Session 6: Myopathies: Mechanisms, modeling, medication

Lectures

L6.1

Disorders of the sarcomere – new phenotypes, genes, and mechanisms

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The traditional classification of (congenital) myopathies is based on their histopathological and ultra-structural features, while more recent approaches highlight genetic, physiological and mechanistic groups. One such example are the disorders of the sarcomere, which subsume disorders caused by genetic abnormalities of the molecular components that make up the sarcomere, including its thin and thick filaments, but still encompass a wide clinical, genetic, and mechanistic heterogeneity. Well-known myopathies within this group include the nemaline myopathies for the thin filament and the skeletal and cardiac myosinopathies for the thick filament. Here I will discuss four new entities that emerged from highly collaborative work, including new phenotypes and mechanisms for known disease genes (TPM3 and MYBPC1) as well as the characterization of phenotypes and mechanisms for new disease genes (TNNC2 and UNC45B). These disorders cover the thin filament (TPM3 and TNNC2) as well as the thick filament (MYBPC1 and UNC45B) and cover mechanistic concepts of hyper-contractility (TPM3), hypo-contractility (TNNC2), myogenic tremor generation (MYBPC1), as well as chaperone-related secondary myosinopathy (UNC45B).

L6.2

Role of nebulin in health and disease

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Nebulin is a not well understood giant protein expressed in skeletal muscle. Nebulin has unique N-and C-termini whereas the majority of the molecule is comprised of small actin-binding domains, organized into super-repeats. The importance of nebulin is shown by the many nebulin mutations that cause nemaline myopathy. Here we focus on recent insights into the function of nebulin including results that are relevant for exon-skipping gene therapy. We also show that in slow muscle, nebulin collaborates with leiomodin-2 (Lmod2), with nebulin regulating a proximal thin filament segment and Lmod2 regulating the length of a distal segment that is nebulin-free.

L6.3

Molecular mechanisms of centronuclear myopathies

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Centronuclear myopathies are a clinically and genetically heterogeneous group of genetic neuromuscular disorders. Clinically, they are associated with severe limb muscle weakness, eye muscle involvement, and multiple technology dependencies (often including ventilator and wheelchair dependence). Genetically, the primary causes are mutations in MTM1, DNM2, RYR1 and SPEG. These genes encode proteins that likely share molecular function(s) in skeletal muscle, and have mutations that likely cause shared pathomechanisms. We have established pre clinical models in cell lines, zebrafish and mice that accurately represent the features of the genetic subtypes of CNM. We have employed these models to define disease pathogenesis and develop therapies. In this presentation, I will describe recent advances from our lab related to identification of novel pathways and potential treatments for CNM.

Oral Presentations

O6.1

The role of the super relaxed state of myosin in the manifestation of diverse cardiomyopathy phenotypes associated with the *MYL3* gene

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The super relaxed (SRX) state is considered central to modulating force production and energy utilization in cardiac muscle and serves as a research tool to study sarcomere energetics. We assessed the SRX state of myosin in two pathological models of human cardiomyopathy expressing hypertrophic (HCM-A57G) or restrictive (RCM-E143K) mutations in the myosin essential light chain (ELC, *MYL3* gene). Mouse papillary muscles (PM) were incubated in 250 μM mant-ATP and decay fluorescence curves were collected during a rapid mant-ATP exchange with 4mM non-labeled ATP. The SRX-data for HCM-A57G and RCM-E143K vs. WT ELC hearts were analyzed alongside their phosphorylation status of myosin regulatory light chain (P-RLC). HCM-A57G mice promoted the SRX-to-DRX transition and showed a 40% increase in P-RLC compared with the RLC of WT-ELC. The RCM-E143K model favored the SRX state and showed ~2-fold lower P-RLC compared with WT-ELC. In agreement with SRX data, electrically stimulated PM from mice either increased (HCM-A57G) or decreased (RCM-E143K) the duration of force transients compared with WT-ELC mice. In summary, both mutations imposed antagonistic effects on the ATP-dependent myosin energetic states, with HCM-A57G fostering the DRX state and signifying the hypercontractility phenotype and RCM-E143K favoring the energy-conserving SRX state. These effects correlated with an up-regulated or downregulated RLC phosphorylation and with observed earlier enhanced or depressed intact heart function in HCM-A57G and RCM-E143K mice, respectively.

O6.2

Zebrafish as a model for dissecting the *in vivo* roles of Collagen VI

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Collagen VI (COL6) is a key extracellular matrix protein, expressed in different tissues where it is involved in a range of physiological and pathological processes. COL6 plays an important role in skeletal muscle, and mutations of COL6 genes are causative for different inherited muscle diseases in humans. To elucidate the pathogenic mechanisms underlying COL6-related disorders, diverse animal models were generated. However, the functions exerted by COL6 during embryogenesis remain to be elucidated. Thanks to its numerous advantages, zebrafish is a powerful model organism for studying vertebrate development and gene function. We generated a novel zebrafish COL6 null line through CRISPR/Cas9 site-specific inactivation of the *col6a1* gene. Phenotypic characterization of zebrafish COL6 null embryos and larvae revealed that lack of COL6 causes defective architecture of slow muscle fibers, with locomotor dysfunctions, together with defects in the motor axons elongation and in acetylcholine receptors patterning. Molecular and ultrastructural analysis showed that *col6a1* null fish display autophagy and organelle defects during development. Notably, treatment with salbutamol, a beta-2 adrenergic receptor agonist, leads to a significant amelioration of the neuromuscular defects in *col6a1* null embryos, thus paving the way for further drug studies in this animal model aimed at finding novel treatments for COL6-related disorders.

O6.3

Kbtbd13^{R408C}*-knockin* mouse model displays muscle-type dependent onset and progression of NEM6 myopathy

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Patients harboring mutations in *KBTBD13* (NEM6) display a impaired muscle relaxation, which compromises normal muscle function daily-life activities. The majority of NEM6 patients harbor the Dutch founder mutation (c.1222C>T, p.Arg408Cys). Recently, we identified that *KBTBD13*^{R408C} slows muscle relaxation through an actin-based mechanism. However, disease onset and progression remains largely uncharacterized. To study this, we characterized Soleus (SOL) and Extensor digitorium longus (EDL) morphology and function of homozygous *Kbtbd13*^{R408C}-knockin and wild type mice at 7 days, and 1, 3, 9 and 18 months of age. Morphological and functional assays of SOL and EDL of *Kbtbd13*^{R408C}-knockin mice showed no differences at 1 month old when compared to wild type mice. Nemaline bodies, slow-twitch fiber predominance, and hypertrophy of slow-twitch and fast-twitch fibers was observed in SOL muscle starting from 3-months-old and progressed over time. SOL intact muscle mechanics assays revealed impaired relaxation kinetics and decreased maximal tension starting at 3-months-old in *Kbtbd13*^{R408C}-knockin mice. Functional assays in EDL showed impaired maximal tetanic force and relaxation kinetics at 18-months-old in *Kbtbd13*^{R408C}-knockin mice, but not in younger mice.

In conclusion, the *Kbtbd13*^{R408C}-knockin mouse model phenocopies human NEM6 hallmarks, displaying a muscle-type dependent onset and disease progression, both structurally and functionally. Hence, this model enables us to further decipher the NEM6 pathomechanism and provides a therapeutic window to test treatment strategies.

Virtual Posters

P6.1

New strategy for utrophin activation in human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) for the studies of its role in Duchenne Muscular Dystrophy-associated cardiomyopathy

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Duchenne muscular dystrophy (DMD) is an X-linked disease caused by mutations in gene encoding dystrophin – component of dystrophin-associated glycoprotein complex present in muscle cells, maintaining a structural function in stabilizing the sarcolemma. Lack of this protein leads to progressive muscle weakness followed by respiratory failure and cardiomyopathy, which is a major cause of death of DMD patients. Mechanisms of cardiomyopathy are not yet fully known and currently there are no available treatment strategies, therefore in our studies we focused on deciphering the role and potential compensatory mechanism of utrophin, a protein which shares a high sequence and protein structural similarity to the dystrophin. Cell therapy based on human induced pluripotent stem cells-derived cardiomyocytes expressing the utrophin represents a new potentially therapeutic approach to the treatment of DMD-associated cardiomyopathy. As the utrophin is present in muscle cells only at the stage of fetal development, our goal was to activate its expression in hiPSC-CMs. For this approach, we utilized the CRISPR/Cas9 strategy for gene activation based on nuclease dead Cas9 (dCas9), transcriptional activators and guide RNA sequences targeting the gene of interest. The system was delivered to hiPSC-CMs with a use of adeno-associated viral vectors (AAV). Measurement of GFP-positive cells after transduction of hiPSC-CMs with AAV serotypes 6 and 9 encoding GFP revealed more efficient gene transfer in case of serotype 6. The strategy proved to be effective and the utrophin activation was confirmed both on mRNA and protein level.

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P6.2

Exercise-activated Ca^{2+} entry and enhanced risk of Heat Stroke

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Exertional/Environmental Heat Strokes (EHSs) are hyperthermic crises triggered by strenuous physical exercise and/or exposure to environmental heat, which are caused by an altered intracellular Ca^{2+} homeostasis in muscle. Store-Operated Ca^{2+} Entry (SOCE) is a mechanism that influences intracellular Ca^{2+} levels, allowing recovery of extracellular Ca^{2+} during prolonged activity. We recently demonstrated that exercise leads to formation of Calcium Entry Units (CEUs), intracellular junctions between stacks of sarcoplasmic reticulum (SR) and transverse tubules (TTs) that promote interaction between STIM1 and Orai1, the two proteins that mediate SOCE.

Here we tested the hypothesis that exercise-induced assembly of CEUs may increase the risk of EHS when physical activity is performed in challenging environmental conditions.

We subjected 4 months old mice from 3 experimental groups to an exertional stress (ES) protocol: incremental 45 min run on treadmill at 34°C and 40% humidity. We then: a) measured the internal temperature of mice during the exertional stress protocol; b) applied an ex-vivo exertional stress protocol to isolated EDL muscles (tetanic stimulation performed at 30°C); c) analyzed CEUs by electron microscopy (EM).

The data collected in this study suggest that assembly of CEUs during exercise could predispose mice to EHS when exercise is performed in hot/humid environmental conditions.

P6.3

Investigating the interaction of calmodulin with $Ca_v1.2$ calcium channels in Long QT syndrome

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Long QT syndrome (LQTS) is a major cause of sudden cardiac death, and is characterised by dysfunctional ion channel activity. Genetic screening of clinical populations has identified mutations in the calcium (Ca^{2+}) sensor calmodulin (CaM), suggesting an important role of CaM in the molecular aetiology of the disease. However, little is currently known about the molecular mechanism of CaM-associated LQTS. CaM is a small Ca^{2+}-binding protein that is involved in the Ca^{2+}-dependent regulation of multiple targets such as $Ca_v1.2$, where it mediates Ca^{2+}-dependent inactivation (CDI) by interaction with the N-terminal spatial Ca^{2+} transforming element (NSCaTE) and the C-terminal IQ regions on the channel. Ergo, CaM mutations that affect binding to $Ca_v1.2$ may affect channel modulation, leading to arrhythmia.

We investigated the structure of clinically relevant CaM variants and their interaction with $Ca_v1.2$. Circular dichroism and ^1H ^{15}N HSQC NMR experiments reveal alterations to protein conformation that are exacerbated in the presence of Ca^{2+}, and only partially ameliorated by interaction with NSCaTE or IQ domains. Furthermore, isothermal titration calorimetry demonstrates that in Ca^{2+}-saturating conditions, LQTS-associated CaM variants have a significantly decreased affinity for NSCaTE, whilst binding to IQ is significantly stronger. CaM mutations also affect $Ca_v1.2$ activity as measured by patch-clamp electrophysiology. Together, this demonstrates the unique mechanisms by which each mutant interacts with $Ca_v1.2$, leading to prolonged Ca^{2+} influx and manifestation of the LQTS phenotype.

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P6.4

The structural and molecular mechanisms by which a pathogenic variant in troponin T tail domain impairs cardiac length-dependent activation

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Missense variant Ile79Asn in human cardiac troponin T (HcTnT-I79N) has been associated with familial hypertrophic cardiomyopathy (HCM), arrhythmia and sudden cardiac death. Little is known about effects of this pathogenic variant on cardiac myofilament function and structure. To fill this gap, cardiac tissues were harvested from non-transgenic (NTg) control mice and transgenic mice bearing HcTnT-I79N. Left ventricular papillary muscle bundles were permeabilized and mounted for mechanical measurements. Sarcomere length (SL 1.9, 2.1 or 2.3 μm) was set at pCa 8 using HeNe laser diffraction and then Ca^{2+}-dependence of isometric force, sinusoidal stiffness (SS, 0.2% PTP length oscillation) and rate of tension redevelopment (k_{TR}) were measured. We found that HcTnT-I79N tissue exhibited increased Ca^{2+}-sensitivity of force, SS, and k_{TR}, slower k_{TR} at all levels of Ca^{2+}-activation, and less length-dependent activation (LDA). Small-angle X-ray diffraction revealed that HcTnT-I79N skinned cardiac muscles exhibit smaller myofilament lattice spacing at longer SLs (2.1 μm and 2.3 μm) compared to NTg. Using 3% Dextran T500 to osmotically compress the myofilament lattice (SL 2.1 μm), HcTnT-I79N showed no change in myofilament lattice spacing and little change in mechanical parameters associated with LDA. Interestingly, upon osmotic compression, HcTnT-I79N displayed a decrease in disordered relaxed state (DRX, ON state) of myosin and an increase in super-relaxed state (SRX, OFF state) of myosin. We conclude that altered muscle mechanics, lack of responsiveness to osmotic compression, and reduced LDA observed with HcTnT-I79N are partially due to a combination of smaller myofilament lattice and disturbed ON and OFF states of myosin.

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P6.5

Dysregulation of angiogenesis contributes to Duchenne muscular dystrophy pathology

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Duchenne muscular dystrophy (DMD), caused by a lack of functional dystrophin, is characterized by progressive muscle degeneration. Dysregulated angiogenesis has already been hypothesized to contribute to DMD pathology, however, its status in *mdx* mice, a model of DMD, is still not fully clear. Our study aimed to investigate angiogenesis-related alterations in skeletal muscles of 6- and 12-week-old *mdx* mice compared to wild-type (WT) counterparts.
We revealed dysregulation of several angiogenic factors including decreased vascular endothelial growth factor A (VEGF) in skeletal muscles of dystrophic animals. Concomitantly, we observed impaired *in vitro* tube formation by human aortic endothelial cells (HAECs) in presence of serum from 12-week-old *mdx,* but not WT mice. Although a higher number of $CD31^{+}\alpha\text{-}SMA^{+}$ double-positive blood vessels and an increased percentage of endothelial cells were found in dystrophic skeletal muscles, the abundance of pericytes was diminished and the blood flow was reduced. Moreover, impeded perfusion recovery after hindlimb ischemia (HLI) associated with a blunted inflammatory and regenerative responses was evident solely in DMD mice. In younger, 6-week-old *mdx* animals, neither basal blood flow nor its restoration after HLI was affected reinforcing the hypothesis of age-dependent angiogenic dysfunction in dystrophic mice.
In conclusion, the complexity of angiogenesis-related alterations in dystrophic animals implicates for testing of vascular-based therapies aiming at the restoration of functional angiogenesis to mitigate DMD severity.

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P6.6

Estimating the impaired lateral force transmission in older humans through an experimentally-based finite elements model

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The decrease of muscle mass with aging is accompanied by a much greater decline in muscle strength. Several studies suggest that this can be associated with an impaired ability to laterally transmit the force generated by muscle fibers, which may be partially due to alterations of the extra-cellular matrix (ECM) with aging. ECM is a multi-level structure from single fibers to the whole muscle. Therefore, the multiscale finite elements (FE) modelling is a powerful tool to quantitatively assess its role.
We experimentally compared the relative contribution of the ECM to passive tension generated by elongation in small bundles of young healthy (Y: 21 y.o.) and elderly (E: 67 y.o.) subjects. Our data suggested that the larger amount of collagen in elderly subjects (Y: 3.3%, E: 8.2%) can fully account for the observed difference in total tension, and that the intrinsic stiffness of the connective material was almost unchanged.
Then, we developed a micromechanical FE model to simulate a muscle bundle formed by a few fibers connected through the ECM. We defined separated meshes for ECM and the contractile material. Using our experimentally based parameters, we quantitatively assessed the influence of ECM age-related modifications on lateral force transmission reproducing two experimental protocols proposed in the literature. The numerical simulations support the evidence that changes of ECM can contribute to the loss of contractile force with aging.

P6.7

Crystallographic structures of titin immunoglobulin-like i21 domains involved in dilated cardiomyopathy

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The protein titin is the main responsible for the passive elasticity of the sarcomere, determining the stiffness of cardiomyocytes. Titin is also a key factor in the etiology of heart disease, given that truncating mutations in titin are the most common cause of dilated cardiomyopathy (DCM). However, missense mutations have commonly been classified as variants of uncertain significance because of their high frequency in the general population and the absence of functional annotation. Recently, a cysteine to serine missense mutation targeting a conserved cysteine in the I21 domain of titin has been identified as a cause for DCM in humans, but the mechanisms triggering the disease are still not clear. Here, we present the structural and biophysical characterization of the wild type and mutated domains. We show that, despite the thermal stability of the mutated domain is severely compromised, its overall structure remains unaltered. We observed that the I21 titin domain contains a second cysteine located 5Å away from the cysteine mutated in dilated cardiomyopathy patients, but our biochemical analyses ruled out the potential contribution of intramolecular disulfide bonds to the higher stability of the wild-type domain. Alternatively, we hypothesize that the strong difference in hydrophobicity between cysteine and serine in the core of the domain can stabilize the unfolded state of the mutant domain. Our results demonstrate that titin domain destabilization by missense variants can lead to DCM, even if the structure is preserved, and highlight the importance of the chemical microenvironment in preserving protein function.

P6.8

The muscle glycogen phosphorylase (PYGM) role in health and disease

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Muscle glycogen phosphorylase (PYGM) is a key enzyme in the first step of glycogenolysis. Muscle isoform differs from other glycogen phosphorylases in expression pattern and biochemical properties. The main role of PYGM is providing sufficient energy for muscle contraction. However, it is expressed in tissues other than muscle, such as the brain, lymphoid tissues, and blood.

We attempt to analyze the available data regarding the features and protein partners of PYGM to shed light on its possible interactions and functions. The analysis of bioinformatics resources shows that PYGM is important not only in glycogen metabolism, but also in such diverse processes as the insulin and glucagon signaling pathway, insulin resistance, necroptosis, immune response, and phototransduction. PYGM is implicated also in several pathological states, such as muscle glycogen phosphorylase deficiency (McArdle disease), schizophrenia, and cancer. The data indicates that PYGM is especially involved in processes demanding rapid supply of energy[1].

Glycogen metabolism, and the subsequent cellular energy balance, is important for all cells. The analysis of research data from databases and publications shows several interesting connections between PYGM and other proteins, which need further investigation.

References:

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P6.9

From mutated gene to protein – investigation of Emery-Dreifuss muscular dystrophy type 1 molecular background using patient's cells

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Emery-Dreifuss muscular dystrophy is a rare genetic disease caused by mutations in genes encoding nuclear proteins lamin (EDMD2, EDMD3), emerin (EDMD1) or associated proteins, characterized by skeletal muscle wasting, contractures of major tendons and cardiac conduction defects. The molecular background is not clear. The mutations found in EDMD1 patients may result in loss of emerin, protein loss of function or gain of toxic properties by changing interaction network. Most of analysed EDMD1 patients' cells were reported to be emerin null, however this conclusion might have come from the single immunostaining approach.

In search for a molecular background, we established and analysed five lines of patient-derived fibroblasts with mutations in EMD gene encoding emerin: 187+1 G>A, Δ153C (2 patients), 399+1 G>C, 450insG. We performed a sequencing of all emerin exons, focusing on splicing sites, to confirm mutations. We analysed emerin transcripts length with one step RT-PCR and expression level of particular regions of emerin transcripts with relative qPCR. We also examined a presence of particular peptides resulting in mutations with western blotting and immunfluorescence using antibodies directed to different emerin epitopes.

The obtained results let us conclude how particular mutations lead to changes in EMD expression, changes in splicing patterns and finally, in protein level and modification. This brought new insight on emerin control and maintenance as well as EDMD1 background, reported before as resulting mostly from lack of protein. This finding would have implication of potential therapy, that supposed to be aimed at reducing expression of dominant negative shorten proteins, instead of overexpression of wild type emerin.

P6.10

A comprehensive guide to genetic variants and post-translational modifications of cardiac troponin C

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Familial cardiomyopathy is an inherited disease that affects the structure and function of heart muscle and has an extreme range of phenotypes. Among the millions of affected individuals, patients with hypertrophic (HCM), dilated (DCM), or left ventricular non-compaction (LVNC) cardiomyopathy can experience morphologic changes of the heart which lead to sudden death in the most detrimental cases. *TNNC1*, the gene that codes for cardiac troponin C (cTnC), is a sarcomere gene associated with cardiomyopathies in which probands exhibit young age of presentation and high death, transplant, or ventricular fibrillation events relative to *TNNT2* and *TNNI3* probands. Using GnomAD, ClinVar, UniProt, and PhosphoSitePlus databases and published literature, an extensive list to date of identified genetic variants in *TNNC1* and post-translational modifications (PTMs) in cTnC was compiled. Additionally, a recent cryo-EM structure of the cardiac thin filament regulatory unit was used to localize each functionally studied amino acid variant and each PTM (acetylation, glycation, s-nitrosylation, phosphorylation) in the structure of cTnC. *TNNC1* has many variants (> 100) relative to other genes of the same transcript size. Surprisingly, the mapped variant amino acids and PTMs are distributed throughout the cTnC structure. While many cardiomyopathy-associated variants are localized in α-helical regions of cTnC, this was not statistically significant $\chi 2$ ($p = 0.72$). Exploring the variants in *TNNC1* and PTMs of cTnC in the contexts of cardiomyopathy association, physiological modulation and potential non-canonical roles provide insights into the normal function of cTnC along with the many facets of *TNNC1* as a cardiomyopathic gene.

P6.11

High-fat diet increases the risk of environmental heatstroke in mice

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Heat-strokes are life-threatening responses to heat characterized by an abnormal increase in body temperature (>40°C) that may end in death. Environmental heat-strokes (EHSs), often triggered by a hot and humid environment, are caused by excessive heat production in muscle, which in turn is the result of an abnormal Ca^{2+} leak from the sarcoplasmic reticulum (SR) and oxidative stress. As a high fat diet is known to increase oxidative stress, the objective of the present study was to investigate the effects of administration of a high-fat diet in c57bl/6 wild type (WT) mice of 4 months of age (adult). Our results show that WT mice fed with high-fat diet (for 3 months) display: a) increased heat generation and energy expenditure when exposed to a heat stress (ES) protocol (41°C for 1h); b) elevated oxidative stress in both EDL and Soleus muscles; and c) enhanced sensitivity to caffeine and temperature of isolated EDL and Soleus muscles during in-vitro contracture test (the gold standard procedure to test EHS susceptibility in-vitro). Our data suggest that a high-fat diet predispose mice to EHS, possibly as a result of increased oxidative stress and excessive Ca^{2+} leak from the SR. This study may have implications for guidelines regarding food intake during heatwaves, periods of intense environmental heat which are becoming more frequent due to climatic changes.

P6.12

Differential expression of Notch pathway genes in iPSC-derived skeletal muscles from Duchenne Muscular Dystrophy patients

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Duchenne muscular dystrophy (DMD) is an X-linked muscle-wasting genetic disease that causes progressive weakness and premature death. Dystrophin absence leads to a constant state of degeneration, requiring persistent Muscle satellite cell (MuSC) activation. This environment leads to MuSC dysfunction and loss of regeneration capability. Notch signaling pathway plays an essential role in MuSC fate, controlling their proliferation, differentiation, and quiescence. We aimed to evaluate Notch pathway regulation in human patients' iPSC-derived skeletal muscles. We generated and characterized human iPSC-derived skeletal muscles from three unrelated DMD patients and three healthy individuals. Then we interrogated the modulation of the Notch pathway-related genes in one early stage of differentiation. We found downregulation of canonical Notch genes as *HES1, NOTCH3,* and *JAG1* while *HEY1* and *NOTCH2* were upregulated in dystrophic iPSC-derived muscles. Then, we performed RNAseq analysis on three later stages of myogenic differentiation. Among enriched pathways, we found Notch pathway, with differentially expressed genes such as *NOTCH1*, *DLL1* and *STAT6*. Our study validates the iPSC model as an important tool to understand molecular mechanisms of human DMD to unveil new therapeutic targets, highlighting the Notch pathway role on disease pathogenesis.

P6.13

Canine mitochondrial myopathy: the current state of knowledge

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Mitochondrial myopathies represent an extremely heterogeneous group of disorders that may result from genetical defects of nuclear or mitochondrial genes. Based on the current state of animal research, there is limited data about mitochondrial DNA (mtDNA) defects and its association with diseases development of domestic dog (*Canis lupus familiaris*) in contrast to human medicine where recently mitochondrial genetics research has been increased. Most of the genetic research is focused on alterations of a nuclear genome (nDNA) and its impact on disease development without considering abnormalities of the mtDNA.
Molecular findings indicate that improper functioning of mitochondria resulted from genetic defects of mtDNA severely impact on cells and tissue levels especially to that which are heavily dependent on oxidative metabolism such as brain, skeletal, and cardiac muscles, and as a result, on a whole organism.
Among animals, mitochondrial myopathies have been identified mainly in horses and dogs. Canine examples of mitochondrial myopathies include: mitochondriopathy with exertional myopathy, lactic acidosis and stroke like symptoms in English sheep dogs, mitochondrial myopathy in German Shepherd in Parson Jack Russell Terrier and Springer Spaniel and regional encephalic mineralisation of cerebellum affected Jack Russell Terrier. Up to now, described canine mitochondrial myopathies were caused by changes of mtDNA in *COXI*, *COXII* genes. Probably, there are more cases of canine mitochondrial myopathies then reported but diagnostic difficulties related to non-specific symptoms of mitochondrial diseases pressure to draw attention to canine mitochondrial myopathies occurrence and its molecular background.

P6.14

Effects of mutations Q93H and E97K in tropomyosin Tpm2.2 on tropomodulin 1 binding to the thin filament and elongation of the thin filament at the pointed end

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Missense mutations in the genes encoding tropomyosin (Tpm) isoforms, thin filament regulatory proteins, are linked to inherited myopathies and cardiomyopathies. Molecular mechanisms underlying these muscle diseases are not fully understood. Previously, we have shown that mutations occurring in slow muscle Tpm3.12 isoform affected actin filaments length (Moraczewska J *et al*., 2019, *FEBS J* **286**: 1877-189). As the length of thin filaments determines the size of the overlap between thin and thick filaments, it affects contractile force. Thin filaments size is maintained by many regulatory proteins. One of them is tropomodulin (Tmod), which inhibits elongation at the pointed end.
In this work we used two substitutions in Tpm2.2: Q93H, causing congenital myopathy, and E97K, linked to distal arthrogryposis, which are manifested by hypo- and hypercontractile phenotypes, respectively. The effects of the mutations on Tmod1 binding to actin and inhibition of thin filament polymerization in the absence and presence of troponin ($\pm Ca^{2+}$) were analyzed. Our goal was to verify the hypothesis that hypo- and hypercontractile phenotypes may arise from variable thin filaments length.
The presence of troponin, especially without calcium ions, significantly decreased affinity of Tmod1 to the thin filament. We did not observe any pronounced impact of Q93H and E97K mutations on Tmod1 affinity for actin. On the other hand, both substitutions interfered with the pointed end elongation.