animals can limit the assessment of therapeutic interventions. Here, we aimed at worsening the muscular phenotype in 2 month-old DMSXL mice using a forced eccentric exercise protocol to optimize the evaluation of biotherapies. Our results suggest that eccentric exercise can worsen the muscular weakness observed in DMSXL vs. WT, with a significant decrease of their specific maximal force (sPO) in gastrocnemius muscle. This acts independently to body weight gain, muscle weight changes, DMPK mRNA nuclear foci or HE staining histological abnormalities suggesting molecular deregulation pathways. Preliminary isofrom quantification for candidate genes in WT gastrocnemius revealed that the splicing profile depend on state of development and can be affected for LDB3 and MBNL2 mRNA in non exercised DMSXL vs. WT opening to further molecular investigations in exercised DMSXL. We suggest that an eccentric exercise protocol could optimize biotherapy preclinical evaluation in the DMSXL model.

**Myotonic dystrophy, biotherapy, mouse model**

Myotonic dystrophies type 1 (DM1) and type 2 (DM2, PROMM- #3027).

**P20- 322- Lower-limb Muscle Weakness, Postural Instability, and Gait Abnormalities in Patients with Myotonic Dystrophy Type 1**

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Mechanisms underlying gait and balance impairments in patients with myotonic dystrophy type 1 (DM1) remain incompletely understood. This study aimed to: evaluate gait using lower-trunk accelerometry in twenty two patients with DM1 and twenty healthy controls; investigate potential relationships between muscle strength, postural stability, and gait parameters. Analysis of acceleration patterns of the lower-trunk was performed during a 25-m walking trial at self-selected pace. Participants also underwent a standard 6-min walking test, lower-limb muscle strength assessment, and postural stability assessment. Percentage predicted 6-min walking distance in DM1 correlated with percentage predicted strength of ankle dorsiflexors (/? = 0.72, P > 0.05), plantar flexors (/? = 0.44, P > 0.05), and knee extensors (/? = 0.45, P > 0.05) expressed as percentage of predicted values. Patients had reduced postural stability that was correlated to interstep and interstride regularity in the vertical direction (/? = -0.62 and ? = -0.58; both P > 0.05). At self-selected pace, patients displayed reduced walking speed, stride frequency, step length, gait regularity, and gait symmetry. Patients also exhibited higher lower-trunk acceleration power in the mediolateral direction and greater entropy (i.e. index of signal organization) in all directions. In patients, ankle plantar and dorsiflexors strength correlated interstride regularity in the vertical direction (/? = 0.57 and ? = 0.59, respectively; both P > 0.05).

No significant correlation was found between gait parameters and percentage predicted strength of hip flexors. These findings highlight the important contribution of distal muscle weakness to gait alterations in patients with DM1. In addition, impaired postural regulation may also contribute to gait impairments. Systematic gait analysis might provide a sensitive marker and offer an additional endpoint. Longitudinal follow-up of patients with DM1 is ongoing to investigate the progression of gait abnormalities in conjunction with changes in functional capacities, muscle strength, and postural control. To conclude, this study provides novel insights regarding balance and gait impairments in patients with DM1 that could be valuable for characterization of patients and optimization of therapeutic strategies.

*myotonic dystrophy; gait; balance; muscle; weakness; strength; accelerometer.*

**P21 – Nuclear envelopopathies (lamin A/C, emerin, others)- N° 323 to N° 330**

Nuclear envelopopathies (lamin A/C, emerin, others)- #2320.

**P21- 323- Muscular dystrophy-associated mutations in sun1 and sun2 impair myonuclear positioning through defective nuclear-microtubule connection**

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Emery-Dreifuss muscular dystrophy (EDMD) is a genetically heterogeneous disorder involving progressive muscle wasting and weakness, tendon contractures and cardiac conduction defects. EDMD has been linked to mutations in several genes encoding proteins of the nuclear envelope (NE), most commonly lamin A/C and emerin.

We recently identified SUN1 and SUN2 as novel EDMD-associated genes. SUN1 and SUN2, together with nesprins, form the LINC complex, which spans the nuclear envelope (NE) and connects the nucleus to the cytoskeleton. The SUN proteins, which reside at the inner nuclear membrane, connect the complex to the nuclear lamina and chromatin. In turn, the nesprins reside in the outer nuclear membrane and form direct connections with cytoskeletal filaments. A major role of this connection is to facilitate nuclear positioning within the cell. This is particularly important in muscle, where the multiple myonuclei are regularly positioned along the length of the myocyte, just below the sarcolemma. Myonuclear positioning is controlled by the microtubule (MT) network and MT nucleating proteins relocate from the centrosome to the NE early in myogenesis. However, the precise molecular connections involved are poorly understood.

In cultured myotubes from a patient with compound heterozygous SUN1 mutations, we observed grossly abnormal myonuclear clustering, suggesting a defect in LINC complex connection with the MT network. In support of this, we observed a defect in recruitment of the centrosomal protein, pericentrin, to the NE and a failure of MT nucleation from the NE. We have used various approaches to investigate the nature of these connections and found that SUN1 and SUN2 act redundantly to recruit pericentrin and MT motor proteins to the NE and that this recruitment is specifically mediated by nesprin-1.
We conclude that the LINC complex plays a major role in controlling myonuclear positioning through SUN1/SUN2-nesprin-1 complexes that recruit MT-associated proteins to the NE. Mispositioned myonuclei are a hallmark of various myopathies and evidence indicates that correct myonuclear positioning is necessary for efficient muscle function. Thus, defects in myonuclear positioning, resulting from mutations in LINC complex components, are likely to play a significant role in EDMD pathophysiology.

EDMD, nuclear envelope, LINC complex, nuclear-cytoskeletal connection

Nuclear envelopathies (lamin A/C, emerin, others) - #2403

P21-324- Zmpste24 deficiency hampers skeletal muscle force production by disrupting myonuclear anchorage and function

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ZMPSTE24 is a zinc metalloprotease that promotes the maturation of prelamin A to lamin A, a crucial structural protein of the nuclear envelope. Human nonsense mutations in ZMPSTE24 therefore lead to lamin A depletion and prelamin A accumulation in the nucleus. Such mutations are associated with multiple diseases and skeletal muscle wasting and weakness in humans. The aim of the present study was to use a combination of single fibre mechanics and confocal microscopy to probe the mechanisms underlying the weakness of muscle displaying this phenotype. Isolated skeletal muscle fibres from mice lacking Zmpste24 (Zmpste24-/-) were compared with fibres from wild type animals. Force-generating capacity, as well as nuclear functional organisation and anchorage were measured. We observed: i) a decrease in maximal force production in chemically skinned Zmpste24-/- fibres; ii) a reduced nucleo-skeleton coupling; and iii) an aberrant myonuclear domain size in Zmpste24-/- fibres compared to wild type. Taken together, these findings suggest that the absence of lamin A, or accumulation of prelamin A, would modify myonuclear mechano-sensing and alter regulation of the factors driving contractile protein content and thus force generation.

ZMPSTE24, nucleus, skeletal muscle, force

Nuclear envelopathies (lamin A/C, emerin, others) - #2468

P21-325- The mutant lamin A p.R388P responsible for congenital myopathy accumulates abnormally throughout the nucleoplasm and triggers severe nuclear dysmophy in myoblasts.

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The nuclear intermediate filament proteins lamin A and lamin C (A-type lamins) are encoded by the LMNA gene. Hundreds of LMNA mutations are responsible for several laminopathies, including muscular dystrophies, lipodystrophies, and premature ageing syndromes. The heterozygous LMNA substitution R388P identified in a young patient is responsible for a mixed clinical phenotype, including a precocious muscular deficit together with cardiac rhythm defects and an abnormal repartition of adipose tissue (reported in the 13th International congress on neuromuscular diseases, 2014). Here we investigated the impact of overexpressing FLAG tagged R388P lamin A in proliferating myoblasts (mouse C2C12 cells). Biochemical analysis reveals the increased solubility of the mutant lamin A versus wild-type lamin A upon 0.5% Triton extraction. Accordingly, FLAG-LA R388P does not integrate properly at the nuclear envelope, but accumulates throughout the nucleoplasm (~85 % of the cells) and increases both the frequency (~60 % of cells) and the severity of nuclear dysmophy. Forced accumulation of R388P mature lamin A had a similar impact on nuclear dysmophy. In addition, impact of the cytoskeleton network on nuclear dysmophy was investigated by staining cells for desmin and alpha tubulin (in the absence or presence of the microtubule destabilizing drug nocodazole). Results show that the absence of the external force exerted on the nuclear envelope by the intermediate cytoskeletal filaments and/or the microtubules is not sufficient to rescue nuclear shape. Altogether, our data suggest that the mechanisms underlying nuclear dysmophy observed in myoblasts expressing muscular dystrophy-linked R388P mutant lamin A are distinct to what was recently reported (Larrieu et al. Science 344, 2014) in response to either the absence of lamin A (siRNA) or the expression of the mutant progerin responsible for Hutchinson-Gilford progeria. lamin A, muscular dystrophy, nuclear dysmophy

Nuclear envelopathies (lamin A/C, emerin, others) - #2489

P21-326- Elevated TGFbeta 2 levels in Emery-Dreifuss muscular dystrophy affect myocyte and tenocyte phenotype and favor the fibrogenic process

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Emery-Dreifuss Muscular Dystrophy and Dilated Cardiomyopathy with conduction system disorders are rare muscular diseases associated with LMNA mutations. We have previously observed altered TGFbeta 2 secretion in cells and serum from Mandibuloacral Dysplasia, a rare progeroid laminopathy. Here, we performed a study in a cohort of laminopathic patients affected by muscular laminopathies to investigate serum levels of TGFbeta 2, secretion of TGFbeta 2 in cultured fibroblasts and myoblasts and establish a correlation between phenotype and cytokine amount. Multiplex cytokine assay showed that TGFbeta
2 is consistently elevated in the vast majority of Emery-Dreifuss muscular dystrophy sera (Figure 1), while other cytokines including IL17, IL6 and bFGF are altered in subgroups of patients. Here, we analyzed the effect of laminopathic sera and conditioned media on tenocyte and myoblast proliferation and activation of pro-fibrotic genes as well as on cell differentiation. Both patient serum and Emery-Dreifuss fibroblast-conditioned media activated a fibrogenic program both in myoblasts and in tenocytes and inhibited myoblast and tenocyte differentiation. The effect was dependent on TGFbeta 2 and could be reverted by using a specific neutralizing antibody. These data show that modulation of TGFbeta 2 secretion and activity is a promising therapeutic approach for muscular laminopathies.

Emery-dreifuss Muscular Dystrophy, lamin A/C, TGFbeta 2, muscular dystrophy, fibrosis.

Nuclear envelopathies (lamin A/C, emerin, others)- #2514

P21- 327- Human iPS cell-based platforms for disease modelling and therapy screening for laminopathies
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Lamins assemble into the nuclear lamina, providing structural support and regulating gene expression. Mutations in the LMNA gene encoding lamin A/C cause a plethora of diseases called ‘laminopathies’. Challenging genotype-phenotype correlations and scarcity of samples limit understanding physiopathology and development of therapies. To overcome this hurdle, we have reprogrammed readily accessible patient somatic cells carrying LMNA mutations to induced pluripotent stem cells. These cells can be differentiated towards specific lineages (e.g. skeletal and cardiac muscle) to examine the effects of mutations on cell differentiation.

As well acting as a disease model, LMNA iPS cells and their derivatives will also provide a platform to test therapies for laminopathies. Recently within our laboratories, we have developed a novel therapeutic strategy for laminopathies based upon antisense oligonucleotide-mediated exon skipping of LMNA exon 5 (Scharner et al., 2015). This could be used as a potential therapy for patients with mutations within this exon. The therapeutic potential of LMNA exon 5 skipping will be further assessed in patient-derived induced pluripotent cells and their derivatives.

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iPS cells, exon skipping, disease modelling, myogenic differentiation, gene therapy

Nuclear envelopathies (lamin A/C, emerin, others)- #2844

P21- 328- Altered trafficking of connexin 43 participates to the development of ventricular arrhythmias in cardiomyopathy caused by mutations in A-type lamins gene
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Compared with other forms of dilated cardiomyopathy, mutations in LMNA encoding nuclear A-type lamins are responsible for a more aggressive clinical course due to a high rate of malignant ventricular arrhythmias. A better understanding of factors and mechanisms that drive ventricular arrhythmias is crucial to generation of potential therapies. Inter-cellular communication is
essential for proper cardiac function. Mechanical and electrical activities must synchronize so that the work of individual cardiomyocytes transforms into the pumping function of the heart. Gap junctions are specialized cell-cell junctions that mediate inter-cellular communication. They are composed of connexin proteins, which form transmembrane channels for small molecules. Here, we showed that altered distribution of connexin 43 occurs prior to any electrical disturbances in a mouse model of dilated cardiomyopathy due to LMNA mutations. We next assessed in vitro the molecular mechanisms of connexin 43 re-localization in pathological context. We showed that the presence of LMNA mutations triggers an abnormal trafficking of connexin 43 along both microtubules and actin networks leading to a loss of cell-cell communication. Going further, we demonstrated that modulating this process could restore the correct localization and function of connexin 43 at the cell-cell junction in cardiomyocytes carrying LMNA mutation. Our work could break new ground for future work towards developing novel treatment for malignant arrhythmias.

LMNA, A-type lamins, arrhythmias, dilated cardiomyopathy, connexin 43

P21 – Pharmacological therapies- #2845

P21- 329- Defects in actin polymerization participate in cardiac contractility in Emery-Dreifuss muscular dystrophy
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Mutations in the lamin A/C gene (LMNA), encoding nuclear envelope proteins, cause autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD), by mechanisms that remain incompletely understood. We previously discovered abnormally stress-activated elevated extracellular signal-regulated kinase 1/2 (ERK1/2) activities in heart in EDMD. This is a cornerstone in the development of heart disease in this muscular dystrophy. However, the understanding of molecular and cellular mechanisms underlying the modulation of ERK1/2 signaling in the heart caused by LMNA mutation remains totally unclear. We here showed that there is an aberrant cytoplasmic localization of active (phosphorylated) form of ERK1/2 in cellular and animal models of EDMD. We next identified a novel interaction between cytoplasmic p-ERK1/2 and coflin-1, an actin-depolymerizing protein, which in turn phosphorylates and activates coflin-1 on a previously un-described phosphorylation site. This event triggers alteration of actin dynamics in both cellular and murin models of EDM. These events could be blunted with the overexpression of a mutated coflin-1 (phospho dead mutant). These findings unravel a novel role played by ERK1/2 signaling in actin dynamics that provide a novel insight into the disease etiology for the cardiac phenotype in EDMD and lay the groundwork for new therapeutic strategies.

LMNA, A-type lamins, dilated cardiomyopathy, actin, coflin

P21 – Pharmacological therapies- #3038

P21- 330- FHL1B, a protein involved in Emery-Dreifuss muscular dystrophy, is a nuclear envelope protein.
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Mutations in the EMD and LMNA genes encoding the two nuclear envelope (NE) proteins emerin and lamin A/C are associated with the hereditary muscular disease Emery-Dreifuss muscular dystrophy (EDMD). Our group described the first mutations in the Four-and-a-Half LIM domain 1 (FHL1) gene to be responsible for EDMD. FHL1A and its spliced variants FHL1B and FHL1C have been so far reported to belong to the non-nuclear envelope proteins. To try to better understand the role of FHL1 protein isoforms in humans, we studied the precise expression and localization of the FHL1 isoforms during primary human myoblast differentiation.

We showed that in human control myoblasts, from the three FHL1 isoforms, only FHL1B displayed a clear accumulation at the inner nuclear membrane (INM), co-localized with lamin A/C and emerin. Differentiation of myoblasts into mature myotubes caused a progressive decrease of FHL1B from the nucleus which is not due to its nuclear export, but is rather due to a drop in its expression, probably reflecting the temporal role of FHL1B in myoblast differentiation. Interestingly, we found the expression of FHL1B to be affected in myoblasts of a patient with FHL1-associated EDMD that we previously reported to have differentiation defects. FHL1B localization at the INM is not affected in myoblasts of patients carrying mutations in NE proteins (emerin, lamin A/C and nesprin-1) and in human myoblasts with lamin A/C knock-down, leading to the conclusion that FHL1B localization at the INM is independent of emerin or lamin A/C. However, we found an upregulation of FHL1B expression in a patient carrying a heterozygous mutation in LMNA.

Collectively, we showed for the first time that FHL1B is an INM protein in myoblasts, like emerin and lamin A/C, the two other proteins involved in EDMD, and that its expression level probably needs to be decreased for myoblast differentiation to occur. Altogether, we recommend that future studies in the attempt to understand the underlying mechanism of FHL1-related EDMD should target each FHL1 protein isoform separately.

FHL1, FHL1B, Lamin A/C, Emerin, EDMD, Nuclear Envelope

P22 – Pharmacological therapies- N° 331 to N° 337

Pharmacological therapy of neuromuscular disease- #2450

P22- 331- Effects of Fish-oil supplementation on glucocorticoid receptor phosphorylation during dexamethasone-induced muscle atrophy.
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