12th Japanese-French Workshop

New insights in personalized medicine for neuromuscular diseases: From Basic to Applied Myology

September 9th – 10th, 2022

Musée des Impressionnismes, Giverny, France.

CENTRE DE RECHERCHE EN MYOLOGIE

MÉDECINE SORBONNE UNIVERSITÉ

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National Center of Neurology and Psychiatry

Welcome Note

The 12th Japanese-French Workshop will be held at the *Musée des Impressionnismes* at Giverny, France on September 9th to 10th, 2022. We hope all participants will enjoy the meeting and will exchange their own ideas with each other.

The conference theme this year is “New insights in personalized medicine for neuromuscular diseases: From Basic to Applied Myology”. Hereditary neuromuscular diseases, including muscular dystrophies, consist of a group of many rare diseases. Elucidation of the etiology and pathophysiology of these diseases would open a new avenue for the development of therapeutic approaches, which remains the greatest challenge of the 21st century. Since the first Japanese-French workshop in 1996, Japanese and French researchers have made a significant contribution in this research field. Accordingly, primary objective of this meeting is to identify the genes that cause inherited neuromuscular diseases. In approximately 40% of hereditary neuromuscular diseases, the causative genes have not been identified. The secondary study objective is to elucidate the pathophysiology of diseases and determine the most appropriate therapeutic approaches.

Since 1996, researchers in this research field of Japan and France have met each other at intervals of two or three years to discuss hereditary neuromuscular diseases with a focus on muscular dystrophy and to facilitate exchanges of information. This process, which was covered in academic journals (Acta Myologica 2005; Neuromuscular Disorders 2006 and others), has an illustrious history of providing the first opportunity to present new findings or for the initiation of joint studies, as well as prefacing exchanges by young researchers from both countries.

In summary, this workshop will facilitate the exchanges of your ideas and the initiation of joint projects between both countries and hopefully lead to the development and promotion of collaborative research and fruitful outcomes.

Gisèle Bonne
Institut de Myologie
Ichizo Nishino
National Center of Neurology and Psychiatry

The head of organizing committee
The 12th Japanese-French Workshop

General Information

Meeting Location

Musée des Impressionnismes

99 rue Claude Monet

27620 Giverny

(https://www.mdig.fr/)

Accommodation - Hôtel

Hôtel Le Normandy

1 avenue Pierre Mendes

27200 Vernon

(https://normandy-hotel.fr/)

Travel - Transportation

From Paris CDG Airport

Shuttle bus from Paris CDG Airport to the Hotel in Vernon is scheduled **at 17h00** with depart from **Terminal 2E** (Bus will wait for passengers of the flight which arrives at 16h45).

From and To Paris City Center:

Train tickets and shuttle bus have been bought for the following journeys:

On Friday September 9th, 2022

Paris Saint Lazare Train Station > Vernon: Train TER 13107 at **8h14,** arrival at Vernon at **9h05**

Shuttle bus from Vernon train station to the Meeting Venue in Giverny

On Saturday September 10th, 2022

Shuttle bus from meeting venue to Vernon train station scheduled **at 17h00**

Vernon Train station > Paris Saint Lazare: Train TER 13172 at **17h56**, arrival at Paris Saint Lazard at **18h48**

From Hotel to Meeting Venue

Shuttle bus between the hotel and the meeting venue in Giverny is organized:

On Friday September 9th: Departure from Meeting venue to the Hotel **at 22h30**

On Saturday September 10th: Departure from the Hotel to the Meeting venue (via the Vernon train station) **at 8h30**

Scientific Program

12th Japanese-French Workshop

"New insights in personalized medicine for neuromuscular diseases: From Basic to Applied Myology"

September 9th – 10th, 2022 – Musée des Impressionnismes, Giverny, France.

Time slots of 20 min: 15 min max talk + 5 min discussion

Friday, September 9th

9:30 **Welcome address:** Ichizo Nishino and Gisèle Bonne

9:45 **Session 1. Clinical & Molecular Deciphering of Neuromuscular Diseases**

9:45 **Satoru Noguchi (NCNP)** Gene hunting in myofibrillar myopathy

10:05 **Rocio Nur Villar-Quiles (Institut de Myologie)** *LAMA2*-related myopathy: brain imaging as a key tool in the diagnosis of LGMD

10:25 **Tetsuya Takeda (Okayama University)** Reconstitution approaches to elucidate pathogenesis of congenital myopathy caused by defective membrane remodeling

10:45 **Coffee break.**

11:05 **Johann Böhm (IGBMC, Illkirch)** Severe *ACTA1*-related nemaline myopathy - intranuclear rods, cytoplasmic bodies, and enlarged perinuclear space as characteristic pathological features on muscle biopsies

11:25 **Session 2. Muscle Homeostasis in Health & Diseases**

11:25 **So-Ichiro Fukada (Osaka University)** New insights in muscle hypertrophy and muscle regeneration

11:45 **Shahragim Tajbakhsh (Pasteur Institute)** Perturbations in muscle stem and niche cell dynamics in pathologies

12:05 **Lunch break**

13h30 **Visit of Claude Monet House & Garden**

15.00 **Norio Motohashi (NCNP)** Clarification of functional differences in satellite cells from different fiber types.

15:20 **Akiyoshi Uezumi (Tokyo Metropolitan Institute of Gerontology)** Mechanisms coupling muscle regeneration and resolution of inflammation.

15:40 **Laura Muraine (Institut de Myologie)** Muscle fibrosis: a vicious circle between human fibroadipogenic progenitors and muscle fibers.

16:00 **Shinichiro Hayashi (NCNP)** Understanding DMD transcriptional networks using single-nuclei RNA-seq

16:20 **Lorenzo Giordani (Institut de Myologie)** Multimodal Single Cell profiling of Duchenne Muscular Dystrophy

16:40 **Coffee break**

17:00 **Session 3. Pathophysiology of Neuromuscular Disorders**

17:00 **Kunihiro Tsuchida (Fujita Health University)** Posttranslational protein modification and pathophysiology of muscular diseases

17:20 **Saline Jabre (Institut de Myologie)** Mechanical stretch impacts chromatin organization: implications in laminopathies.

17:40 **Tatsushi Toda (The University of Tokyo)** Recent Advance in Fukuyama Muscular Dystrophy and Dystroglycanopathies

18h00-19h30 Visite musée des impressionnismes et ses jardins

Diner > 19h30 at Brasserie des Artistes at the Meeting venue

Saturday, September 10th

9:00 **Session 3 (cont'd).**

9.00 **Ichizo Nishino (NCNP)** Inflammatory myopathy

9:20 **Julian Dal Cin (Institut de Myologie)** Description of macrophages in idiopathic inflammatory myopathies using in-situ RNAseq

9:40 **Hiroyuki Ishiura (The University of Tokyo)** Expanded CGG repeats in oculopharyngodistal myopathy and related disorders

10:00 **Mario Gomes Pereira (Institut de Myologie)** Glial cell dysfunction in myotonic dystrophy brain disease.

10:30 Coffee break

11:00 **Session 4. Development of Therapeutic Approaches**

11:00 **Denis Furling (Institut de Myologie)** Gene therapy for DM1

11:20 **Hidetoshi Sakurai (Kyoto University, CiRA)** Establishment of protocols for the induction of myogenic progenitor cells from patients-derived iPS cells and development of therapeutic approaches

11:40 **Alexandra Bayer Wildberger (Institut de Myologie**) Cell therapy approaches for Myasthenia gravis: *in vitro* and *in vivo* evaluations of conditioned mesenchymal stem cells

12:00 Lunch break

14:00 **Session 4. (cont'd).**

14.00 **France Piétri-Rouxel (Institut de Myologie**) GDF5 therapeutic potential for Duchenne Muscular Dystrophy gene therapy optimization

14:20 **Yoshitsugu Aoki (NCNP)** Brain Dp140 alters glutamatergic transmission and social behavior in Duchenne muscular dystrophy mouse mod

14:40 **Mariko Okubo (Institut de Myologie**) Gene therapy for Striated muscle Laminopathies

15:00 Coffee break

15:30 Sessions 5. Outcomes measures and Standards of Care

15:30 **Shotaro Tachibana (Institut de Myologie**) Perceptions of key stakeholder groups in Gene Therapy for DMD patients: the place of educational therapy in a new care pathway

16:00 **Harmen Reyngoudt (Institut de Myologie**) Prediction of disease progression in neuromuscular disorders using quantitative MRI and 31P MRS

16:20 **Valérie Decostre (Institut de Myologie**) Relationship between hand strength and function in patients with Duchenne Muscular Dystrophy or Spinal Muscular Atrophy

16:40 General discussion

17: 00 Closing remarks& Departure

Abstracts

Session 1

Clinical & Molecular Deciphering of Neuromuscular Diseases

Gene hunting in myofibrillar myopathy

Satoru Noguchi

Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

The diseases representing the myofibril degeneration with presence of protein aggregation in myofibers are collectively referred to as myofibrillar myopathies. Recent widespread use of next generation sequencing has enabled the identification of causative genes in myofibrillar myopathies. So far, more than 10 genes have been identified as causative genes. However, more than half of the patients remain to be genetically diagnosed in Japan. Recently, we identified a variant in *DNAJB4* in a family with a dominantly inherited distal myopathy, in which affected members have specific features on muscle pathology represented by the presence of cytoplasmic inclusions and the accumulation of desmin, p62, HSP70, predominantly in type 1 fibers. Both Dnajb4-F90L knock-in and knockout mice developed muscle weakness and recapitulated the patient muscle pathology in the soleus muscle, where DNAJB4 has the highest expression. These data indicate that the identified variant is causative resulting in defective chaperone function and selective muscle degeneration in specific muscle fibers. Our results demonstrate the importance of DNAJB4 in skeletal muscle proteostasis by identifying the associated chaperonopathy.

LAMA2-related myopathy: brain imaging as a key tool in the diagnosis of LGMD

Villar-Quiles RN[a,b], Masingue M[a], Romero NB[b,c], Eymard B[a], Metay C[d], Nelson I[b], Bonne G[b], Allamand V, [b] Stojkovic T[a,b]

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LAMA2-related dystrophies' spectrum ranges from severe forms of congenital muscular dystrophy to milder late-onset phenotypes with predominant limb-girdle muscular dystrophy (LGMD), typically associated with white-matter abnormalities which can be asymptomatic. We report here three siblings from a non-consanguineous family presenting with a slowly progressive retractile LGMD phenotype with marked intrafamilial variability. Two of them presented since early-childhood with difficulty running and poor sports performance associated with prominent and diffuse joint contractures and slowly progressive proximal weakness. The third one had only Achilles' contractures in childhood while muscle weakness was noted from the third decade. CK levels were elevated in the three patients (399-1200 U/L) and muscle biopsies from 2 patients revealed dystrophic features. Muscle MRI from one of them revealed atrophy and fatty infiltration mainly affecting the posterior thigh. Fatty replacement of the quadriceps with a *COL6*-like sandwich sign was also remarkable. Whole-exome sequencing revealed two missense *TTN* variants, segregating with the phenotype in this family. Three years later, white matter abnormalities found on brain MRI from patient III.4, and subsequently found in the two remaining patients, allowed to re-orientate the genetic analysis leading to the diagnosis of *LAMA2*-RD. This work highlights the importance of a thorough clinical phenotyping and the performance of brain imaging in the diagnostic workup of limb-girdle muscular dystrophies, in order to accurately address the genetic workup.

Reconstitution approaches to elucidate pathogenesis of congenital myopathy caused by defective membrane remodeling

Kenshiro Fujise[1], Kohji Takei[1], Mariko Okubo[2], Ichizo Nishino[2], Satoru Noguchi[2], and **Tetsuya Takeda[1]**

1. Faculty of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan;
2. National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Kodaira, Tokyo, Japan

Centronuclear myopathy (CNM) is a congenital myopathy characterized by centralized nuclei in skeletal myofibers. T-tubules, sarcolemmal invaginations required for excitation-contraction coupling, are disorganized in the skeletal muscles in CNM patients. Previous studies showed that various endocytic proteins are involved in T-tubule biogenesis and their dysfunction is tightly associated with CNM pathogenesis. *DNM2* and *BIN1* are two causative genes for CNM that encode essential membrane remodelling proteins in endocytosis, dynamin 2 and BIN1, respectively. In this talk, I will overview how the ordered assembly of these membrane remodelling proteins exert their functions in T-tubule biogenesis and discuss how their defective assembly leads to CNM pathogenesis based on our recent studies using reconstitution approaches both *in cellulo* and *in vitro*.

Severe *ACTA1*-related nemaline myopathy - intranuclear rods, cytoplasmic bodies, and enlarged perinuclear space as characteristic pathological features on muscle biopsies

Clémence Labasse[1*], Guy Brochier[1*], Ana-Lia Taratuto[2], Bruno Cadot[3], John Rendu[4,5], Soledad Monges[6], Susana Quijano-Roy[7], Valérie Biancalana[8,9], Mai Thao Bui[1], Anaïs Chanut[1], Angéline Madelaine[1], Emmanuelle Lacène[1], Maud Beuvin[1,3], Helge Amthor[7], Laurent Servais[10,11,], Lionel Van Maldergem[12], Marcela Erro[13], Maria Saccoliti[4], Osorio Abath Neto[9], Béatrice Lannes[14], Vincent Laugel[15], Sandra Coppens[16], Fabiana Lubieniecki[6], Nigel Laing[17], Ana BujBello[18], Teresinha Evangelista[1,3], Jocelyn Laporte[9], **Johann Böhm[9]**, Norma B. Romero[1,3]

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Nemaline myopathies (NM) form a genetically and clinically heterogeneous group of congenital myopathies (CMs) characterized by early-onset hypotonia, muscle and facial weakness, respiratory distress, swallowing difficulties, delayed motor milestones and skeletal deformities. To date, 14 causative NM genes have been identified, and they primarily code for components of the contractile unit, the sarcomere. As a direct consequence of pathogenic changes in the NM genes, muscle biopsies from affected individuals typically show abnormal accumulations of sarcomeric structures known as nemaline rods. Mutations in *ACTA1*, encoding α-actin, account for more than half of the genetically characterized cases with severe NM. Besides classical nemaline rods, histological investigations of muscle sections from ACTA1 patients often reveal other structural anomalies including intranuclear rods, zebra bodies, and focal disorganizations (core-like areas) with or without inclusion of rods. Of note, α-actin has been shown to connect the cytoskeleton with the nuclear envelope through interaction with Nespin-2, and thereby contributes to the control of nuclear plasticity. However, an abnormal shape of the nucleus or the nuclear envelope has never been observed in *ACTA1* patients.

Here, we provide the clinical description of a series of ten unreported patients with severe NM together with a thorough analysis of the muscle morphology and ultrastructure. Molecular investigations detected known or novel *ACTA1* mutations in all affected individuals. Histological and ultrastructural investigations of the muscle biopsies uncovered *ACTA1*-typical anomalies such as clusters of rods, intranuclear rods and cytoplasmic bodies, and we detected hitherto non-reported structure abnormalities affecting the perinuclear space and the neuromuscular junction. Immunocytochemistry demonstrated an abnormal distribution pattern of Lamin-A, Nesprin-1, and Nesprin-2, confirming an impaired nuclear envelope integrity. Overall, this study expands the morphological spectrum of structural abnormalities in NM muscles and indicates a disease signature of *ACTA1*-related severe nemaline myopathy.=

Session 2

Muscle Homeostasis in Health & Diseases

New insights in muscle hypertrophy and muscle regeneration

So-ichiro Fukada

Osaka University

Skeletal muscle has abilities to regenerate and adapt to mechanical loading. Muscle satellite cells play an essential role in the both abilities, because depletion of muscle satellite cells leads to the impaired muscle regeneration and blunted muscle hypertrophy. In addition, orchestrated cellular networks are critical for the efficient proliferation and differentiation of muscle satellite cell. Recently, we reported the indispensable roles of mesenchymal progenitors (also known as FAPs) in muscle satellite proliferation in loaded muscles[1]. However, compared to the regenerative process, less is known about the cellular networks during muscle hypertrophy[2]. In this symposium, we will present our unpublished data indicating the cellular networks during muscle hypertrophy based on scRNA-seq data and interactome analyses of overloaded muscles.

Concerning muscle regeneration processes, since the ability of muscle regeneration decreases with the progression of muscular dystrophy, elucidation of the self-renewal mechanism of muscle satellite cells is one of the most important issues for the treatment of muscular dystrophy. It has been believed that the muscle satellite cell abilities are exhausted by repeated damages in dystrophic conditions, but the exact mechanism is unknown. In this symposium, we share our exciting data indicating that the regenerative ability of muscle satellite cell is dramatically disrupted by pathogenic niche cells. These results suggest that elimination of pathogenic cells or suppression of the disrupted factor might be a therapeutic approach for the treatment of muscular dystrophies.

References

1 Kaneshige, A. *et al.* Relayed signaling between mesenchymal progenitors and muscle stem cells ensures adaptive stem cell response to increased mechanical load. *Cell Stem Cell* **29**, 265-280 e266, doi:10.1016/j.stem.2021.11.003 (2022).

2 Fukada, S. I. & Ito, N. Regulation of muscle hypertrophy: Involvement of the Akt-independent pathway and satellite cells in muscle hypertrophy. *Exp Cell Res* **409**, 112907, doi:10.1016/j.yexcr.2021.112907 (2021).

Perturbations in muscle stem and niche cell dynamics in pathologies

Barbara Gayraud-Morel[1,2], Daniela Di Girolamo[1,2], Thibaud Metais[1,2], Pierre-Henri Commere[3], Sheung Wai[4], Leo Poon[5,6], Roberto Bruzzone[6], Thomas Cheung[4], **Shahragim Tajbakhsh[1,2]**

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Viral infections, such as the flu, induce a host immune response to combat pathogens, which are associated with systemic inflammation. Influenza virus infects primarily the respiratory tract and not skeletal muscle, yet it induces secondary symptoms such as muscle weakness, which has severe consequences in populations with preconditions. Our study focuses on the indirect effects of viral infections on muscle stem cells (MuSCs) exposed to systemic inflammation following intranasal infection of mice.

We show that MuSCs respond to the distal influenza viral infection and alter their properties including a reduced size in vivo. Using EdU pulses in vivo and in vitro to monitor cell proliferation, we show that MuSCs are impaired in their ability to enter the cell cycle. Surprisingly, regulators or markers of quiescence, such as HeyL, Calcitonin receptor, and Pax7 are downregulated, but commitment and differentiation are not induced as shown by the absence of Myod protein. Further, MuSCs from influenza virus infected mice have a reduced metabolism during homeostasis. These alterations result in delayed and compromised muscle regeneration following injury. We propose that MuSCs exposed to virus-induced systemic inflammation adopt a novel quiescent cell state.

Clarification of functional differences in satellite cells from different fiber types

Norio Motohashi

National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP)

Skeletal muscle can be classified into slow- and fast-type muscles, which possess distinct contractile and metabolic properties. Myogenic progenitors associated with each muscle fiber type are known to intrinsically commit to specific muscle fiber lineage during embryonic development. However, it was unclear whether the functionality of postnatal adult myogenic cells is attributable to the muscle fiber type in which they reside, and whether the characteristics of myogenic cells derived from slow- and fast-type fibers can be distinguished at the genetic level. Elucidating the diversity of muscle stem cells will be crucial not only for understanding muscle biology, but also for clarifying the differences in disease severity of skeletal muscle observed in sarcopenia and DMD. Previously, we isolated adult satellite cells from slow and fast muscle separately and found that each type of muscle satellite cell generates myotubes expressing the same myosin heavy chain isoforms as the original muscle. Further, we identified that slow muscle-derived cells are less likely to differentiate but more likely to self-renew compared to the fast muscle-derived cells, and that these are regulated by Tbx1 expression. These data suggested that metabolic and myogenic properties of myogenic progenitor cells vary depending on the type of muscle from which they originate. Additionally, our data along with recent study have shown that myogenic cells derived from different regions of muscle with the same myofiber type were functionally and genetically different, indicating that the diversity of muscle stem cells may be highly dependent on the original tissue from which they are derived. These insights may provide novel therapeutic approaches for muscular diseases.

Mechanisms coupling muscle regeneration and resolution of inflammation

Madoka Ikemoto-Uezumi[1], Tamaki Kurosawa[1], So-Ichiro Fukada[2], Glenda Comai[3], Shahragim Tajbakhsh[3], Andreas Schedl[4], Keisuke Hitachi[5], Kunihiro Tsuchida[5], **Akiyoshi Uezumi**[1]

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Inflammatory response and macrophages are required for successful muscle regeneration. However, inflammation must be resolved, and prolonged inflammation interferes with regeneration and impairs tissue function. How inflammation and regeneration are coordinately regulated is poorly understood. Mesenchymal progenitors are important regulator of muscle regeneration, but they also contribute to pathological degeneration such as adipogenesis and fibrosis. A recent study reported the importance of retinoic acid (RA) signaling in mesenchymal progenitors, and we have also focused on this signaling pathway as a regulator of mesenchymal progenitors. In this talk, we will show that cell-autonomous RA signaling in mesenchymal progenitors plays a critical role in the regulation of macrophage phenotype. We generated mice expressing dominant negative RA receptor specifically in mesenchymal progenitors (Pα/RAR403) and found severe impairment in conversion of macrophages from pro-inflammatory to pro-reparative phenotype. Recruitment of pro-inflammatory monocyte/macrophage population was severely impaired in mesenchymal progenitor-depleted mice but not affected in Pα/RAR403. These results reveal a novel role for mesenchymal progenitors as crucial inflammatory regulators and provide insight into the mechanism by which cell-autonomous RA signaling in mesenchymal progenitors can favorably influence muscle regeneration.

Muscle fibrosis: a vicious circle between human fibroadipogenic progenitors and muscle fibers

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Fibrosis, characterized by an excessive accumulation of extracellular matrix (ECM) components, is one of the main complications in many muscular dystrophies. This heterogeneous group of disorders is characterized by weakness and/or progressive degeneration of skeletal muscle with a wide clinical presentation and severity. In Oculopharyngeal muscular dystrophy (OPMD), a late-onset disorder, only a small group of specific muscles are primarily affected. Cricopharyngeal muscle of OPMD patients is characterized by an exacerbated fibrosis, fiber atrophy, inflammation and on-going regeneration. While the cellular and molecular mechanisms regulating muscle fibrosis has been extensively studied in mouse, our understanding of the exact nature and role of mesenchymal cells involved in fibrosis is limited in human and their implication in dystrophic muscle progression remains to be clarified.

For this, we characterized human fibroadipogenic progenitor (FAPs) cells from control and affected fibrotic muscles using transcriptomic and mass cytometry analysis. We looked at their effect on muscle by coculturing them with myotubes. Our results show that human FAPs in OPMD fibrotic conditions display a strikingly different profile than in control muscles; those cells show an exacerbated proliferation and ECM secretion, and when activated, have a detrimental effect on muscle differentiation. We also demonstrated the role of endothelin, a new targetable regulator involved in this process.

We propose that this cell phenotype in fibrotic conditions establishes a vicious circle with muscle fibers leading to the development of fibrous tissue. Our results underline the importance of a coordinated crosstalk between multiple cell actors to ensure muscle homeostasis and leads the way to the identification of targetable pathways for anti-fibrosis therapies.

Understanding DMD transcriptional networks using single-nuclei RNA-seq

Shinichiro Hayashi

Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

Duchenne muscular dystrophy (DMD) is the most common and severe fatal muscle disorder characterized by cycles of degeneration and regeneration of muscle fibers. The extent to which different nuclei sharing a common cytoplasm within muscle fibers exhibit transcriptional diversity in DMD is not well understood. It is also not clear how these individual myonuclei respond to the changes related to the pathology of the disease. Similarly, little is known about the transcriptional differences that occur among the non-muscle cell types within the muscle and their interactions with the muscle cells. In this study, we performed single-nuclei RNA-seq on healthy control and DMD patient muscles. We found that the expression of FBXO32 (Atrogin 1) and TRIM63 (MuRF1) in the muscle of DMD patients increased, and this was associated with the increased expression of FOXO1/3, especially in the fast-type myonuclei. Interestingly, most of the muscle stem cells in the DMD muscle were not proliferating, and the majority of them were in the quiescent state. The absence of proliferation was most remarkable in the advanced stages of DMD. Cell-cell interaction analysis revealed that interactions between myofibers and non-muscle cells change over time as the disease progresses, with a decrease in the interactions with the muscle stem cells and an increase in interactions with the mesenchymal progenitor cells. These results reveal the cellular dynamics within muscle fibers during DMD progression and provide insights into the transcriptional pathways of degeneration and regeneration that underlie the pathogenesis of the disease. Our data sheds light into the molecular mechanisms of DMD that are regulated by the transcriptional activity between muscle and non-muscle cells.

Multimodal Single Cell profiling of Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is one of the most severe pediatric degenerative myopathies. In the initial phase of the disease, muscle is exposed to continuous cycles of degeneration and regeneration; over time, regenerative potential is exhausted, and necrosis prevails. As of today, the cellular and molecular determinants responsible for this functional exhaustion remain largely uncharacterized.

Adult tissue repair requires the activation of resident stem cells that can both self-renew and generate differentiated progeny. To establish and maintain their properties, stem cells require constant interactions with their microenvironment and their neighboring cells that altogether constitute the niche. The stem cell and its niche form as a whole the minimum functional unit of adult tissue repair. Any given perturbation affecting either the stem cell or the molecular/cellular components of the niche will invariantly impact repair potential. Therefore, in DMD the changes hindering the correct execution of the repair process must therefore occur either in the stem cell or in its niche.

Here we present a multi-omic approach to elucidate the determinants interfering with regeneration in the dystrophic muscle and study the niche-stem cell interactions. Taking advantage of different single cell technologies, we have profiled during disease progression the evolution of muscle-resident cellular populations to identify dysfunctional subfractions and deregulated crosstalk.

Our data could serve as basis for future studies aimed at the identification of novel biomarkers and lay the foundation for new therapeutic approaches to promote muscle regeneration.

Session 3

Pathophysiology of Neuromuscular Disorders

Posttranslational protein modification and pathophysiology of muscular diseases

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The skeletal muscle is a pivotal organ regulating movement, posture, and whole-body metabolism. Skeletal muscle mass decreases by immobilization, disuse, malnutrition, and aging. Skeletal muscle mass is also affected and becomes atrophied by muscular dystrophies, neurodegenerative disorders, cancers, and cardiovascular diseases. Skeletal muscle atrophy by various diseases is related to the quality of life, survival rates, and healthy life expectancy in humans. Skeletal muscle constriction and relaxation are controlled by actomyosin system. Myosins, core components of thick filaments, are composed of heavy chains, and regulatory and essential light chains. Myosin heavy chains are divided into several categories: fast, slow, embryonic, and perinatal subtypes. Fast types of MyHCs (myosin heavy chains) contain MyHC-IIa (Myh2), MyHC-IIX (Myh1), and MyHC-IIb (Myh4). MyHC-IIb is considered not to be fully functional in humans. We have recently established double knockout mice deficient both of Myh1 and Myh4. The dKO mice show a drastic decrease in muscle mass and altered myofiber areas. Fibrosis in skeletal muscle is observed. During the identification of posttranslational protein modification of muscle proteins using a doubling muscle model lacking myostatin, we found that fast type of muscle myosins are monomethylated by a specific protein methyltransferase in a normal state. We further show that methylation affects the dynamics of actomyosin. In this workshop, I would like to present our recent findings related to the regulation of skeletal muscle atrophy, posttranslational protein modification of myosins, and their possible involvement in the pathophysiology of muscular diseases.

Mechanical stretch impacts chromatin organization: implications in laminopathies

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Background. An integrated network of nuclear envelope proteins defines the ensemble mechanical properties of the nucleus and regulates cell mechanical signalling in response to mechanical challenges. In muscle cells, A-type lamins and chromatin compaction could be critically involved in the nuclear responses to mechanical stress.

Aim. To investigate how A-type lamins and chromatin compaction affected the nuclear responses to mechanical stress in muscle cell precursors (MuSCs) and myotubes.

Methods. Static stress was analyzed by cultivating MuSCs on rigid and soft substrates (glass and 12kPa matrix). Chromatin decompaction was induced by treating MuSCs and myotubes with Dezanoplacin A (0.5µM, 48h) or Trichostatin A (0.1µM, 48h). Lamin or SUN2 knockouts were done by using Si RNA against Lamin A/C or Si RNA against SUN2. PCR were used to define the optimal concentration of Si RNA. IF, WB and image analysis (Image J and Imaris) were used to analyze the effects of stress or drugs on nuclear envelope proteins and nuclear characteristics.

Results. In myoblasts, static stress was associated with significantly higher protein expression of A-type lamins (1.08 ± 0.07, $p<0.05$) and SUN2 (4.4±1.6 vs 0.5±0.1, $p<0.05$). In myoblasts and myotubes, chromatin decompaction induced a significant increase in the expression of A-type lamins (1.1 ± 0.2 vs 3.9 ± 0.7, $p<0.05$), and a decrease in the expression of phospho-lamin/lamin ratio (0.77 ± 0.25 vs 0.09 ± 0.03, $p<0.05$) and SUN2 protein (3.1 ± 0.2 vs 1.9 ± 0.1, $p<0.05$). Fusion and differentiation indices significantly increase after treatment ($p<0.05$). The nucleus volume, thickness and area were significantly increased also.

Conclusion. These results suggest that increased in nuclear volume and thickness induced by chromatin decompaction decreases the expression of the Sun2 and the phospho-lamin/lamin ratio. Additional experiments are in process to analyse the effects of dynamic stress on the nuclear envelope proteins.

Recent Advance in Fukuyama Muscular Dystrophy and Dystroglycanopathies

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Fukuyama muscular dystrophy (FCMD) and muscle-eye-brain (MEB) disease are similar disorders characterized by congenital muscular dystrophy, brain and eye anomalies. Hypoglycosylation of α-dystroglycan (α-DG) are common characteristics of these dystroglycanopathies. We identified the genes for FCMD (fukutin) and MEB (POMGnT1). FCMD is the first human disease found to result from ancestral insertion of a SVA retrotransposon. We show that aberrant mRNA splicing, induced by SVA exon-trapping, underlies the molecular pathogenesis of FCMD. Introduction of antisense oligonucleotides (AONs) targeting the splice acceptor, the predicted exonic splicing enhancer and the intronic splicing enhancer prevented pathogenic exon-trapping by SVA in cells of patients with FCMD and model mice, rescuing normal fukutin mRNA expression and protein production. AON treatment also restored fukutin functions, including *O*-glycosylation of α-DG and laminin binding by α-DG. We further optimized it to one nucleic acid NS-035, completed toxicity-safety-efficacy studies, and initiated investigator-initiated clinical trials with the support of AMED.

We further identified the previously unknown glycan unit ribitol 5-phosphate (Rbo5P), a phosphoric ester of pentose alcohol, as a tandem repeat that functions as a scaffold for the formation of the ligand-binding moiety of α-DG. We determined the enzyme activities of three major α-DGpathy-causing proteins to be involved in the synthesis of tandem Rbo5P. ISPD is cytidine diphosphate ribitol (CDP-Rbo) synthase. Fukutin and fukutin-related protein are Rbo5P transferases that use CDP-Rbo. Consequently, Rbo5P glycosylation is defective in α-DGpathy models. We further demonstrate that prodrug treatments (tetra-acetylated CDP-ribitol) can ameliorate muscular dystrophy caused by defects in ISPD.

Pathological features of dermatomyositis and antisynthetase syndrome

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Since the discovery of the upregulation of type I interferon (IFN1)-inducible genes in muscles from dermatomyositis (DM) patients, DM has been increasingly recognized as type I interferonopathy in muscle. In line with this finding, we have shown that the expression of myxovirus resistance protein A (MxA), a representative IFN1-inducible protein, in myofibers on immunohistochemistry is the most sensitive and specific diagnostic marker on muscle pathology. However, even among DM, clinical features seem to be different depending on positive DM-specific autoantibodies (DMSAs), e.g., cancer association in anti-TIF1-γ DM, clinically amyopathic DM in anti-MDA5 DM, and juvenile DM in anti-NXP-2 DM. This naturally raises a question whether there is difference in the pathological phenotype among different subtypes according to positive DMSAs. We therefore evaluated myopathological features of the 256 patients who had been pathologically diagnosed with DM based upon myofiber MxA expression. As expected, there were certain features depending on the positive DMSAs: anti-TIF1-γ DM was associated with vacuolated punched-out fibers, anti-Mi-2 DM with perifascicular necrosis and perimysial pathology and sarcolemmal membrane attack complex (MAC) deposition, anti-MD5 DM with scattered/diffuse distribution of MxA-positive fibers, and anti-NXP-2 with microinfarcion. We further extended this pathological study to antisynthetase syndrome (ASS) by evaluating 212 ASS muscle biopsies. ASS was associated with HLA-DR expression in myofibers. Antii-Jo-1 and anti-OJ ASS were associated with perifascicular necrosis while only anti-OJ ASS was associated with higher inflammatory score, suggesting that ASS also shows different pathological features depending on positive anti-ARS antibodies.

Description of macrophages in idiopathic inflammatory myopathies using in-situ RNAseq

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Idiopathic inflammatory myopathies are a heterogenous group of pathologies characterized by autoimmunity mainly targeted at the skeletal muscle. Each subgroup of myositis shows specific clinical manifestations, autoreactive antibodies, and pathological features. Subgroups include inclusion body myositis, dermatomyositis, anti-synthetase syndrome, immune-mediated necrotizing myopathies, and lastly immune checkpoint inhibitor-induced myositis (ICI-myositis). These disorders exhibit different levels and composition of inflammatory infiltrates in skeletal muscle, invariably including macrophages.

Macrophages represent a heterogeneous and plastic population adapting its phenotype to the cytokine environment and are known to play a role in the muscle regeneration and differentiation process.

We wanted to further characterize macrophage population in myositis and describe their potential pathologic role.

Spatial transcriptomics (Visium, 10X Genomics) were performed, sequencing RNA information from pathological muscle biopsies sampled for each idiopathic inflammatory myopathy subgroup as well as healthy donor. Considering the high density of inflammatory infiltrate in ICI-myositis, single-cell RNAseq was performed on digested inflammatory cells from biopsies to further corroborate *in situ* results.

We isolated different phenotypes corresponding to bone-marrow-derived macrophages ($CD14^+$ $CCR2^+$ $CD68^+$ $IRF4^+$), and resident macrophages ($CD68^+$ $CD163^+$ $MAFB^+$ $SPI1^+$ $MARCO^+$ $VSIG4^+$ $APOE^+$ $TIMD4^+$). Pro-inflammatory mediators *TNF*, *CXCL9*, *CXCL10*, *NOS2* and *IL1B* were overexpressed in specific tissue domains. Conversely, IL10 and TGFB1 were found upregulated across other tissue domains. Resident macrophages were split into two subgroups defined by key genes as *MRC1*, *CCL18*, *SELENOP* using single-cell RNAseq in muscular inflammatory infiltrate.

In this work, the main advantage of *in situ* sequencing is the possibility to study macrophages in their tissue-specific state, avoiding the plasticity changes linked to other methods.

Expanded CGG repeats in oculopharyngodistal myopathy and related disorders

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Oculopharyngodistal myopathy (OPDM) is a muscular disorder characterized by ocular, facial, pharyngeal, and distal limb muscle weakness. Japanese neurologists, Drs. Satoyoshi and Kinoshita, originally described the disease in 1977. Most families are consistent with an autosomal dominant mode of inheritance, while some patients are sporadic. Most of the case reports have been from Japan and China, and only a limited number of cases were reported from Europe and other regions.

While the genetic basis of OPDM had long been unknown, a family was the clue. Patients in the family present oculopharyngeal weakness. The index patient additionally showed cardiomyopathy, leukoencephalopathy, and gastrointestinal dysmotility, and we named the disease oculopharyngeal myopathy with leukoencephalopathy (OPML). MRI scan of the patient showed diffusion hyperintensity in the corticomedullary junction, reminiscent of those observed in patients with neuronal intranuclear inclusion disease (NIID) and fragile X-associated tremor/ataxia syndrome (FXTAS). The observation prompted us to search for CGG repeats, and expanded CGG repeats were identified in NIID, OPML, and OPDM, thus suggesting a new disease spectrum from leukoencephalopathy and neuropathy to oculopharyngeal myopathy. Updates of studies on expanded CGG repeats will be presented.

Glial cell dysfunction in myotonic dystrophy brain disease

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The proper function of the central nervous system (CNS) relies on the complex interplay between different cell types, each with differing contributions in neurological disease. In myotonic dystrophy type 1 (DM1), expanded CTG repeats are transcribed into toxic CUG RNA that accumulates in multiple cell types of the brain, resulting in cognitive and behavioural impairment. While main DM1 symptoms are established to result from sequestration and/or dysregulation of multiple RNA binding proteins (such as splicing regulators), a major gap exists in our understanding of how molecular events are connected to neurological symptoms, and how each cell type in the CNS drives pathology.

We sought to investigate how toxic RNA expression perturbs the function of specific brain cell types, through the combination of live cell imaging and RNA sequencing of individual cell populations. We used the DMSXL mice, a transgenic model of the disease presenting relevant CNS alterations, as a source of homogenous cultures of primary neurons, astrocytes, oligodendrocyte precursor cells (OPCs) and mature oligodendrocytes. Careful cell phenotyping together with the analysis of alternative splicing and gene expression, revealed marked deleterious effects of the DM1 repeat expansion on glial cell biology. Cultured DMSXL astroglia and oligodendroglia exhibit abnormal cell morphology, adhesion and migration, in association with pronounced spliceopathy of cytoskeleton-related transcripts, when compared with DMSXL neurons. Glia phenotypes *in vivo* include reduced astrocyte density and branching, as well as defective myelination in DMSXL mouse brains. We suggest that pronounced RNA toxicity in glia cells affect neuronal physiology through defective glial-neuronal crosstalk, possibly contributing to the abnormal neurotransmission and synaptic plasticity that we have detected in DMSXL mice. Future therapeutic strategies must consider restoring of glia cell function, in conjunction with neuronal rescue and repair strategies.

Session 4

Development of Therapeutic Approaches

Gene therapy for Myotonic Dystrophy

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Myotonic Dystrophy type 1 (DM1) is characterized by progressive muscle weakness and wasting, myotonia, cardiac defects, endocrine troubles and cognitive impairments. DM1 is caused by an expanded tract of CTG repeats within the 3'UTR of the *DMPK* gene and a toxic RNA gain-of-function mechanism triggered by the expression of mutant transcripts containing expanded CUG repeats (CUGexp-RNA). Thus, abnormal binding of MBNL RNA binding proteins (RBPs) to CUGexp leads to their sequestration within nuclear foci. As MBNL1 is involved in the control of alternative splicing events during development, its functional loss in DM1 condition results in splicing misregulations and some of them were associated with clinical symptoms. To date, there is no cure for DM1 but several therapeutic strategies are under development. Here we assessed a gene therapy approach using a modified RBP with a high affinity for CUGexp that could act as a decoy to reverse RNA toxicity. For this purpose, we engineered a truncated MBNLΔ protein that keeps its zing finger domains required for the binding to CUGexp but lacks the C-terminal domain involved in splicing activity and homodimerization. MBNLΔ-decoy was next assessed in human DM1 muscle cells and DM1 mice. We showed that the binding of the decoy to CUGexp allows the release of sequestered endogenous MBNL1 from nuclear foci, restores MBNL1 activity and corrects the transcriptomic signature of DM1 muscle cells. Moreover, CUGexp-nucleoprotein complexes or foci formed by the decoy are less stable than those formed with MBNL1 resulting in reduce levels of CUGexp-RNA. *In vivo*, local or systemic delivery of the decoy into skeletal muscles of DM1 mice using AAV9 vectors leads to long-lasting correction of both splicing defects and myotonia, hallmarks of DM1. This study supports the development of decoy-RBPs with high binding affinities for CUGexp as a therapeutic strategy for DM1.

Establishment of protocols for the induction of myogenic progenitor cells from patients-derived iPS cells and development of therapeutic approaches

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Induced pluripotent stem cells (iPSCs) have been used in research for the development of treatments for various intractable diseases due to their unlimited proliferative and multipotent potential. We are aiming to develop novel therapies for intractable muscular diseases using iPS cells by two approaches.
One is cell transplantation therapy. We have developed a differentiation induction method that mimics the developmental stages and have succeeded in inducing skeletal muscle stem cells that are applicable to cell transplantation therapy. We have found that cell transplantation into Duchenne muscular dystrophy model mice is effective in regenerating more than 10% of dystrophin-positive fibers. In addition, some of the cells have been engrafted as satellite cells in vivo, and it is expected that the therapeutic effect will continue for a long period of time. We have also developed a differentiation method to induce mesenchymal stromal cells (MSCs) from iPSCs. Transplantation of iPSC-derived MSCs into Ullrich congenital muscular dystrophy (UCMD) model mice enabled the restoration of collagen type VI which resulted in the amelioration of UCMD phenotypes.

The other strategy is to generate iPS cells derived from patients with muscle diseases for pathological analysis and drug discovery. We have also developed another method to induce muscle cell differentiation with high efficiency and in a short period of time by transient overexpression of MyoD. Using this MyoD-mediated differentiation system, we reported that an oxidative stress is one of the exacerbating factors for aberrant DUX4 expression in facioscapulohumeral muscular dystrophy. However, the induced myocytes showed immature characteristics, many of muscular disease phenotypes could not be recapitulated only by this MyoD-mediated system. Recently, we have developed a novel maturing method of iPSC-derived myocytes by combination of optimization of MyoD induction and electrical stimulation. A fatigue-like contractile decline could be recapitulated by this maturing system in DMD patient-derived iPSCs.

Cell therapy approaches for Myasthenia gravis: *in vitro* and *in vivo* evaluations of conditioned mesenchymal stem cells.

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Myasthenia gravis (MG) is a chronic autoimmune disease characterized by autoantibodies targeting the neuromuscular junction and precluding normal nerve-to-muscle communication. Conventional treatments are limited by severe side effects mandating the set-up of novel therapies. Mesenchymal Stromal Cells (MSC) are multipotent progenitors presenting immunomodulatory capacities. Research grade (RG-) MSC conditioned with peripheral blood mononucleated cells (PBMC) improved clinical outcomes in our humanized MG mouse model (NSG-MG) (Sudres *et al*., JCI Insight, 2017). In clinical perspective, RG-MSC should be replaced by clinical grade (CG-) MSC. Here, we characterized and compared phenotypes, gene expression profiles and functional, *in vitro* and *in vivo,* capacities of resting and conditioned cells. Therefore, CG-MSC derived from adipose tissue were conditioned by PBMC (cMSC), left untreated (rMSC), or activated by IFN-γ (γ-MSC) as a control. Flow cytometry phenotyping showed that cMSC most remarkable phenotypic traits include increased CD54, CD273 and CD49a and reduced HLA-DR expression. At variance, IFNγ activation induced major changes in MSC phenotype in agreement with literature (increased CD54, HLA-ABC, HLA-DR, reduced CD59). Single cell clustering by mass cytometry suggested cMSC and rMSC proximity and underlined the phenotypic alterations induced by IFNγ. Gene expression study by RNA-Seq showed differential expression of 244 genes between rMSC and cMSC, while 2089 and 3614 genes were differentially expressed when comparing γ-MSC with rMSC and cMSC, respectively. Each conditioning involved particular pathways. *In vitro* immunomodulating capacities were evaluated by PBMC proliferation inhibition assays that showed that the cMSC supernatant reduced proliferation by at least 50%. Finally, cMSC were challenged in our NSG-MG mouse model, and cMSC-treated mice presented MG scores lowered by 50% compared to untreated mice from 2 weeks post-injection. To sum-up, this work unveiled treatment-dependent phenotypic markers of MSC and demonstrated that immunomodulation capacities *in vitro* and *in vivo* are enhanced by cellular conditioning.

GDF5 therapeutic potential for Duchenne Muscular Dystrophy gene therapy optimization

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Duchenne muscular dystrophy (DMD) is a lethal disorder characterized by the lack of dystrophin, which is essential for muscle fibers integrity as its absence results in muscle necrosis followed by cyclic degeneration and regeneration. Initially, regeneration in DMD disease is supported by the proliferation and differentiation of muscle precursor cells, as satellite cells (SCs). However, they progressively exhausted, rendering muscle repair inefficient and leading to muscular dysfunction. Among the therapeutic strategies developed for DMD, clinical trials with AAV administration of microdystrophins (AAV-microDys) are underway and the first data indicate the potential of gene therapy as treatment to respond to unmet need for DMD. However, these approaches target the degenerating muscle, so their long-term success heavily depends on maintaining muscle mass for as long as possible. Emerging relevance, in terms of muscle mass preservation, is observed for Growth Differentiation Factor 5 (GDF5) which has been shown to prevent muscle mass loss and force decline during ageing. In addition, it has been described as a positive regulator of muscle homeostasis. We investigated its role in DMD progression using mdx mice and showed that GDF5 over-expression modulates regeneration process and induces hyperplasia. Of relevance, we propose to investigate the benefits of a combination of GDF5 with AAV-microdystrophin in improving gene therapy by preserving myofibers integrity and increasing muscle mass.

Brain Dp140 alters glutamatergic transmission and social behavior in Duchenne muscular dystrophy mouse model

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Duchenne muscular dystrophy (DMD), caused by mutations in the DMD gene, shows progressive muscle degeneration as well as neurobehavioral comorbidities due to dystrophin deficiency. The lack of Dp140, a dystrophin short brain isoform, is clinically associated with autism spectrum disorders (ASDs), but its postnatal function is not well understood. Here, we aim to investigate synaptic function in the presence or absence of Dp140 in murine brains. We utilized C57BL/6 (wild-type) and two DMD mouse models, including mdx23 lacking Dp427 and mdx52 lacking both Dp427 and Dp140, at the age of 7 days or 8 weeks. Social behavior was evaluated, and then protein levels of dystrophin brain isoforms, synaptic function and morphology in the basolateral amygdala (BLA) were assessed by molecular biological techniques, electrophysiological tests and electron microscopy in the mice. We found that social interaction deteriorated in mdx52 mice ($p < 0.005$ versus mdx23 mice), and the glutamatergic transmission was altered in BLA pyramidal neurons of mdx52 mice ($p < 0.005$ versus mdx23 mice). Restoration of internally deleted Dp140 isoform by exon 53 skipping using antisense oligonucleotides or Dp140 mRNA overexpression in the BLA ameliorated deficits in social behavior and glutamatergic transmission in treated mdx52 mice. Our results provide the first evidence of synaptic functional improvement following postnatal Dp140 restoration, highlighting its therapeutic potential for DMD patients with ASD-like symptoms.

In vivo gene therapy for *LMNA*-related congenital muscle dystrophy

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LMNA-related Congenital Muscular Dystrophy (L-CMD) is the most severe form of striated muscle laminopathies caused by mutations in *LMNA*. Our purpose is to development gene therapy for L-CMD to correct *LMNA* mutations at mRNA level and improve the function of affected organs. Homozygous *Lmna*^{delk32} mice model L-CMD, pathology caused by both the expression of toxic mutant lamin A/C and absence of WT lamin A/C expression, while heterozygous mice, a model of dilated cardiomyopathy, present with reduced WT lamin A/C expression and residual mutant lamin A/C expression in the heart. Based on these facts, we aim at optimizing a therapeutic approach that both reduces the expression of the mutant proteins and restores the normal lamin A/C levels. To achieve such strategy, we developed AAV2/9 vector containing either human mature lamin A under control of a CMV promoter, or a combination of shRNA against either only mutant mouse *Lmna* mRNA or both alleles. These AAVs were injected intravenously at 10^{11} viral genomes for WT, homozygous and heterozygous *Lmna*^{delk32} mice at 2 days of age. All these treatments showed benefits in terms of max survival of homozygous mice. While, only transient benefit was shown for heterozygous mice. In addition, we could not find clear effects on cardiac function for heterozygous mice. Interestingly, 6 mice had liver nodule of total 50 treated mice, and three of them were diagnosed with hepatocellular carcinoma. From molecular analysis at end stage, expression of human lamin A mRNA was increased in both heart and liver. While, expression of endogenous mouse *Lmna* mRNA was decreased only in liver. Furthermore, we detected more quantity of viral genome in liver than heart. From this *in vivo* study, we will optimize and improve the therapeutic cassette to increase the efficacy of tissue targeting of these tools in the affected organs.

Sessions 5

Outcomes measures and Standards of Care

Representations of key stakeholder groups in Gene Therapy for DMD patients: the place of educational therapy in a new care pathway

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Introduction: Duchenne muscular dystrophy (DMD) has been a major target for gene therapy development for nearly 30 years. Currently, few clinical trials are underway in order to give new perspectives to patients. However, gene therapy treatment is a topic of discussion within key stakeholders about a possible change in care pathway. In fact, it is essential for scientists and clinicians to be clear about the risks and benefits of these technologies while encouraging education about genetic therapies to patients and their families. Establishing a therapeutic patient education (TPE) program would seem important to strengthen the therapeutic alliance and compliance in the future.

Objective: The objective of the study is to collect information about the expectations of genetic therapies from key stakeholder groups including clinicians, patients and their families concerned by DMD.

Methods: Two distinct group will participate in a questionnaire based on the educational diagnosis model of TPE. The first group will be represented by clinicians and caregivers experienced in DMD care. The second group will be represented by DMD patients and their families. The questions will concern expectations about gene therapy in biomedical, cognitive, socio-professional, psycho-affective and motivational dimensions. The questionnaire will be distributed the end of the first quarter 2022, and collected in the beginning of the second quarter 2022. The study will be conducted in France.

Results: This research is conducted as part of a Master's Degree, the results of which are expected in June 2022.

Discussion: Depend of the results, we hope that study will provide an initial survey of representations of key stakeholders in gene therapy for DMD and help to conceptualize a TPE program.

Prediction of disease progression in neuromuscular disorders using quantitative MRI and 31P MRS

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Quantitative magnetic resonance imaging (MRI) is used nowadays in many studies of neuromuscular diseases, often in the evaluation over time of muscle fat replacement (muscle fat fraction or FF) or the residual muscle mass. Another quantitative MRI outcome measure is the assessment of water T_2, which reflects the disease activity linked to pathophysiological mechanisms such as inflammation, oedema, necrosis, … The use of phosphorus magnetic resonance spectroscopy (^{31}P MRS) leads to the measurement of pH and indices of energy and phospholipid metabolism. We demonstrate here the use of quantitative MRI and ^{31}P MRS in the evaluation and even prediction of disease evolution.

First of all, muscle FF is a cumulative outcome measure sensitive to temporal evolutions over the course of 1 year, giving rise to standardized response means > 0.8 when using the global thigh or leg muscle segment, as shown in different neuromuscular diseases. Modelisation of FF trajectories in Duchenne muscular dystrophy have shown they can add prognostic value to the time of loss of ambulation, as well as to predict the FF at the next time point within a certain error margin. In Pompe disease, dysferlinopathy and GNEM myopathy, water T_2 and ^{31}P MRS indices such as pH, measured at a certain time point, have shown to be significantly correlated to the extent of the increase in FF at the subsequent time of evaluation. Correlating FF or the residual mass with function or strength have already shown the added value of multi-parametric evaluations to clinically meaningful endpoints such as loss of ambulation, loss of upper limb function and general loss of quality of life. Quantitative MRI and ^{31}P MRS can be perceived as a ‘swiss knife’ in the evaluation of neuromuscular diseases and potentially sensitive to therapeutic treatment, especially in combination with functional assessments.

Relationship between hand strength and function in patients with Duchenne Muscular Dystrophy or Spinal Muscular Atrophy

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This study aims to investigate the relationships between hand strength and function in ambulant and non-ambulant patients with Duchenne Muscular Dystrophy (DMD) or Spinal Muscular Atrophy (SMA).

The maximal strength of handgrip and key pinch was measured with the MyoGrip and MyoPinch dynamometers, respectively, while hand function was assessed with the MoviPlate, the Motor Function Measure items for distal upper limb (MFM D3 UL) and the Cochin scale.

The data of 91 DMD and 77 SMA patients (age 6–31 years) from 3 natural history studies were analysed.

The MoviPlate, MFM D3 UL and Cochin scale scores were strongly correlated with the strength expressed as a percentage of the predicted value ($P < 10^{-80}$). However, the Spearman correlation coefficients varied between 0.765 and 0.435 in absolute value, suggesting that some functions like those reflected by the Cochin score require strength much more than other tasks like the MoviPlate test, respectively.

Hand function was rather preserved when handgrip and key pinch strength were higher than a strength cut-off delimiting normal and altered hand functions. For hand strengths lower than the cut-off, hand function scores decreased with decreasing strength although a large variability was observed.

SMA patients demonstrated more performant strength – function relationships than DMD patients for the MoviPlate, the MFM D3 UL and the Cochin scale, possibly because they developed less contractures.

In conclusion, the function of the hand depends on the strength. Above the strength cut-off, hand is almost fully or fully functional. Below the strength cut-off, the hand function scores decrease with the decrease in strength, but a large variability in the relationships prevents individual prediction of the functional consequences of a strength modification, possibly due to the contribution of contractures, motor compensations and psychological state.

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