments indicate that CD8-induced motoneuron death occurs in a contact dependent manner, via the recognition of the MHC-I complex exposed by motoneurons. Our results suggest that IFNg, Fas-Fas ligand and perforin-granzyme pathways are implicated in this neurotoxicity.

Beside this in vitro analysis, one of the main objective of this project is to assess the therapeutic effect of a specific immunodepletion of CD8+ T cells in SOD1G93A mice. To this aim, we developed a protocol of long term immunodepletion of CD8 cells.

**ALS, CD8+ T cells, neuroimmunity**

Young Investigator Poster- #2568

**P26- 401- Simultaneous parametric mapping of water T2 and fat fraction for skeletal muscle tissue characterization by NMR imaging**

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The last decade have seen a growing use of quantitative nuclear magnetic resonance imaging for studying neuromuscular disorders. Skeletal muscle inflammation, necrosis, hydrostatic edema and fatty infiltration are strong indicators of disease activity and progression, respectively. Muscle transverse relaxometry and water/fat separation techniques have proven to be efficient non-invasive methods for assessing and monitoring these phenomena. While reflecting inflammatory infiltration, myocyte swelling, sarcoplasmic leakiness, cell necrosis, or simply hydrostatic edema, and therefore being nonspecific, muscle water transverse relaxation time (T2H2O) changes provide relevant information about disease activity, and about muscle physiological status. Fatty infiltration and replacement in muscles, however, reveal the extent and severity of muscle destruction and loss in chronic conditions.

Transverse relaxation time and FF are usually measured separately, using dedicated NMR sequences. The muscle T2H2O estimation is most often derived from standard Multi-Slice Multi-Echo (MSME) acquisitions and exponential fitting of the temporal signal decay, while methods exploiting the chemical shift between water and fat protons (e.g. Dixon techniques) are the most popular approaches for FF estimation. In practice, instrumental imperfections such as non-ideal slice pulse profiles, radiofrequency and static magnetic field inhomogeneities contaminate the MSME sequence and render T2H2O determination highly unreliable using exponential models.

In the present study, we propose to apply a bi-component extended phase graph model that takes into account all the instrumental imperfections to simultaneously and accurately quantify the muscle water T2 and fat fraction from a unique MSME acquisition. The interest of this methodology over more standard approaches was demonstrated in term of accuracy and precision on parameters estimation. This simultaneous evaluation of T2H2O and FF also represents a real advantage for whole-body investigations as it allows a decrease in acquisition time and improve patient comfort.

**quantitative NMR imaging, fat fraction, water T2**

Young Investigator Poster- #2573

**P26- 402- Exploiting CRISPR-Cas9 nuclease to study specific alternative splicing titin isoform in vivo.**

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Titin (TTN), forming the third filament system in the myofibrils, has structural, mechanical and regulatory functions in skeletal and cardiac muscle. Its importance for the muscle function was underlined by the identification of titin mutations in a range of heart and skeletal diseases. The N-terminal ends of titin molecules are anchored to the Z-disc, while the C-terminal ends are embedded in the M-band in the middle of the sarcomere. The M-band part of titin contains a kinase domain and 10 Ig-like
Dystroglycanopathies constitute a group of genetic diseases caused by defective glycosylation of alpha-dystroglycan (aDG), a membrane glycoprotein involved in the cell/matrix anchoring of muscle fibers. The aDG glycosylation, a very complex process, requires many proteins whose functions are not fully elucidated. In particular, mutations in the FKRP gene encoding Fukutin related protein, lead to hypoglycosylation of aDG, resulting in different forms of dystroglycanopathies, among which Limb Girdle Muscular Dystrophy type 2I (LGMD2I).

A knock-in mouse model was generated in our lab, presenting with abnormal glycosylation of aDG, reduction of its binding to the extracellular matrix protein laminin, presence of central nuclei and low resistance to eccentric mechanical stress. Using this mouse model, we performed FKRP gene transfer, by means of a rAAV2/9 vector expressing the murine FKRP under the transcriptional control of the desmin promoter. The rAAV vector was injected intramuscularly or intravenously. Expression of the FKRP transgene was obtained, both at RNA and protein levels. The glycosylation of aDG was restored, as well as binding to laminin. A histological rescue was observed by the decrease of central nuclei. The AAV vector also improved the muscle function, since it conferred a better resistance to eccentric stress to the injected muscles. In parallel with these promising results, we uncovered that increasing the rAAV-FKRP dose was leading to a decrease of aDG glycosylation and of its binding to laminin. Moreover, an immune response against the transgene was revealed in some of the injected mice. Altogether, these data highlight the interest of using the CRISPR/Cas9 system to study in vivo the function of specific RNA isoforms generated by alternative splicing.

Titin, muscular dystrophy, CrispR-Cas9 nucleases, alternative splicing, mouse model

Young Investigator Poster- #2583
P26- 403- AAV-mediated gene transfer of FKRP in mice
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Dystroglycanopathies constitute a group of genetic diseases caused by defective glycosylation of alpha-dystroglycan (aDG), a membrane glycoprotein involved in the cell/matrix anchoring of muscle fibers. The aDG glycosylation, a very complex process, requires many proteins whose functions are not fully elucidated. In particular, mutations in the FKRP gene encoding Fukutin related protein, lead to hypoglycosylation of aDG, resulting in different forms of dystroglycanopathies, among which Limb Girdle Muscular Dystrophy type 2I (LGMD2I).

A knock-in mouse model was generated in our lab, presenting with abnormal glycosylation of aDG, reduction of its binding to the extracellular matrix protein laminin, presence of central nuclei and low resistance to eccentric mechanical stress. Using this mouse model, we performed FKRP gene transfer, by means of a rAAV2/9 vector expressing the murine FKRP under the transcriptional control of the desmin promoter. The rAAV vector was injected intramuscularly or intravenously. Expression of the FKRP transgene was obtained, both at RNA and protein levels. The glycosylation of aDG was restored, as well as binding to laminin. A histological rescue was observed by the decrease of central nuclei. The AAV vector also improved the muscle function, since it conferred a better resistance to eccentric stress to the injected muscles. In parallel with these promising results, we uncovered that increasing the rAAV-FKRP dose was leading to a decrease of aDG glycosylation and of its binding to laminin. Moreover, an immune response against the transgene was revealed in some of the injected mice. Altogether, these data support the possibility to use the AAV-mediated transfer of FKRP but call for a control of gene expression.

limb girdle muscular dystrophy, dystroglycanopathy, gene transfer

Young Investigator Poster- #2606
P26- 404- Duchenne muscular dystrophy: an easy, fast and robust cell platform for developing therapeutic strategies
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Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD) are two recessive X-linked monogenic myopathies caused by mutations in the dystrophin gene. The main difference between DMD and BMD is the phenotype severity which is linked to the level of dystrophin expression. BMD has a shorter but functional dystrophin while DMD is usually characterized by the loss of the open reading frame (ORF) leading to the loss of a functional protein. These DMD patients, with a worldwide prevalence around 1 in 21,000 boys, display severe phenotypes. DMD symptoms usually appear in early childhood (2-5 years old) with a progressive loss of muscle strength implying the use of wheelchair by age 12 and leading to premature death due to cardiac and respiratory failures by age 30 in average.

There is no treatment available yet to stop the progression of the disease. However, developments of several therapeutic approaches are in progress. Among them, there is the exon skipping strategy where small antisense molecules (PMOs) enable the restoration of ORFs in the dystrophin transcript and, therefore, the translation of a truncated but potentially functional protein. This approach has already reached the clinical trial stage. However, two main issues remain: 1) the in vitro DMD model using myoblasts for the validation of gene therapies is laborious and time-consuming; 2) the current exon skipping method seems to be safe but its efficacy is not sufficient to restore enough functionality in DMD patients.

Our laboratory has developed an easy, fast and robust cell platform using BMP4-treated human pluripotent stem cells (induced, hiPSCs, from healthy and DMD patients, and embryonic, hESCs) which express high level of embryonic dystrophin (Dp412e), a newly identified long isoform of dystrophin similar to a functional isoform found in a BMD muscle. This platform could be used for developing novel therapies (like gene editing) or for improving the current ones. As a proof of concept, we are working on the improvement of the exon skipping strategy by the use of a high-throughput screening of PMOs/small molecules combination.

Duchenne muscular dystrophy, human pluripotent stem cells, embryonic dystrophin, high-throughput screening, exon skipping
Asymptomatic hyperckemia (CK) requires extensive evaluation to determine the underlying cause. The aim of this study is to analyze the causes of asymptomatic or oligosymptomatic hyperckemia, to help in the long term follow up and prevention of possible complications.

METHODS: Retrospective study.

Inclusion criteria:
Patients under 18 years with CK levels ≥ 1.5 times normal in two separate controls separated by at least a month.
Asymptomatic or oligosymptomatic forms including myalgia, fatigue, cramps or muscle stiffness.

Testing performed:
a) In all cases: manual test of muscle strength, cardiologic evaluation and familial CK levels.
b) According to the case: Dystrophin MLPA, EMG, metabolic studies, acid alpha-glucosidase, studies for McArdle disease, MRI and / or muscle biopsy.

RESULTS:
1) n = 26 patients, 4-18 years of age. 16 males. 19 asymptomatic, 7 oligosymptomatic. CK between 400 and 5900 IU / L. Follow-up: 2-5 years.
2) 61.5% (16) had family history of Dystrophinopathy, cramps and fatigue.
3) Diagnosis was obtained in 18 cases (69%): 6 dystrophinopathy, 3 CPT II, 3 organic acidurias, 1 dysferlinopathy, 1 RYR-1, 1 McArdle, 1 statin myopathy, 1 mitochondrial myopathy and 1 muscular dystrophy.
4) In the 8 patients without diagnosis, they had normal EMG, 3 with non-specific changes in biopsy. They remained asymptomatic but with high CK.

CONCLUSIONS:
In our patients most cases of asymptomatic/oligosymptomatic hyperckemia were due to dystrophinopathies or metabolic disorders. We were unable to establish a correlation between CK blood levels and diagnosis. Most patients remained asymptomatic without clinical changes during the follow up time.

*dystrophinopathy, CPTII, organic acidurias, dysferlinopathy, McArdle disease*
Amyotrophic lateral sclerosis (ALS) motor neuron disease is uniformly fatal, usually within five years. Most ALS cases are sporadic (SALS) whereas ~10% are familial (FALS). Mutations in PFN1 (profilin 1) have recently been identified in some ALS patients however only few genetic studies confirmed that PFN1 mutations could cause ALS. PFN1 is a nucleotide exchange factor regulating the polymerization of actin network and is involved in multiple cellular pathways. In order to support the role of PFN1 in the pathogenesis of ALS we sequenced PFN1 in 720 French ALS patients: 120 FALS and 600 SALS. Two mutations especially in motor neurons carrying the E117G variant showed the presence of neuronal intranuclear protein inclusions, immunopositive for alpha-actin, E117G variant in two SALS (0.3%) and 1/500 control (0.2%). Interestingly, neuropathological analyses performed on a patient carrying the E117G variant showed the presence of neuronal intranuclear protein inclusions, immunopositive for alpha-actin, which could result from a disruption of PFN1-mediated nuclear actin export. To study the functional impact of these mutations in vitro and in vivo we (i) established lymphoblast cell lines from patients carrying PFN1 mutations and (ii) produced 3 lentiviral vectors expressing wild-type or mutant PFN1. These constructs were used to overexpress PFN1 in HEK293T cells: PFN1 mutants induced the formation of cytoplasmic TDP43 positive inclusions. These vectors have also been used to generate new mouse models using lentiviral transgenesis technology with high efficiency: 80% of the progeny carried the transgenes. Phenotypes in these transgenic mice are currently followed using body weight, motor behavior and electromyography recordings. Quantitative analysis of motor neuron survival, motor axon diameter and neuromuscular junctions will be performed as well as aggregate formation and glosis. Results will be correlated for each animal to the level of transgene expression, especially in motor neurons.

ALS, profilin, mutations, mouse models

Inflammatory idiopathic myopathies (myositis) are a group of acquired muscle diseases which are characterized by a disabling muscular weakness and the presence of inflammatory immune responses within the skeletal muscle. The clinical, histological and immunological heterogeneity observed in these diseases has led to its classification into different sub-categories. Before 50 years old, the most common are polymyositis (PM), whereas sporadic inclusion body myositis (sIBM) is the most frequent in patients older than 50 years old. Dermatomyositis (DM), and necrotizing autoimmune myositis (NAM) are the two other forms of myositis. In PM and sIBM patients, CD8+ T cells invade muscle fibers which abnormally over-express MHC-I molecules. Here, these CD8+ T cells secrete cytotoxic enzymes leading to cell death. These observations suggest the presentation of muscular autoantigens by MHC class I molecules to CD8+ T cells, thereby leading to their activation and the secretion of perforines and granzymes at the origin of muscle destruction. Nevertheless, till date there exists no efficacious treatment for sIBM patients. Our research has three main objectives: firstly to confirm the presence of an ongoing immune response in sIBM patients and to identify the autoantigens targeted during the disease, and lastly to obtain a refine characterization of the various cell populations in these four groups of myositis patients. Based on an interferon gamma Enzyme linked ImmunoSpot technique (ELISpot), we cultured peripheral blood lymphocytes (PBMC) of patients and healthy donors (HDs) in the presence of native myosin extracted from different HDs skeletal muscle tissue. Prior to our Elispot assay we realized an accelerated co-culture of dendritic cells (acDc) in order to optimize the immune responses. Here, we demonstrate an active immune response mediated by the secretion of interferon gamma in 5 out of 9 sIBM patients compared to 5 HD. Nevertheless, the specific autoantigen responsible for this immune response is still to be identified. On the other hand, we are presently optimizing a mass cytometry assay (CyTOF). This assay will enable us to phenotype up to 34 specific markers on PBMC, thus allowing us to distinctly characterize each population of T cells, B cells, natural killer cells and monocytes thereby distinctively separating the different groups of myositis patients.

myositis, T cells, B cells, autoantigens, autoimmunity, cytof, elispot
Myotonic Dystrophy including both type 1 (DM1) and type 2 (DM2) is one of the most prevalent muscular dystrophies in adults. DM1 is caused by the abnormal expansion of a repeated CTG trinucleotide whereas the less common form DM2 is due to a large CCTG expansion. There is currently no cure for DM however several therapeutic strategies are under development. Before in vivo assessment using diseased animal models, therapeutic compounds are tested in vitro using cellular models, as an initial step. For this purpose, human primary cells derived from biopsies of DM1 patients represent a valuable model since the mutation remains in its natural genomic context. Human myoblast cell cultures are quite easy to set up and manipulate however two major difficulties hamper their use: i) accessibility and availability of muscle biopsies from patients affected with muscular dystrophies, ii) limited proliferative capacity of adult human myoblasts.

To overcome these limitations that are worsen for DM myoblasts since they enter prematurely in replicative senescence, we have developed immortalized DM myoblast cell lines. Primary myoblasts isolated from DM patients were transduced with lentiviral vectors expressing telomerase and cyclin-dependent kinase 4. Immortalized DM1 and DM2 myoblasts recapitulate primary DM muscle cells features: DM1 myoblasts show impaired differentiation, nuclear foci of CUGexp-RNA and alternative splicing misregulations whereas DM2 myoblasts show nuclear foci of CCUGexp-RNA but no splicing or differentiation alteration when compared to control or DM1 myoblast cell lines. These DM cellular models represent a new resource and reliable tools for the assessment of therapeutic strategies. As example, inhibition of CUGexp-RNA toxicity using antisense strategies reverses both differentiation and alternative splicing defects in DM1 cell lines. Also, these models can be used for high-throughput screening aimed at identifying compounds that reduce deleterious effects of the DM mutation. Finally, to further characterize DM1 myoblasts cell lines, RNA-seq experiments were performed to obtain a complete overview of transcriptome expression, to pull out new targets/pathways involved in DM pathophysiology and to reveal the whole of restored events giving us an insight of efficacy or potential specific targets of dedicated therapeutic approaches.

DM1, splicing, therapy, RNAseq

Young Investigator Poster- #2879
P26- 410- Immortalized human myoblasts isolated from patients with neuromuscular disorders: an ideal tool for therapeutic strategies
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The exponential development of innovative therapies, eg gene-based, genome editing, exon-skipping, and the orientation towards a personalized medicine adapted to each mutation require models which can be adapted to each patient. In the context of neuromuscular disorders, patient cells and especially human myoblasts derived from muscle biopsies represent an ideal in vitro model to test therapeutic strategies. So far the restricted access to patient tissue and the limited proliferative capacity of human myoblasts restricts their use.

By the transduction of telomerase (hTERT) and cyclin-dependent kinase 4 (cdk4) into patient primary myoblasts, we have developed an innovative strategy to immortalize human myoblasts derived from muscle biopsies of dystrophic patients. These cells maintain their original behavior since they keep their potential to differentiate and to regenerate in vivo after injection into the TA muscle of immunodeficient mice (Mamchaoui et al., 2011). Ninety human immortalized myoblast lines from twenty-seven different neuromuscular disorders including DMD, LGMD2B, OPMD and FSHD have now been generated. We have also developed control cell lines from newborn to elderly subjects. For some muscle diseases, access to muscle biopsies was not feasible. For these patients, we have also developed an alternative cellular model based on the myogenic conversion of fibroblasts. Fibroblasts are isolated from skin biopsies, then immortalized by transduction of hTERT, and converted by transduction of inducible MyoD.

All these cell lines from various neuromuscular disorders are accessible to the scientific community on a collaborative basis. We have also developed recently cell lines (Control, DMD, FSHD) for which patient consent specifically permits their use by private companies.

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human myoblast, neuromuscular disorders

Young Investigator Poster- #2891
P26- 411- Functional analysis of caveolin-3 mutations in muscular dystrophy diseases
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Caveolae are 60-80 nm cup-shaped membrane invaginations, rich in sphingolipids and cholesterol and mainly constituted of caveolins (Cav1, 2 or 3). Caveolae have been described to play a role in endocytosis, cell signaling, lipid homeostasis. More recently, my laboratory established a new function for caveolae in the cell response to mechanical stress: caveolae that are invaginated at steady state flatten out under mechanical stress thereby playing a role of membrane reservoir which buffers membrane tension variations due to mechanical stress. Furthermore, caveolae flattening leads to the release of caveolae components into the cytosol and the plasma membrane. In some muscular dystrophies, the muscle-specific caveolin-3 (Cav3) isoform is mutated which results in Cav3 trapping in the Golgi apparatus.
As AChRs are conserved in many organisms, we use the model organism Caenorhabditis elegans to identify new mechanisms of AChR metabolism or function are considered. So far, however, only few cellular factors are known to regulate AChRs.

Overall, our results indicate that the absence of functional assembled caveolae in the Cav3 mutants leads to a defect in both membrane integrity and signaling response to an IL6 stimulus, a pathway that has been associated with muscle membrane repair.

caveolin-3, caveolae, mechanical stress, mechanosensingsensing, myotubes

Young Investigator Poster- #2909

P26- 412- Human Neuromuscular Integrative System for drug discovery
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Neuromuscular diseases (NMD) correspond to a vast group of diseases that perturbs or even progressively blocks the control and the force of muscle voluntary movement by affecting motoneurons and NMJs. To date, no efficient curative treatments have been identified for NMD. Progresses towards identification of new treatments have been hampered by the incomplete understanding of the molecular mechanisms that control synapse formation and maintenance as well as the availability of relevant screening tools. Indeed, research in NMD field are facing several challenges: (1) difficulty in examining the NMJs of patients with NMD, and absence of reliable biomarkers revealing disease status and evolution, (2) lack of reliable in vitro models recapitulating functional human NMJ, (3) animal models that poorly phenocopy the human diseases because of genetic and physiological discrepancies between the neuromuscular systems of mouse and man, (4) in vivo models that are complex to analyze and do not allow the study of each partner cell at the synapse. Altogether, these bottlenecks largely block the deployment of drug discovery campaigns and therefore abrogate the development of new medicines curing NMD early on in the drug development process. The aims of this project is to establish mature human neuromuscular junction (NMJ) in vitro models representative of different neuromuscular diseases (NMD) and to develop new screening tools for drug discovery approaches. We propose to take advantage of the recent improvement of established human pluripotent stem cells-MN system combined with micropattern cultures of human primary myotubes to implement a new robust system of human NMJ which will permit the study of the normal and pathological NMJ formation and maintenance. Preliminary experiments indicate the presence of numerous clustering of acetylcholine receptors in close proximity of MNs in a reproducible way through micropatterned. The alignment of motor neuron projections to the postsynaptic acetylcholine receptor (AChR) endplates was observed by visualizing the apposition of presynaptic nerves with synaptophysin antibodies and AChR clusters with ?-BTX.

Young Investigator Poster- #2932

P26- 413- Coping with cholinergic overstimulation is a multimodal process
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Myasthenia gravis is the most prevalent form of neuromuscular transmission troubles, and is often due to a downregulation of the acetylcholine receptors (AChRs) expressed at the muscle cell surface. Current treatments use acetylcholine esterase inhibitors to restore efficient neuromuscular transmission. Unfortunately these inhibitors can sometimes lead to a cholinergic overstimulation, a condition also known as Ach toxicity. To prevent these crises, alternative therapeutic strategies relying on the modulation of AChR metabolism or function are considered. So far, however, only few cellular factors are known to regulate AChRs.

As AChRs are expressed in many organisms, we use the model organism Caenorhabditis elegans to identify cholinergic overstimulation through the use of the GCAmp indicator expressed in muscle cells. We showed that i) the increase of calcium concentration upon levamisole exposure relies on the canonical pathway involving action potential firing, ii) long-term adaptation is determined early and depends on the continuity of this pathway, iii) this early step requires calcium-dependent phosphatase calcineurin.

By studying mutations in AChR partners conferring hyperadaptability to levamisole, we have raised a model for levamisole-mediated adaptation, in which the AChR cluster signaling is decoupled from the downstream mechanisms leading to global calcium variations. We suggest that a modulator being part of the clusters inhibits AChR signaling by shutting down the firing of action potentials.
In mouse experimentally induced autoimmune myasthenia gravis (EAMG), we performed a glucose tolerance test, evaluating muscle cells. Akt pathway is involved in insulin-mediated glucose uptake, so we investigated this aspect in MG models.

Muscle is a major insulin-dependent organ that uses and stocks large amounts of glucose and thus participates in glucose homeostasis. We previously described a decrease of Akt phosphorylation by insulin in the presence of AChR antibodies in muscle cells. Akt pathway is involved in insulin-mediated glucose uptake, so we investigated this aspect in MG models.

In mouse experimentally induced autoimmune myasthenia gravis (EAMG), we performed a glucose tolerance test, evaluating glycaemia at regular intervals after an injection of glucose in overnight fasted mice. In EAMG mice, the glycaemia peaked higher than in control mice at 30min post-injection, then decreased faster, so that in the next intervals, the glycaemia of both groups were the same. We also measured insulin in the serum during this test at 0 and 75min. Insulin levels were the same in fasted mice, but were higher in EAMG at 75min (p> 0.02). These results evoke an insulin resistance. Preliminary results also suggest that anti-AChR antibodies would be sufficient to decrease glucose capture of muscle cells in vitro, so that this metabolic phenotype could be generated from a muscle insulin resistance. Insulin resistance is the main feature of the metabolic syndrome, also known as pre-diabetes. It has also been described as inversely correlated to muscle force. Thus, insulin resistance could participate in the pathogenesis in MG and therapies aiming at improving glucose capture could complement existing treatments.

In conclusion, by using 23Na NMR spectroscopy, indices sensitive to changes in sodium biodistribution and interaction with macromolecules can be acquired in human skeletal muscles with acquisition times compatible with investigation of patients in a clinical research setting (>15min).

**Young Investigator Poster- #2948**

P26- 415- Acetylcholine Receptor (AChR) antibodies cause alterations of glucose homeostasis in Myasthenia Gravis (MG) models

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Myasthenia Gravis (MG) is an autoimmune disease in which most patients have antibodies targeting post-synaptic proteins. Clinical features comprise muscle weakness and fatigability, which are attributed to neuromuscular junction defects. However, little is known about muscle physiology in MG, although it is now accepted that muscle is not a passive target.

In mouse experimentally induced autoimmune myasthenia gravis (EAMG), we performed a glucose tolerance test, evaluating glycaemia at regular intervals after an injection of glucose in overnight fasted mice. In EAMG mice, the glycaemia peaked higher than in control mice at 30min post-injection, then decreased faster, so that in the next intervals, the glycaemia of both groups were the same. We also measured insulin in the serum during this test at 0 and 75min. Insulin levels were the same in fasted mice, but were higher in EAMG at 75min (p> 0.02). These results evoke an insulin resistance. Preliminary results also suggest that anti-AChR antibodies would be sufficient to decrease glucose capture of muscle cells in vitro, so that this metabolic phenotype could be generated from a muscle insulin resistance. Insulin resistance is the main feature of the metabolic syndrome, also known as pre-diabetes. It has also been described as inversely correlated to muscle force. Thus, insulin resistance could participate in the pathogenesis in MG and therapies aiming at improving glucose capture could complement existing treatments.

**myasthenia gravis, AChR, glucose homeostasis**

**Young Investigator Poster- #2965**

P26- 416- Accelerated NMR imaging schemes for multi-parametric skeletal muscle characterization

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Lately, quantitative nuclear magnetic resonance imaging has been increasingly used for studying neuromuscular disorders. It provides efficient non-invasive methods, like muscle transverse relaxometry and water/fat separation techniques, to assess phenomena such as skeletal muscle inflammation, necrosis, hydrostatic edema and fat infiltration, which are strong indicators of disease activity and progression, respectively. Muscle water transverse relaxation time (T2H2O) changes provide relevant information about disease activity, and about muscle physiological status. It is however non-specific as it reflects inflammatory infiltration, myocyte swelling, sarcoplasmic leakiness, cell necrosis, or simply hydrostatic edema. Fat infiltration and replacement in muscles reveal the extent and severity of muscle destruction and loss in chronic conditions. Transverse relaxation time and fat fraction (FF) can be simultaneously measured from a unique standard Multi-Slice Multi-Echo (MSME) acquisition, instead of the usually separate sequences of MSME for T2H2O and Dixon techniques for FF. To do this, a...
bi-component extended phase graph model that takes into account all the instrumental imperfections is used to simultaneously and accurately quantify the muscle water T2 and fat fraction. This methodology was demonstrated to deliver accurate and precise parameter estimations. Furthermore, this simultaneous evaluation of T2H2O and FF also represents a real advantage for whole-body investigations as it allows a decrease in acquisition time and improve patient comfort.

In our study, we aim to further reduce the acquisition time by undersampling a radial MSME sequence. To recover the image, the use of non-linear algorithms that makes use of assumptions (e.g. mostly homogeneous image, locally similar variations in the time domain) is required. The main drawback is that the reconstruction takes longer than in a traditional imaging scheme, but this has no effect on the patient comfort as it is done afterwards. The radial scheme is also more robust to subject motion and using a golden-angle pattern, the acquisition can be stopped at any time and still be usable, if the patient has moved. Using this scheme, we are able to have an even shorter acquisition time while maintaining the precision and accuracy of the T2H2O and FF simultaneous estimation.

**quantitative NMR imaging, fat fraction, water T2, compressed sensing acceleration**

Young Investigator Poster- #2991  
P26- 417- Comparing the mechanical properties of myoblasts and myotubes in physiological and pathological conditions by atomic force microscopy

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The aim of this research is the biophysical study of mechanical properties of muscle cells in order to understand how these properties are altered in certain myopathies. We developed an atomic force microscopy (AFM) method, which provides a quantitative characterization of mechanical response of living adherent cells to a mechanical stress. AFM, originally introduced in the physicist community in the late eighties has been more recently applied to the mechanical characterization of living cells in biology. We coupled AFM indentation experiments with an alternative method of data analysis based on a wavelet tool and nonlinear parametrisation of the AFM indentation data (force curves). From the mechanical characterization of murine myoblasts C2C12, we observed that the mechanical properties of myoblasts are not uniform but vary depending on the probed cell region. Pharmacological ATP depletion and inhibition of myosin II activity via blebbistatin proved that C2C12 myoblast mechanical response changes when actin cytoskeleton dynamics are perturbed. Indeed, the structural study performed by fluorescent labeling of the cytoskeleton has clarified that such energy stress causes a complete reorganization of the actin filament network modifying the rigidity of actin cortex. By inducing the differentiation of myoblasts in myotubes we also characterized how the mechanical parameters evolves during this physiological process. Finally, this AFM-method was applied on human primary cells from patients affected by Duchenne Muscular Dystrophy to quantify the impact of this pathology on the mechanical properties of myoblasts and myotubes.

**Atomic force microscopy, cell mechanics, myoblasts, myotubes, cytoskeleton, Duchenne muscular dystrophy**

Young Investigator Poster- #3008  
P26- 418- Immune response developed against enzyme replacement therapies in lysosomal storage diseases: the difficulty to assess serologic status.

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Fabry disease is a rare lysosomal storage disease (LSD) with a worldwide incidence estimated from 1/40.000 to 1/117.000 live births. Mutations in the GLA gene on the X-chromosome lead to defects in the lysosomal alpha-galactosidase enzyme activity causing diffuse intracellular glycosphingolipids accumulation. Unclear mechanisms lead to the development of diffuse vasculopathy with a wide range of symptoms such as hypertrophic cardiomyopathy, renal failure or strokes that decrease life expectancy. Available since 2001, enzyme replacement therapy (ERT) with either agalsidase alfa (Replagal, Shire) or beta (Fabrazyme, Genzyme) have revolutionized the management of the disease. Nevertheless, recent reports conclude that ERT would only allow mild benefits. One of the causes of these mild effects could be the development of an immune response against ERT. Humoral response with anti-agalsidase antibodies (Abs) are described in Fabry disease but their clinical impact remains unclear. To date serological status can only be assessed by pharmaceutical industries as no independent assay exists. Therefore we aimed to develop an independent tool to assess the serological status and clarify the role of such Abs.

We built up an independent cohort of French treated and untreated Fabry patients and collected blood samples from 43 individuals. We've developed an ELISA assay to identify anti-agalsidase Abs by testing sera samples from patients on agalsidase (alfa or beta) coated-plates. Despite multiple coating, blocking, washing and buffers recipes, we couldn't get out of important background making results aspecific. We then developed an immuno-dot assay that was tested on 29 patients and revealed 3 positive sera (2 reactive against both agalsidase alfa and beta, 1 against agalsidase beta only). Among patients who received agalsidase alfa, seropositivity was 1/17 (5.8%) whereas it was 3/8 (37.5%) among patients who received agalsidase beta. Of notes 3 sera showed doubtful results (2 against both enzyme, 1 against agalsidase alfa) but 2 of these 3 patients haven't received ERT.

These results showed a less frequent seroprevalence among treated patients than what is usually considered in literature. The high background observed in ELISA was already mentioned by industries in their phase 3 trials and highlights the difficulty to determine humoral response with reliable techniques and clearly identify its impact.

**lysosomal storage disease, cardiomyopathy, enzyme replacement therapy, immune response, Fabry disease**
P26- 419- Interplay between chromatin topology and regulation of alternative splicing by DDX5 and DDX17 during myogenesis
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The goal of this project is to better understand the interplay between genome architecture and alternative splicing in the context of myogenesis.

Our team has previously identified two new regulators of myogenesis, namely the two paralog RNA helicases DDX5 and DDX17, and have demonstrated that these proteins coordinate key aspects of gene expression during myoblast differentiation. For example, their miR-1/206-induced downregulation during differentiation contributes to establish a muscle-specific splicing program (Dardenne E. et al. Cell Reports 2014). It also was shown that DDX5 functionally interacts with MBNL1, a major splicing regulator of which altered function is the main cause of myotonic dystrophy (Laurent F.-X. et al. Nucleic Acids Res. 2012).

Interestingly, DDX5/17 interact with several regulators of 3-dimensional chromatin architecture such as CTCF and Cohesin that are involved in gene insulation (Yao, H. et al. Genes Dev. 2010), Condensin (preliminary data) and Matrin-3 (Fujita T et al. PloS One 2011) that are involved in DNA topology and looping. This suggests a role for DDX5/17 in chromatin-genome architecture. Preliminary results from our team indicate that depletion of those partners impact on alternative splicing of exons regulated by DDX5/17, suggesting an interconnection between chromatin architecture and alternative splicing that could be mediated by DDX5/17.

My PhD project will address for the first time the link between chromatin architecture and alternative splicing in human models of muscle differentiation. This question is important since abnormalities of chromatin and splicing are common in muscle diseases, although no clear connection has been established so far between these two levels of regulation.

alternative splicing, chromatin, RNA helicases, myogenesis

P26- 420- Continuous home recording of non-ambulant patients with Duchenne Muscular Dystrophy using magneto-inertial technology
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Continuous recording at home in neuromuscular non-ambulant patients is a clinical necessity raised by clinicians to adapt the needs in terms of rehabilitation, but also as part of research and development of outcome measures. Based on a magneto-inertial technology, ActiMyo, is an innovant system that assess the patients’ activity in their daily life. Using a dedicated software to process data, ActiMyo precisely detects and characterizes through computed variables specific movements of the upper limbs of the patients, their phases of immobility and the use of the wheelchair.

The present study is part of a proof of concept protocol (PreActi).

Seven non-ambulant patients with Duchenne muscular dystrophy were recorded for 2 weeks in their daily lives wearing ActiMyo at the wrist. On average, ActiMyo was worn during 8.7 hours per day with a good tolerability. Patients with a Brooke score of less than 4 had an upper limb motor activity for more than 30% of the time; very weak patients (Brooke score of 6) had a poor upper limb motor activity (between 10 to 18% of the time). For the very weak patients (Brooke score superior to 5), there was no upper limb movements, nor wheelchair movement for 61 to 87% of the recorded time.

Variables were determined to appropriately characterize the continuous activity of patients. Durations of upper limb activity phases were correlated with the following variables: parameter of angular velocity (?r2 = 0.91), the parameter for elevation rate d7 (r = 0.94) and an estimate of the power developed to move the forearm P (r = 0.92). These same variables were also correlated with the patient's immobility phases durations: ? (r2 = 0.82), d7 (r2 = 0.77) and P(r2 = 0.80).

ActiMyo is an innovative tool for characterizing different activities of a non-ambulant patient in daily life. Continuous home recording allows better follow-up of the neuromuscular patient and at the same time, can help to evaluate changes if a new therapy is administrated.

actimetry, Duchenne Muscular Dystrophy, monitoring

P26- 421- Does the Myosin heavy chain central enhancer organize the (3D) chromatin structure of the locus ?
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Adult skeletal muscles are composed of slow and fast myofiber subtypes. Fast twitch myofibers are classified in 3 types following their properties and myosin heavy chain (Myh IIA, IIX or IIB) expression. Myh are the primary determinant of muscle contraction efficiency. We currently do not know how the spatio-temporal expression of the fast Myh genes is controlled. Six Homeoproteins promote the genesis (during embryogenesis) and phenotype (in adult) of fast-type myofibers in mouse. Previous work has identified an enhancer at the fast Myh locus, and shown that Six homeoproteins bind to it to positively regulate the expression of the fast Myh genes. The identified enhancer may act as a locus control region (LCR) to direct and restrict the transcription of each fast Myh during development and in the adult.

We obtained transgenic mice carrying a 250kb BAC of the Myh locus including the enhancer and Myh2, Myh1 and Myh4 recombinated genes respectively with the cDNAs of YFP at the ATG of Myh2, of Tomato at the ATG of Myh1 and CFP at the ATG of Myh4.
of Myh4. Efficient YFP, Tomato and CFP staining in several adult muscles was observed. We are testing if the transgenes recapitulate the specific interactions and expression observed for the endogenous locus. Our goal is to study the links between Six proteins, higher order chromatin conformation, in adult myofiber specialization and vulnerability. Preliminary data will be presented.

Six1, Muscle, Myosin, LCR

Young Investigator Poster- #3288


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The whole research project takes place in the context of evaluating the functional motor abilities of a person affected by a neuromuscular disease (NMD), with the Motor Function Measure (MFM) quantitative scale. The final aim is to propose more low-cost and easy-to-use technology precise and reproducible tools to be used as outcome measure in clinical trial. In our context, tools are defined as the combinations of technological facilities and their software. Consequences will be to avoid the hazardous characteristic of the human evaluation and increase patients' compliance for the evaluation.

Authors propose to implement the Microsoft Kinect, which presents interesting measurement advantages in the context described: inexpensive, non-invasive, non-intrusive and allowing rapid data collection. The first step was to develop a software named KinectLAB that allow to capture the Kinect digital skeleton and particularly anatomical joints of a patient. Then, experiments were proposed to evaluate if the Kinect with KinectLAB software can be used to obtain a digital skeleton even in the very specific and restrictive conditions of use imposed by users in wheelchairs and clinical services facilities. The results shown that KinectLAB was able to detect one user for three hundred test in manual/electric wheelchair and limited space. Moreover, segmental length measurement dispersions of the digital skeleton are low and stable for a user posture with outstretched arms. Finally, experiments were proposed to adjust the KinectLAB computing algorithm to measure the activity limitation in this research context. The first experiments made on 5 items from the MFM with 3 volunteers' patients (2 with muscular dystrophy and 1 with congenital myopathy) showed that the score generated by our computing algorithm and the practitioners' evaluation score are exactly the same.

Works are in progress specifically on Spinal Muscular Atrophy patients to develop a usable tool in clinical trials.

Neuromuscular Disease, Functional Motor, Kinect, Digital Skeleton, Wheelchair

Young Investigator Poster- #3645

P26- 423- Impaired interneurons activity lead to motoneurons hyper-excitability in ALS patients

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Amyotrophic lateral sclerosis (ALS) is an adulthood neurodegenerative disease characterized by loss of motor neurons (pyramidal cells & motoneurons), leading to atrophy, paralysis and finally death. Recently, we have shown in ALS patients by non-invasive electrophysiology techniques that reduced somatosensory potential from muscle clinically affected (intrinsic hand muscles) leads to motoneurons hyper-excitability of muscle clinically unaffected (triceps brachial). While corticospinal volley from primary motor area onto motoneurons were not deleterious, these results bring the possibility of interneurons involvement in the modification of motoneuron's intrinsic properties. Thus, we investigated electrophysiologically some excitatory (propriospinal system) and inhibitory (inhibitory interneuron of the propriospinal system, Ib interneuron, Renshaw cell) interneurons at upper and lower limb of ALS patients. Preliminary results suggest that motoneuron's hyper-excitability is due to a higher excitatory activity associate with a decrease of inhibitory activity of these interneurons.

ALS, human, non-invasive electrophysiology

Young Investigator Poster- #2426 – Oral Presentation

P26- 424- Study of physio-pathological mechanisms implicated in sarcoglycanopathies and design of new pharmacological approaches.

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Limb Girdle Muscular Dystrophies are rare genetic diseases, characterized by weakness and progressive muscular atrophy. A subfamily of LGMD2 regroups sarcoglycanopathies caused by mutations in genes coding for α/β/γ or δ sarcoglycans (SG). These transmembrane proteins are part of the dystrophin complex which is an important link between the actin cytoskeleton, the sarcosome and the extracellular matrix that protects muscle fibers against mechanical stress due to contraction. There is no treatment available for these diseases.

More than 65% of mutations in SG genes are missense. One of the most frequent mutations is R77C in α-SG gene. This mutation accounts for up to one-third of all mutations in European populations except in Finland where it is found in every LGMD2D patients often in both alleles.
Previous studies demonstrated that SG missense mutants are not present at the muscle fiber membrane because they are retained in the endoplasmic reticulum by the quality control (ERQC) and they are prematurely degraded by the proteasome.

In order to understand the mechanisms implicated in sarcoglycanopathies and to identify new therapeutic targets, we decided (i) to study, at the molecular level, the ERQC pathways responsible for sarcoglycan disposal and susceptible of pharmacological interference and (ii) to test small molecules modulating ERQC pathways and promoting rescue of disease related mutations. We first generated in vitro and in vivo models to investigate the SGs cellular trafficking mechanisms. Cell lines expressing one α-SG mutant were used to test approved drugs selected towards their known action of the ERQC. We identified promising pharmacological compounds promoting α-SG mutants membrane localization. We are now developing a cytometry protocol using Imagestream technology in order to quantify the effect of the molecules on the α-SG mutants retargeting.

In parallel, we will take advantage of these in vitro and in vivo models to screen small molecules libraries for compounds being able to rescue the targeting or the folding of the mutated proteins.

P26- 425- Pre-mRNA trapping in PABPN1 nuclear aggregate induces splicing defect in OPMD
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Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant, late-onset degenerative muscle disorder characterized by progressive eyelid drooping (ptosis), swallowing difficulties (dysphagia) and proximal limb weakness. OPMD is caused by a short trinucleotide repeat expansion in the polyadenylate-binding protein nuclear 1 (PABPN1) gene that results in an N-terminal expanded polyalanine tract. The mutated expanded PABPN1 proteins accumulate as insoluble intranuclear aggregate in muscles of OPMD patients. While the roles of PABPN1 in nuclear polyadenylation and regulation of alternative poly(A) site choice are established, the molecular mechanisms behind OPMD and the exact contribution of these aggregate remain undetermined. Using exon array, we identified splicing defects in OPMD. We demonstrated a defect in the splicing regulation of the Troponin T3 mRNA in OPMD muscle samples. This splicing defect was directly linked to SRSF2 splicing factor and to the presence of nuclear aggregates. As reported here, these aggregate are able to trap Troponin T3 pre-mRNA, driving it outside of nuclear speckles leading to an altered SRSF2-mediated splicing. This resulted in a decreased calcium sensitivity of muscle fibers, which could in turn play a role in muscle pathology. Alternative splicing defects have previously been observed in other trinucleotide repeat disorders including myotonic dystrophy. This is the first time that a splicing defect is identified in OPMD. In spliceopathies described so far, alternative splicing misregulation is in most cases due to an abnormal activity of an RNA splicing factor. Here in OPMD we describe a different mechanism directly linked to the presence of aggregates: the splicing defect is due to the spatial separation of the deregulated RNA from its splicing factor. Although the generic nature of this alternative mechanism remains to be demonstrated, it may play a role in various other diseases with nuclear inclusions or foci, inducing physiological consequences as shown here in OPMD.

Young Investigator Poster- #2860- Oral Presentation
P26- 428- Bcl11b/CTIP2, a transcriptional repressor : its role in transcriptionnal regulation of cardiac hypertrophy
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Diverse forms of cardiomyopathies, result in cardiac compensatory remodelling that may progress to ventricular dilation and heart failure. This remodeling process is characterised by an increase in the size of cardiomyocytes associated with elevated rates of RNA synthesis, the activation of a foetal cardiac gene program and concomitant
repression of corresponding adult cardiac genes that regulate cardiac contractility. It has been shown that Bcl11b, a C2H2 zinc finger protein, interacts with the RNApolymerasell regulatory complex pTefb and exert an inhibitory action on it. Microarray data comparisons showed that Bcl11b could modulate the expression of genes during cardiac hypertrophy. Furthermore, a genetic study revealed that common genetic variations in a locus harboring this gene is associated with increased risk for cardiovascular disease making it relevant to study the role of Bcl11b in the heart under normal and pathological conditions. Our aim is to determine the role of bcl11b in regulating transcription during cardiac hypertrophy. Through the use of various techniques (Western Blot, immunoprecipitation), we were first able to characterize a post-translational modification (SUMOylation) of Bcl11b in the context of cardiac hypertrophy. This SUMOylation was specific to the hypertrophic state of the heart both under physiological and pathological conditions. In addition, using the chromatin immunoprecipitation (ChiP) technique on cardiac tissue, the binding of Bcl11b on the promoters of genes involved in cardiac hypertrophy was studied (βMHC, skeletal and cardiac actin, ANF). An enrichment of Bcl11b recruitment at the level of βMHC, skeletal and cardiac actin promoters was observed in mice with induced hypertrophy (phenylephrin osmotic mini-pump treatment) as compared to control. This enrichment was correlated with an increase in the expression of the βMHC and skeletal actin genes and anti-correlated with the expression of the cardiac actin gene suggesting a dual role for Bcl11b (activator versus inhibitor). Based on these preliminary data and on the literature, we speculate that the status of Bcl11b (sumoylated versus unsumoylated state) could be implicated in this dual response. Our results will allow gaining further insights into the molecular mechanisms that govern the onset of pathological cardiac hypertrophy and more specifically the role of Bcl11b and its SUMOylation in the regulation of gene expression during cardiac hypertrophy.

SUMOylation, Hypertrophy, Cardiac remodeling

Young Investigator Poster- #2884- Oral Presentation

**P26- 427- Dynamics of triad organization**

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Excitation-contraction coupling relies on spatial organization of triad membranes and on the precise localization of proteins of the Calcium Release Complex (CRC) in these membranes. Sarcoplasmic Reticulum (SR) proteins of the CRC are exclusively found in terminal cisternae (TC) of the reticulum, but the mechanisms responsible for their traffic and retention in this compartment are poorly defined. Among CRC proteins, triadin was proposed to act as an anchor for the other reticulum proteins at triads. To investigate the mechanisms leading to protein targeting to triads and to the organization of triad membranes in muscle cells, we have expressed fluorescent chimeras of triadin in differencing primary myotubes from triadin KO mice.

The mobility of GFP-triadin was recorded during cell differentiation. Although fixed at late differentiation stages, SR membranes labeled with GFP-Triadin can undergo short and long distance movements at earlier stages. These movements of SR membranes require intact microtubule cytoskeleton, and may be necessary for triad organization at the A-I junctions. A photoactivatable version of Triadin (PAGFP-Triadin) was used to study the dynamics of limited pools of activated molecules. At both early and late myotube differentiation stages a continuous diffusion of triadin molecules in the SR was revealed. Triadin accumulation in the TC of SR was shown to depend on its C-terminal luminal domain. Overall, our experiments reveal that clusters of triadin move along microtubules during early differentiation stage. These movements could reflect TC formation and organization along the sarcomeres during myotube differentiation. During this process, and also at later differentiation stages when TC have a fixed localization, a continuous diffusion of triadin allows its traffic to the TC. We show that the exclusive localization of Triadin in TC rely, at least in part, on a retention-based mechanism mediated by its C-terminal part.

Triads, Photoactivation, Traffic, Anchorage