Calsequestrin (CASQ) is the main Ca2+ buffering protein in the terminal cisternae of sarcoplasmic reticulum (SR) and a regulator of ryanodine receptor (RYR1)-mediated Ca2+ release. Recently a recurrent mutation, c.731A>G (p.Asp244Gly), in the CASQ1 gene has been reported in Italian patients presenting with a proximal myopathy or fatigue. Muscle histopathology showed the presence of large vacuoles containing aggregates of sarcoplasmic reticulum proteins.
Objective of this study was to identify and characterize CASQ1-mutated patients among patients diagnosed with a vacuolar myopathy with histopathological features resembling the original description by Rossi et al. Clinical, radiological, molecular and histopathological features of patients with identified mutations in CASQ1 gene were collected
Seventeen CASQ1 mutated patients belonging to 10 families were included, all but one sharing the same heterozygous mutation (p.Asp244Gly). A novel p.Gly103Asp was identified in 1 patient. Patients usually presented in the 5th decade with exercise intolerance and myalgias, and later developed mild-to-moderate lower limb proximal weakness with scapular winging and quadriceps hypotrophy with slow evolution over time. CK levels were markedly elevated in all patients (>1000 U/L). Muscle MRI (n=7) showed variable fibro-fatty substitution and muscle oedema in posterior and anterior thigh, posterior leg and triceps brachii. One patient presented with a hypertrophic cardiomyopathy, suggesting that CASQ1 may be involved in heart function. Muscle histopathology showed in all large vacuoles containing aggregates of sarcoplasmic reticulum protein.
In conclusion, we present a large series of patients affected by CASQ1 related myopathy, a recently identified neuromuscular diseases characterized by myalgias and mild proximal weakness with retained ambulation till late age. A possible heart involvement is presented. One mutation is recurrent in Italian patients possibly due to a founder effect, but the identification of a novel missense mutation suggest that CASQ1 related myopathy may be underdiagnosed. Our study suggest to include CASQ1 gene analysis in the process of molecular characterization of patients presenting with myalgia or cramps showing a vacuolar myopathy at muscle histopathology.

myopathy, Calsequestrin 1, vacuolar myopathy

P18 – Muscle function- N° 275 to N° 283

Muscle function- #2486

P18- 275- Implication of NO and ROS in enhanced sarcoplasmic reticulum Ca2+ leak. New mechanism for statin induced myopathy.

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The number of people taking statins is likely to increase in the future as cardiovascular risk thresholds for statin prescription are reduced across the globe. Therefore the need to understand statin induced myopathy, the most common side-effects of statins, is paramount. We have previously shown that statin treatment in vivo increases the frequency and duration of Ca2+ sparks/embers in intact skeletal muscle fibres from the rat, consistent with increased sarcoplasmic reticulum Ca2+ leak. Here we investigate a role for nitric oxide (NO) and reactive oxygen species (ROS) in this increased leak. Male Wistar rats were treated with simvastatin 40 mg/kg/day by oral gavage over 28 days. Intact skeletal muscle fibres from flexor digitorum brevis (FDB) were isolated and loaded with fluorescent dyes. Inhibition of NOS with L-NAME (1 mM) had a greater impact (P>0.05) on NO (indexed with DAF-2) in statin fibres compared with controls, consistent with increased NOS activity with statin treatment. Inhibition of NOS reduced spark frequency by 60% (P>0.01) in fibres from statin-treated animals, but was without effect in controls, suggesting a role for NO in increased leak. Moreover, expression of caveolin 1 and 3 was reduced by 47% (P>0.05) and 15% (P>0.05) while we noted an increase in nNOS (37% P>0.05) and eNOS (97% P>0.005) ingastrocnemius from statin treated animals. Mitochondrial membrane potential (indexed with JC-1) was reduced in statin fibres compared with controls (1.1±0.1 vs. 1.7±0.2 AU; P>0.05); this uncoupling of the mitochondrial respiratory chain would be predicted to increase ROS production. In support of this, there was evidence of increased ROS production with statin treatment- a trend for a decrease in the ratio of reduced:oxidised gluthatione (GSH:GSSG) in GAS from statin-treated animals (P>0.05). Importantly, the superoxide dismutase mimetic MnTMPyP (0.1 mM) and the mitochondrial ROS scavenger Mitotempo (25 μM) both significantly reduced (P>0.05) spark frequency in statin fibres but were without effect in controls. Together these data show that increased SR Ca2+ leak seen in intact muscle fibres from statin-treated rats is linked to an increase in NO and ROS. We propose that caveolin-regulated NO and Ca2+-dependent mitochondrial ROS production modify the RyR to effect this leak. Defining the cellular processes that underlie statin induced myopathy is the first step in the development of co-therapies to improve statin compliance. Sponsored by the BHF

Muscle function- #2510

P18- 276- Muscle-specific alternative splicing regulator Rbm24 is a target of microRNA-222 in skeletal muscle cells

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During skeletal muscle differentiation, gene expression is tightly regulated at both transcriptional and post-transcriptional levels. microRNAs (miRNAs) have recently emerged as key post-transcriptional modulators of gene expression in virtually all biological processes, including myogenesis. A number of miRNAs have been shown to regulate skeletal muscle development and differentiation. Our previous studies on skeletal muscle cells, led to the identification of microRNA-222 (miR-222) that is downregulated during myogenic differentiation and its overexpression leads to alteration of muscle differentiation process and specialized structures. By using in silico miRNA target prediction tools combined with biochemical pulldown techniques followed by RNA sequencing we have identified two new targets of miR-222 involved in regulation of myogenic differentiation, Ahnak and Rbm24. The RNA binding protein Rbm24 is a major regulator of muscle-specific alternative splicing and its down regulation by miR-222 results in defective exon inclusion, impairing the production of muscle-specific isoforms of Coro6, Fxr1 and NACA transcripts, that play important roles in skeletal muscle formation and regeneration. Reconstitution of normal levels of Rbm24 in cells overexpressing miR-222 recovers muscle-specific splicing capacity. We have identified a new function of miR-222 leading

to alteration of myogenic differentiation at the level of alternative splicing, and we provide evidence that this effect is mediated by Rbm24 protein. Interestingly, some forms of muscular dystrophies are associated to aberrant alternative splicing and miR-222 expression levels were found increased in these pathologies. Therefore, we suggest that dysregulation of alternative splicing by miR-222 may play a crucial role in muscle disease pathogenesis.

microRNAs, RISC pulldown, alternative splicing

Muscle function- #2609

P18- 277- O-GlcNAcylation in skeletal muscle: a key regulator of the sarcomeric structure?

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In skeletal muscle, sarcomere is characterized by an accurate structural organization through highly regulated interactions between myofilament proteins. It has been shown that many myofilament proteins are modified by O-N-acetyl-glucosaminylation (O-GlcNAcylation), an atypical glycosylation. Akin to phosphorylation, O-GlcNAcylation is involved in numerous cellular processes, including protein-protein interactions. Although it has been described as a modulator of contractile activity, the role of this glycosylation in sarcomeric organization remains to be considered.

To investigate the O-GlcNAcylation role in sarcomeric structure, C2C12 myotubes were treated with Thiamet-G (Thiazoline amino-ethyl gluco-configured) or DON (6-Diazo-5-Oxo-L-Norleucine) to increase or decrease the global O-GlcNAcylation level respectively. Using western blot, we showed that the myofilament proteome was more finely modulated than the whole proteome since O-GlcNAcylation variations were more sensitive and dynamic on myofilament proteins. Through confocal microscopy after MHC labeling, we pointed out morphometric changes of the sarcomere directly correlated to these O-GlcNAcylation levels. We observed an increase in the dark and M-band width and a decrease in the I-band width and sarcomere length. Clearly, we showed that the sarcomeric structure was modulated according to the O-GlcNAc level.
To determine if protein-protein interactions were modulated consecutively to O-GlcNAc variations, we performed Red-Native PAGE and noted changes in protein complexes profiles, suggesting that protein interactions were modified. We identified, through mass spectrometry, proteins belonging to these modified proteins complexes, and among them, desmin, ?B-crystallin, ?-actinin, filamin-C and moesin, key structural proteins involved in sarcomeric architecture. Native western blot and co-immunoprecipitations were carried out, and we showed that protein expressions in some complexes were modified. So, localization, polymerization and/or interaction changes of these proteins, within the sarcomere, could be discussed.

Taken together, these results suggested that O-GlcNAcylation is involved in sarcomeric structure, protein-protein interactions and in sarcomere remodeling. This work may provide new insights in the understanding of molecular mechanisms of diseases such as myofibrillar myopathies where sarcomere is disorganized, and many protein-protein interactions are disrupted.
O-GlcNAcylation, skeletal muscle, sarcomere structure, protein-protein interactions

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Muscle function- #2616

P18- 278- Voltage gated sodium channels expression in muscles during sepsis

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Voltage gated sodium channels (NaV) are responsible of membrane excitability, and are involved in triggering and propagation of muscle action potential. They influence the excitation/contraction coupling. NaV 1.4 and NaV 1.5 are the mains ? isoforms in skeletal muscles and in cardiomyocytes with a greater number of NaV 1.4 in muscle conversely to heart. Moreover ? subunits (?1-?4), which can modulate the sodium current, are differently represented in muscle and heart. Muscle excitability is decreased during sepsis by inflammation and especially by the pro-inflammatory cytokines released, but the impact of sepsis on channel expression (? and ? proteins) is still not clear.
After seven days of sepsis induced by caecal ligature and perforation in rat, heart, diaphragm (Diaph), soleus (Sol) a typical slow type muscle, peroneus longus (Pl) (mixte muscle) and extensor digitorum longus (EDL) a typical fast type muscle, were dissected free for protein extraction. The quantification of NaV 1.4, NaV 1.5 and ?1-?4 subunits was done by western blotting on membrane protein extracts.
Heart presents less NaV 1.4, ?1 and ?4 subunits, but the same amount of NaV 1.5 that other muscles. After sepsis, NaV 1.4 is decreased in all muscles until -34% in the Pl, whereas sepsis has no effect on NaV 1.5 population. Sepsis has a more pronounced effect on Sol, Pl and EDL, especially for beta subunits with a decrease up to -68% for ?1 in Pl and -56% of ?3 in EDL.
In conclusion, we have evidenced a difference in the repartition of NaV 1.4, NaV 1.5 and ?1-?4 subunits in five different types of muscles in control condition. Moreover we demonstrate that sepsis has a transductionnal effect on channel population as well in ? proteins type as in ? subunits. These effects are depending on the type of muscle. These effects could be involved in the decreased excitability observed during sepsis.

Voltage gated sodium channels, Sepsis, Protein expression, Skeletal muscle, Heart

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Muscle function- #2893

P18- 279- Single muscle immobilization decreases single-fibre Myosin Heavy Chain polymorphism

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Introduction
Muscle contractile phenotype is affected during immobilization. Myosin heavy chain (MHC) isoforms are the major determinant of the muscle contractile phenotype. We therefore sought to evaluate the effects of muscle immobilization on both the MHC composition at single-fibre level and the mitogen-activated protein kinases (MAPK), a family of intracellular signalling pathways involved in the stress-induced muscle plasticity.
Methods
The distal tendon of Wistar rat left Peroneus Longus (PL) was cut and fixed to the adjacent bone at neutral muscle length. Four weeks after the surgery, immobilized and contralateral PL were dissociated using enzymatic method (collagenase 0.3%) and the isolated fibres were sampled. MHC composition of single fibres was assessed using the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method. p38, Jun-c phosphorylation (JNK), and ERK1/2 were measured in 6- and 15-days immobilized PL and compared with contralateral muscle.
Results
MHC distribution in immobilized PL was as follows: I = 0%, IIa = 11.8 ± 2.8%, IIx = 53.0 ± 6.1%, IIb = 35.3 ± 7.3% and I = 6.1 ± 3.9%, IIa = 22.1 ± 3.4%, IIx = 46.6 ± 4.5%, IIb = 25.2 ± 6.6% in contralateral muscle. The MHC composition in immobilized muscle is consistent with a faster contractile phenotype according to the Hill's model of the force-velocity relationship compared with contralateral muscle. Immobilized and contralateral muscles displayed a polymorphism index of 31.1 ± 5.7 and 39.3 ± 2.5%, respectively. Unlike ERK, significant increases in p38 and JNK phosphorylation were observed following 6 and 15 days of immobilization.
Conclusions
Despite the preservation of neural influences, single muscle immobilization at neutral length induces a shift of MHC composition toward a faster phenotype. The decrease in the proportion of hybrid fibres may result from the restriction in the length variations of immobilized muscle.

Muscle immobilization, Myosin Heavy Chain, Fibre polymorphism, Muscle Fibre Plasticity, Contractile phenotype, Mitogen-Activated Protein Kinases

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Muscle function- #2906

P18- 280- Role of Rev-erb-alpha in the control of skeletal muscle lipid metabolism

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Many organs are involved in the regulation of metabolism, although the metabolic role of skeletal muscle has long been underestimated. However, it represents 40% of the total body weight and uses over 80% of available glucose of the body. Skeletal muscle exercise capacity is genetically regulated and follows a circadian rhythm. The Rev-erb-alpha nuclear receptor is a key regulator of the molecular clock, which also plays an important role in the regulation of skeletal muscle metabolism. Pharmacological activation of Rev-erb-alpha leads to better exercise performances. In addition, we have recently observed that it could also play a role in skeletal muscle lipid metabolism.
Histological analysis of different skeletal muscles of Rev-erb-alpha deficient mice shows less lipid droplets within muscle fibers, while more lipid droplets are observed in myogenic cells overexpressing Rev-erb-alpha. These observations were confirmed by Thin Layer Chromatography experiments in skeletal muscle, where the total amount of triacylglycerides is decreased in Rev-erb-alpha deficient mice in the morning, while the phospholipid content is increased in the afternoon compared to wild type mice. In addition, our data reveal a potential role of Rev-erb-alpha in myoblast fusion during myogenic differentiation, which requires plasma membrane reorganisation and therefore lipid rearrangement.
These data suggest a new role for Rev-erb-alpha in the circadian control of skeletal muscle lipid dynamic. Lipidomic analysis of skeletal muscle isolated from Rev-erb-alpha deficient mice at different times of the day is in progress, in order to characterise the role played by Rev-erb-alpha in skeletal muscle lipid dynamic.

Nuclear receptor, lipid metabolism, circadian ryhtm

Muscle function- #3006

P18- 281- MSY3-myogenin network controls neuromuscular junction disruption and muscle wasting progression upon skeletal muscle denervation

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The Y-box protein MSY3/Csda regulates postnatal repression of the myogenic transcription factor myogenin. In postnatal muscle, myogenin plays a key role in regulating pathways involved in muscle maturation and degeneration. MSY3 binds a highly conserved DNA cis-acting element located upstream of the myogenin promoter (MyogHCE) and its activity is regulated in adult muscle by Akt. Our preliminary evidence shows that MSY3 knock down affects AChR expression in muscle fibers and alters in vitro and in vivo AChR distribution in the synaptic and extra-synaptic area of the muscle fibers. Moreover, MSY3

deficiency exacerbates the atrophy induced by denervation, indicating a direct involvement of MSY3 in the muscle-nerve interaction. Since MSY3 controls proper myogenin expression along the muscle fiber and consequently the correct AChR distribution at the end-plates, our working hypothesis is that MSY3-myogenin-AChR and other myogenin targets is a crucial network that regulates NMJ structure and function, and muscle mass, in normal and diseased muscles. By analyzing the distribution of myofibers with an oxidative or glycolitic metabolism in muscles of old WT and hom MSY3 KO mice, we observed a strong increase of oxidative type fibers in the mutant hom mice, suggesting a role of this protein in muscle metabolism regulation, especially in elderly mice. Moreover, analysis of the MSY3 binding sites in adult muscle, shows a preferential occupancy in proximity of genes that control mitochondrial oxidative phosphorylation. We also have evidence that HDACs control MSY3 activity in response to nerve signals. Indeed upon denervation we observed a strong enrichment of the MSY3 occupancy at the myogenin promoter when HDAC4 is genetically ablated, suggesting a role of HDAC4 in inhibiting MSY3 DNA binding capacity. All together these results suggest that MSY3 can mediate modulation of the myogenin regulatory pathway during muscle atrophy and it is a potential therapeutic target for therapies for muscle degeneration-associated diseases.

MSY3/Csda, myogenin, neuromuscular junction, muscle wasting, denervation

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Muscle function- #3015

P18- 282- Mechanosensing and mechanosignaling by caveolae: A new role in human muscular dystrophy diseases

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Cells perceive their microenvironment not only through signaling receptors, but also through physical and mechanical cues. Cells translate these stimuli by mechanotransduction into biochemical signals controlling multiple cellular functions. We recently established caveolae as mechano-sensors that play a major role in the homeostasis of the membrane tension of the cell membrane. Caveolae are 60-80 nm cup-shaped membrane invaginations, rich in sphingolipids and cholesterol and made of oligomerized caveolins (Cav1, 2 or 3). Caveolae are particularly abundant in muscle cells. We have investigated the role that caveolae play in the physical and biological responses to mechanical stress at the plasma membrane of human muscle cells. Based on membrane tension measurements and mechano-signaling responses to cyclic stretching, we show that caveolae are not mechanically functional in several limb-girdle muscular dystrophies. These studies open the way to a new mechanistic understanding of human muscular dystrophies and to the elaboration of new therapeutics to correct caveolae dysfunction in muscular dystrophies.

caveolae, mechanics, myotube, mechanosignaling

Muscle function- #4467

P18- 283- Muscle contraction is required to maintain the pool of muscle progenitors via the transcriptional co-activator YAP and NOTCH during fetal myogenesis

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Skeletal muscle is a plastic tissue that can adapt its size according to changes in mechanical loading. The variations in muscle mass can occur by changes in fibre size and/or by changes in satellite cell number (stem cells). The development, homeostasis and regeneration of skeletal muscle rely on progenitor/stem cells. Although, the respective contributions of muscle fibres and stem cells in muscle wasting in unloading conditions and disease-mediated atrophy is not well established.
We used the chick as a model to study the role of mechanical forces in the maintenance of skeletal muscle mass and progenitor cells during foetal myogenesis.
We demonstrated that the activity of NOTCH signalling pathway, known to be a central regulator of muscle stem cells, was decreased in foetal muscles following immobilization. Moreover, the inhibition of muscle contraction mimicked a NOTCH loss-of-function phenotype, i.e. dramatically decreased the number of foetal muscle progenitors (PAX7+ cells) that shifted towards a differentiation fate (increased MYOD and MYOG expression). Forced-NOTCH activation prevented the diminution in the number of muscle progenitors in immobilized embryos. We also provide evidence that immobilization reduces the amount of the transcriptional co-activator YAP in nuclei of post-mitotic muscle fibres and results in reduced JAG2 expression. Our results identify a novel mechanism acting downstream of mechanical forces and indicate that muscle activity signals via YAP and NOTCH to regulate the pool of foetal muscle progenitors.

P19 – Myasthenia (immune & congenital)- N° 284 to N° 298

Congenital myasthenic syndromes- #2458

P19- 284- Identification of MYO9A as a novel causative gene in congenital myasthenic syndrome

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Background: Congenital myasthenic syndromes (CMS) are a group of genetically heterogeneous disorders characterised by compromised function at the NMJ. CMS manifests in childhood with fatigable weakness of limb, ocular and bulbar muscles.
Aims: To identify novel CMS genes by whole exome sequencing (WES).
Methods: DNA from a cohort of patients with a clinical diagnosis of CMS with suspected autosomal recessive inheritance was sent to deCODE genetics for WES. Variants in the exome were filtered to exclude those with a frequency greater than 1% in control populations, unlikely to significantly impact the protein structure and not compatible with the inheritance model. Genes that had not been excluded were then segregated within the families. Identified candidates were subject to functional analysis in