fondation suisse de recherche sur les maladies musculaires
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14th Swiss Meeting on Muscle Research

Macolin / Magglingen

3rd-5th December 2023

Program and Abstracts

The Swiss Meeting on Muscle Research was launched by the FSRMM in 1996 based on the initiative of its former Scientific Director, Prof. Denis Monard, as a biannual meeting to learn about the progress of funded projects.

Meanwhile, it has developed into a meeting for all researchers working in basic science and clinics who are devoted to the understanding and treatment of neuromuscular diseases in Switzerland. The purpose of the meeting is to discuss the latest results, initiate collaborations, exchange samples and ideas and to get to know each other better.

To foster scientific exchange and to promote young scientists, the FSRMM keeps the participation in this meeting free of charge.

Program

Sunday, December 3rd

16:00-17:30 Arrival, Check-in

17:30-18:30 Welcome Apero

18:30-19:30 Dinner

19:30-19:35	Meeting opening (Alain Pfulg and Markus Rüegg)
19:35-20:20	Keynote lecture: Challenges in ALS: From Diagnosis to Drug Development Special guest: Markus Weber, Neuromuscular centre/ALS clinic, Kantonsspital St. Gallen

Session 1: New developments for ALS **Chair: Markus Rüegg**

20:20-20:50	Sphingolipids as emerging players in amyotrophic lateral sclerosis Museer Lone, University Hospital Zürich
20:50-21:20	Precision amidst dynamic chaos: Unveiling moving targets of ALS Leonidas Emmanouilidis, ETH Zürich

Monday, December 4th

Session 1: New developments for NMDs **Chair: Susan Treves**

8:30-9:00	Pathomechanisms involved in the selective affection of skeletal muscle in X-linked Myopathy with Excessive Autophagy Ilaria Cocchiararo, University of Geneva
9:00-9:30	RyR1 content decrease-induced ER stress is a hallmark of myopathies Nadège Zanou, University of Lausanne

9:30-10:00 AAV-mediated therapeutic effect of linker protein-mediated gene therapy on muscle and nerve pathology in mouse models for LAMA2 MD
Judith Reinhard, University of Basel

10:00-10:30 Uncovering Wif1 Contribution to Muscular Dystrophy Phenotypes
Eleonora Maino, University of Basel

10:30-11:00 Coffee break

Session 2: Calcium signalling **Chair: Francesca Amati**

11:00-11:30 Interaction between the Ca2+ channel Orai3 and the scaffold protein AHNAK2: implication during muscle stem cell activation
Stephane König, University of Geneva

11:30-12:00 Store Operated Calcium Entry regulates synaptic gene expression and muscle response to denervation
Alexandre Prola, University of Geneva

12:00-12:30 Exploiting S-acylation of STIM proteins in muscle
Amado Carreras Sureda, University of Geneva

12:30-13:30 Lunch

Poster session

13:30-15:30 ALL POSTERS, coffee and tea

Session 3: Muscle function **Chair: Thomas Laumonier**

15:30-16:00 Biofabrication of a 3D microphysiological model of human skeletal muscle for the study of fibrosis in aging and disease
Simone Bersini, Università della Svizzera italiana and Ente Ospedaliero Cantonale

16:00-16:30 The neuromuscular junction: the chink in skeletal muscle's armor in neuromuscular pathologies and other diseases
Christoph Handschin, University of Basel

16:30-17:00 New aspects of TGFβ signaling in muscle regeneration
Jérémy Kessler, University of Geneva

17:00-17:30 Exploring the multifaceted signaling in skeletal muscle of geriatric mice
Daniel Ham, University of Basel

17:30-17:50 Break

Session 4: Stem cells — Chair: Frédéric Allain

17:50-18:20	Interspecies generation of iPSC-derived functional muscle stem cells Ori Bar-Nur, ETH Zürich
18:20-18:50	Uncovering a novel myogenic regulator that facilitates reprogramming of fibroblasts into functional myogenic progenitor cells Giada Bacchin, ETH Zürich
18:50-19:20	Intracellular autofluorescence allows the isolation of functional human MuRC subpopulations with distinct cell states Axelle Bouche, University of Geneva

19:30-20:30 Dinner

Poster prize committee meets over dinner

Evening program

20:30-23:00 free beer at poster site

Tuesday, December 5th

Session 1: Mitochondrial involvement in disease mechanisms — Chair: Maud Frieden

9:00-9:30	Integrated nutrient-sensitized screens predict mitochondrial disease genes Alexis Jourdain, University of Lausanne
9:30-10:00	LACTB, a new player in lipid modulation Sylviane Lagarrigue, University of Lausanne
10:00-10:30	MitoBooster: a novel strategy for inducing mitophagy and enhancing cellular homeostasis Ammar Ebrahimi, University of Lausanne

10:30-10:45 Concluding remarks and poster prizes (Markus Rüegg)

10:45-11:15 Coffee Break and Poster removal

Departure

Abstracts

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Sphingolipids as emerging players in amyotrophic lateral sclerosis

Museer A. Lone

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Amyotrophic lateral sclerosis (ALS) is a devastating neurological disorder. Clinically, it is characterized by severe muscle wasting finally leading to paralysis and death. Etiologies for ALS are heterogeneous but rarely genetic. Recently, we identified a set of missense and deletion mutations in the SPTLC1 subunit of the enzyme, Serine-palmitoyltransferase (SPT) that are associated with juvenile ALS. Extending this analysis, we now show that recurring mutations in another subunit of the enzyme, SPTLC2 also cause juvenile ALS. SPT catalyzes the first and the rate-limiting step in the de novo synthesis of sphingolipids (SL). SPT conjugates palmitoyl-CoA with L-serine to form long chain bases (LCB), the identifying structural moieties of SL. SPT is composed of SPTLC1-SPTLC2 dimers that interact with negative regulators, ORMDL-1, -2, and -3. SPT-ORMDL interaction is essential for SL homeostasis. Using HEK293 SPTLC1 and SPTLC2 knockout cells, we showed that ORMDL binding to the holoenzyme complex is impaired in cells expressing pathogenic SPT-ALS alleles, leading to complex disassembly. SPT-ALS mutations thus lead to impairment in the feedback inhibition of the enzyme and unregulated canonical SL synthesis. Previously, specific SPTLC1 and SPTLC2 variants were associated with peripheral sensory neuropathy, HSAN1 due to the synthesis of 1-deoxysphingolipids (1-deoxySLs) that form when SPT metabolizes L-alanine instead of L-serine. We deduced lipid signatures specific to SPT-ALS and HSAN1 mutations in HEK293 cells, patient derived primary fibroblasts and plasma. These SL species could serve as markers for identifying the disease form in patients carrying SPT mutations. Limiting L-serine availability in SPTLC1-ALS expressing cells increased 1-deoxySL and shifted the SL profile from an ALS to an HSAN1-like signature. This effect was corroborated in an SPTLC1-ALS pedigree in which the index patient uniquely presented with an HSAN1 phenotype, increased 1-deoxySL levels, and an L-serine deficiency. These data demonstrate how pathogenic variants in different domains of SPTLC1 give rise to distinct clinical presentations that are nonetheless modifiable by substrate availability.

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Precision amidst dynamic chaos: Unveiling moving targets of ALS

Leonidas Emmanouilidis

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Neuromuscular diseases constitute a complex challenge for healthcare and society, characterized by the progressive degeneration of neurons. Amyotrophic Lateral Sclerosis (ALS) exemplifies the intricate interplay between genetic susceptibility, cellular dysfunction, and clinical manifestation. This devastating disorder leads to motor neuron degeneration, paralysis, and fatality, highlighting the need for innovative therapies. Genetic predisposition and environmental factors contribute to ALS etiology, with mutations in genes like *SOD1, TDP-43, C9orf72*, and FUS being implicated in familial cases. Recent advances highlight the significance of phase transitions in neuromuscular diseases. ALS-associated mutations induce the formation of cytoplasmic condensates through liquid-liquid phase separation (LLPS). LLPS involves molecular assembly into distinct phases within cells, playing pivotal role in compartmentalization and function of the molecules involved. Familial ALS-linked mutations, particularly in *FUS* and *TDP-43*, accelerate liquid-to-solid transitions, leading to potentially toxic protein network formation. This insight unveils new opportunities for intervention, suggesting small peptides to disrupt aberrant phase separation events. Our project objectives encompass identifying peptides and small molecules targeting FUS and TDP-43, to prevent aberrant LLPS or to dissolve preexisting condensates. Preliminary experiments indicate that our peptides indeed inhibit FUS droplet formation. Furthermore, high resolution solution Nuclear Magnetic Resonance (NMR) spectroscopy allowed us to confirm the mode of interaction. Even though, the affinity of these peptides is very low for the target protein this is very early stage in our research. We will optimize further the peptides and even convert them to small molecules to achieve higher efficiency in LLPS inhibition. In conclusion, by designing peptides and small molecules targeting critical aromatic motifs, we aim to interfere with aberrant LLPS, offering a potential therapeutic avenue to disrupt the formation of toxic protein networks, ultimately contributing to the development of novel ALS treatments.

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Pathomechanisms involved in the selective affection of skeletal muscle in X-linked Myopathy with Excessive Autophagy

Ilaria Cocchiararo

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Over the last decades, an increasing number of diseases have been linked to lysosomal dysfunction. In particular, defective lysosomal acidification triggers accumulation of undigested content by disrupting autophagy. Previous reports identified VMA21 as a chaperone of the v-ATPase pump, responsible for organelle acidification. Mutations in the *VMA21* gene cause X-linked myopathy with excessive autophagy (XMEA), characterized by muscle atrophy and fatigue. Although XMEA is likely to arise from autophagy defects, the pathomechanisms leading to selective dysfunction of skeletal muscles in the patients remain unknown. We unveiled that *VMA21* encodes two differently expressed isoforms (referred to as VMA21a and VMA21b). While VMA21a is expressed ubiquitously, the yet-unknown VMA21b is specific to muscle. Moreover, VMA21b is strongly up-regulated upon muscle cell differentiation. Interestingly, both isoforms interacted with sub-units of the v-ATPase, suggesting a common role for VMA21a and VMA21b in regulating its assembly. Depletion of both VMA21 isoforms impaired autophagic flux in skeletal muscle cells. In contrast, overexpression of VMA21a or VMA21b did not affect autophagy in vitro. To assess the pathological role of each isoform, we analyzed their expression in muscle cells from two XMEA patients (P) carrying distinct mutations. Both P1 and P2 showed decreased expression of VMA21a and VMA21b, indicating that the deficiency of both isoforms may contribute to the muscle pathology in XMEA. Of note, cells from both patients showed autophagy defects. Ongoing experiments aim at restoring the expression of VMA21a and VMA21b in XMEA cells to assess their role in autophagy deregulation. Moreover, the mouse model of XMEA, recently generated in the lab, will help us deciphering the different functions of VMA21 isoforms and identify potential therapeutic targets.

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RyR1 content decrease-induced ER stress is a hallmark of myopathies

Nadège Zanou

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Background: Decreased ryanodine receptor type 1 (RyR1) protein levels are a well-described feature of recessive RYR1-related myopathies. The aim of the present study was twofold: 1) to determine whether RyR1 content is also decreased in other myopathies and 2) to investigate the mechanisms by which decreased RyR1 protein triggers muscular disorders. Methods: We used publicly available datasets, muscles from human inflammatory and mitochondrial myopathies, an inducible muscle specific *RYR1* recessive mouse model and *RyR1* knockdown in C2C12 muscle cells to measure RyR1 content and ER stress markers. Proteomics, lipidomics, molecular biology and transmission electron microscopy approaches were used to decipher the alterations associated with the reduction of RyR1 protein levels. Results: *RYR1* transcripts were reduced in muscle samples of patients suffering from necrotizing myopathy ($p=0.026$), inclusion body myopathy ($p=0.003$), polymyositis ($p<0.001$) and juvenile dermatomyositis ($p<0.001$), and in muscle samples of myotonic dystrophy type 2 ($p<0.001$), presmyptomatic ($p<0.001$) and symptomatic ($p<0.001$) Duchenne muscular dystrophy, Becker muscular dystrophy ($p=0.004$) and limb-girdle muscular dystrophy type 2A ($p=0.004$). RyR1 protein content was also significantly decreased in inflammatory myopathy (-75%, $p<0.001$) and mitochondrial myopathy (-71%, $p<0.001$) muscles. Proteomics data showed that depletion of RyR1 protein in C2C12 myoblasts leads to myotubes recapitulating the common molecular alterations observed in myopathies. Mechanistically, RyR1 protein depletion reduces endoplasmic reticulum (ER)-mitochondria contact length (-26%, $p<0.001$), Ca^{2+} transfer to mitochondria (-48%, $p=0.002$), the mitophagy gene Parkinson protein 2 transcripts ($p=0.037$), and induces mitochondrial accumulation (+99%, $p=0.005$) and dysfunction ($p<0.001$). This was associated to the accumulation of deleterious sphingolipid species. Our data showed increased levels of the ER stress marker chaperone-binding protein/glucose regulated protein 78, GRP78-Bip, in *RyR1* knockdown myotubes (+45%, $p=0.046$), in mouse *RyR1* recessive muscles (+58%, $p=0.001$) and in human inflammatory (+96%, $p=0.006$) and mitochondrial (+64%, $p=0.049$) myopathy muscles. This was accompanied by increased protein levels of the pro-apoptotic protein CCAAT-enhancer-binding protein homologous protein, CHOP-DDIT3 in *RyR1* knockdown myotubes (+27% $p<0.001$), mouse RyR1 recessive muscles (+63% $p=0.009$), human inflammatory (+50% $p=0.038$) and mitochondrial (+51% $p=0.035$) myopathy muscles. In publicly available datasets the decrease in RYR1 content in myopathies was also associated to increased ER stress markers and *RYR1* transcript levels are inversely correlated with ER stress markers in the control population. Conclusions: Decreased RyR1 is commonly observed in myopathies and associated to ER stress in vitro, in mouse muscle and in human myopathy muscles, suggesting a potent role of RyR1 depletion-induced ER stress in the pathogenesis of myopathies.

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AAV-mediated therapeutic effect of linker protein-mediated gene therapy on muscle and nerve pathology in mouse models for LAMA2 MD

Judith Reinhard

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LAMA2-related muscular dystrophy (LAMA2 MD or MDC1A) is the most frequent form of congenital muscular dystrophies. It is caused by mutations in *LAMA2*, the gene encoding laminin-α2, one chain of the heterotrimeric extracellular matrix protein laminin-211 (α2β1γ1). The large size of the coding sequence for laminin-α2 (~ 9.4kb) and the heterotrimeric structure of laminin-211 present a challenge for AAV-mediated gene replacement or gene editing strategies. Here, we describe the development of an AAV-based gene therapy to functionally replace laminin-211 by two small linker proteins. Prior work in transgenic mice has demonstrated that this Simultaneous Expression of Artificial Linkers (SEAL) in skeletal muscle of LAMA2 MD mice has a tremendous ameliorative effect on the muscular dystrophy (Reinhard et al., 2017). Recently, we have demonstrated that transgenic expression of the linkers proteins by ubiquitous promoters also prevents nerve pathology (Reinhard et al., 2023). As both linkers, mini-agrin (mag) and αLNNd, are small enough to be packed into AAV, we intravenously co-injected mag- and αLNNd-expressing AAVs into a LAMA2 MD mice. We compared the disease ameliorative effects by the use of a muscle-specific or ubiquitous promoter. The linkers were highly expressed in skeletal muscle or peripheral nerves. And led to a strong improvement of the muscular dystrophy disease phenotypes, including an increase in body- and muscle weights, grip strength and myofiber size and the reduction of fibrosis. The use of a ubiquitous promoter additionally ameliorated the nerve pathology presented as a progressive hindlimb paralysis in LAMA2 MD mice. These studies thus establish that systemic delivery of AAVs expressing the two linkers might be a possible strategy to treat LAMA2 MD patients. As the linkers are designed from proteins (agrin, nidogen-1 and laminin-α1) that are all expressed in LAMA2 MD patients, this treatment is expected to be well tolerated.

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Uncovering Wif1 contribution to muscular dystrophy phenotypes

Eleonora Maino

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Muscular dystrophies (MDs) are a group of genetic disorders characterized by progressive muscle degeneration and strongly accompanied by fibrosis. The spectrum of phenotypic manifestations in MDs emerges from the complex interplay among distinct cell types constituting skeletal muscle. To elucidate the molecular mechanisms underpinning compromised muscle function in MDs, we conducted an extensive transcriptomic profiling in muscles of a mouse model representative of the severe LAMA2-related muscular dystrophy (LAMA2 MD), the dy^{W}/dy^{W} mouse. Our analysis unveiled the downregulation of Wif1, an inhibitor of the Wnt pathway, in the muscles of dy^{W}/dy^{W} mice. Through the use of single nucleus-RNAseq, we found that Wif1 is expressed in tenocytes. Most importantly, Wif1 overexpression mitigated fibrotic disease manifestations in the muscles of dy^{W}/dy^{W} mice. Collectively, our findings provide strong evidence for a pivotal role of the Wnt pathway in MD progression, and they emphasize the significant impact of non-muscle cell populations on disease manifestations, suggesting potential targets for therapeutic development in MDs.

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Interaction between the Ca2+ channel Orai3 and the scaffold protein AHNAK2: implication during muscle stem cell activation

Stéphane König

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Our work aims to study the activation of quiescent muscle stem cells obtained in vitro, called reserve cells (RC). Stimulation for 24 hours with medium containing FCS activates approximately 30% of RC and elicits a robust calcium response. We assessed the involvement of Orai calcium channels in RC activation and found that Orai3 downregulation led to a 40% decrease in this process, while Orai1 downregulation had no effect. On the contrary, Orai1 knockdown reduced both store-operated calcium entry (SOCE) and the calcium response induced by FCS stimulation, while downregulating Orai3 affected none of these responses. The absence of Orai3 led to a decrease in the RC population, which was not observed when Orai1 was downregulated. The re-expression of Orai3 wt in the Orai3 downregulation experiments allowed the rescue of the phenotype. A non-permeable Orai3 channel mutant also rescued the RC activation. We conclude that RC activation, while regulated by the calcium channel Orai3, is unexpectedly independent of calcium signals. To identify the pathways regulated by Orai3, we performed a protein proximity assay (Orai3-BioID). We identified AHNAK2 scaffold protein as potentially interacting with Orai3. The downregulation of AHNAK2 decreases the number of RC as well as the capacity of these cells to be activated by FCS, in a similar way as what we obtained after Orai3 downregulation. The knockdown of both proteins did not enhance the unitary effect, suggesting a role in the same pathway. We have shown that the function of the calcium channel Orai3 in activating human muscle stem cells does not depend on its function as an ion channel but could be associated with its interaction with AHNAK2 protein. Our hypothesis is that Orai3/ AHNAK2 must be necessary for the maintenance of the quiescent state. Interestingly, dystrophin downregulation increases the expression of Orai3 and AHNAK2. These results suggest that Orai3/AHNAK2 may be involved in MuSC dysregulation in Duchenne muscular dystrophy (DMD). Our work opens essential perspectives on understanding human muscle stem cells and the new calcium-independent emerging role of calcium channels.

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Store Operated Calcium Entry regulates synaptic gene expression and muscle response to denervation

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Muscle fiber contraction is governed by motoneuron innervation at the level of a unique synapse called the neuromuscular junction (NMJ). In the muscle fiber, the expression of synaptic genes including genes encoding acetylcholine receptor subunits is restricted to a cluster of nuclei, named sub-synaptic nuclei, localized under the post-synaptic part of the NMJ. This regionalized expression pattern within muscle fibers is known to rely on calcium-dependent pathways. However, mechanisms inducing local calcium regulations remains to be identified. Moreover, this regionalized expression pattern is lost in response to denervation, but whether a modification of calcium fluxes triggers these changes is still unknown. Here, we identify a regionalized expression of the main components of store operated calcium entry (SOCE): while STIM1 and ORAI1 are enriched in the sub-synaptic region of muscle fibers, STIM2 and TRPC3 are more expressed in extra-synaptic regions. Consistently, using isolated muscle fibers, we found higher SOCE in the sub-synaptic region of the fiber. In response to muscle denervation, extra-synaptic SOCE capacity was rapidly increased (12h after sciatic nerve cut) while sub-synaptic SOCE remained unchanged. After 7 days of denervation, both extra- and sub-synaptic SOCE capacities were increased. Denervation-induced changes in SOCE were associated with increased expression of STIM2 and TRPC3 and modification of STIM1 localization. These results suggest activity-dependent SOCE regulation and a potential role of SOCE in muscle response to denervation. Accordingly, Stim1 knockdown reduced denervation-induced up-regulation of synaptic gene expression. Inversely, overexpression of STIM1 was sufficient to induce an upregulation of synaptic genes in innervated muscles. Finally, we observed that chronic activation of mTORC1 pathway in TSCmKO mice, a condition known to hinder muscle response to denervation, results in a major increase of SOCE capacity, especially in extra-synaptic region. Together, our results revealed a yet-unknown role of SOCE in sensing neural activity, which may be regulated by the mTORC1 pathway. SOCE inhibition could represent a new strategy to mitigate neuromuscular affliction in pathological conditions associated with mTORC1 overactivation, such as sarcopenia.

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Exploiting S-acylation of STIM proteins in muscle

Amado Carreras Sureda

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Skeletal muscle contraction relies on action potentials, which trigger depolarization of the plasma membrane (PM) and activate voltage-gated ion channels. These channels lead to the release of calcium (Ca2+) from the sarcoplasmic reticulum (SR) via Ryanodine receptors (RYR) into the cytosol. To sustain muscle contraction and prevent fatigue, it's essential to replenish intracellular SR Ca2+ stores, achieved through Store-Operated Ca2+ Entry (SOCE). Stromal Interacting Molecules 1 and 2 (STIM) sense SR Ca2+ depletion, causing them to change conformation and move to SR-PM junctions, where they regulate PM ORAI1 channels to induce Ca2+ entry which is then pumped back into the SR. Gain-of-function mutations in *STIM1* and *ORAI1* can lead to excessive SOCE and SR Ca2+ overload, resulting in Tubular Aggregate Myopathy (TAM), a rare myopathy disorder with no current treatment. Recent research suggests that ORAI1 s-acylation promotes SOCE, however, if global s-acylation is chemically promoted SOCE is reduced. This event made us propose that SOCE has several layers of regulation via s-acylation and we aimed to explore them aiming to use this knowledge for TAM treatment. Our first observations showed that all SOCE members are s-acylated during muscular differentiation and that s-acylation of STIM1 and the muscle specific isoform STIM1L have opposite roles on SOCE, opening a therapeutic avenue for the specific treatment of SOCE in muscle tissue on the context of TAM. These findings are crucial for understanding how the SOCE machinery is regulated by lipid post-translational modifications and may offer a novel therapeutic approach for TAM patients.

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Biofabrication of a 3D microphysiological model of human skeletal muscle for the study of fibrosis in aging and disease

Simone Bersini

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Tissue fibrosis is a hallmark of organ degeneration during aging and disease. However, our knowledge of the biological mechanisms regulating the progression of fibrosis is still limited and a few therapeutic treatments are available [1]. Skeletal muscle fibrosis characterizes physiological muscle aging and incurable muscle diseases including dystrophies. Current pre-clinical in vitro models do not faithfully recapitulate the human fibrotic muscle microenvironment, since they neglect key cellular populations (e.g. blood vessels) that play a central role during disease progression. Hence, the development of more relevant disease models could help understanding the complex pathological mechanisms of fibrosis and identify new therapies [2]. Here, we aimed at biofabricating a human fibrotic skeletal muscle where a suspended myobundle is surrounded by a vascularized matrix. Based on our previous model [3], a miniaturized culture system was microfabricated using high-resolution 3D printing. This system embeds micropillars which anchor the myobundle. A custom-made electrical stimulation system based on silicone-carbon electrodes was setup to trigger contraction. A 1-cm long, free-standing myobundle (diameter: 500µm) was biofabricated and cultured for two weeks. Multinucleated myotubes and key muscle-specific markers (e.g. striated sarcomeric alpha actin) were observed. Electrical stimulation triggered muscle contraction with a force of 100-200µN. To test the model in fibrotic conditions, a 3D ECM containing muscle-specific fibroblasts (from Duchenne patients or healthy donors), endothelial cells and macrophages was cast around the muscle and cultured for 1 week. The presence of Duchenne fibroblasts was sufficient to trigger an early signature of vascular damage including endothelial-to-mesenchymal transition (i.e. PAI1 and CDH2 upregulation). Similar results were achieved in presence of an inflammatory microenvironment generated by M1 macrophages. Furthermore, Duchenne fibroblasts impaired the contractility of the myobundle and incubation with the anti-fibrotic drug Nintedanib partially restored this function. Overall, this miniaturized system allowed to form suspended myobundles that mimicked their native tissue counterpart. Once validated, this technology will allow to study the interactions among different cell populations in the context of muscle fibrosis and muscle wasting during aging and disease.

1-Rockey, 2015, N.Engl.J.Med.
2-Bersini, 2018, Adv.Drug.Deliv.Rev.
3-Bersini, 2018, Cell Reports.

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The neuromuscular junction: the chink in skeletal muscle's armor in neuromuscular pathologies and other diseases

Christoph Handschin

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The neuromuscular junction (NMJ) is a specialized region on the muscle fiber, not only indispensable for the regulation of normal muscle contractions, but also one of the hotspots of disease etiology in a number of pathologies, from sarcopenia to cachexia and numerous neuromuscular dystrophies. The transcriptional specification of the 3-5 subsynaptic nuclei in comparison to the extrasynaptic counterparts is of great interest, yet poorly understood. For example, it is unclear how local signals control the transcription of acetylcholine subunit-encoding and other subsynaptic genes. Similarly, even though mounting evidence hints at a substantial retrograde signaling from the muscle fiber to the motor neuron in the adult muscle post-development, the nature of this crosstalk still is nebulous. We therefore embarked on a spatial analysis of myonuclear transcription, comparing subsynaptic and extrasynaptic transcriptomes. A large list of genes could be identified with high enrichment in expression at the NMJ. We currently are characterizing such potential candidates to maintain and inform NMJ function in different contexts. As example, we found the ribosomal protein S6 kinase A1 (RSK1 protein, *Rps6ka1* gene/transcript) not only to be almost exclusively expressed in the subsynaptic region of muscle fibers, but also, based on loss-of-function experiments, to substantially contribute to the maintenance of normal NMJ morphology and function. We have tested this in the context of cancer cachexia: in this pathology, *Rps6ka1* expression is reduced, and various molecular and functional parameters can be improved by targeted overexpression of this gene. We therefore hope that *Rps6ka1* and other NMJ-enriched genes will help to elucidate NMJ stability, morphology and function in health and disease.

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New aspects of TGFβ signaling in muscle regeneration

Jérémy Kessler

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Muscle regeneration is a multistep process that is tightly regulated by different factors. Cytokines of the transforming growth factor β (TGFβ) family like myostatin, but also the actual TGFβ cytokines, are well established as such factors. Surprisingly, we recently identified an undescribed heterodimeric protein within the TGFβ family. Transcriptomic data indicated that the genes responsible for this heterodimer are well expressed in muscle and genetic data from mice and men suggested that the heterodimer plays a role in muscle regeneration. With support from the FSRMM, we were able to analyze this heterodimer and its effect on muscle cells in detail. We could demonstrate that the heterodimeric TGFβ cytokine is preferentially formed compared to homodimeric variants. Biochemical and structural analysis (cryogenic electron microscopy) highlighted that this preference is due to specific cysteines in the latent TGFβ complex; only the heterodimeric TGFβ allows stable disulfide-bonds. Homodimer expression, in contrast, causes protein agglomeration, lack of latency, and increased risk of fibrosis formation. We currently study whether homodimers are physiologically expressed at all or if their formation is linked to pathologies. All latent TGFβs, including this heterodimer, require a specific activation mechanism to become signaling competent. We will show that the heterodimeric TGFβ binds with high affinity to the integrin αVβ6 that is expressed in mature myocytes. Additionally, we show robust activation of the heterodimeric TGFβ by myocytes, as well as myoblasts. By adding heterodimeric TGFβ to differentiating primary human muscle cells, we could show that this new TGFβ reduces cell fusion in a dose-dependent manner. Further, we discovered differences in the signaling outcome of heterodimeric TGFβ compared to the well-established TGFβ1 that is associated with fibrosis in pathologies including myopathies like Duchenne. Moreover, TGFβ1 expression is closely linked to infiltrating immune cells, while the genes expressing this heterodimer are expressed by myogenic and tendon cells and fibro-adipogenic progenitors. Thus, a picture emerges in which heterodimeric TGFβ signaling is a physiological regulator of muscle regeneration and homeostasis while TGFβ1 is linked to immune invasion. This opens new possibilities for a safer therapeutic targeting of the TGFβ pathway in order to treat fibrotic diseases.

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Exploring the multifaceted signaling in skeletal muscle of geriatric mice

Daniel Ham

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As global life expectancy continues to climb, maintaining skeletal muscle function is increasingly essential to ensure a good life quality for aging populations. We recently identified mTORC1 activation as a hallmark of sarcopenia, the age-related loss of muscle mass and function. While continuous, long-term treatment with the mTORC1 inhibitor rapamycin predominately exerted anti-sarcopenic effects in mice, we also discovered a pro-aging phenotype and gene expression signature specifically in the gastrocnemius (GAS) muscle. The heightened pro-aging gene expression signature is a unique opportunity to further characterize the mechanisms contributing to sarcopenia. After a thorough initial phenotypic, physiologic and bulk mRNA-seq profiling (Ham, 2020; Ham, 2022), we have now generated a large single nuclei (sn) RNA-seq (>100k nuclei) data-set from GAS muscle of 10-month-old adult and 28-month-old control and rapamycin-treated mice. UMAPs identified several aging-specific myonuclei clusters, whose prevalence were strongly increased in rapamycin-treated mice. Gene expression patterns of these nuclei were very distinct from those seen in myonuclei from adult muscle. Further sub-clustering of this 'Aging' cluster identified a small group of nuclei enriched for genes involved in 'muscle development', one enriched for 'denervation' markers and another, larger group that displayed both overlapping and distinct marker genes for 'senescence' and 'atrophy'. Importantly, while the gene expression signature of the 'Aging' cluster aligned well with the rapamycin-induced pro-aging signature from bulk-seq, each sequencing technique identified candidate genes missed by the other. To link muscle fiber phenotypes with the presence of 'Aging' cluster signatures, we now use highly multiplexed single molecule (sm) RNA-fluorescence in situ hybridization (FISH) to visualize the expression of genes associated with 'denervation', 'senescence' and 'atrophy' clusters in GAS muscle cross sections. In summary, our results support the notion that local expression changes in multiple genes contribute to the initiation of sarcopenia and they highlight the likely need for multiple future treatment strategies.

Ham DJ and Börsch A, et al. The neuromuscular junction is a focal point of mTORC1 signaling in sarcopenia. Nat. Commun. 11, 4510 (2020).
Ham DJ and Börsch A, et al. Distinct and additive effects of calorie restriction and rapamycin in aging skeletal muscle. Nat. Commun. 13, 2025 (2022).

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Interspecies generation of iPSC-derived functional muscle stem cells

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Satellite cells, the stem cells of skeletal muscle tissue, hold a prodigious regeneration capacity. However, low satellite cell yield from autologous or donor-derived muscles precludes adoption of satellite cell transplantation for the treatment of muscle diseases such as Duchenne muscular dystrophy (DMD). Furthermore, directed differentiation of induced pluripotent stem cells (iPSCs) into adult and quiescent satellite cells in vitro has been challenging. To address these limitations, we investigated whether sufficient quantity of iPSC-derived satellite cells can be produced in allogeneic or xenogeneic animal hosts in vivo. First, we generated intraspecies mouse chimeras by injection of CRISPR/Cas9-corrected DMD-iPSCs into mouse blastocysts carrying an ablation system of host satellite cells. Extensive analysis of skeletal muscles from adult chimeras revealed exclusive generation of iPSC-derived satellite cells and derivative myoblasts. Similarly, injection of genetically-corrected mouse DMD-iPSCs into rat blastocysts produced interspecies rat-mouse chimeras harboring mouse satellite cells. Notably, iPSC-derived muscle stem cells produced in either allogenic or xenogeneic animal hosts efficiently restored dystrophin expression in limb muscles of DMD mice following intramuscular transplantation, and contributed to the stem cell reservoir. Collectively, our study provides a proof-of-principle for the generation of therapeutically-competent stem cells between divergent animal species, raising the possibility of procuring human stem cells in large animals for regenerative medicine purposes.

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Uncovering a novel myogenic regulator that facilitates reprogramming of fibroblasts into functional myogenic progenitor cells

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Conversion of fibroblasts into muscle cells by ectopic overexpression of MyoD has been widely used in past decades to study myogenic cellular conversion, or generate muscle cells in vitro. Contrastingly, the conversion of fibroblasts into muscle cells by other myogenic regulatory factors (MRFs), including Myf5, Myf6 and Myog, has been significantly less studied. To address this disparity, here we set out to investigate the role of MRFs during transdifferentiation of fibroblasts into myotubes, or direct reprogramming into induced myogenic progenitor cells (iMPCs) by small molecule treatment. Surprisingly, we found that Myf6, a transcription factor that is associated with late-stage myogenesis, can efficiently convert fibroblasts into either postmitotic myotubes alone, or iMPCs with compound treatment. The Myf6-derived iMPCs expressed differentiation markers including MyHC and Myogenin, in addition to muscle stem cell markers including Pax7 and Myf5. Following clonal isolation, Myf6-derived iMPCs were highly proliferative and could be expanded extensively for more than 10 passages. As a next step, we generated a dox-inducible Myf6 overexpression mouse model to investigate in a defined system the reprogramming kinetics and transcriptomic changes which occur in fibroblast during the conversion into the myogenic lineage by Myf6 overexpression. Mechanistically, we demonstrate that Myf6, in concert with small molecule treatment, can generate expandable Pax7-positive cells in the absence of endogenous MyoD, demonstrating MyoD is dispensable for Myf6-iMPC production and maintenance. Collectively, these findings report an unexpected role for Myf6 in the generation of expandable Pax7-positive stem cells from fibroblasts, highlighting an alternative path for the induction of the muscle stem cell fate in vitro.

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Intracellular autofluorescence allows the isolation of functional human MuRC subpopulations with distinct cell states

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Adult muscle stem cells (MuSC) are essential for skeletal muscle regeneration. The MuSC pool displays a continuum of cell states, with subpopulations competent for self-renewal ($Pax7^{High}$) while others are more restricted to proliferation and differentiation ($Pax7^{Low}$). We recently demonstrated that human MuRC are heterogenous for Pax7 expression with a $Pax7^{High}$ subpopulation in a deeper quiescent state and with a reduced metabolic activity. These data suggest that $Pax7^{High}$ MuRC may constitute an appropriate stem cell source for potential therapeutic applications in muscle diseases. Nevertheless, there is no tool to isolate viable human $Pax7^{High}$ subpopulations. In the present study, we evaluated cellular autofluorescence (AF) and investigated it as a tool to isolate viable $Pax7^{High}$ and $Pax7^{Low}$ MuRC. Using a standard violet laser (excitation 405nm and emission 450/50nm), we observed that human MuRC are highly autofluorescent as compared to proliferating myoblasts. Human MuRC were then sorted by flow cytometry based on their AF level (AF^{High}: 10% of the highest AF and AF^{Low}: 10% of the lowest AF) and analysed by flow cytometry for Pax7 expression. We observed a significant increase in the proportion of $Pax7^{High}$ cells in MuRC A^{High} population (68% of $Pax7^{High}$) as compared to MuRC AF^{Low} population (35% of $Pax7^{High}$). We then tested their capacity to re-enter the cell cycle and we observed that MuRC AF^{High} take significantly longer time to enter the cell cycle than do MuRC AF^{Low} or myoblasts with respectively 5%, 31%, and 51% of EdU positive cells after 24h of reactivation. We also noticed that freshly isolated MuRC AF^{High} and MuRC AF^{Low} displayed similar capacities to form myotubes as compared to myoblasts after 48h in differentiation conditions. Together, these results demonstrate that cellular AF can be used to isolate viable MuRC subpopulations with distinct Pax7 expression levels. Moreover, we observed that freshly isolated MuRC AF^{High} are functional myogenic cells in vitro, in a deeper quiescent state. In vivo experiments are currently underway to evaluate the therapeutic potential of MuRC AF^{High} and MuRC AF^{Low} subpopulations after transplantation in immunodeficient mice.

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Integrated nutrient-sensitized screens predict mitochondrial disease genes

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Mitochondrial disorders are a group of rare genetic conditions that primarily affect mitochondria, the energy-producing structures within our cells. These disorders result from mutations in either nuclear or mitochondrial DNA, causing disruption in the mitochondria's capacity to generate energy in the form of ATP, resulting in a marked reliance on glucose for energy production, or "glucose auxotrophy". As a result, various bodily systems can be severely impacted, particularly those with high energy demands such as muscles. Symptoms of mitochondrial disorders can vary widely, encompassing fatigue, muscle weakness, developmental delays, and even organ failure. Diagnosis proves challenging due to their heterogeneous nature, dual genomic inheritance, and the high number of genes required for mitochondrial function. Consequently, about 50% of the patients with suspected mitochondrial disorders have inconclusive molecular diagnosis, both in Switzerland and abroad. Here, we harness glucose auxotrophy and report a series of nutrient-sensitized proteomics and CRISPR/Cas9 screens that identify genes likely to cause mitochondrial disorders at a genome-scale. We use machine learning to integrate our datasets with other resources and compile a list of candidate mitochondrial disease genes, which we validate experimentally. We discover new genes required for mitochondrial function, mutations of which are highly likely to cause mitochondrial disorders in patients. We propose our list to be prioritized for the diagnosis of mitochondrial disorders, and we are currently working with clinicians at the Lausanne Hospital (CHUV) to help diagnose patients with suspected mitochondrial disorders but inconclusive molecular diagnosis.

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LACTB, a new player in lipid modulation

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Mitochondria evolved from alpha-proteobacteria through endosymbiosis. Several mitochondrial proteins are evolutionarily related to bacterial proteins although not always keeping similar functional properties. Lactamase B (LACTB) derives from the penicillin-binding/beta-lactamase protein family involved in peptidoglycan synthesis of bacteria. In eukaryotic cells LACTB is localized in the mitochondrial intermembrane space. Since mitochondria do not synthesize peptidoglycan, LACTB may have gained one or more novel function(s). Using the CRISPR-Cas9 technology, we generated a *LACTB* knock-out zebrafish and *LACTB* KO fibroblasts. *LACTB* deletion altered proteins of the mitochondrial electron transport chain, particularly complex I, and decrease mitochondrial function. Skeletal muscle lipidomics evidenced modifications of specific glycerophopholipids. In parallel, changes in skeletal muscle lipidomics, show modifications of particularly of specific glycerophopholipids. In *LACTB* KO cells, we observed increases in lipid droplets, changes in lysosomal numbers, size and function, as well as altered LC3B flux. Taken together, our results reveal the potential role of LACTB in lysosomal lipid storage myopathies associated with mitochondrial dysfunction, recalling the constellation of observations in diseases related to specific mutations and their effect on the autophagy pathway such as Fabry disease and other spingolipidoses.

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MitoBooster: a novel strategy for inducing mitophagy and enhancing cellular homeostasis

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Mitophagy is a cellular process that involves the selective degradation and removal of damaged or dysfunctional mitochondria. It plays an important role in cellular homeostasis, maintaining energy balance and metabolism and preventing cellular stress. Impaired mitophagy has been implicated in the pathogenesis of several disorders, including neurodegenerative diseases, metabolic disorders, cancer, and cardiovascular diseases. While there have been promising findings regarding the potential therapeutic applications of mitophagy modulation, the translation of these discoveries into clinical practice requires further research and development. This includes optimizing delivery methods, specifically targeting mitochondria, and assessing efficacy in proper animal or cell models. The goal of this study was designing chimeric peptides (MitoBoosters) containing 3 different regions: 1. CPP for cell internalization, 2. MTS for Mitochondrial targeting, and 3. LC3 interacting region to recruit the autophagy machinery. Our results from electron microscopy, MitoKeima, Mito-GR and autophagosome reporter cell lines confirmed significantly increased mitophagy in cellular or zebrafish model. We also confirmed biogenesis of mitochondria and higher mitochondrial respiration after the mitophagy stage in healthy and parkinsonian patient-derived fibroblast cells. Our data also showed that MitoBooster (MB)-treated Zebra fish larvae had significantly higher net velocity, active time, and movement distance compared to untreated controls. The mito stress test revealed increased ATP production in MB-treated C2C12 myotubes compared to untreated control and EPS-trained myotubes, indicating that MBs may have an exercise-mimicking effect. We believe that this novel strategy for peptide delivery and mitophagy induction can be served as a promising therapeutic to tackle diseases with impaired mitochondria and by removing dysfunctional mitochondria through mitophagy, making room for the generation of new, healthy mitochondria which can function more efficiently, leading to improved endurance and energy metabolism.

Posters

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Structural analysis of synapse impairment in cells derived from ALS patients with C9ORF72 mutation using cryo-electron tomography

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Altered synaptic function is one of the hallmarks of numerous neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). It is known that a hexanucleotide repeat expansion (HRE) in *C9orf72* gene is a major cause of familial ALS. According to clinical and experimental studies, multiple pathogenic mechanisms have been postulated by the dysfunction of the C9ORF72 protein, such as impaired autophagy, sequestration of RNA-binding proteins by the transcribed RNA repeats, and the formation of toxic dipeptide aggregates. However, the cause of synaptic dysfunction in ALS is not well understood. The aim of my study is to gain more insight into neurotransmission deficiencies in ALS cases by examining the effect of the diminution of C9orf72 function on neuronal connectivity, synaptic vesicle (SV) architecture, and function in neuronal-like cell models. We will use transmission electron microscopy (EM) and in-situ cryo-electron tomography (ET) techniques to study synaptic structure of motor neurons (MNs) differentiated from induced pluripotent stem cells (iPSCs) derived from both patients and healthy controls. This will provide us with information to identify changes in the conformation and population of SVs. The EM/ET data will be supported with immunocytochemistry, molecular biology, and biochemical assessments of candidate genes and proteins. In this proposal, we aim to 1) develop a protocol that effectively produces mature synapses from immortalized cell lines; 2) study the morphological changes in SVs in MNs from C9orf72 ALS specimens; and 3) investigate if patient-derived cell lines exhibit other pathological characteristics such aggregates in synaptic regions that contribute to synaptopathy.

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Investigation of SRSF1 interactions with GC-rich RNA repeats and consequences on phase separation in the context of ALS/FTD

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of the motor system [1]. Frontotemporal dementia (FTD) is a disorder characterized by behavioural changes, aphasia [2]. The primary genetic cause of these diseases is the expansion of (G4C2) hexanucleotide repeats (HRE) in the first intron of *C9ORF72* gene, with more than 30 repeats deemed to be pathological [3]. Serine-arginine rich splicing factor 1 (SRSF1) in complex with NXF1 exports HRE RNAs from the nucleus into the cytoplasm, where they are translated into toxic dipeptide repeats, which is seen as the main cause of ALS/FTD development in the presence of GC-repeats [4]. SRSF1 is a protein composed by two RNA recognition motifs (RRM1+2) which are linked by a flexible linker, and a C-terminal disordered RS domain [5]. HRE RNAs can also condense into foci in the nuclei of patient neurons [6], and SRSF1 co-localizes with these foci [4]. We investigate the effect of HRE RNA length and secondary structure on its interaction with SRSF1, formation and maturation of condensates with SRSF1 in vitro. For our project, we could successfully produce (G4C2)14 and (G4C2)43 RNAs representing normal and pathological transcripts respectively. We purified full-length (FL) SRSF1 protein and its RRM1+2 domains to better characterize the importance of the RS domain in this context. We could demonstrate that both (G4C2)14 and (G4C2)43 HRE transcripts form G-quadruplexes (G-Qs) as well as hairpins in vitro. RRM1+2 domains of SRSF1 showed higher binding affinity to the G-Q forming RNAs compared to hairpin structures. However, the SRSF1 FL protein displayed structure-independent interaction with the HRE RNAs which can be explained by the presence of the disordered RS domain. Moreover, the RS domain of SRSF1 induces liquid-liquid phase separation (LLPS) of the FL protein in the presence of HRE RNAs. Interestingly, this effect depends on the protein:RNA stoichiometry, which could have some consequences on ALS/FTD development. Now, we would like to understand how these SRSF1- HRE RNA droplets can evolve into RNA foci as they may prevent SRSF1 to favour HRE RNA export to the cytoplasm. In addition, we will try to obtain some structural information about the interaction of SRSF1 with these repeats with the hope of finding a therapeutic strategy to block these interactions.

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Understanding the selective neurotoxicity of serine-palmitoyltransferase (SPT) mutations towards motor and sensory neurons

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Amyotrophic lateral sclerosis (ALS) is a progressive, neurodegenerative disease of the lower and upper motor neurons, characterized by severe muscle wasting, eventually leading to paralysis and death. ALS is genetically heterogeneous and the etiology remains unclear in the majority of cases.

Serine–palmitoyltransferase (SPT) is the key enzyme in the de novo synthesis of sphingolipids (SL). Missense mutations in SPT have been previously linked to the hereditary sensory and autonomous neuropathy type 1 (HSAN1) - a predominantly sensory neuropathy that is caused by the pathological formation and accumulation of an atypical class of neurotoxic 1-deoxysphingolipids. Although associated with the same enzyme, the entity of the SPT-ALS mutations is different. It is associated with an altered homeostatic control, and an excessive formation of canonical ceramides and complex SL species. Motor neurons seem to be particularly susceptible to these changes whereas sensory neurons appear to be specifically sensitive to 1-deoxysphingolipid accumulation. Within this project, we want to understand why and how the respective SPT mutations affect sensory and motor neurons so differently. Therefore, induced pluripotent stem cell (iPSC) lines have been generated from patient derived fibroblasts carrying either SPT-ALS or SPT-HSAN1 mutations. These HSAN1- or SPT-ALS patient specific derived iPSC lines will be differentiated into sensory (iPSCdSNs) or motor neurons (iPSCdMNs). The primary aspect of the iPSC differentiation experiments will be to compare the capability of the SPT-ALS and SPT-HSAN1 mutant expressing lines to develop, respectively, a motor- or sensory phenotype. The phenotypic changes will be followed along the differentiation process by monitoring the expression of specific development markers at different developmental checkpoints. We will compare the sphingolipid profiles of newly, not yet characterized mutants to previously known variants. The ALS associated species could be used as potential biomarker assisting in the diagnosis and might give some information about the disease mechanism. These experiments will provide insights into the underlying pathomechanisms but also in the metabolic differences between sensory and motor neurons, eventually enabling novel therapeutic targets.

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Characterization of mouse model knocked in for the *RyR1* p.F4976L mutation identified in a severely affected child: physiological, biochemical and structural features

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Mutations in *RYR1*, the gene encoding the Ryanodine Receptor 1 (RyR1) Ca2+ channel, are the most common cause of congenital myopathies, accounting for approximately 30% of all human congenital myopathies. RYR1 related myopathies (RYR1-RMs) can be divided into subgroups depending on inheritance pattern, histological muscle appearance and pathological mode of action of the mutations. Dominant RYR1 mutations, those associated with Malignant hyperthermia (MH) and Central core diseases (CCD), generally affect the biophysical properties of the channel. MH is caused by gain of function mutations whereby the RyR1 becomes hypersensitive to activation, whereas CCD is caused by loss of function mutations, whereby the channels become leaky or exhibit reduced capacity to transport calcium. Recessive RYR1 mutations linked to Multiminicore disease (MmD) and Centronuclear myopathy (CNM), present as compound heterozygous or homozygous mutations, generally lead to a reduction of RyR1 protein content in the patient's muscle. To expand the knowledge on RYR1-RMs with the long term aim of developing a therapeutic approach for patients, we recently created a new transgenic *Ryr1* mutant mouse model carrying the p.F4976L mutation at the homozygous state. This mutation was initially identified in a severely affected child who was born prematurely and demonstrated severe muscle impairment soon after birth. A multilevel approach using in vivo, ex vivo and in vitro methods was used to determine phenotypical, functional and biological alterations of skeletal muscles from homozygous (Ho) mice compared to wild type (WT) and heterozygous (Het) littermates. In vivo experiments showed that Ho mice exhausted significantly sooner than WT or Het littermates during treadmill exhaustion test. Moreover, fast- and slow-twitch muscles from Ho mice generated less force compared to their WT and Het counterparts. They also exhibited a reduction of electrically evoked calcium transients. While no fiber-type switch was reported in Ho muscles, EM analysis showed the presence of ultrastructural abnormalities namely misorientation of the calcium release units, and myofibrillar degenerations. These as well as additional experimental results using the *Ryr1* p.F4976L mutant mouse demonstrate that our model recapitulates in part the clinical phenotype of the human proband and offers a useful experimental model for future clinical studies.

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Functional consequences of MH-causative *RYR1* mutations on the immune system

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The Ryanodine receptor 1 (RyR1) is an intracellular Ca2+ release channel expressed in skeletal muscle where it releases the calcium necessary for muscle contraction. To date, more than 700 *RYR1* variants have been identified in humans, of which 165 are considered pathogenic and have been shown to cause a number of skeletal muscle diseases, including malignant hyperthermia (MH), central core disease (CCD), multiminicore disease (MmD) and centronuclear myopathy (CNM). Past work by our laboratory has revealed a possible link between *RYR1* mutations found in MH individuals and alterations of the immune system.

To better understand the consequences of *RYR1* mutations and their effect on the human immune system, we are now focusing on a large cohort of MH patients and healthy donors (HDs) from Brazil. Results from a Questionnaire from 20 patients shows that the incidence of allergic disease in RYR1 mutation carriers is 45%, with 8 cases of allergic rhinitis and 1 case of asthma. Another 25% of the mutation carriers showed food allergies, while the remaining mutation carriers (41%) did not show any allergic symptoms. In comparison, among the total population of Brazil, allergies are reported to affect 14% of the population.

To study the functional effect of the mutations directly, serum samples have been collected from patients and HDs at rest and after vaccination and serological cytokine levels and Igs are being analyzed by ELISA and Mass spectrometry. In addition, peripheral B cells from MH patients and HDs were immortalized with Epstein-Barr virus. These cells are currently being assessed for their sensitivity to RyR1 stimulation using the RyR1 agonist 4-chloro-m-cresol. The results obtained so far indicate that cells from MH patients are more sensitive to 4-chloro-m-cresol stimulation in terms of calcium release. Since calcium signaling is involved in a number of intracellular signaling pathways in B cells, such changes in calcium homeostasis may affect cellular immune responses including secretion of cytokines or expression of surface receptors. The increased sensitivity of MH mutant cells to activation may thus explain why MH patients are more likely to experience allergic symptoms.

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Genotypic and transcriptomic characterization of patients with King-Denborough Syndrome

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King-Denborough syndrome (KDS) is a rare genetic disorder characterized by dysmorphic features, short stature, myopathy and malignant hyperthermia susceptibility (MHS). Most patients carry autosomal dominant mutations in *RYR1*, the gene encoding the ryanodine receptor calcium release channel (RyR1). However, in most cases the parents are clinically unaffected even though one of them carries the same *RYR1* mutation and may be MHS. Such findings have puzzled clinicians and geneticists leading to the hypothesis that the patients may carry a second yet to be identified null or hypomorphic RYR1 mutation. This hypothesis would be compatible with the myopathic phenotype of the patients but is more difficult to associate with their characteristic short stature, profound scoliosis, low set ears and neck webbing. In the present study we enrolled three patients with genetically confirmed dominant *RYR1* mutations clinically affected by KDS. Their ages ranged between 11 and 20 years of age, 2 were males and one was a female. We performed RNAseq analysis using RNA isolated from the patient's muscle biopsies and compared the results to those from age and sex-matched healthy controls. Our results show that muscles from KDS patients express significantly lower amounts of RYR1 transcripts as well as decreased levels of many transcripts including, ATP2A1, MYH1, DHRS7C, TMEM52 and PRKAG3. Gene Ontology Gene set analysis further revealed significant downregulation of genes encoding ribosomal proteins, constituents and subunits as well as genes encoding mitochondrial enzymes, proteins involved in oxidative phosphorylation and cellular respiration. More than 100 other mutations were identified in the patient's RNA and we are currently searching for the causative second mutation.

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Effect of RYR1 mutations on muscle spindle function and their impact on the musculoskeletal system

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Mutations in *RYR1*, the gene encoding the ryanodine receptor 1 (RyR1), the calcium channel of the sarcoplasmic reticulum, are the underlying cause of approximately 30% of all congenital myopathies. Patients usually present developmental delays, muscle weakness, hypotonia and muscle stiffness that are caused by defects in channel conductance or decreased amounts of RyR1 protein leading to less calcium being released from the sarcoplasmic reticulum. However, in addition to the skeletal muscle phenotype, many patients also present musculoskeletal defects including scoliosis, congenital hip dislocation, foot deformities, joint laxity etc. whose underlying causes have not been investigated. We hypothesized that muscle spindle malfunction caused by mutant RyR1 channels in intrafusal muscle fibers, could be the link between mutations in the *RYR1* gene and the musculoskeletal changes observed in patients. Muscle spindles convey spatial information relating to muscle stretch and velocity of contraction to the central nervous system, which responds by adjusting the tonic response of agonistic and antagonistic muscle groups. Here we studied the role of RyR1s in intrafusal muscle fibers in a mouse model we created knocked in for compound heterozygous Ryr1 mutations (dHT). Our results support the hypothesis that the skeletal deformities observed in many patients with *RYR1* mutations are due at least in part, to the malfunction of intrafusal muscle fibres, which make up muscle spindles. Firstly, we obtained immunohistochemical evidence that RyR1s are located in the polar region of muscle spindles. The polar region is the contractile domain endowed with fusimotor activity. Secondly, muscle spindles from dHT mice showed altered architecture, particularly the capsules surrounding the spindle and nuclei was 30 % large than that present in WT littermates. Thirdly, biomechanical mobility tests using the Beam Walk and CatWalk tests revealed significant changes in gait and locomotion. For example, the Terminal Dual Stance parameters which translate into a lack of synchronicity between the limbs, was significantly higher in dHT mice compared to WT littermates. Fourthly, microtomography studies revealed dramatic changes in the curvature of the spine resulting in kyphosis and scoliosis (Cobb angle= 20) in dHT mice. This study provide evidence for a causative role of mutant RyR1 in the skeletal malformation observed in patients with *RYR1* mutations.

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ANTXR2 regulates Collagen VI homeostasis in skeletal muscle

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In skeletal muscle tissue, a specialized and highly organized extracellular matrix (ECM) guarantees mechanical support for force transmission and provides appropriate environmental signals for muscle development, homeostasis, and repair. Collagen VI (COL6) plays critical roles in the ECM of skeletal muscles, and mutations of COL6 genes in humans are causative for a distinct class of congenital muscle diseases, whose major forms are Bethlem myopathy and Ullrich congenital muscular dystrophy. Maintaining a proper and at the same time highly dynamic ECM structure is mandatory for muscle fibers and muscle stem cells. Therefore, the synthesis, deposition and degradation of ECM proteins are tightly regulated under physiological conditions as well as during adaptation to physical exercise, disuse or disease.

The recently characterized ECM receptor anthrax toxin receptor 2 (ANTXR2, also known as CMG2) is involved in the binding and endocytosis of B. anthracis toxins but also of COL6. Mutations of ANTXR2 gene in humans result in hyaline fibromatosis syndrome, a systemic disease characterized by the formation of subcutaneous nodules enriched with ECM components in several tissues. However, the function of ANTXR2 in skeletal muscle was never investigated thus far.

We found that muscles of ANTXR2 null ($Antxr2^{-/-}$) mice display a progressive accumulation of COL6 in the endomysium, accompanied by an expansion of the perimysium and by muscle stiffening. Of note, $Antxr2^{-/-}$ mice undergo age-dependent muscle hypertrophy and myofiber remodelling with increased protein synthesis, thus highlighting how increased COL6 deposition and altered ECM organization have a major impact on muscle homeostasis. Interestingly, $Antxr2^{-/-}$ male mice display phenotypic muscle defects resembling human tubular aggregates myopathies, with an accumulation of tubular aggregates within myofibers accompanied by decreased strength, but with no signs of muscle degeneration.

Altogether, these data candidate ANTXR2 as a novel player involved in COL6 and ECM homeostasis in skeletal muscle, paving the way for further work aimed at dissecting in detail the underlying mechanisms involved in the phenotypic defects of ANTXR2 null mice, as well as for studies aimed at investigating the involvement of ANTXR2 in human muscle diseases.

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Metabolic dysregulation contributes to the development of dysferlinopathy

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Dysferlin is a transmembrane protein that plays a prominent role in the control of membrane repair in damaged muscle fibers. Accordingly, mutations of the dysferlin gene cause progressive muscular dystrophies collectively referred to as dysferlinopathies for which no effective treatment exists. Unexpectedly, even though experimental approaches can successfully restore membrane repair, muscles remain dystrophic, suggesting that additional, hitherto unknown dysferlin-dependent functions contribute to the development of the pathological phenotype. Therefore, alternative or complementary therapeutic strategies are needed. In many muscle diseases including Duchenne muscular dystrophy, overexpression of PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1α) in skeletal muscle ameliorates muscle fiber atrophy, integrity and function. Therefore, we were interested in the therapeutic effect of muscle PGC-1α on dysferlinopathy. Surprisingly however, the pathology of dysferlin-deficient mice was exacerbated by elevated levels of PGC-1α. This accelerated disease progression unveiled novel aspects that could be crucial for the development of the dystrophy. Our experiments revealed an altered metabolic phenotype in dysferlin-deficient muscles, which might contribute to disease etiology. More specifically, we observed increased glucose uptake and a pronounced glycogen accumulation in dysferlin-deficient muscles in the presence of throttled glycolysis. We could demonstrate that by further elevating muscle glycogen disease progression is accelerated. Therefore, targeting glucose and glycogen metabolism by for instance dietary or non-damaging exercise interventions could alleviate disease progression. Collectively, our results not only shed light on novel aspects of dysferlin function, but also propose new therapeutic avenues to ameliorate these muscular dystrophies, complementing those targeting membrane repair.

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Molecular mechanisms of complement activation and neuromuscular disruption by combinations of autoantibodies from patients with Myasthenia Gravis

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In patients with myasthenia gravis, acetylcholine signaling from motor nerves to muscles is disrupted by autoantibodies against postsynaptic membrane proteins, most commonly the acetylcholine receptor (AChR). Studies of patients' sera, which typically contain complex mixtures of anti-AChR antibodies, have provided support for the existence of several pathomechanisms, of which the most important is complement-mediated destruction of the receptor-bearing postsynaptic membrane. However, there is no correlation between the titers of anti-AChR antibodies and the severity of the disease, or response to treatment, suggesting a large variability in the pathogenicity of individual antibodies. Understanding this variability, however, requires the ability to isolate individual antibodies, which, until recently, was rarely achieved. We therefore developed a suite of techniques to enable isolation of monoclonal AChR-binding antibodies from single B cells. Initial experiments with antibodies from patients with myasthenia gravis have yielded the unexpected finding that their pathogenicity is dependent on synergistic combinations of antibodies targeting disparate subunits of the receptor, and that single antibodies are almost harmless. The effect appears to be mediated by complement, because complement blockade in vivo prevents the pathogenic effect of the antibody combinations. The objective of this study is to elucidate why combinations of antibodies against different subunits of the AChR are so much more effective in triggering complement activation and tissue destruction than single antibodies. This phenomenon has clear implications not only for the treatment of myasthenia gravis, but for our basic understanding of the interaction between antibodies, complement and cells in health and disease.

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Integrative analysis of the single nucleotide polymorphism and inflammatory cytokine responses in patients with Myasthenia Gravis

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Autoimmune Myasthenia gravis (MG) is characterized by muscle weakness due to impaired neuromuscular transmission. Autoantibodies against components of the neuromuscular junction (AChR, MuSK, or LRP4) play an important role in the pathogenesis, often in addition to thymic abnormalities. Several studies of genome-wide association (GWAS) have provided evidence for genetic variants linked to MG, such as single nucleotide polymorphisms (SNPs) within CTLA4, HLA-DQA1, and TNFRSF11A. Recently, we have reported that subsets of not only B cells but also inflammatory T helper cells correlate with MG severity. However, the connection between SNPs and immune system features - that could potentially serve as indicators for identifying individuals at risk of MG - require further investigation. Here, we examined the correlation between the MG-associated SNPs (based on available GWAS data) and immune phenotypes with global serum cytokine profiling in the patients with MG.

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Three heterozygous GAA cases mimicking late-onset Pompe disease

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Background: Late-onset Pompe disease (LOPD) is a recessive disorder caused by acid α-glucosidase (GAA) deficiency. Carriers of one GAA pathogenic variant are asymptomatic. There are several cases reported, where only one pathogenic GAA variant has been identified. Here, we present three unrelated cases with suspected LOPD carrying one pathogenic GAA variant together with other heterozygous variant(s) related to glycogen storage or structural muscle protein.

Methods: All patients were examined by a neurologist and underwent WGS (60x, PCR-free, PE150). The GAA enzyme activity was measured in dried blood spot, leukocytes and/or in fibroblasts (also used for total RNA sequencing, PE75).

Results: The phenotype of all three patients included adolescent to adult disease onset, proximal weakness and myalgia. GAA was decreased, and creatine kinase was normal to mildly elevated. WGS revealed following heterozygous phenotype-related variants: Patient 1 maternal GAA c.-32-13T>G (pathogenic), paternal PHKB (VUS) and AMPD1 (VUS); Patient 2 GAA c.-32-13T>G and AMPD1 (VUS; same variant as in Patient 1); Patient 3 GAA c.1082C>T p.(Pro361Leu) (pathogenic) and FHL1 (VUS). In fibroblasts of Patient 1, the activity of GAA was 0.83 (NR 6.04-17.06) nmol/min/mg protein and RNA sequencing confirmed abnormal transcripts due to c.-32-13T>G but no other splicing defects in GAA.

Conclusion: There may be a small cohort of LOPD-like cases where a symptomatic heterozygosity or digenic/oligogenic inheritance can be considered.

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Acute modulation of VMA21 leads to skeletal muscle degeneration

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Autophagy, a central catabolic process involving lysosomes, is essential for skeletal muscle homeostasis (1). X-linked myopathy with excessive autophagy (XMEA) is a rare myopathy characterized by accumulation of autophagic vacuoles in skeletal muscle and caused by mutations in the *VMA21* gene (2). VMA21 is a chaperone of the vacuolar (v)-ATPase proton pump, required for lysosomal acidification (3). VMA21 deficiency would reduce v-ATPase activity and thereby increase lysosomal pH, ultimately leading to autophagy blockade. Interestingly, we recently showed that Vma21 muscle-specific knockout mice display early lethality marked by severe skeletal muscle degeneration. Moreover, besides the short, ubiquitous VMA21 isoform (VMA21a), our lab identified a long VMA21 isoform, predominantly expressed in skeletal muscle (VMA21b). The physiological roles of each VMA21 isoform and their contribution to XMEA remain unknown. To better understand the roles of VMA21 isoforms, we modulated their expression in vivo using adeno-associated virus (AAV) vectors. Interestingly, acute expression of the Cre recombinase in skeletal muscle from Vma21flox/flox mice led to complete tissue degeneration, indicating that VMA21 is required for adult muscle homeostasis. Surprisingly, overexpression of VMA21a isoform also caused rapid, pronounced muscle degeneration. Partial and complete muscle regeneration was detected 4 and 8 weeks after AAV injection, respectively. In contrast, VMA21b overexpression did not lead to major histological defects, suggesting that the two isoforms have distinct pathophysiological roles in skeletal muscle. Ongoing experiments aim at understanding the mechanisms leading to muscle degeneration upon VMA21 depletion and VMA21a overexpression, and at further characterizing the effect of VMA21b overexpression on skeletal muscle (e.g., changes in fiber type/size, autophagic flux, mTORC1/Akt activity). Unveiling yet-unknown functions of VMA21 will help to better understand XMEA pathogenesis and identify therapeutic approaches to counteract muscle dysfunction in the disease.

1. Castets, P., et al., J. Neuromuscul. Dis., 2016. 3(2): p.127-155.
2. Hirano, M. and S. DiMauro, Cell, 2009. 137(2): p.213-5.
3. Ramachandran, N., et al., Acta Neuropathol., 2013. 125(3): p. 439-57.

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Studying the role of S-Acylation of ORAI1 channel and STIM1 protein in tubular aggregate myopathies

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Background: Skeletal muscle contraction relies on depolarization of the plasma membrane (PM) and activation of Voltage-gated calcium (Ca2+) channels (VGCCs). VGCCs induce Ryanodine receptors (RYR) in the sarcoplasmic reticulum (SR) to release Ca2+ into the cytosol. To sustain muscular contraction and prevent muscular fatigue, intracellular SR Ca2+ replenishing is critical and requires the activation of STIM-ORAI mediated Store-Operated Ca2+ Entry (SOCE). Stromal Interacting Molecule proteins (STIMs) are Ca2+ sensors located in the SR. They are activated upon Ca2+depletion from the SR lumen, which leads to their oligomerization and translocation to SR-PM junctions where they gate PM ORAI channels. Ca2+ is pumped back into the SR, which causes STIM de-oligomerization and prevents Ca2+ overload. Gain of function (GoF) mutations in STIM1 and ORAI1 cause a non-treatable rare genetic muscle disorder named Tubular Aggregate Myopathy (TAM), due to excessive SOCE and tubular aggregate formation.
Aims: We aim to develop a new therapeutic strategy to target STIM-ORAI GoF in TAM, by focusing on their lipid postranslational modifications (PTM). A recent work from our group has identified a new pathway to modulate SOCE by S-Acylation of ORAI1 on Cysteine residue C143. S-Acylation is a highly dynamic and reversible lipid PTM that consists of the attachment of a long-chain fatty acid (most commonly palmitate) onto free thiols of cysteines. The reaction is mediated by a family of membrane protein Acyltransferases (PATs) of which, 23 isoforms have been identified in humans. S-Acylation of ORAI1 by PAT20 promotes its activity.
Methods and Results: Using the biochemical AcylRAC (Resin Assisted Capture) assay, we found that STIM1 and its muscle specific longer isoform STIM1L are S-Acylated at Cysteine C437. In HEK cells, STIM1 S-Acylation is mediated by PAT20, which enhances SOCE (intracellular Ca2+measurements); however, S-Acylation of STIM1L has an opposite role. To assess the relevance of STIM-ORAI S-Acylation during muscle differentiation, we used primary human myoblasts. In nascent Myotubes, all SOCE proteins were S-Acylated and PAT20 upregulation enhances muscle differentiation, revealed by an increased expression of MEF2c and Myosin heavy chain (Immunofluorescence).
Conclusion: We are currently investigating the molecular mechanism behind the opposite effects of STIM1 vs STIM1L S-Acylation in the context of muscle physiology. Unveiling those molecular mechanisms is crucial to understand how the SOCE machinery is regulated by S-Acylation during muscle differentiation and to propose it as a novel therapeutic targeting approach to treat TAM patients in the future.

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Fumaric acid in adults with Becker muscular dystrophy: A single-centre, open-label, safety, and efficacy proof of concept 28-days trial

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No abstract

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The masseter muscle, an often-neglected muscle in Duchenne muscular dystrophy: a pilot study using mouse models

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Clinical studies show that patients with Duchenne muscular dystrophy (DMD) develop orofacial dysfunction. Currently, the majority of studies using DMD mouse models focus on limb and respiratory muscles and the craniofacial muscles are neglected. The primary aim of this pilot study was to investigate how the lack of dystrophin affects orofacial function, with emphasis on the changes in the masseter muscles. Two different mouse models for DMD, namely the mdx^{5Cv} model (8 mice, 13-week-old), representing mild disease, and the *D2/5Cv* model (8 mice, 13-week-old) representing severe disease, were used. Histological sections of masseter muscles and of two exemplary limb muscles (extensor digitorum longus and soleus) were examined for inflammation; frequency of centralised nuclei; presence of degenerative or regenerative fibres; and fibrosis. Observations were compared to wild-type mice in the respective genetic backgrounds. Results suggest that masseter muscles in the mild mdx^{5Cv} model are more severely affected in term of myonecrotic lesions and inflammation when compared to the *D2/5Cv* model at 13 weeks. However, masseter muscles in *D2/5Cv* mice exhibit greater histopathological changes than those of mdx^{5Cv} mice, namely fibrosis, as determined by trichrome Masson and collagen staining. The pathological fibrosis of the masseter muscles is comparable to that of the limb muscles. In conclusion, the masseter muscles of DMD mice showed dystrophic changes, comparable to those in the limb muscles. These insights may be highly relevant for the future development of anti-fibrotic therapy for patients with DMD, where the target should not be limited to the skeletal but also to the craniofacial muscles.

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Investigating the myotendinous junction in the context of LAMA2-related muscular dystrophy

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The myotendinous junction (MTJ) is the site where muscle fiber tips attach to tendons. To warrant strong attachment between muscle fibers and tendons, foldings of the tendon protrude into invaginations of the muscle fiber membrane to allow robust cell-matrix contact under mechanical stress and strain. Interestingly, single nuclei RNA-sequencing shows that the nuclei at the MTJ are transcriptionally distinct from myonuclei. Moreover, proteins selectively accumulate at the MTJ. However, the significance of this local specialization is understudied and hence poorly understood.

One of the components that accumulates at the MTJ basement membrane is laminin-α2, the long chain of the heterotrimeric laminin isoform laminin-211. Mutations in the *LAMA2* gene coding for laminin-α2, cause a severe, early-onset muscular dystrophy characterized by a reduction in muscle mass and function. To elucidate the role of laminin-α2 and to investigate its role for MTJ function, we use LAMA2-deficient dy^{W}/dy^{W} mice. In a first exploratory approach, we investigate the cellular and molecular composition of the MTJ combining morphological techniques with proteomics analysis.

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LIN28 inhibition reverses miRNA expression and disease hallmarks in myotonic dystrophy

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Sequestration of MBNL1 by expanded CUG or CCUG repeats causes myotonic dystrophy (DM) [1-3]. Besides its activity as a splicing factor, MBNL1 binds to a UGC motif located within the terminal loop region of pre-miR-1 and prevents access to the RNA from LIN28, an RNA binding protein which mediates uridylation and subsequent degradation of miRNA precursors by TUT4 [4]. Several groups are investigating peptides or small molecules as possible inhibitors of the MBNL1.CUGexp interaction as potential therapeutic approaches for treatment of DM [5-6]. However, targeting of MBNL1 might also interfere with non-pathological MBNL1-RNA interactions. Therefore, we are investigating an alternative high risk approach by inhibition of LIN28. Cardiac defects and myotonic dystrophies in DM patients are directly associated with dysregulation of a small number of ion channels that are targets of miRNAs, e.g. miR-1, miR-9, miR-30, miR-107 and miR-181. We have shown that precursors of these miRNAs bind to MBNL1 and LIN28 in in vitro assays, and furthermore are lowly expressed in myotubes from DM1 and DM2 patients compared to those from healthy volunteers. Two prominent ion channels in cardiac defects are CACNAC1 and KCNJ2 [4, 7], both of which have conserved predicted binding sites for some of these miRNAs in their 3'UTRs. Treatment of cells with NTPA an inhibitor of LIN28, increased levels of several of these miRNAs in DM1 and DM2 myotubes, and reduced levels of KCNJ2, possibly via altered biogenesis of miR-1 and miR-9, both of which are predicted to regulate KCNJ2. In a separate study, it has also been shown that reduced expression of MEF2 transcription factor in human heart sample from DM1 model alters miR-1 expression levels [8]. We observed in human skeletal myotubes from DM1 and DM2 patients that indeed MEF2A expression level is low compared to cells from healthy volunteers. Therefore, we checked the effects of compound NTPA on MEF2A mRNA and protein levels in myotubes from DM1 and DM2 patients. NTPA treatment increased both MEF2A protein and mRNA levels and would be expected to partially correct the disease phenotype. An additional consequence of expanded CUG repeats in myotonic dystrophies is a reduction of the CLC-1 chloride channel at mRNA and protein levels [9] by a mechanism which is not understood. Consistent with previous observations, preliminary experiments have shown that NTPA increases expression of CLC-1 in DM1 and DM2 myotubes. We are currently testing other small molecules which has similar LIN28 inhibition activity as a RNA therapeutics strategy towards pathophysiology of myotonic dystrophies or cardiac defects.

[1] Ho T.H. et al. (2004) EMBO J 23, 3103-12
[2] Kanadia R.N. et al. (2006) Proc Natl Acad Sci U S A 103, 11748-53
[3] Miller J.W. et al. (2000) EMBO J 19, 4439-48
[4] Rau F. et al. (2011) Nat Struct Mol Biol 18, 840-45
[5] Garcia-Lopez, A. et al. (2011) Proc Natl Acad Sci U S A 108, 11866-71
[6] Jharomi AH. et al. (2013) ACS Chem Biol 5, 1037-43
[7] Yang, B. et al. (2007) Nat. Med. 13, 486–491.
[8] Kalsotra, A. et al. (2014) Cell Reports 6,1-10
[9] Mankodi, A. et al. (2002) Mol Cell 10, 35-44

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Cultivated Meat

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The emerging field of cultivated meat production offers a sustainable and ethical alternative to traditional livestock farming. However, challenges in achieving cost-effective and efficient meat production have limited its scalability. Muscle stem cells are the natural source of muscle tissue in animals, making them the ideal starting point for cultivated meat without the need for traditional livestock. Within this study we aim to further characterise bovine muscle stem cells for cultivated meat purposes.

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“Oh my DAG”, unveiling the specific link between human insulin resistance and lipotoxicity

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Ectopic lipid depositions, defined as an excess accumulation of lipids in non-adipose tissues, are positively associated with obesity and are key determinants of insulin resistance (IR). Among other lipids, diacylglycerols (DAG) have been incriminated in driving a large part of the lipotoxicity theory, but which DAG moieties are responsible of this negative effect is not yet known. In this project, we will describe the distribution of DAG moieties in the different organelle of muscle from donors with and without IR to unveil which DAG species are associated with IR. To this aim, organelle fractions will be extracted from muscle biopsies of lean and obese volunteers. Custom isomer specific standards will be used as reference for the quantification of organelle-specific DAG in a precision medicine approach, permitting determination of fatty acid chains position, chain length and saturation degree. After the identification of target DAG moieties, we will characterize their mechanisms of actions. For this, specific caged-DAG will be specifically delivered to organelles, i.e. mitochondria, lipid droplets (LD) and plasma membranes, to allow a local increase of the targeted DAG species. This will be performed in human primary muscle cells of insulin resistant and insulin sensitive individuals. Phosphorylation pathways activated by DAG, as well as insulin sensitivity and mitochondrial capacity will be measured as main outcomes. This project will allow to describe the causal paradigm and identify those DAG species that are “lipotoxic”.

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A novel PLIN3 splicing variant reveals a conserved mitochondrial targeting of perilipin protein family members

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Perilipin3 (PLIN3) is a ubiquitous member of the Perilipins lipid droplet-coating protein family. Due to its lipid binding function, PLIN3 contributes to lipid droplet growth, lipophagy, phosphatidylcholine synthesis and to lipotoxicity cellular protection. PLIN3 is highly expressed in skeletal muscle, where its levels have been correlated with fatty acid oxidation and exercise training. We identified a PLIN3 splicing variant, hence named PLIN3B. Expression of the canonical PLIN3A and PLIN3B in cells highlighted a specific mitochondrial targeting of the novel isoform. PLIN3B lipid droplet and mitochondria targeting is shared with zebrafish zPlin2 and zPlin3, suggesting a conserved double targeting feature. PLIN3B expression in HeLa cells led to a reorganization of the mitochondrial network, with swollen mitochondria clustered in the perinuclear area. At the ultrastructural level, electron micrographs revealed alterations of the mitochondrial suborganellar organization. In accordance with the morphological phenotype, mass spectrometry analyses of PLIN3B interactors identified several mitochondrial partners, with a particular enrichment of intermembrane space proteins. zPlin2 and zPlin3 morpholino and CRISPR/Cas9 knocked down zebrafish presented lower spontaneous locomotion and reduced mitochondrial respiration. We are currently investigating the role of PLIN3B in intraorganellar lipid trafficking and in the modulation of mitochondrial phospholipid balance. In addition, cellular and in vivo models are under development. The discovery of PLIN3B indicates the existence of a splicing-dependent regulation of PLIN3 targeting and a possible ancestral mitochondrial function of the perilipins family members.

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More than the clock: distinct regulation of muscle function and metabolism by PER2 and RORα

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Circadian rhythms, governed by the dominant central as well as various peripheral clocks, regulate almost all biological processes, including sleep-wake cycles, hormone secretion, and metabolism. In certain contexts, regulation and function of the peripheral oscillations can be decoupled from the central clock. However, the specific mechanisms underlying muscle-intrinsic clock-dependent modulation of muscle function and metabolism remain unclear. We investigated the outcome of perturbations of the primary and secondary feedback loops of the molecular clock in skeletal muscle by specific gene ablation of Period 2 (Per2) and RAR-related orphan receptor alpha (RORα), respectively. In both models, a dampening of core clock gene oscillation was observed, while the phase was preserved. Moreover, both loops seem involved in the homeostasis of amine groups. Very divergent outcomes were seen for overall muscle gene expression, primarily affecting circadian rhythmicity in the Per2, and non-oscillating genes in the RORα knockouts, leading to distinct outcomes in terms of metabolome and phenotype. These results highlight the entanglement of the molecular clock and muscle plasticity, and allude to specific functions of different clock components, i.e. the primary and secondary feedback loops, in this context. The reciprocal interaction between muscle contractility and circadian clocks might therefore be instrumental to determine a finely tuned adaptation of muscle tissue to perturbations in health and disease.

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STIM1 and STIM1L in skeletal muscle: central regulators of calcium circuitry

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In most cell types, the depletion of the endoplasmic reticulum (ER) Ca2+ stores leads to a mechanism called the Store Operated Ca2+ Entry (SOCE). The main players of this mechanism are STIM1, a Ca2+ sensor of the ER, and Orai1, a plasma membrane Ca2+ channel. Our group previously reported that two isoforms of STIM1 are highly expressed in skeletal muscle: STIM1 and a longer splice variant, STIM1L. Since Ca2+ signals are essential for proper muscle differentiation, especially at an early stage of development, our aim was to determine the specific functions of STIM1 and STIM1L during myogenesis. To do so, we infected human primary myoblasts with a lentivirus encoding a doxycycline-inducible shRNAmir expression to downregulate both STIM1/1L isoforms or only STIM1L. The knock-down was triggered simultaneously with the differentiation of the myoblasts or after 4 days of differentiation, during the maturation process. Electrical stimulations-induced Ca2+ transients revealed that STIM1/1L and STIM1L down-regulation impacts the amplitude of Ca2+ transients only at an early stage of myogenesis, while the cytosolic Ca2+ clearance was slowdown upon both down-regulations and at both early and late stages of differentiation. To investigate this defect, we divided the Ca2+ clearance into 2 different mechanisms: Ca2+ extrusion and Ca2+ repumping within the stores. First, we observed that STIM1 but not STIM1L affected the Ca2+ extrusion, and using siRNA against the different extrusion systems (PMCA1/4 and NCX3), we showed that only PMCA1 is of importance in myotubes. Preliminary results of co-IP experiments showed indeed an interaction between STIM1 and PMCA1 in myotubes, that could explain the modulation of PMCA1 activity by STIM1. Then, surprisingly, only STIM1L downregulation increased the SR Ca2+ content and accelerated the rate of SR Ca2+ repumping, pointing to a negative effect of STIM1L on SERCA activity. This remains however to be confirmed. Overall, our data reveal an alteration of the Ca2+ circuitry upon down-regulation of STIM1 and STIM1L. The Ca2+ clearance is impaired during early and late stages of myogenesis with evidence that STIM1 and STIM1L are modulators of Ca2+ extrusion and Ca2+ repumping, respectively.

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Orai3 and its partner AHNAK2 regulate the activation of human skeletal muscle stem cells in vitro

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Skeletal muscle repair and maintenance rely primarily on the activation of quiescent muscle stem cells (MuSC). Upon activation, the skeletal MuSC re-enter the cell cycle and proliferate as myoblasts, which subsequently either differentiate and fuse to form new fibers, or return to quiescence to replenish the MuSC pool. Mechanisms underlying human MuSC activation and self-renewal remain poorly understood, largely due to the difficulty of monitoring these processes in humans. In our work, we used an in vitro model of human primary muscle cells where we can study the activation of stem cell-like cells called reserve cells (RC). We demonstrated that the activation of RC is promoted by the Ca2+ channel Orai3, but surprisingly, in a Ca2+-independent manner. Indeed, Orai3 downregulation decreased the ability of RC to activate in response to serum stimulation, while an Orai3 mutant protein not permeable to Ca2+ rescued RC activation. Moreover, Orai3 depletion was associated with a reduced RC population and an increased myotube differentiation after 48h. Proximity-dependent biotin identification (BioID) revealed a large scaffold protein, AHNAK2, as a new potential partner of Orai3. When downregulated, AHNAK2 demonstrated similar effects on RC activation and myotube differentiation than Orai3 knockdown. The downregulation of both Orai3 and AHNAK2 did not potentiate the effects suggesting a role in the same signaling pathway. We are now generating different constructs of AHNAK2 to determine the interaction site(s) between both proteins. Furthermore, we postulate that Orai3 and AHNAK2 may act either early during differentiation by promoting commitment to myotubes or play a role in the maintenance of RC quiescent state at later stages. We are currently exploring both hypotheses. These studies on Orai3 and AHNAK2 shall provide new insights into the molecular process that underlies human RC activation and quiescence. In addition, we found that dystrophin downregulation increases the expression of Orai3 and AHNAK2 suggesting that these proteins may be involved in the pathological mechanisms of Duchenne muscular dystrophy (DMD). Thus, our work shall open up a new understanding of MuSC dysregulation during the process of DMD.

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Roles of the two STIM2 isoforms in human myotube formation and function

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Store-operated Ca2+-entry (SOCE) is fundamental during myogenesis and involves the plasma membrane Ca2+ channel Orai, and the sarcoplasmic reticulum (SR) resident Ca2+ sensor STIM. Ca2+ store depletion activates SOCE, and cytosolic Ca2+ is eventually pumped back into the SR by the SERCA pumps. Skeletal muscle cells express STIM1 and STIM2, and both have as well splicing isoforms. Thus, STIM2.1 was shown to act as a negative regulator of SOCE while STIM2.2 acts classically as an activator. We confirmed by qPCR the presence of both STIM2 isoforms with STIM2.1 being more expressed in myotubes compared to myoblasts. The aim of our study is to understand the implication of STIM2.1 and STIM2.2 on human myogenesis, and their impact on cell proliferation, differentiation and function of human myotubes. We showed that myoblast proliferation was slowed down upon STIM2.1 but not STIM2.2 downregulation. While STIM2.2 downregulation led to the formation of larger myotubes with a higher percentage of MEF2C-positive nuclei, no major changes were observed in siSTIM2.1 condition. However, when we assessed the Ca2+ response in myotubes, we noticed an increased basal Ca2+ level upon STIM2.1 knockdown only, which was associated with a nuclear localization of NFATc1 at rest. This result, which confirms the negative regulatory role of STIM2.1 on SOCE, shows however that the activation of NFATc1 by SOCE is not necessary for the myotube fusion steps. To mimic excitation-contraction (EC) coupling, we stimulated the cells with high K+. The cytosolic Ca2+ peak level was reduced when STIM2.1 or STIM2.2 were downregulated. In line, STIM2.1 and STIM2.2 downregulation decreased Ca2+ level within the SR (measured with the ER-targeted Cameleon probe D1ER). We also assessed the expression level (mRNA) of the three main players in EC coupling, namely DHPR, RyR1 and STAC3 and all three were decreased in STIM2.1 downregulation condition only. We conclude that, although STIM2.1 and STIM2.2 are involved in SR filling, they play very different roles during myotube differentiation. STIM2.1 plays a role in EC coupling protein expression and STIM2.2 in the control of myotube formation. These results show that STIM2.1 and STIM2.2 are involved in mechanisms other than SR filling.

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From nerve to muscle and vice versa: exploring how exercise shapes the motor unit and muscle-nerve communication

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The degeneration of the motor unit, the functional entity responsible for muscle contraction, is a common hallmark of different pathological conditions, like neuromuscular diseases, cancer cachexia and sarcopenia. A deeper understanding of the mechanistic and functional aspects of motor unit plasticity are, hence, of high interest to understand the physiology and pathophysiology of this structure. We already know that exercise confers strong beneficial outcomes in various neuromuscular pathologies by remodeling the motor unit.

However, it is not known whether exercise directly affects motor neurons or if the changes observed at the pre-synaptic level are due to muscle retrograde signalling. In addition, we want also to understand whether and how exercise influences nerve-muscle crosstalk.

In order to address these questions, we performed an acute bout of exercise, by letting adult mice run on a treadmill until exhaustion. We then analyzed neuromuscular junction (NMJ) morphology and muscle innervation at different timepoints after the end of the running protocol.

Here we show that a single exercise session boosts genes linked to axon guidance in untrained mice. This is accompanied with a decrease in the number of axons innervating hindlimb muscles, and an increase in the amount of denervated fibers. These initial findings suggest that exercise triggers a rapid axonal retraction, similar to what happens after peripheral nerve injury, indicating an immediate NMJ remodeling in untrained animals. Additionally, we are exploring the entire motor unit's transcriptomic profile using single-nucleus RNAseq to understand its complex cellular interactions.

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Molecular mechanisms modulating muscle protein synthesis.

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The imbalance between protein synthesis and protein degradation is a common pathogenic mechanism (muscle wasting) occurring in myopathy, neuromuscular disorders, and chronic diseases, and is also a hallmark of aging. Recently, we observed that the activation status of the serine/threonine kinase mammalian target of rapamycin (mTOR) modulates the translation landscape of the aging muscle. Notably, inhibition of MTORC1 with rapamycin (RM) anti-aging treatment results in the redistribution of ribosome towards the 5'UTR of RNA, accompanied by the increased expression of specific translation initiation factors such as EIF3F. In order to better understand the molecular regulation of muscle protein synthesis we decided to identify EIF3F proximal/transient interactome. We employed the proximity labelling properties of BirA biotine ligase (BioID) and established functional EIF3F-BioID chimers. The biotinylated proteins captured upon expression of EIF3F-BioID show a significant enrichment in translation regulation, mRNA stability, ubiquitin/proteasome machinery, and the WNT pathway. We used Crispr/Cas editing tool to successfully engineer knock-in human muscle cell lines expressing endogenous level of functional EIF3F-BioID (at EIF3F locus). We demonstrate with these cells that EIF3F mainly associates with components of the pre-initiation complex of translation (EIF3A/B/CL/E, EIF4E, EIF4G, EIF5) irrespectively of the cell differentiation status. We identify a core EIF3F-EIF3 complex where EIF3F strongly associates with LAMP1 (lysosomal-associated membrane protein1) in terminally differentiated cells suggesting a possible involvement of EIF3F in local protein synthesis (LAMP1 positive vesicles). Surprisingly, we show that EIF3F exhibits both a nuclear and a cytosolic distribution (diffuse; LAMP1+/- vesicles) in proliferating and terminally differentiated cells. Moreover, half of the identified proximal interactor are nuclear proteins and reflect translation independent function of EIF3F. In summary, we unravel that endogenous EIF3F mainly associates with translation regulatory proteins in muscle cells and identify LAMP1 as a new binding partner, suggesting the possible involvement of EIF3F in spatial regulation of protein synthesis.

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