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Abstracts

✶PF1

PS1-1 / #138

Theme: *1.1 - Basic sciences in NMD: Muscle homeostasis / Muscle regeneration*

Role of Serum response factor in muscular satellite cells

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In the muscular system, thetranscription factor Srf (Serum response factor) controls the expression of a wide range of genes including those involved in proliferation (immediate early genes) and myogenic differentiation (MyoD, a-actin). Indeed, inhibition of Srf in cultured myogenic cell lines C2C12 was shown to impede myoblast's proliferation and differentiation into myotubes. However data are lacking regarding the role of Srf in muscle stem cells behavior *in vivo*. For this purpose, we generated Pax7-CreERT2;$Srf^{flx/flx}$ mice in which Srf loss is induced in satellite cells (SC) after tamoxifen injection. In parallel we conducted ex vivo cultures of primary myoblasts expressing or not Srf ($Srf^{flx/flx}$ myoblasts transduced with adenoviral vectors expressing the recombinase Cre, and Pax7-CreERT2;$Srf^{flx/flx}$;Pax7-GFP sorted cells) to further study the cellular and molecular processes involved.

We demonstrated :

- that Srf is very faintly expressed in quiescent SC while prominently in activated/proliferative SC.
- that Srf deletion in SC did not alter their number nor their quiescent state suggesting that Srf is not crucial for the maintenance of SC quiescence in steady state conditions. In contrast, following cardiotoxin CTX-induced muscle regeneration or overload-induced hypertrophy, Srf loss in SC strongly affected muscle regeneration and muscle growth indicating a contribution of Srf activity to SC fate in stress conditions.
- that upon injury or overload, SC proliferation and early differentiation were not affected by Srf loss. These unexpected results were confirmed *in vitro* in cultured myoblasts. Accordingly MyoD and MyoG expressions were not altered by Srf loss.
- that SC late differentiation and fiber growth were altered *in vivo* and *in vitro*.

Further characterization of SC cell behaviour (self-renewal, motility, survival...) will be presented. In addition, we performed transcriptomic studies and identified the set of genes whose expressions are altered by Srf loss in proliferating and differentiating myoblasts. Genes identified in this screen will aid deciphering the underlying molecular mechanism.

PS1-2 / #247

Theme: *1.1 - Basic sciences in NMD: Muscle homeostasis / Muscle regeneration*

Sdf-1 promotes BMSCs participation in regeneration of Pax7-/- mouse skeletal muscles

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The skeletal muscle is characterized by an unique ability to reconstruct its structure and regain functionality after the injury. Regeneration is mediated by the muscle specific stem cells, i.e. satellite cells. However, in case of extensive damage, satellite cells cannot fully reconstruct muscle architecture. In such case other stem cell populations can serve as a potential source of myogenic cells. Previously, it was shown that bone marrow stem cells (BMSCs) manifest the ability to follow the myogenic program *in vitro*. However, the process of BMSC engraftment into injured muscle is inefficient and only few percent of new muscle fibers is formed with the participation of BMSCs. In our study we focus at chemokine Sdf-1 (stromal derived factor-1) which is one of the crucial factors participating in the regulation of cells migration during development and regeneration. Previously, we showed that Sdf-1 improves the regeneration of skeletal muscles. In the present study we take advantage of mice lacking Pax7 transcription factor

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(Pax7-/-) to test whether stem cells other than satellite cells could participate in the reconstruction of skeletal muscle. Skeletal muscles of these mice are severely depleted of satellite cells and for this reason cannot efficiently regenerate. We used Sdf-1 to impact at the stem cells mobilization and G-CSF to increase the number of mobilized cells.

Our results show that regenerating, Sdf-1 treated Pax7-/- muscles were characterized by increased weight, increased expression of muscle proteins and lower fibrosis, as compared with control muscles. This effect can be explained by the infiltration of regenerating muscle with mononucleated cells expressing Cxcr4 and Cd34. Next, we use G-CSF to increase the number of Sdf-1 mobilized cells. After such treatment we observed increased number of Cd34+and Cxcr4+ cells in regenerating Pax7-/- muscles as compared with control ones. Since, the number of satellite cells did not change after Sdf-1 administration we suggested that Cd34+and Cxcr4+ cells were mobilized from the bone marrow. Importantly, more new myofibers was formed in the muscles of Pax7-/- mice injected with G-CSF and Sdf-1. We suggested, that in the absence of satellite cells Cd34+ and Cxcr4+cells, mobilized from the bone marrow, can efficiently participated in the skeletal muscle reconstruction.

PS1-3 / #303

Theme: *1.1 - Basic sciences in NMD: Muscle homeostasis / Muscle regeneration*

Control of the fate of human muscle stem cell by modulation of the *in vitro* microenvironment

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Several therapeutic approaches are currently developed for the treatment of the loss of skeletal muscle tissue function. Cell therapy, which consists in the isolation, *in vitro* expansion and intramuscular transplantation of muscle progenitor cells (satellite cells) isolated from the patient or from a healthy donor, is limited by the amount of isolated cells and the genotypic shift that the cells undergo when kept on typical culture dishes.

We are currently developing a biomaterial adapted to the culture of satellite cells. Our aim is to reconstitute *in vitro* a cellular environment capable to provide satellite cells specific signals that mimic their natural niche. There are now several experimental evidences that muscle cells in their native microenvironments are sensitive to mechanical, biochemical and topographical cues. The satellite cell is indeed confined in

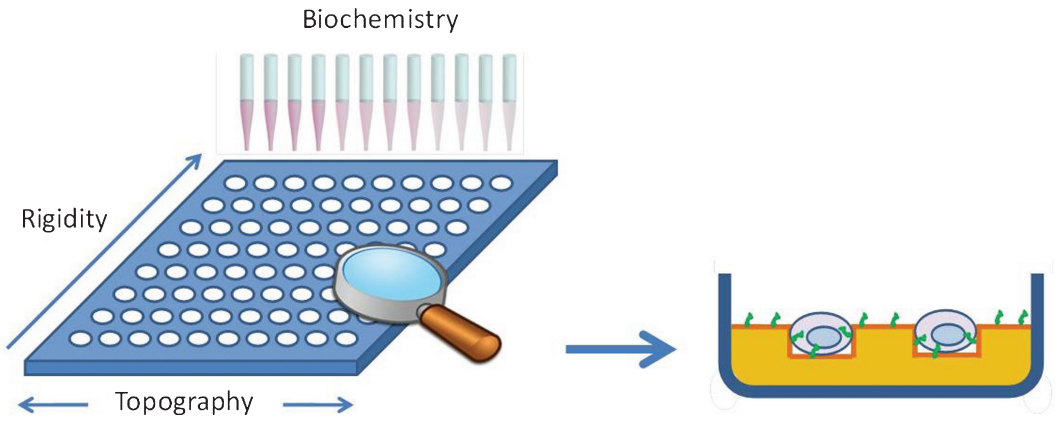

Fig. 1. Miniaturized culture plateform for the screening of conditions for the culture of satellite cells. Rigidity, biochemistry and topography can be modulated separately or combined together. The rigidity (▬) is varied by chemical cross-linking of the PEM film. The biochemistry (◠) of the substrate is modulated by grafting of peptide or incorporation of growth factors. The topography (⌴) is provided by microsctucturation of the underlying substracte.

its niche and surrounded by extracellular matrix (ECM) components as laminin or collagen and bioactive molecules as growth factors. The development of innovative biomaterials is then of great interest in order to overcome the limitations faced in the *in vitro* culture of satellite cells.

By the mean of the polyelectrolyte multilayer (PEM) films, we develop new culture platforms for human muscle stem cell maintaining or expansion for either therapeutic purpose but also for fundamental studies on muscle development in physiological and pathological conditions. The tools are developed by micro- and nano-engineering for the study of cellular and multi-cellular responses. The innovation of our biomaterial relies on the combination of mechanical (stiffness), biochemical (peptide grafting to target specific surface receptors, growth factor presentation) or topographical stimuli that can be spatially controlled (figure 1). Our work is at the fundament level to further understand muscle development in physiological and pathological conditions but also in view of future therapeutic trials.

PS1-4 / #344

Theme: *1.1 - Basic sciences in NMD: Muscle homeostasis / Muscle regeneration*

Changes in ALDH+ muscle cell populations with ageing in healthy and DMD patients and models

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A new category of resident muscle progenitors has been described on the basis of expression of Aldehyde Dehydrogenase (ALDH). Two sub-populations could be discriminated by their phenotypes and functional characteristics. Indeed, the ALDH+/CD34+ cells display a mesenchymal-like profile and are unable to participate to muscle regeneration *in vivo*, while the ALDH+/CD34- cells differentiate towards the myogenic lineage and participate to the regeneration of injured muscles *in vivo*. These populations may be involved in muscle homeostasis, but there is little information regarding their presence and evolution in pathological conditions or during ageing. We analyzed the presence and evolution of these populations using muscle biopsies obtained from healthy donors of various ages, or presenting with Duchenne muscular dystrophy, and using biopsies from normal and mdx mice of various ages. In Human, we observed a trend toward a decrease then a stabilization in the proportion of ALDH+ cells with age, both sub-populations of CD34+ and CD34- evolving in parallel. Classical endothelial (CD31) or myogenic (CD56) markers were maintained during ageing. In DMD patients, however, despite their young age, the ALDH+/CD34- sub-population is decreased as compared to controls, and the proportion of CD45+ cells (hematopoietic cells, monocytes) is increased. Cell cultures prepared from these DMD biopsies yielded far less ALDH+/CD56+ progenitors than controls. In young control and mdx Mice, the proportions of ALDH+ cells were comparable, but they decreased in mdx after the classical acute cycle of degeneration-regeneration observed around the fourth week.Taken together, these results support the hypothesis that these cell populations are associated with muscle homeostasis and regeneration. Other cell types expressing adhesion, interaction and migration markers such as CD29, CD44, CD49, and CD146 are under study. These results will be challenged in otherpathological situations and animal models. This study suggests a role played by some ALDH+ populations in muscle repair, that may become promising new tools for therapeutic strategies.

✶PF1

PS1-5 / #487

Theme: *1.1 - Basic sciences in NMD: Muscle homeostasis / Muscle regeneration*

Muscle Ring Finger-1 (MuRF1) activity is present in cardiac mitochondria and regulates reactive oxygen species production *in vivo*

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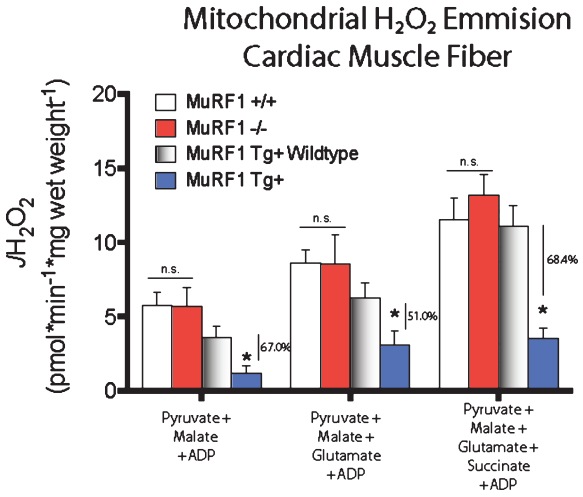
Recent studies have used a proteomics approach to identify that most ubiquitinated proteins are found in the mitochondria (38.0%), followed by the cytosol (27.3%). Despite the fact that the mitochondria contain most of the ubiquitinated proteins in the heart, few ubiquitin ligases that drive these processes have been identified in the mitochondria in any cell type. Muscle Ring Finger-1 (MuRF1) is a ubiquitin-ligase found in striated muscle that targets troponin I and myosin heavy chain for degradation in skeletal muscle and cardiac atrophy. While MuRF1 has been reported to interact with mitochondrial substrates in yeast two-hybrid studies, no studies have identified MuRF1's role in regulating mitochondrial function to date. In the present study, we measured cardiac mitochondrial function from isolated permeabilized muscle fibers in previously phenotyped MuRF1 Tg and MuRF1-/- mouse models to determine the role of MuRF1 in intermediate energy metabolism and ROS production. We identified a significant decrease in reactive oxygen species production in cardiac muscle fibers from MuRF1 transgenic mice with increased alpha-MHC driven MuRF1 expression (Figure 1). MuRF1 expression in ex vivo and *in vitro* experiments revealed no alterations in the respiratory chain complex I and II function. Working perfusion experiments on MuRF1 transgenic hearts demonstrated significant decreases in glucose oxidation, but no changes in oleate oxidation. Parallel studies of cardiac power and developed pressure found that MuRF1 Tg+ hearts had decreased function but used significantly less oxygen (total oxygen consumption). Conversely, working perfusion experiments on MuRF1-/- hearts demonstrated an enhanced glucose oxidation, but no changes in oleate oxidation or oxygen consumption. MuRF1-/- hearts had significantly more cardiac power and developed pressure without any differences in oxygen consumption. These studies provide evidence that MuRF1 is a novel regulator of cardiac ROS and glucose oxidation, offering additional mechanisms by which increased MuRF1 expression may be cardioprotective in ischemia reperfusion injury by inhibiting apoptosis via proteasome-mediated degradation of c-Jun. The lack of mitochondrial function phenotype identified in MuRF1-/- hearts may be due to the overlapping interactions of MuRF1 and MuRF2 with energy regulating proteins found by yeast two-hybrid studies reported here, implying a duplicity in MuRF1 and MuRF2's regulation of mitochondrial function.



PS1-6 / #488

Theme: *1.1 - Basic sciences in NMD: Muscle homeostasis / Muscle regeneration*

Non-targeted metabolomics analysis of Muscle Ring Finger-1 (MuRF1), MuRF2, and MuRF3 *in vivo*

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The muscle-specific ubiquitin ligases MuRF1, MuRF2, MuRF3 have been reported to have overlapping substrate specificities, interacting with each other as well as proteins involved in metabolism and cardiac function. In the heart, all three MuRF family proteins have proven critical to cardiac responses to ischemia and heart failure. The non-targeted metabolomics analysis of MuRF1-/-, MuRF2-/-, and MuRF3-/- hearts was initiated to investigate the hypothesis that MuRF1, MuRF2, and MuRF3 have a similarly altered metabolome, representing alterations in overlapping metabolic processes. Ventricular tissue was flash frozen and quantitatively analyzed by GC/MS using a library built upon the Fiehn GC/MS Metabolomics RTL Library. Non-targeted metabolomic

analysis (Table 1) identified taurine, myoinositol, stearic acid as significantly different from sibling-matched wildtype MuRF1-/-, MuRF2-/-, and MuRF3-/- ventricles (via VIP statistical analysis). Moreover, pathway enrichment analysis of MuRF1-/-, MuRF2-/-, and MuRF3-/- ventricles demonstrated that MuRF1-/- and MuRF2-/- ventricles had significant changes in metabolite(s) involved in the biosynthesis of unsaturated fatty acids and ascorbic acid/aldarate metabolism (via VIP and *t*-test analysis vs. sibling-matched wildtype ventricles). Despite these many similarities, unique metabolites in were found in MuRF1-/-, MuRF2-/-, and MuRF3-/- ventricles (Table 1), with MuRF3 demonstrating the least overlap and most unique metabolites (e.g. aldohexose1, palmitic acid, linoleic acid). By identifying the functional metabolic consequences of MuRF1, MuRF2, and MuRF3 in the intact heart, including multiple overlapping metabolites and metabolic pathways, non-targeted metabolomics analysis discovered common pathways functionally affected by cardiac MuRF family proteins *in vivo*. Follow up molecular studies are needed to delineate mechanistically if MuRF1, MuRF2, and MuRF3 are directly or indirectly linked to these changes in these metabolic pathways and how these might affect the heart in protective and/or adverse ways during cardiac disease.

PS1-7 / #547

Theme: *1.1 - Basic sciences in NMD: Muscle homeostasis / Muscle regeneration*

Identify which population of muscle stem cells harbor the capacity to differentiate into autonomously beating pacemaker-like myocytes ?

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Mammalian heart pacemaker and conduction system is orchestrated by a specialized cell population: the pacemaker myocytes, distinct from "working" cardiomyocytes and responsible for initiating and propagating the electrical impulse driving cardiac rhythmic contraction. As such, this conduction system is regarded as the "intrinsic innervation" of cardiac muscle. Neuromuscular diseases are often accompanied by cardiac conduction disorders causing life-threatening arrhythmias that require electronic pacemaker implantation through surgical intervention. Whereas biological repair of pacemaker activity by engraftment of progenitor cells may represent a good alternative, it requires the identification of a suitable cell source for transplantation.

Adult skeletal muscle retains a lifelong plasticity and ability to regenerate skeletal myofibers but also their innervation and vascularisation. This regenerative potential implies significant remodelling whereby the satellite cell niche supporting myofiber regeneration co-operates with vascular, neural and connective tissue-derived stem cells, thus representing additional muscle-resident sources of stem cells.

We have previously shown that adult skeletal muscle contains a population of multipotent Muscle Derived Stem Cells (MDSC) isolated on the basis of their low adherence and capable of differentiating into autonomously beating pacemaker cells.

In order to effectively define the muscle stem cell population differentiating into pacemaker-like cells, we used a transgenic mouse model with a GFP reporter for nestin expression. Using cell tracking of GFP-expressing cells, immunohisto and cytochemistry, electrophysiology, and fluorescence-activated cell sorting (FACS), we show that a Nestin-GFP-positive population present in skeletal muscle can differentiate *in vitro* into beating pacemaker-like myocytes. Nestin-GFP-positive muscle stem cells isolated by FACS differentiated within 10 days and were cultured for up to 4 months, showing the typical morphological, molecular and electrophysiological features of native sino-atrial pacemaker cells including co-expression of HCN4 and connexin45 with alpha-actinin, and Troponin I.

Whole mount immuno-histochemistry and video time lapse experiments on muscle fibers isolated from different GFP-reporter transgenic mice will enable us to further identify the cellular origin of this muscle stem cell population with a promising potential for regenerative cell therapy of neuromuscular diseases.

PS1-8 / #562

Theme: *1.1 - Basic sciences in NMD: Muscle homeostasis / Muscle regeneration*

Potential Link Between Alternative Splicing of Histidyl-tRNA Synthetase (HARS) and Inflammatory Myopathy

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Recent evidence of a genetic link between histidyl-tRNA synthetase (HARS) and neuromuscular disease has emerged 1) a HARS missense variant associated with Usher syndrome type III, an autosomal recessive disease characterized by inflammatory retinal disease, sensory neural hearing loss and myopathy [1] and 2) 6 HARS missense variants found in 8 patients with axonal peripheral neuropathy [2]. Historically the HARS protein was identified as the sole target of the anti-Jo-1 autoantibodies (Jo-1 Abs) which are detected in a high proportion of patients with both rare autoimmune inflammatory myopathy (IM) and interstitial lung disease (ILD), progressive and debilitating acquired myopathies for which no drugs are specifically approved [3]. Immunization with murine HARS fused to MBP to generate high-titer antibodies targeting HARS resulted in skeletal muscle and lung inflammation characterized by infiltrating immune cells (including T cells) [4, 5], pathologies reminiscent of observations in inflammatory myopathy patients with Jo-1 Abs. Taken together, this data suggests that generation of antibodies specifically targeting HARS disrupts immune and muscle homeostasis.

To further our investigations into the role of HARS blockade in inflammatory myopathies and of HARS in immune and muscle homeostasis, we utilized x-ray crystallography to generate the structure of homodimeric human HARS protein. Detailed characterization of Jo-1 Abs present in diverse populations of patients revealed a broad range of titers and concentrations. Specific epitopes recognized by Jo-1 Abs were distributed across the entire HARS protein and were well separated spatially in reference to the 3D structure. Epitopes recognized varied considerably among subjects, but serum from a majority of patients exhibited cross-reactivity with the N-terminal portion of HARS. In addition, our discovery efforts identified a natural splice variant of HARS specifically enriched in lung, but found at low levels in skeletal muscle, encoding only the N-terminal portion of the protein. Relative to healthy controls, we found mRNA transcripts for HARS and the splice variant were significantly upregulated in muscle biopsies from patients diagnosed with dermatomyositis (DM). Increasing association of HARS with neuromuscular disease and discovery of a splice variant that isolates the major epitope of Jo-1 Abs warrants further investigation to gain understanding of the possible role of HARS in immune and muscle homeostasis.

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PS1-9 / #71

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

Function of the H19 non coding RNA in muscle stem cells

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The H19 imprinted gene is located on mouse chromosome 7, close to the Igf2 (insulin-like growth factor 2) gene. These two genes are regulated by a differentially methylated region, the imprinting control region (ICR) located 2kb upstream of the H19 gene. The maternal H19 allele expresses a 2.3kb non coding RNA, as well as a micro RNA, the miR-675. We have shown that H19 acts as a transregulator of an imprinted gene network (IGN) involved in growth control of the embryo. We have recently shown that the H19 lncRNA binds the MBD1 protein and that this complex recruits histone methyl-transferases on DMRs of genes of the IGN.

The H19 gene is highly expressed during embryonic growth, but its expression is repressed after birth in all tissues except skeletal muscles, suggesting a function in this tissue.

In limb muscles of adult mice lacking the H19 gene, we observed an increased number of fibers (hyperplasia) that are larger (hypertrophy) compared with wt mice muscles. Moreover, a delay in regeneration time of muscles injured by cardiotoxin injection is observed. This delay could be linked to the decrease in the number of satellite cells (muscle stem cells) that we detected in H19 knock-out mice compare to wt mice. These results suggest direct or indirect H19 function in adult muscle regeneration. We established myoblast cell lines from mice lacking the H19 gene or wt mice, and we observed that the expression of three IGN genes is increased in absence of H19 expression. Interestingly, these genes are implicated in cell cycle, growth and differentiation.

PS1-10 / #115

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

Key role of EGFR in human primary myoblasts differentiation

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Human adult skeletal muscle possesses a remarkable capacity to regenerate its damaged fibers thanks to the presence of satellite cells. Once activated, those muscle stem cells are able to proliferate as myoblasts, differentiate and to fuse into myotubes. Our goal is to decipher the molecular events initiating myoblast differentiation prior to their fusion. For that purpose, we use a well-established *in vitro* model based on primary myoblast cell cultures isolated from human muscle biopsies which mimics the myoblast differentiation process.To our knowledge, one of the earliest steps of the differentiation process known so far is the activation of Kir2.1, an inward rectifier K+ channel. This channel, already present at the plasma membrane, is activated during the first hours of differentiation. Kir2.1 activation produces a hyperpolarization of membrane resting potential which in turn plays an important role in the initiation of myoblast differentiation as it allows the activation of calcium-dependent intracellular pathways by increasing the driving force for Ca2+ ions. We previously showed that Kir2.1 is maintained inactive during proliferation by the phosphorylation of its tyrosine 242. One possibility is that, in proliferation condition, an active tyrosine kinase receptor (RTK) keeps Kir2.1 channels phosphorylated and thus inactive. Among 43 receptors tyrosine kinase, epidermal growth factor receptor (EGFR) turn out to be the only one to be strongly active in proliferation conditions and down-regulated when differentiation is induced. Pharmacological inhibitions or silencing of EGFR were able to induce myoblast differentiation in proliferation conditions. Indeed, key early events of differentiation such as Kir2.1 channels activation and myogenic transcription factors expression (Myogenin, MEF2) were observed. We found using p42/p44 MAPK activity as a reporter of EGFR activity that EGFR activity is decreased during the first hour of differentiation and precedes Kir2.1 activation. Furthermore EGFR expression at the plasma membrane is not detected anymore after nine hours of differentiation. We propose that EGFR is a regulator

of Kir2.1 activity during myoblast differentiation. However, what triggers EGFR inactivation/degradation during the first hours of differentiation and how EGFR controls Kir2.1 activity (direct or indirect mechanisms) remains to be elucidated.

PS1-11 / #211

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

Control of transcription elongation is essential for cardiac and skeletal muscle development

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Background: Gene expression is often regulated at the level of transcriptional elongation, when RNA polymerase II pauses along nascent mRNAs, effectively arresting gene transcription. Its activity is resumed following the recruitment of transcription elongation factors (TEFs). This level of regulation is widespread in particular among genes that are developmentally regulated, but its role in tissue specific gene expression is not well understood.

Aims and methods: To determine whether the control of transcriptional elongation plays a role in development of cardiac and skeletal muscle *in vivo*, using zebrafish as a model organism.

Results: We knocked down expression of a specific TEF using antisense morpholino oligonucleotides in zebrafish. Morphants present with severe defects in cardiac development, including oedema, looping defects and absent circulation. In skeletal muscle, myofibres detach from the myosepta. These defects are similar to those observed in mutants for dystrophin and integrins. Surprisingly, expression of these genes was maintained in TEF morphants, suggesting that novel candidates may be involved. Global analysis of gene expression via microarray and RT-PCR shows deregulated expression of numerous genes involved in heart and skeletal muscle development.

Conclusions: The control of transcriptional elongation plays crucial roles in the development of cardiac and skeletal muscle, and suggests that alterations in this process may underlie cardiomyopathies and muscular dystrophy in patients.

PS1-12 / #245

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

Pericytes induce both satellite cell quiescence and differentiation during post-natal muscle growth

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Muscle microvasculature is often considered solely as a source of nutrients and oxygen for growing muscle cells. However, muscle microvascular cells are also important cell players in muscle satellite cells (mSCs) niche, which represents a major aspect of muscle stem cell biology. How mSCs interplay with their vascularniche is poorly understood. We previously reported that adult mSCs are closely associated with capillaries, functionally interact with endothelial cells (ECs), and self-renew in response to local release of Angiopoietin-1 (Angpt-1).

The objective of this study is to understand the influence of microvascular cells on the behavior of the mSC during the post-natal development until homeostasis and during the regeneration process or post-injury regeneration.

We used C57Bl/6 mice to study the post-natal development. *In vitro*, we did indirect co-cultures, muscle myofibers culture in conditioned media and long term culture in conditioned media to study the effect of pericytes and ECs on the behavior of mpc (mSC in culture). *In vivo*, we used two transgenic mouse strains: Tg:NG2-Cre:iDTRmice and Tg:TNAP-CreERT2:Angpt1 mice to study the effect of pericyte ablation or pericyte-derived Angpt-1 depletion on the behavior of mSC during post-natal development and regeneration.

We show that 80–90% of adult muscle capillary ECs are tightly associated with a pericyte. During post-natal mouse muscle development, NG2+pericytes are initially remote from cycling mSCs, and progressively move towards mSCs, entering in their vicinity as myofibers increase in size and mSCs become quiescent. *In vitro*, ECs stimulated mSC proliferation

through PDGF-BB and Angpt-2, whereas pericytes had dual effects, promoting myogenic cell differentiation through IGF1 and inducing mSC quiescence through Angpt-1. Consistently, DT-induced ablation of muscle pericytes in adult muscle ofTg:NG2-Cre:iDTRmiceand early conditional inhibition of pericyte Angpt-1 production inTg:TNAP-CreERT2:Angpt1mice, induced the release of mSCs from homeostatic quiescence.

Thus, besides their previously described mesenchymal stem cell potential, muscle pericytes exert paracrine effects on adjacent myogenic cells, conspicuously involved in muscle post-natal growth and homeostasis.

✶PF1

PS1-13 / #379

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

Cytoskeleton network regulation and nuclear positioning during muscle development

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Centronuclear myopathies are diseases with weakened muscles thought to be caused by nuclei mispositioned to the middle of the muscle. In the normal course of development or regeneration of damaged muscles, myoblasts fuse to form a syncytial myotube. Nuclei of the newly formed myotubes are originally found in the center of the fiber, but during development, they migrate to the periphery of the fiber. The molecular machinery for nuclear positioning is poorly understood.

Recent advance in understanding nuclei movement and localization in developing muscle cells reveals the key role of microtubules, microtubule binding proteins and molecular motors. Those complexes drive different nuclei movement just after fusion and also inside the formed myotubes allowing correctly localized nuclei in mature fibers, contributing to muscle function. The comprehensive analyses of the contribution of each motor on the regulation of nuclei localization remind to be elucidated.

We conduct a drug approach to elucidate which cytoskeleton was responsible for nuclei movement during myotube formation and find that microtubules is the preferential path used by nuclei to position. We used a library of small interfering RNAs (siRNAs) that target molecular motors and microtubule interacting members. We explored the involvement of each individual motor using this siRNA screen and quantify different parameters regarding nuclei localization into myotube. Results highlight the key role of some molecular motors on particulars mechanisms like nuclei alignment organization, time in motion and speed of nuclei inside myotube. We also identified a microtubule binding protein regulating microtubule network that control myonuclear positioning involved in the establishment of neuro-muscular junction as well as musculo-tendinous junction.

PS1-14 / #394

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

A modified cysteine knot ligand trap of the TGFB superfamily, ACE-083, increases muscle mass locally in mice

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To investigate a new therapeutic strategy for asymmetrical myopathies, we generated a modified cysteine knot ligand trap of TGFβ superfamily, ACE-083 which acts locally and increases muscle mass. ACE-083 binds to activin and myostatin and inhibits their signaling. Unlike ActRIIb/Fc, ACE-083 does not bind to BMP9/10.

To demonstrate the ability of ACE-083 to increase muscle mass locally, we used eight week old C57BL/6 mice that received intramuscular injections of either ACE-083 (1–300µg, 50µl, biw) or vehicle (PBS, 50µl, biw) for four weeks. All animals received an equivalent injection volume of 50µl with a range of ACE-083 protein that was independent of weight. The

effective doses of ACE-083 escalated from 3 to 300 µg (approximately 0.05 mg/kg to 15 mg/kg). Over the course of the study there were no significant differences in body weight between the ACE-083 or vehicle treated mice.

After 4 weeks of treatment muscles from the animals were collected to evaluate the effect of ACE-083. Mice injected with 300µg of ACE-083 had increased muscle mass in the injected gastrocnemius muscle of 121% ($p < 0.0001$) compared to mice injected with PBS. A significant increase in gastrocnemius size was seen with as little as 3µg of ACE-083 (+20%, $p = 0.0017$). In the gastrocnemius of the contralateral, non-injected leg, there was no significant difference in the muscle mass between the ACE-083 and PBS cohorts. Histologically, muscles injected with ACE-083 underwent hypertrophy with no signs of hyperplasia and there was no alteration in muscle fiber type distribution.

To determine if ACE-083 was acting systemically on non-injected muscles we examined the pectoral and femoris muscles. In the highest dose cohort there was no significant difference between the pectoral muscles of animals in either treatment group ($p = 0.838$). There was no effect on the femoris muscle in the injected leg ($p = 0.620$) or the non-injected contralateral leg ($p = 0.244$). Importantly, we determined there was no active ACE-083 detected in the serum following IM or IV injections potentially limiting unwanted effects in other organs.

These preclinical results demonstrate ACE-083 can effectively double muscle mass locally following direct, intramuscular injections. Therefore, local inhibition of Myostatin using a soluble myostatin inhibitor represents a promising therapeutic approach for the treatment of asymmetric myopathies

PS1-15 / #398

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

Dynamic of Triadin, a protein of the Calcium Release Complex

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The macromolecular calcium release complex (CRC) plays a central role in striated muscle contraction and is responsible for excitation-contraction coupling. In skeletal muscle cells, it is localized in a specific compartment, the triad, composed of one plasma membrane Transverse-Tubule (TT) in close contact with two Sarcoplasmic Reticulum (SR) terminal cisternae (TC). The Calcium Release Complex is composed of two calcium channels with associated proteins (Triadin, Junctin, Calsequestrin...). All these proteins are exclusively localised in the triad. The molecular mechanisms leading to the traffic and exclusive localization of CRC proteins at the triads are so far unknown. Triadin is a transmembrane protein of the CRC residing in SR membrane and proposed to anchor other members of the complex at the triad. To investigate the mechanisms responsible for Triadin localization in skeletal muscle we have expressed a photoactivatable version of the protein in primary myotubes cultivated from triadin KO mice. Video recording of the traffic of activated pools of triadin show it diffuses in SR and progressively accumulates in triads. Moreover, the traffic of photoactivatable triadin seems isotropic and able to span distances representing several sarcomeres. Our data suggest synthesis of triadin can occur in all SR, and that the molecule is able to traffic over long range before specific molecular determinants triggers its immobilization in triads.

PS1-16 / #415

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

Cytoskeleton composition of the human extraocular muscles

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The extraocular muscles (EOMs) are extremely fast and differ significantly from other skeletal muscles in the body, particularly with regard to fiber type composition and response to disease. We have investigated the distribution of a large number of cytoskeletal proteins in the human EOMs using immunohistochemistry in 5- and 1-micron thick sections as well as electron microscopy.

The majority of the muscle fibers containing myosin heavy chain (MyHC) slow tonic were only very weakly labelled or were unlabelled with three different antibodies (two monoclonal and a polyclonal antibody) against desmin. These muscle fibers had a well-preserved sarcomeric structure and basement membrane. The presence of desmin was restricted to the subsarcolemmal cytoskeleton only in some muscle fibers. The remaining fibers showed staining patterns for desmin identical to those previously reported for limb muscle fibers. Nestin was present in a large number of muscle fibers of the EOMs, with no correlation with the distribution of desmin or MyHCembryonic. Vimentin was not present in any muscle fibers in the adult EOMs. The patterns of distribution of intermediate filament proteins at the myotendinous junctions differed from those typical for the other muscles in the body, e.g. absence of nestin in some fibers.

Titin, nebulin and obscurin were present in all muscle fibers, with some degree of heterogeneity that was not apparently related to fiber types. In the majority of the muscle fibers in the EOMs, M-protein was not present or only very weak labelling was seen, although they were fast muscle fibers. In contrast, the muscle fibers containing MyHC slow tonic were strongly labelled with Abs against M-protein.

In summary, the human EOMs differed significantly from limb and trunk muscles with respect to the composition and distribution of key cytoskeletal proteins such as desmin, nestin and M-protein. The absence of desmin in normal human muscle fibers is a unique finding. Further studies are needed to elucidate the functional impact of this diversity and to determine whether they are relevant for the distinct behaviour of the EOMs in neuromuscular disease.

PS1-17 / #442

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

Impairment of HACD1-dependent regulation of myoblast fusion leads to muscle hypotrophy

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Myoblast fusion allows the formation of large, multinucleated muscle fibers and promotes developmental muscle growth. Although fundamental aspects underlying this process are emerging, pathogenic implications of its primary impairment are unknown. In humans and dogs, HACD1 loss-of-function mutations cause congenital myopathies characterized by fiber size disproportion and muscle weakness. HACD1 encodes an enzyme that elongates very long chain fatty acids (C≥18) and *in vitro*, its knockdownaffects myoblast development. Here we show that a muscle-specific transcript encoding the active HACD1 isoform was up-regulated during muscle development and myoblast differentiation. *In vitro*, we demonstrated that the HACD1 active isoform was specifically required for efficient myoblast fusion. Consistently with an *in vivo* impairment of myoblast fusion, we show that HACD1 deficiency in our dog and knockout mouse models resulted in myofiber hypotrophy and defective regenerative capacities, eventually leading to fiber size disproportion and reduced muscle mass. Mechanistically, we proved that HACD1 active isoform provoked an increase in ≥C18 and monounsaturated fatty acids, concomitantly with a raise in membrane fluidity. We thus propose that in skeletal muscles, splice regulation of HACD1 controls lipid balance and promotes membrane permissiveness required for myoblast fusion. We further propose that impairment of myoblast fusion constitutes a novel primary pathogenic mechanism in hypotrophic congenital myopathies.

PS1-18 / #473

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

Changes in skeletal muscle structure and function following genetic inactivation of myostatin in rats

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Myostatin (GDF-8) is a negative regulator of skeletal muscle mass. Inactivation of myostatin in mice causes increased muscle size and isometric force production (P^o), but a decrease in specific force (sP^o). There have been limited analyses of the impact of myostatin on the mechanics and biochemistry of muscles from organisms other than mice. We sought to evaluate the effect of myostatin deficiency on the structure and function of skeletal muscles from rats with a targeted inactivation of myostatin. We hypothesized that, compared to controls ($MSTN^{+/+}$), rats in which the myostatin gene was inactivated using zinc-finger nucleases ($MSTN^{\Delta/\Delta}$) would exhibit an increase in muscle mass and P^o, a reduction in sP^o, and an increase in type II fibers. $MSTN^{\Delta/\Delta}$ animals had a 21% increase in body mass, and a 37% and 45% increase in fibers per muscle and a 32% and a 19% increase in mass for the EDL and soleus, respectively. EDL and soleus muscles from $MSTN^{\Delta/\Delta}$ animals also displayed a 57% and 20% increase in P^o while exhibiting a 31% and 21% increase in PCSA, respectively, which led to no substantive changes in sP^o. EDL fiber area was increased in $MSTN^{\Delta/\Delta}$ animals in all type II fibers and hybrid fibers. Soleus fiber area was not significantly increased in either type I or type IIA fibers, however in both the EDL and soleus, $MSTN^{\Delta/\Delta}$ animals displayed a decrease in the percentage of type I and IIA/IIX fibers. $MSTN^{\Delta/\Delta}$ animals had distinct expression profiles for several mRNAs and miRNAs involved in atrophy, ECM remodeling and lipid synthesis. The soleus muscles of $MSTN^{\Delta/\Delta}$ rats had increased levels of phosphorylated GSK3β, IGF1R and mTOR compared to controls. The results from this study indicate there are species-specific consequences of myostatin inactivation, and further examination into the physiology of $MSTN^{\Delta/\Delta}$ rats is warranted.

PS1-19 / #557

Theme: *1.2 - Basic sciences in NMD: Muscle structure / Muscle development / Muscle growth*

A new phenotype-modifying mechanism that buffers nonsense mutation and avoids the extreme overgrowth of muscular tissue in the cattle

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We identified an unexpected founder mutation in the myostatin gene associated to muscle phenotype in the Blonde d'Aquitaine cattle breed. In skeletal muscle, the mutant allele was highly expressed leading to an abnormal transcript consisting of 41-bp inclusion and premature termination codons and to residual levels of a correctly spliced transcript. This expression pattern, caused by a leaky intronic mutation with regard to spliceosome's activities and its stability with regard to surveillance mechanisms, could explain both the moderate muscle hypertrophy and variations of muscle conformation between animals. This molecular study reveals a naturally occurring mechanism where the effect of either modifier genes or an epigenetic mechanism could be suspected. This finding is of importance for genetic counseling for meat quantity and quality in livestock production and possibly to manipulate myostatin pre-mRNA in human muscle diseases.

✶PF1

PS1-20 / #107

Theme: *1.3 - Basic sciences in NMD: Muscle atrophy / Degeneration*

The Effect of Aerobic Training and Detraining on Mitochondrial Function In Aging

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Introduction: Age-related loss of muscle mass (sarcopenia) results in decline in physical function and frailty in the elderly. Mitochondrial dysfunction has been proposed to play an important role for the development of sarcopenia. Since exercise improves mitochondrial function, it has been suggested that exercise could counter-act sarcopenia, but very few studies have investigated the effect of exercise on mitochondrial function in elderly. Thus, the aim of this study was to investigate the effect of exercise in elderly compared to young healthy subjects on mitochondrial dynamics and function.

Methods: Eleven healthy, elderly (80±4 Y.O) and ten young (24±3 Y.O) subjects participated in six weeks of supervised training followed by an 8-week deconditioning period. Each training session, which lasted 35 minutes, was performed on a stationary bicycle 4 times weekly, alternating between continuous exercise (70 % of VO_{2max}) and interval exercise (90% of VO_{2max}). Before and after the training period and four and eight weeks after training had ended (deconditioning), VO_{2max} and endurance capacity at 80 % of VO_{2max} (pre-training value) was tested and a muscle biopsy was performed from a trained muscle (m. vastus lateralis). Muscle biopsies were analyzed for mitochondrial proteins (e.g. Complex I-V, Bax, Bcl-2), proteins involved in mitochondrial biogenesis (e.g. PGC-1alpha, TFAM), mitochondrial DNA copy number, enzyme activities of Complex I-V and Citrate Synthase (CS) and markers for reactive oxygen species.

Results: Training improved VO_{2max}, and endurance capacity equally in the two groups (13.0% and 287% in the elderly, compared to 8.5% and 175% in the young subjects). After eight weeks of deconditioning, VO_{2max} returned to pre-training level, while endurance capacity decreased to 200% and 97% respectively of pre-training level in both groups. The results on mitochondrial content and dynamic are currently under investigation, and will be presented at the conference.

Conclusion: The preliminary results show that aerobic training in elderly persons improves VO_{2max} and endurance to the same extent as in young subjects. The findings suggest that exercise in advanced age is a putative tool to decrease muscular aging. The molecular findings on protein signaling and mitochondrial function will be presented at the conference.

PS1-21 / #163

Theme: *1.3 - Basic sciences in NMD: Muscle atrophy / Degeneration*

New balances to control muscle mass and function: cross-talk of DHPR/ActIIBR in skeletal muscle pathophysiology

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Muscle is an extremely highly adaptable tissue that responds to different mechanical and biochemical stimuli inducing either hypertrophy or atrophy.

Muscle mass and fiber size undergo to rapid and significant changes according to physiological and pathological conditions.

Among the various tropic signals, intrinsic muscle contractile activity, neurotransmission and neurotrophic factors are crucial components regulating the integrity of muscle mass.

We already described how the *in vivo* downregulation of a1S- subunit of DHPR (ΔDHPR), the voltage sensor of the triads, induced profound muscle atrophy. This effect was mediated by nNOS redistribution within the muscle fiber and activation of FoxO3A, with consequent expression of autophagy-related proteins.

The observation that the sarcolemmal fraction of DHPR is mostly affected during a1S down-regulation process, compared to the DHPR clusters at the T-tubules, suggested a specific role for the sarcolemmal DHPR channel in the maintenance of muscle mass. To go further in analyzing this role, we investigated here if DHPR loss could affect the expression of other proteins regulating atrophy/hypertrophy in muscle. We

found a deregulation of ActivinIIB Receptor expression in ΔDHPR muscles, in resting or electrically stimulated conditions, suggesting the existence of a cross talk between DHPR and myostatin pathways. To better understand the link between DHPR/Myostatin/ActIIBR we either downregulated DHPR in $KO^{mst-/-}$ mice or we injected a myostatin inhibitor (pro-peptide) in DDHPR muscles. In both models we found that the inhibition of myostatin pathway ameliorates atrophic phenotype induced by DHPR downregulation, included a slight increase of muscle mass and increase of specific force. In parallel, we found an increased basal expression of DHPR in $KO^{mst-/-}$ mice. Finally, we measured that myostatin inhibition rescued the activation Akt and mTor (implicated in autophagic response) and Cathepsin L increase, caused by DHPR downregulation.

Taken together our results show for the first time a close cross talk between hypertrophic and atrophic signaling, and drawn new mechanisms in the homeostatic control of muscle physiology.

PS1-22 / #305

Theme: *1.3 - Basic sciences in NMD: Muscle atrophy / Degeneration*

Protein biomarkers of Duchenne Muscular Dystrophy indicate a new component of the cellular pathogenesis : desintegration of the elastic mechanosensor network

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Several clinical trials are underway for Duchenne muscular dystrophy (DMD) and there is an urgent need for the valuable biomarkers to follow up the short and long time effects of the treatments. In this context urine and serum samples from DMD patients and respective controls were collected in United States (147 individuals) and European Union (209 individuals) in the frame of ADNA program (Advanced Diagnostic for New therapeutic Approaches). Using a multidisciplinary tactic comprising antibody arrays and a bottom-up proteomic approach we identified several new blood and urine biomarkers differentially expressed between healthy individuals and DMD patients. These studies identified myomesin-3 as the most prominent abnormally excreted protein which is absent from normal serum but abundantly present in DMD serum. Myomesin-3 is also increased in serum from the two dystrophin-deficient animal models, mdx and GRMD. Forced exercise in normal and mdx mice revealed that myomesin-3 is not increased in normal mice following strenuous exercise, and remains increased without additional peak (in contrast to serum CK) in mdx mice. Myomeosin 3 was also found in serum of patients with alpha-sarcoglycanopathy (LGMD2D) and from mice deficient in alpha-sarcoglycan (Sgca-null). Importantly, gene therapy treatment of Sgca-null mice model permitted to establish a tight correlation between the efficiency of the treatment and myomesin 3 levels. Urine analysis revealed titin N- and C-terminal fragments as the most prominently abnormal peptides found exclusively in all DMD patients. The combined abnormal excretion of titin and myomesin-3 which directly binds titin to the contractile apparatus via myosin suggests that the mechanosensor elastic scaffold in dystrophin-deficient muscle is severely perturbed throughout the disease process. This observation adds a new element to the pathophysiology of Duchenne muscular dystrophy.

✶PF1

PS1-23 / #464

Theme: *1.3 - Basic sciences in NMD: Muscle atrophy / Degeneration*

Novel gene networks and biological pathways regulated by FoxO in skeletal muscle during cancer cachexia

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Muscle wasting is a devastating complication of cancer that results in weakness of both locomotor and respiratory muscles, and may be responsible for up to a third of cancer-related deaths. Therefore, targeting muscle wasting during cancer is essential for both the quality of life and survival of cancer patients. However, in order for this to occur, a better understanding of the complex mechanisms which drive cancer-induced wasting is needed. Published and unpublished work from our lab using animal models of cancer demonstrates that the Forkhead BoxO (FoxO) transcription factors drive muscle wasting and weakness. In order to gain a more comprehensive understanding of the genes and biological pathways targeted by FoxO during cancer, in the current study we performed a genome-wide microarray analysis of muscles from control and Colon-26 (C26) mice in which FoxO DNA binding was either intact or blocked via AAV9-mediated delivery d.n.FoxO (or control). Of the 1,945 genes identified to be differentially expressed in response to C26 ($q \leq 0.01$ and fold change ≥ 1.5-fold), 543 were differentially regulated by d.n.FoxO ($q \leq 0.01$ and fold change ≥ 1.5-fold) and thus labeled as FoxO target genes. Bioinformatic analysis of target genes upregulated in response to C26 revealed the AP1 and IL-6 pathways as top canonical pathways upregulated by FoxO. Further analysis of the -2kb to 2kb cis-regulatory regions of upregulated FoxO target genes revealed conserved transcription factor (TF) consensus motifs corresponding to FoxO, AP1, and STAT (which acts downstream of IL-6) to be among the most highly overrepresented TF motifs. Thus, these data together indicate that FoxO acts as an upstream regulator of both AP1 and IL-6/STAT signaling during cancer. Bioinformatic analysis of the FoxO target genes downregulated during C26 revealed the extracellular matrix (ECM) and ECM-mediated pathways as the most dominant biological networks downregulated by FoxO during cancer. Several of these ECM proteins play known roles in regulating myogenic activity and muscle force transmission, both of which are compromised during cancer. In summary this is the first body of work to identify the genome-wide target genes & biological pathways regulated by FoxO in skeletal muscle during cancer which may offer novel insight into the mechanisms of muscle wasting.

✶PF1

PS1-24 / #486

Theme: *1.3 - Basic sciences in NMD: Muscle atrophy / Degeneration*

Mrtf/Srf are mediators of mechanotransduction in skeletal muscle

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Muscle tissue depends on mechanical stimulation for its homeostasis: chronically decreased mechanical loads lead to muscle atrophy and increased loads to muscle hypertrophy. While significant progress has been made in understanding the signaling pathways that control muscle mass, the molecules that translate muscle load into signals that support hypertrophy/atrophy are unclear.

Cells can sense environmental mechanical properties directly or through intermediate biochemical reactions such as changes in F-actin polymerization/

depolymerization. Actin dynamics have been shown to regulate the activity of the transcription factor Srf (Serum response factor). Indeed, the ability of Srf to regulate downstream target genes depends on its association with coactivators, among which the Myocardin-related transcription factors, whose nuclear localization is regulated by G actin levels in the cell. In a search for novel factors involved in controlling muscle mass in response to workload, we identified the transcription factor Srf. In the present study, we investigate the role played by the actin/Mrtfs/Srf pathway in muscle atrophies of differing origin. We demonstrate that the actin/Mrtfs/Srf axis is downregulated in disuse atrophy (denervation, tenotomy and immobilization) but not affected by caloric restriction. The absence of mechanical signals causes an increase in nuclear G-actin and a rapid shuttling of the Srf coactivator Mrtf-A from myonuclei to a perinuclear area. This shuttling leads to Srf activity downregulation, resulting in a decrease in Srf-dependent transcription, which contributes to atrophy. Furthermore, the downregulation or the maintenance of either Srf or Mrtfs activity in denervated muscle aggravates or counteracts atrophy respectively. Collectively these *in vivo* results highlight Srf as a crucial mediator of mechanotransduction in skeletal muscle tissue and a key transcription factor regulating skeletal muscle mass as a function of mechanical activity. To gain further insights in the inter-relations between Srf and mechanotransduction, we use original ex vivo cellular models allowing biophysical and molecular studies. In particular, primary myoblast/myotubes were cultured on flexible substrates to which variable stains were applied. Our results confirm that mechanical stretchinduces the activation of actin/Mrtfs/Srf pathway through MrtfA nuclear translocation allowing now the study of the underlying signaling/sensor molecules involved.

✶PF1

PS1-25 / #258

Theme: *1.4 - Basic sciences in NMD: Nuclear envelope / Nuclear matrix of muscle cell*

Defective mechanosensing responses are associated with altered Yes-associated protein (YAP) signaling in myoblasts from human muscular dystrophies resulting from mutations in LINC-complex associated proteins

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Background: Mechanisms underlying the cellular response to mechanical forces are critical for muscle development and functionality. The LINC (LInker of the Nucleoskeleton and Cytoskeleton) complex may directly contribute to the cell's ability to probe its mechanical environment. Mutations in LINC-complex associated proteins, including lamins and nesprins cause human muscular dystrophies but disease mechanisms still remain to be elucidated. Recently, Yes-Associated Protein (YAP) signaling has emerged as a particularly important regulator of the mechano-response. Whether and how human muscular dystrophies resulting from mutations in LINC-complex proteins cause mechanosensing defects still remain to be elucidated.

Objectives: Our first aim was to determine whether human muscular dystrophies resulting from mutations in some of the LINC-complex associated proteins (A-type lamin and nesprin1) affected the capacity of myoblasts to sense the stiffness of the extracellular matrix. Our second aim was to determine whether defects in YAP signaling were involved in the observed mechanosensing defects.

Methods: Myoblasts with various mutations in the A-type lamins encoded by LMNA (LMNA),and nesprin1 mutant myoblasts (SYNE1) were obtained from patients exhibiting muscular dystrophies and compared to control human myoblasts (WT). Immortalized myoblasts were cultured on 2D soft surfaces

(12 kPa) or on 2D hard conventional glass (~MkPa) surfaces. Focal adhesions, actin cytoskeleton, and YAP signaling pathways were investigated.

Results: On 2D hard surface, there was no obvious difference in actin cytoskeleton and focal adhesion between WT, LMNA and SYNE1 myoblasts. LMNA and SYNE1 cells on soft surface exhibited enlarged focal adhesions and stress fibers compared with WT. Cytoplasmic translocation of YAP observed in WT in response to reduced stiffness matrix was absent in LMNA and SYNE1 cells, suggesting a permanent activation of YAP in mutant cells.

In conclusion, our data indicate that cell culture stiffness is critical to reveal mechanosensing defects in dystrophic muscle cells. Deregulation of YAP signaling pathways could be implicated in the mechanosensing defects in human muscular dystrophies resulting from mutations in LINC-complex associated proteins.

PS1-26 / #309

Theme: *1.4 - Basic sciences in NMD: Nuclear envelope / Nuclear matrix of muscle cell*

The modulation of the NAD+ metabolism participates in the oxidative type fiber phenotype in skeletal muscle dysfunction in a mouse model of Emery-Dreifuss muscular dystrophy

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Emery-Dreifuss muscular dystrophy (EDMD) is characterized by early joint contractures, slowly progressive muscular wasting and weakness and development of cardiac conduction defects, arrhythmias, left ventricular dysfunction and dilation with heart failure. The autosomal dominant form of EDMD is caused by mutations in LMNA gene, which encodes A-type lamins, two components of the nuclear envelope. Despite many efforts from the scientific community, the pathogenesis behind EDMD remains a riddle. While the genetic mutations and phenotypic abnormalities in EDMD have been well described, a central question about the pathogenesis of this muscular dystrophy remains unanswered: How do mutations in genes encoding nuclear proteins cause skeletal muscle pathology? Our novel hypothesis is that alteration in fiber type contributes to the pathology of EDMD.

We developed a knock-in mouse model, $Lmna^{H222P}$ reproducing a missense LMNA mutation (p.H222P) identified in an EDMD family. The homozygous mice develop phenotype mimicking the EDMD disease. Skeletal muscles from young (2–4 months) male $Lmna^{H222P/H222P}$ mice showed no alteration of tension and of kinetics of contraction and relaxation. However, in older $Lmna^{H222P/H222P}$ mice (5–7 months), the slow-twitch soleus muscle presented a 50% reduction of tension compared to WT soleus muscle. We hypothesized that this lowering of tension could reflect differences in fiber type content of the soleus muscle.

We i/stained soleus sections with antibodies recognizing the different MHC isoforms, ii/ studied MHC content by electrophoretic migration and iii/ run transcriptome analysis on 6-month old $Lmna^{H222P/H222P}$ mice.

We found that soleus muscle of 6-month old $Lmna^{H222P/H222P}$ mice exhibited a decrease in the relative content of MHC IIa isoforms (oxidative). Transcriptomic analysis of soleus of old (6 months) $Lmna^{H222P/H222P}$ mice identified metabolic dysfunctions (e.g., NAD metabolism) as one of the highest hits. We showed by qPCR from soleus of thesemice the downregulation of Nampt and Nmnat, two major actors of the nicotinamide adenine dinucleotide (NAD) metabolism. We measured the NAD^+ and NADH content in the soleus 6-month old mice and reported a 20% decrease in NAD^+.

In conclusion, we suggest that the decrease of tension in the soleus muscle of the 6-months old $Lmna^{H222P/H222P}$ mice could be partially related to defective oxidative type fibers, and seems mediated through new signalling pathway involving the NAD^+ metabolism.

★PF1

PS1-27 / #406

Theme: *1.5 - Basic sciences in NMD: Ionic exchanges in neuron and muscle*

Characterization of the trafficking and functional properties of the muscle specific long STIM1 isoform

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Store-Operated Ca^{2+} entry (SOCE) is a ubiquitous Ca^{2+} influx mechanism of particular importance for skeletal muscle and patients harboring mutations in SOCE genes suffer from muscular weakness and myopathies. SOCE is triggered by the Ca^{2+} depletion of the ER which initiates the oligomerization of the ER Ca^{2+} sensor STIM1 and its translocation to the plasma membrane (PM) where it aggregates into punctate structures corresponding to cortical ER subdomains (cER). Whereas in non-excitable cells SOCE takes 1–2 min to develop, in muscle cells influx occurs within 15–20 sec. The mechanism explaining the rapid Ca^{2+} influx of muscle remained unknown until our recent discovery of a longer STIM1L splice variant containing 106 additional amino acids. STIM1L interacts with actin filaments to form permanent clusters close to the PM that colocalize with Orai1 channels before ER depletion. Whether STIM1L remodels the ER to gate Orai channels is not established yet. As STIM1 and STIM1L are both expressed in muscle cells, we used a MEF cell line knocked-out for STIM1 and re-expressed either YFP-STIM1 or YFP-STIM1L to study the trafficking of each isoform independently. Using Ca^{2+} imaging and TIRF microscopy we observed that although STIM1L elicited a Ca^{2+} influx comparable to STIM1, it recruited much less additional PM clusters upon ER depletion and completely failed to enlarge PM clusters. Electron microscopy showed that unlike STIM1, STIM1L did not enlarge cER cisternae. Disruption of the actin cytoskeleton or expression of a STIM1L mutant lacking the actin binding domain partially recapitulated the STIM1 phenotype, with no cluster enlargement but an increase of new PM clusters. Unexpectedly, Mn2+ quench experiments revealed that SOCE activation was slow (2 min) in MEF cells expressing either STIM1 or STIM1L together with Orai1. Orai1-GFP channels were diffusely distributed at the PM in these cells and clustered with identical kinetics (4 min) upon store depletion. These results indicate that 1) STIM1L mediates SOCE without enlarging PM clusters or elongating cER cisternae, 2) cluster appearance and enlargement are 2 separable mechanisms, and 3) Orai1 clustering, rather than STIM1L recruitment at the PM, appears to be the rate-limiting step for SOCE. This latter finding suggests that, in skeletal muscle, scaffolding proteins maintain STIM1L-Orai1 clusters preassembled at the PM to enable rapid SOCE activation upon store depletion.

PS1-28 / #507

Theme: *1.5 - Basic sciences in NMD: Ionic exchanges in neuron and muscle*

Depression of voltage-activated Ca2+ release in skeletal muscle by activation of a voltage-sensing phosphatase

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Phosphoinositides (PtdInsPs) act as major signaling molecules in a vast diversity of cellular transduction processes and PtdIns(4,5)P2 is known to regulate the function of several types of ion channels in the plasma membrane of various cell types. We investigated the potential role of PtdIns(4,5)P2 in the regulation of Ca2+ homeostasis and excitation-contraction (E-C) coupling in adult mouse muscle fibers by using *in vivo* expression of the voltage-sensitive phosphatases Ci-VSP and Dr-VSP. Confocal images of EGFP-tagged Dr-VSP revealed a double-banded pattern consistent with localization of the protein within the triadic region. In both Ci-VSP and Dr-VSP expressing fibers, membrane current measurements from a depolarized holding potential established the presence of a gating current component consistent with the voltage-sensitive domain of the VSP being active in the t-tubule membrane, with a mid-activation voltage of +60 mV

and +90 mV, respectively. Rhod-2 Ca2+ transients generated by 0.5 s-long voltage-clamp depolarizing pulses to voltages below the range of activation of VSPs were unaffected by their presence in the membrane. However, upon repeated application of 200 ms depolarizing pulses to +100 mV, Ci-VSP expressing fibers exhibited a depression of Ca2+ release with, in average, the peak Ca2+ transient being depressed by 30 % after 10 pulses to +100 mV as compared to when pulses to +10 mV were applied. No concurrent change in the membrane current was observed. The phenomenon was reversible and could also be detected in Dr-VSP expressing fibers. Results strongly suggest that depletion of t-tubule PtdIns(4,5)P 2 alters voltage-activated Ca2+ release. As InsP3 signaling is unlikely to play a role in E-C coupling, the effect may rather result from a regulatory interaction between PtdIns(4,5) P 2 and a t-tubule membrane protein involved in the activation of the ryanodine receptors.

PS1-29 / #43

Theme: *1.6 - Basic sciences in NMD: Immune mechanisms in neuromuscular diseases*

Targeting miR-155 restores dysfunctional microglia and ameliorates disease in the SOD1 model of ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease. In the SOD1 model of ALS we found loss of the unique molecular signature that characterizes microglia in association with increased-expression of miR-155. There was loss ofunique microglial moleculesP2ry12,Tmem119,Olfml3, microglial transcription factorsEgr1,Atf3,Jun,Fos,Mafb and upstream regulatorsCsf1r, Tgfb1andTgfbr1essential for microglial survival.Major microglia biological functionsincluding phagocytosis were suppressed.Genetic ablation of miR-155 increased survival in SOD1 mice and reversed abnormal microglial and monocyte molecular signatures.We found increased expression of miR-155 in the spinal cord of ALS subjects. Dysregulated proteins in SOD1 spinal cord that we identified in human ALS spinal cord and CSF were restored in $SOD1^{G93A}$/$miR\text{-}155^{-/-}$mice. Treatment with anti-miR-155 injected systemically or into the CSF-prolongedsurvivalandderepressedmicroglialmiR-155 targeted genes.Our findings identify a new avenue for immune based therapy of ALS by targeting miR-155.

This work was supported by by NIH Grant AG027437, DOD grant 109712, a grant from the Amyotrophic Lateral Sclerosis Association and philanthropic support. We thank Prize4Life for providing SOD1 mice.

PS1-30 / #183

Theme: *1.6 - Basic sciences in NMD: Immune mechanisms in neuromuscular diseases*

Decreased number of circulating NK cells and dramatic lack of INFgamma production in patients with antisynthetase syndrome

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Background: Antisynthetase syndrome (aSS) is an autoimmune myositis associated with interstitial lung disease (ILD) and specific anti-tRNA-synthetase antibodies. To date, the pathogenesis of aSS remains unknown and the involvement of Natural Killer (NK) cells is not described.The aim of this study was to conduct the first extensive analysis of the phenotype and functional properties of NK cells in aSS.

Methods: Nine active and 9 inactive patients (median age 50, range 25–77) were included and compared to healthy controls. $CD3^{-}CD56^{+}$ NK cell phenotype was performed by flow cytometry. Assessment of NK cell functions (degranulation & cytokine production) was performed spontaneously or after interleukin(IL)-12 and IL-18 stimulation, in the presence of K562 target cells. Appropriate statistical tests were performed.

Results: Myositis and ILD occurred each in 16/18 patients and was the first cause of active aSS ($n=7/9$). Most patients ($n=14$) were receiving stable and low doses of steroids and/or immunosuppressive drugs. Circulating NK cell count was lower in aSS patients vs controls (127 ± 85 vs 243 ± 95/mm3, $p<0.001$). Proportion of the two NK cells subtypes (cytotoxic $CD56^{dim}$ & immunoregulatory $CD56^{bright}$) was normal. NK cells from all aSS patients were undistinguishable from those of controls in terms of the cell surface expression of NK cell receptors, including those associated with activity (CD69, HLA-DR, NKp44) or maturation (CD16, NKG2A, CD8alpha and CD161). KIR, ILT2 and NKG2C-D were also similar to controls. Importantly, NKp30, a natural cytotoxicity receptor involved in NK cell-Dentritic cell (DC) crosstalk was significantly decreased in aSS patients vs controls and also in active vs inactive aSS patients (53 ± 26 vs 79 ± 18%, $p=0.034$). Functional activities revealed no differences between aSS patients and controls, regarding both spontaneous degranulation capacities and intracellular TNFalpha production. However, IFNgamma production was dramatically decreased after stimulation by IL12 + IL18 (6 ± 10% vs 24 ± 13%, $p<0.003$) and correlated positively with the expression of NKp30 (r2=0.32, $p<0.03$), independently of the treatments.

Conclusions: Circulating NK cells from aSS patients had a decreased capacity to produce IFNgamma due to a lower expression of NKp30. These data suggested an involvement of NK cells and NK-DC crosstalk in aSS pathogenesis.

PS1-31 / #191

Theme: *1.6 - Basic sciences in NMD: Immune mechanisms in neuromuscular diseases*

Myasthenia Gravis associated with autoimmune Idiopathic Pulmonary Fibrosis

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Myasthenia gravis (MG) as an autoimmune neuromuscular junction disease can be rarely associated with other autoimmune conditions. When in association, MG is frequently restricted to the eye muscles, has onset at young age and positive anti-acetylcholine receptor antibodies. Epidemiologically, MG rarely associates with autoimmune Idiopathic Pulmonary Fibrosis. The reason is unknown, but hypothesized as immunological cross-reactivity.

Eighty-nine year old lady presented with generalized weakness, worsening shortness of breath and head drop that have been progressive for the past five months. She has been diagnosed with autoimmune Idiopathic Pulmonary Fibrosis twelve years prior. At the time of our exam, five months from the head drop onset, she had severe axial muscle weakness along with respiratory muscle weakness. Her neck extensors, facial muscles, hip flexors and shoulder abductors were significantly weak, but not the extraocular muscles. There was no ptosis or diplopia. She was using accessory respiratory muscles at rest. There were high titer anti-acetylcholine receptor antibodies in the blood. MUSK antibodies were absent. Repetitive nerve stimulation of the facial nerve at 3 Hz revealed significant decrement and needle EMG revealed short-duration potentials in the face and deltoid. No thymic mass was identified on chest CT. After plasma exchange, steroids, immunosupressants and anti-cholinesterase therapy she improved clinically. Immunoglobulin infusions had no significant benefit.

The association of MG and other autoimmune conditions is rare, but represents a serious diagnostic and therapeutic challenge. MG was difficult to diagnose in the context of chronic respiratory insufficiency secondary to the Idiopathic Pulmonary Fibrosis. Opposite than previous reports, our patient had no eye muscles involvement. This case is also unique due to the new onset MG in an eighty-nine years old lady. To our knowledge, one of the oldest female patients ever reported with new onset MG. Further research is required for better understanding of the phenomena.

PS1-32 / #403

Theme: *1.6 - Basic sciences in NMD: Immune mechanisms in neuromuscular diseases*

Thymectomy and immune mechanisms in patients with myasthenia gravis. Cohort study in Czech Republic

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Background: Myasthenia gravis (MG) is a disease mediated by autoantibodies, where an important role is played by the dysregulation of Th and Treg cells. The immunity organ thymus, which is probably directly responsible for autoagression, is supposed to affect immunopathogenesis of MG . Therefore, after induction of clinical remission in patients up to 55 years of age, thymectomy is recommended.

Material, methods, pilot results: The project lasted 3 years (from November 2010 till December 2013) and was included 65 patients who underwent thymectomy. In all patients we firstly investigate markers of humoral immunity (antibodies antiAChR, ScMAb, IgG with subclass IgG1–4, IgA, IgM, IgD), cellular immunity markers (31 subpopulations of lymphocytes in total including Treg cells and complement markers) and selected cytokines before and after thymectomy in a clearly defined time interval - 1 month, 6 months and 1 year . In parallel in all patients, in time intervals mentioned above, we measure quantified myasthenic score (QMGS) and we compare clinical effect of the treatment (based on QMGS) with the level of laboratory parameters. We are going to present our final results, which are currently being processed.

Discussion and conclusion: Our goal is to find out the correlation between clinical state improvement and laboratory changes of markers. Clinical effect of thymectomy in patients with MG is obvious (except from anti-MUSK positive patients where thymectomy brings no effect). We still don't know, in what time interval from surgery the positive effect occurs and what it depends on.

This longitudinal study is going to show in what time interval after thymectomy the improvement (quantified by - QMGS) and the significant changes in markers of both humoral and cellular immunity in patients with generalized MG occur.

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PS1-33 / #424

Theme: *1.6 - Basic sciences in NMD: Immune mechanisms in neuromuscular diseases*

Danger signals promoting innate immunity activation in Dermatomyositis

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Inflammatory myopathies are an heterogenous group of diseases that include dermatomyositis (DM), polymyositis (PM) or inclusion body myositis (IBM) characterized by acquired muscle weakness. Expression of MHC-I in the muscle fibers is a landmark for these diseases and is considered to actively participate in their immunopathogenesis. Previous results from our group have demonstrated the upregulation of innate immunity receptors in the muscle fibers of these diseases. In particular, RIG-I overexpression was only found in perifascicular atrophic areas of DM biopsies. Functional *in vitro* studies using human primary muscle cultures, set RIG-I as an effector that induce the secretion of IFNbeta and consequently the overexpression of MHC-I and RIG-I. These mechanisms may be responsible of perpetuating the immune

response after a danger signal trigger. However the sources of danger signals that trigger the immune response are unknown. A role for ischaemia-reperfusion has been proposed to have a role in the muscle damage in DM.

We have studied in human primary muscle cultures, the effect of hypoxic microenvironment and damaged-cells derived particles in enhanced RIG-I signaling and sustained immune response against muscle fibers. We have also analyzed the effect of these factors in muscle atrophy.

The regulation of this altered pathway could be the target of new therapies in this inflammatory myopathy.

PS1-34 / #290

Theme: *1.7 - Basic sciences in NMD: Fundamental approaches of motor neuron, axon and related*

Age-related changes in central and peripheral nervous system associated with sarcopenia

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Ageing is associated with a significant decline in skeletal muscle mass and performance, known as sarcopenia that often leads to progressive disability and loss of independence. Loss of neuromuscular junctions on the surface of myofibres is associated with sarcopenia, and molecular analyses of aged muscles in our lab have shown striking alteration of gene expression related to denervation. These neuromuscular changes that contribute to myofibre denervation occur not only within the skeletal muscle tissue, but also in the central nervous system (CNS) and peripheral nervous system (PNS).The age-related cellular and molecular changes in the CNS and PNS are the focus of this study. Exercise reduces the progression of sarcopenia and the impact of resistance exercise on the parameters measured in the CNS and PNS is a central component of this project.

Spinal cord and sciatic nerve samples were collected from sedentary male C57Bl/6J mice aged 4, 15, 18, 22 and 24 months. The sciatic nerve samples have been snap frozen to quantify expression of various nerve-related proteins upon ageing by western blotting. In addition sciatic nerve samples have also been snap frozen from female C57Bl/6J mice aged 3 and 27 months. Immunofluorescence studies have been carried out on another set of paraformaldehayde fixed sciatic nerve samples from male C57Bl/6J mice aged 3, 6 and 27 months. Preliminary results from these studies show a decrease in the number of sensory axons upon ageing. Light microscopic studies from semi thin transverse sections stained with toluidine blue show no significant change in the total axon numbers. 1mm blocks of distal peripheral nerve were fixed in glutaraldehyde for transmission electron microscopy sections to document age-related ultra structural changes that include myelin sheath thickness, axonal numbers cross sectional area and g-ratio. The spinal cord samples will be used for immunohistochemical and molecular biology studies to analyse the expression changes in genes associated with ageing. These combined studies aim to identify the changes in the central and peripheral nervous system associated with sarcopenia.

PS1-35 / #363

Theme: *1.7 - Basic sciences in NMD: Fundamental approaches of motor neuron, axon and related*

Pre-symptomatic transcriptional profiling of differentially vulnerable motor neurons in the Smn2B/- mouse model of SMA

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Spinal Muscular Atrophy (SMA) is a neuromuscular disorder manifesting as muscle weakness and corresponding loss of lower motor neurons from the spinal cord. Despite the ubiquitous expression of the disease causing gene, SMN1, lower motor neurons appear to be primary targets in SMA, with degeneration of neuromuscular junctions representing an early and significant event in pathogenesis. Furthermore, not all motor units appear equally affected, with a selective vulnerability of slow twitch fatigue resistant postural groups. Although the reasons for this selectivity are unclear, this differential vulnerability

represents an excellent opportunity to investigate the underlying mechanisms of pathology and their critical regulators. In this study, we have exploited the selective vulnerability observed in neuromuscular junction pathology to perform a rigorous assessment of the mechanisms which both render specific motor neurons more vulnerable to low levels of Smn and the pathways responsible for the initiation of neuronal pathology. We have utilized the extended life span of the $Smn^{2B/-}$ mouse model to label and trace selectively vulnerable motor neuron populations at pre-symptomatic time points. We then used laser capture micro-dissection to isolate differentially vulnerable populations of motor neurons and perform RNA seq analysis, a highly sensitive and rigorous assessment of the transcriptome. We have then used a 4 way comparative analysis with functional clustering to reveal a number of cellular pathways which are perturbed when Smn levels are reduced in motor neurons, and pathways which are selectively perturbed vulnerable motor neuron populations.

PS1-36 / #561

Theme: *1.8 - Basic sciences in NMD: Neuromuscular junction: Basic Aspects*

Probing the Effect of Electrical Stimulation on the Formation of Neuromuscular Junctions in 2D and 3D

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Many neuromuscular disorders have been identified and are classified based on the site of the neuromuscular junction defect, with non-kinetic post-synaptic generally resulting from a decreased number of functional acetylcholine receptors (AChR). It has recently been shown that formation of neuromuscular junctions can be influenced by electrical stimulation (Fukazawa, 2013), however, the mechanism of how the neuromuscular complex, and specifically the AChR, respond to electrical stimulation is not fully understood.

We have developed a 2D and 3D *in vitro* model for studying the effect of electrical stimulation on the formation of neuromuscular junctions. This model uses a flexible electrical stimulation set up that provides either direct or field electrical stimulation to the cells, and allows many stimulation strategies to be assessed simultaneously. Additionally, multiple conducting materials can be used, allowing us to assess effects of changing the electrode material to include metals, conducting polymers (polypyrrole and PEDOT) and graphene to deliver the stimulation. Preliminary results suggest that some electrical stimulation protocols enhance the number of neuromuscular junctions formed, and the important electrical parameters for enhancing formation of neuromuscular junctions will be discussed.

✶PF1

PS1-37 / #84

Theme: *1.8 - Basic sciences in NMD: Neuromuscular junction: Basic Aspects*

The Wnt receptor Frizzled 3 is required for nerve-muscle target recognition process during neuromuscular junction formation

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The formation of the neuromuscular junction (NMJ) is based on the establishment of a trans-synaptic dialogue between presynaptic motor axons and postsynaptic muscle fibers. NMJ development can be divided into two distinct phases: an early one, nerve independent, which prepare the muscle to receive the synaptic contacts and a late phase characterised by the differentiation of both pre and postsynaptic domains and maturation of the synapse. Many teams have focused on the late phase mechanisms of postsynaptic differentiation including acetylcholine receptor aggregation in the postsynaptic muscle membrane. In contrast, the early phase of nerve-muscle target recognition process is much less understood. How motor axons recognize and stop within a specific muscle domain where the synapse is to be formed? How the

muscle gets ready to receive the first synaptic contact? These are key questions regarding synapse development remaining to be investigated.

Wnts proteins are well-known synaptogenic factors and their role in postsynaptic differentiation at the NMJ begins to be decrypted. We previously demonstrated that Wnt4 is required for NMJ innervation and signals via MuSK activation. However, Wnt4 being able to interact with the Wnt receptor Frizzled (Fz) 3 in the brain, a core component of the Wnt Planar Cell polarity (PCP) pathway, we hypothesised a role for Fz3 in NMJ formation. Fz3 mRNA is expressed in developing diaphragms at a time where the synapse is forming. Moreover, Fz3 protein specifically accumulates at the NMJ in the presynaptic compartment and is localised in developing neurites from cultured ventral spinal cord explants. Diaphragms embryos from Fz3 knock out mice displayed severe innervation defects with reduced nerve arborisation and non innervated AChR clusters. Taken together, our data indicate that at the NMJ, Wnt proteins signal both through MuSK and Fz receptors and suggest that the Wnt PCP signalling is required during nerve muscle recognition process.

This work is supported by the Centre National de la Recherche Scientifique.

PS1-38 / #156

Theme: *1.8 - Basic sciences in NMD: Neuromuscular junction: Basic Aspects*

Wnt elicited molecular mechanisms during neuromuscular junction formation.

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In mammals, the neuromuscular junction (NMJ), a cholinergic synapse between motoneurons and muscle cells, forms in several consecutive steps. This developmental process starts with the differentiation of a postsynaptic domain in a prospective synaptic region, prepatterning the muscle to attract the motor axon. Axons grow toward this postsynaptic domain and once its target reached, neurite extension stops. Increasing evidence in the literature highlight the instructive role of Muscle-specific kinase (MuSK) and its coreceptor Lrp4 in NMJ formation. Interestingly, MuSKcontains in its extracellular region a Cystein Rich Domain (CRD) homologous to the Frizzled-like domain, known to interact with Wnt molecules, a large family of synaptogenic proteins involved in synaptic differentiation and axon guidance. Moreover, Lrp4 is highly homologous to the Wnt co-receptors Lrp5/6. At the NMJ, two Wnts proteins, Wnt4 (in mammals) and Wnt11 (in zebrafish) bind to the CRD of MuSK and are required for muscle prepatterning and innervation. However, the underlying mechanisms and the role of Wnt/MuSK interaction in the development of the NMJ remain to be investigated.

We showed that similarly to Wnt4, Wnt11 is able to induce acetylcholine receptor (AChR) clustering *in vitro* in a dose- and time-dependant manner. Moreover, by using canonical and non canonical Wnt signalling inhibitors, we demonstrated that (i) the clustering activity of Wnt4 act through the canonical pathway while (ii) Wnt11 signal through both canonical and PCP pathways. In addition, ultrasound guided injections of Wnt signalling inhibitors in mouse live embryos lead to severe and specific defects in NMJ formation. We are currently analysing the Wnt11 knock-out mice and will discuss our results in the poster.

Collectively, our data strongly suggest that the interaction between Wnt proteins and MuSK is critical for NMJ formation.

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✶PF2

PS1-39 / #215

Theme: *1.8 - Basic sciences in NMD: Neuromuscular junction: Basic Aspects*

Integrins are required for synaptic transmission and development of the neuromuscular junction

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Background: Development of the neuromuscular junction (NMJ) depends on interactions between proteins in the nerve terminal, muscle fibre and in the synaptic cleft. Laminins play a central role, as they bind to presynaptic voltage-gated calcium channels and to dystroglycan in the muscle fibre. Integrin-a3 is a laminin receptor expressed in the presynaptic active zones, the sites of neurotransmitter release across the synaptic cleft. Aims and methods: To determine the function of integrin-a3 at the NMJ, using mice with a genetic ablation of the integrin-a3 gene. Results: At embryonic day E18.5, $a3^{-/-}$ mice present with abnormal assembly of active zone proteins and reduced synaptic transmission, as determined by electrophysiology. Ultrastructural studies reveal defective deposition of the basal lamina at the synaptic cleft. As $a3^{-/-}$ mice die at birth due to the failure of multiple systems, we used $a3^{+/-}$ mice to study the role of this protein in postnatal maturation of the NMJ. In adult $a3^{+/-}$ mice, we find aberrant NMJ morphology and reduced expression of several active zone proteins. Conclusions: Integrin-a3 is important for the assembly of active zones and of the synaptic basal lamina. The data suggests that defects in this protein may be associated with defect of neuromuscular junction transmission in humans.

PS1-40 / #248

Theme: *1.8 - Basic sciences in NMD: Neuromuscular junction: Basic Aspects*

MuSK-ColQ interaction and signalisation in synaptogenesis of the neuromuscular junction

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Collagen Q (ColQ) is a specific collagen expressed by muscles at the neuromuscular junction (NMJ), where it plays a crucial role by anchoring and accumulating acetylcholinesterase (AChE) in the synaptic basal lamina. Patients bearing ColQ mutations and mice deficient for ColQ present a congenital myasthenic syndrome with AChE deficiency, highlighting the physiological importance of this collagen. The accumulation of the AChE-ColQ complex at the synapse requires the interaction of ColQ COOH terminus with muscle-specific receptor tyrosine kinase (MuSK), a key protein for NMJ formation. In particular, MuSK mediates the actions of the proteoglycan, agrin, on postsynaptic differentiation.

In addition to its structural function, we showed previously that ColQ has important regulatory functions at the synapse by controling acetylcholine receptor (AChR) clustering and synaptic gene expression. At least part of ColQ effects, are mediated by ColQ-MuSK interaction. Moreover, cell surface levels of MuSK are decreased <em style=”mso-bidi-font-style: normal;”>*in vitro* and <em style=”mso-bidi-font-style: normal;”>*in vivo* in cells lacking ColQ, suggesting that ColQ controls MuSK sorting or stabilisation in the muscle membrane.

In an attempt to understand how ColQ exerts its regulatory functions, we are investigating the properties of ColQ-MuSK interaction and the molecular events induced by the binding of ColQ to MuSK. To identify the domains of MuSK required for association with ColQ, we generated MuSK mutants deleted of the extracellular Ig-like domains 1, 2, 3 or the cysteine-rich domain (CRD). Using co-immunoprecipitation experiments, we obtained preliminary results, which suggest that ColQ-MuSK interaction might involve Ig-like domain 1 and part of the CRD. This has now to be confirmed by other approaches such as <em style=”mso-bidi-font-style: normal;”>*in vitro* binding assays. We also studied if ColQ modulates agrin-

induced activation and phosphorylation of MuSK. Using ColQ-transfected and ColQ-deficient muscle cell lines, we show that ColQ inhibits agrin-induced MuSK phosphorylation. These results indicate that ColQ regulates MuSK activation and agrin function. The underlying molecular mechanisms as well as the influence of ColQ on the signaling pathways initiated by MuSK are under current investigations.

✶PF2

PS1-41 / #326

Theme: *1.8 - Basic sciences in NMD: Neuromuscular junction: Basic Aspects*

The Molecular Machinery that Orchestrates Neuromuscular Junction Remodeling

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Neuromuscular junctions (NMJs) in mammalian skeletal muscle undergo a postnatal topological transformation from a simple oval plaque to a complex branch-shaped structure. Disruptions of NMJ maturation and/or maintenance are frequently observed in neuromuscular disorders such as congenital myasthenic syndromes (CMSs).

We previously demonstrated that podosomes, actin-rich adhesive organelles, promote the remodeling process and showed a key role for one podosome component, LL5β. To better understand molecular mechanisms of postsynaptic maturation, we purified LL5β protein complex from myotubes and showed that three regulators of the actin cytoskeleton -Amotl2, Asef2 and Flii- interact with LL5β. These and other LL5β-interacting proteins are associated with podosomes in various cell types strengthening the close relationship between synaptic and non-synaptic podosomes. We then focused on Amotl2, showing that it is associated with synaptic podosomes in cultured myotubes and with NMJs *in vivo*. Depletion of Amotl2 in myotubes leads to increased size of synaptic podosomes and corresponding alterations in postsynaptic topology. These results demonstrate the role Amotl2 plays in synaptic remodeling and support the involvement of podosomes in this process.

✶PF1

PS1-42 / #422

Theme: *1.8 - Basic sciences in NMD: Neuromuscular junction: Basic Aspects*

Neuromuscular junction integrity is dependent on rapsyn/AChR complex-intermediate filament linkage via plectin

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Plectin is a versatile cytolinker protein that acts as a networking and anchoring element of cytoskeletal filaments. Mutations in the plectin gene cause a number of diseases, among them epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) conjugated with a myasthenic syndrome (MyS). EBS-MD-MyS manifests with impaired neuromuscular transmission, abnormal muscle contraction and muscle weakness. In accordance, diaphragm muscle isolated from plectin knock out neonatal mice show changes in synaptic properties, including prolongation of rise times, delayed decays, and reduced amplitudes of end-plate currents. To study the impact of postsynaptic plectin deficiency on neuromuscular junction (NMJ) formation and maintenance we generated a new muscle conditional knock out (cKO) mouse model in which plectin is missing from adult muscle, as well as during myogenesis and regeneration. For this, plectin gene was specifically targeted under the expression of the Pax7 promoter (Pax7-Cre/cKO) in myofibers and satellite cells. Pax7-Cre/cKO mice were found to suffer from progressive muscle degeneration, profound kyphosis and severe muscle weakness. Moreover, muscle fibers derived from adult Pax7-Cre/cKO mice showed pronounced dispersion of acetylcholine receptors (AChRs) along the

sarcolemma and a marked reduction in the postsynaptic infolding pattern. While in wild-type muscle we observed AChRs to be embedded in a condensed desmin lattice, in cKO muscle, desmin IFs were detached from NMJs and showed collapse into aggregates in the proximity of synaptic nuclei. Furthermore, using plectin or desmin-deficient myocytes differentiated ex vivo to myotubes, we could show diminished agrin-inducible AChR clustering and reduced stability of clusters. Moreover, we found one of the 4 major skeletal muscle isoforms of plectin (P1f) to interact with rapsyn, the key AChR-scaffolding molecule, and simultaneously to anchor desmin IFs at the AChR complex. These findings strongly suggest that the lack of plectin affects NMJ-integrity due to dislocalization of IFs from the postsynaptic apparatus.

PS1-43 / #45

Theme: *1.9 - Basic sciences in NMD: Others*

Functional Motor Neuron Subtypes Generated from neurosphere drived adipose tissue for treatment of degenerative Motor Neuron diseases

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Introduction: In the current study, showed that RA +GDNF+BDNF a key differentiation factor allowed cultured adipose drived stem cells (ADSCs) to form motor neuron like cells with characteristics corresponding to form functional connections with muscle fibers. Our study provides a new fundamental basis for autologous cell replacement therapy to treat degenerative MN diseases, ALS and spinal cord injury.

Material & Method : Rat visceral fat was enzymatically digested to yield rapidly proliferating fibroblast-like cells(ADSCs) , a proportion of which expressed the mesenchymal stem cell marker. Cells induced with amixture of motoneuron factors (RA,SHH,GDNF,BDNF) adopted a morphology similar to motoneuron cells.

This cells evaluated by Immunocytochemical staining and RT-PCR ; also,the cells of functionality indicated with ability innervated myotubes and release of synaptic vesicles using FM1-43.

Result & Conclusion: the treated cells with motoneuron factors expressed markers, HB9,Islet1,oligo2 indicative of differentiation. When co-cultured with C2C12(myotube cells), These cells were established synaptic contacts with muscle like cells and these cells increased in number and neuritis. These results indicate adipose tissue contains a pool of regenerative stem cells which can be differentiated to a motoneuron cell phenotype and functional may be of benefit for treatment of degenerative MN diseases.

PS1-44 / #46

Theme: *1.9 - Basic sciences in NMD: Others*

In vitro induction of adipose derived stem cells into motoneurons characterized by calcium waves coupled/and with voltage dependent fluorescence

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Cell replacement therapy has provided the basis for future clinical applications to treat neurodegenerative diseases, such as motoneuron diseases. Induced functional motoneurons are an option for replacing the lost motoneurons. Adipose derived stem cells (ADSCs) are feasible autologous source. The present study, neurospheres (NS) were derived from ADSCs using B27,EGF and bFGF, neural stem cells (NSCs) were generated from neurospheres and were induced into motoneuron-like cells (MNLCs) by Shh and retinoic acid (RA). ADSCs lineage was evaluated by their osteogenic, lipogenic and chondrogenic differentiation, moreover, they were immunostained for CD90, CD44, CD 49d, CD106,CD31and CD45; and stemnss gene (Oct-4, Nanong and SOX2). The neurospheres and NSCs were evaluated by immunostaining (NF 200,NF 60and nestin) and RT-PCR , while MNLCs were evaluated using islet-1, oligo-2 and HLXB9 genes. The yield of MNLCs was 95%. The myotubes were innervated by MNLCs using co-culturing system. Calcium ion imaging, voltage dependent fluorescence and synaptic vesicle release suggested that the MNLCs were functional. The induced ADSCs adopted multipolar motoneuron morphology, and they expressed islet-1, oligo-2 and HLXB9. Moreover a calcium imaging

coupled with voltage dependent fluorescence with the release of the synaptic vesicles evoked by potassium ions

PS1-45 / #112

Theme: *1.9 - Basic sciences in NMD: Others*

AAV9-mediated gene transfer of ?ARKct efficiently ameliorates cardiomyopathy in mice lacking dystrophin but not delta-sarcoglycan

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In Duchenne muscular dystrophy (DMD) and limb girdle muscular dystrophy type 2 F (LGMD2F), absence of dystrophin and Δ-sarcoglycan (Sgcd), respectively, cause instability of the dystrophin-glycoprotein-complex (DGC) and increased vulnerability of cardiomyocytes towards contraction-induced damage. In almost every patient with DMD or LGMD2F subclinical or clinical cardiomyopathy can be found with strong influence on mortality. It is well known that chronic overstimulation of the beta-adrenergic receptor (βAR) by excessive adrenergic stimulation and consecutive upregulation of the G-protein-coupled-receptor kinase 2 (GRK2) plays a key role in the pathogenesis and progression of cardiomyopathies of any genesis. However, the benefit in treating the specific subgroup of patients with muscular dystrophy and cardiomyopathy is not yet established and therefore we do not know if genotypes predict the response to beta-blockers or competitive GRK2 inhibition. Therefore, we have investigated the efficiency of inhibition of GRK2 through adeno-associated virus (AAV9) -mediated cardiac overexpression of the carboxyl-terminusof GRK2 (βARKct) to prevent the development of cardiomyopathy in dystrophin-deficient (mdx) and Sgcd-deficient mice, mouse models for DMD and LGMD2F, respectively. We found that long-term treatment with AAV-9 vectors containing βARKct cDNA under transcriptional control of a CMV-MLC promoter (AAV9/βARKct) profoundly improved systolic left ventricular function and ameliorated myocardial hypertrophy in mdx mice, whereas beneficial effects on cardiac function in Sgcd-/- mice were mild. In contrast to mdx mice neither GRK2 nor NFκB were upregulated in Sgcd-/- mice, indicating the existence of distinct pathogenetic mechanisms for development of cardiomyopathy in these mouse models. Taken together, therapeutic inhibition of GRK2 through AAV-mediated cardiac overexpression of βARKct represents a promising tool to treat cardiomyopathy due to lack of dystrophin. However, effectiveness of this therapy may vary between different forms of muscular dystrophy-associated cardiomyopathies. Despite the tight biochemical association of dystrophin and Δ-sarcoglycan, there are distinct differences in the pathomechanism of cardiomyopathy in Sgcd-/- mice that might explain the insufficient response to AAV9/βARKct treatment.

PS1-46 / #142

Theme: *1.9 - Basic sciences in NMD: Others*

Mybpc3-targeted mice with hypertrophic cardiomyopathy show an impaired autophagic flux

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Background: The autophagy-lysosome pathway (ALP) is one of the major systems for degradation of cellular proteins and organelles. Our previous data indicated that protein levels of autophagic markers, such as LC3 or p62, were elevated inMybpc3-targeted knock-in (KI) and knock-out (KO) mice with hypertrophic cardiomyopathy (HCM). Here, we investigated the autophagic flux in vivoin KI, KO and wild-type (WT) mice andin vitroin KI and WT neonatal mouse cardiomyocytes (NMCMs).

Methods: KI, KO and WT mice of different postnatal ages (10, 30, and 57-week-old) were treated with 40 mg/kg ALP inhibitor leupeptin for 1 hour. NMCMs

were treated with 10 μM ALP inhibitor chloroquine for 24 h. Autophagic flux was determined by Western blot detecting the turnover of cytosolic LC3-I to autophagosome membrane-bound LC3-II. Lysosomal function was assayed by cathepsin D protein level and activity.Autophagosome-lysosome fusion was indirectly measured by Rab7 protein level.

Results: Leupeptin induced less increase in LC3-II/LC3-I ratio in KI than WT mice, suggesting ALP impairment in KI. The extent of impairment increased with age of the mice, being absent in NMCMs, low in 10, moderate in 30 and severe in 57-week-old KI mice. Similar results were obtained in 57-week-old KO mice, excluding mutant cMyBP-C as a trigger. Cathepsin D protein level and activity did not differ between groups, indicating intact lysosomal function. In contrast, Rab7 protein level was lower in KI and KO mice than in WT, hinting at a block in fusion of autophagosomes and lysosomes.

Conclusion: We provide evidence for an impaired autophagic flux that becomes more severe with age in HCM mice lacking functional cMyBP-C. This impairment is probably caused by a block in autophagosome-lysosome fusion rather than a lysosomal dysfunction.

PS1-47 / #152

Theme: *1.9 - Basic sciences in NMD: Others*

Localization of Ankrd2 in PML bodies of skeletal muscle capillary cells

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PML nuclear bodies (PML NBs) are specialized proteinaceous structures proposed to fine-tune a wide variety of processes. SUMO modified PML protein is the key organizer and forms the outer shell of the bodies while its interaction partners are usually inside. Previously we showed that skeletal muscle transcriptional co-factor Ankrd2 is localized in PML NBs of human myoblasts in the nucleus, whereas on differentiation it is up-regulated and predominantly expressed in the cytoplasm of myotubes. Apart from its localization in both cytoplasm and nuclei of myofibers of adult muscle, we have also detected Ankrd2 in PML NBs. The percentage of PML positive nuclei was similar among different muscle types. Surprisingly, PML NBs resided outside of the myofibers, in CD-34 positive cells. This result implicates localization of Ankrd2 in the PML NBs of endothelial cells of the skeletal muscle capillary network. Here we demonstrate for the first time that Ankrd2 has multiple cell type expression in skeletal muscle.We found that human EA.hy926 cells, isolated from human umbilical vein and fused with carcinomic human alveolar basal epithelial cells, were a good *in vitro* model system for studying the function of Ankrd2 in endothelial cells, since it was expressed in these cells and also localized in the PML NBs. Given that PML bodies act as stress sensors, the cells were exposed to As_2O_3, Doxorubicin and H_2O_2 in order to assess Ankrd2 response upon treatments which affect PML NBs. Using immunofluorescence and Western blot we demonstrated that Ankrd2 remained in nuclear bodies, but its expression level was diminished. These results raise the question about the role of Ankrd2 in the non-muscle cells of muscle tissue and in processes such as angiogenesis and neovascularization.

PS1-48 / #199

Theme: *1.9 - Basic sciences in NMD: Others*

Electromechanical delay components during skeletal muscle contraction and relaxation: novel physiological insights and possible application in Myotonic Dystrophies

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Muscle contraction and relaxation are physiological events which involve several mechanisms that are electrochemical and mechanical in nature. The functional insights into these processes can provide useful information in both clinical and physiotherapeutic

fields. During contraction, the time lag between the onset of muscle electrical activation and the onset of force production was traditionally defined as electromechanical delay (EMD). A delay between the cessation of muscle electrical activity and the beginning of force decay can be observed during the relaxation phase (R-EMD). By an electromyographic (EMG), mechanomyographic (MMG) and force (F) combined approach, both EMD and R-EMD can be partitioned into two components, containing one the electrochemical and the other one the mechanical processes underlying muscle contraction and relaxation. The aim of the study was to assess the effects of fatigue on the different electrochemical and mechanical components of EMD and R-EMD. Inter- and intra-operator reliability of the measurements were also evaluated. Twenty-five participants underwent two sets of tetanic stimulations of the gastrocnemius medialis muscle, with 10 min of rest in between. After a fatiguing protocol of 120 s, tetanic stimulations were replicated. The same protocol was repeated on a different day. EMG, MMG and F signals were recorded during contraction. EMD and its two components (between EMG and MMG onset, delta-t EMG-MMG, and between MMG and F onset, delta-t MMG-F), together with R-EMD with its two components (between EMG cessation and the beginning of force decay, R-delta-t EMG-F, and from the initial force decrease to the negative peak of MMG, R-delta-t F-MMG) were calculated. After fatigue: (i) EMD, delta-t EMG-MMG and delta-t MMG-F lengthened by 18%, 16% and 22%, respectively; (ii) R-EMD, R-delta-t EMG-F, and R-delta-t F-MMG lengthened by 11%, 41%, and 67%. Reliability was always from high to very high. Fatigue altered the electrochemical and mechanical processes during both muscle contraction and relaxation.This EMG, MMG and F combined approach provided reliable measurements of the different delay components and may represent a valid tool also to investigate the electrochemical and mechanical involvement in some neuromuscular disorders, including myotonic dystrophies, where muscle contraction and/or relaxation are compromised. The progression of the pathology and the effects of therapeutic interventions could also be monitored.

PS1-49 / #260

Theme: *1.9 - Basic sciences in NMD: Others*

Induction of Neotendon Formation and Scleraxis Expression in a Supraphysiological Model of Tendon Growth

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In response to mechanical loading, tendons grow by increasing their cross-sectional area (CSA). It is not known whether tendons grow by adding additional fibrils (hyperplasia) or by increasing the size of existing fibrils (hypertrophy). To gain a greater understanding of the cellular mechanisms of adult tendon growth in response to mechanical loading, we used a synergist ablation model whereby a tenectomy of the Achilles tendon was performed to induce growth of the synergist plantaris (Pln) tendon. We hypothesized that following synergist ablation, cells in the epitenon of the tendon would migrate toward the interior of the tendon and increase the size of existing tendon fibrils. Six-month old male mice ($N=20$) that express GFP

CSA (mm^2)	Control	2 Day	7 Day	14 Day	28 Day
Original Tendon	0.105±0.016	0.096±0.017	0.154±0.021	0.107±0.011	0.136±0.011
Neotendon	0.000±0.000	0.047±0.031	0.242±0.088*	0.256±0.056*	0.336±0.032*#
Total Tendon	0.105±0.016	0.144±0.047	0.396±0.107*#	0.363±0.045*	0.498±0.036*#
* Significantly different form control (p<0.05) # Significantly different from 2 days (p<0.05)					

Cell Density ($cells/mm^2$)	Control	2 Day	7 Day	14 Day	28 Day
Original Tendon	1980±295	2721±374	1622±151	1954±304	2210±100
Neotendon	0.000±0.000	4845±896*	1948±447#	3236±1031*	2381±187
Total Tendon	1980±295	3198±445	1853±283	2637±649	1560±228.5
* Significantly different form control (p<0.05) # Significantly different from 2 days (p<0.05)					

under the control of the scleraxis promoter (ScxGFP), kindly provided by Dr. Ronen Schweitzer were used in this study. Mice were subjected to a bilateral Achilles tenectomy, with four mice serving as non-ablated controls. Mice received IP injections of the thymidine analog EdU 1 and 2 days prior to sacrafice. Pln tendons were isolated from mice 2, 7, 14, and 28 days after surgery. Tendons were sectioned stained with either hematoxylin and eosin, or prepared for fluorescent microscopy. RNA was isolated from plantaris tendons, reverse transcribed and real time qPCR was used to measure the expression of scleraxis, type I collagen, and PCNA, which were normalized to β2-microglobulin. Following overload, there was a dramatic increase in total CSA of tendons, while the original tendon matrix was not changed. Growth primarily occurred in a neotendon matrix, between the original tendon and epitenon region of the tendon. Despite this marked increase of Pln tendon CSA, there was no difference in cell density. There was a significant increase in scleraxis expression at 2 days that increased further until 14 days, and decreased at 28 days. Collagen expression increased at 7 and 14 days and remained increased at 28 days. The results from this study provide new insight into the mechanisms of tendon growth. While further studies are needed, the results from this study suggest that under supraphysiological loads, tendons may grow via hyperplasia.

PS1-50 / #358

Theme: *1.9 - Basic sciences in NMD: Others*

Comparisons between cellular candidates for mending cardiomyopathy in murine models

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Cell transplantation is considered a therapeutic avenue for the treatment of cardiopathies, but it has been less explored in the setting of nonischemic dilated cardiomyopathies (DCM) which are associated to neuromuscular disorders, than in the context of post-ischemic heart failure. In the few models developed, there is no clear evidence of the integration within the myocardium of injected cell candidates as bona fide cardiomyocytes. We assessed and compared the integration of distinct categories of progenitors upon injection into the myocardium of healthy animals and/or an animal model of dilated cardiomyopathy. Although these cells are not able to differentiate into cardiomyocyte lineage, myoblasts are considered a "gold standard" in cardiac cell therapy and the mouse myogenic cell line D7 was used as an experimental control. Embryonic stem cells (ESC) were committed towards a cardiomyogenic fate using BMP. Finally, we tested cardiac and skeletal cells expressing the enzyme Aldehyde Deshydrogenase (ALDH) enzyme, since we observed their strong commitment into the myogenic pathway and contribution to skeletal muscle regeneration *in vivo*. Immunodeficient healthy mice accomodated xenogenic transplantations, and allogenic transplantations were performed under immunosuppression into $Lmna^{H222P/H222P}$ mice, a faithful genetic mouse model of laminopathy exhibiting a progressive and lethal DCM. Echocardiography documented a functional stabilisation in Mb-transplanted $Lmna^{H222P/H222P}$ mice two months after transplantation, when compared to ESC-transplanted and control-injected animals. Mb consistently engrafted and formed skeletal myotubes (but no cardiomyocytes) in injected myocardia, while no cells were detected in hearts of mice receiving cardiac-committed ESC. The engraftment capacities of ALDH+ cell populations are under study and the results will be discussed. While the functional benefits of Mb transplantation might extend to nonischemic DCM, committed ESC failed to integrate in this myocardial environment, thus making the identification of true cardiac progenitors of crucial importance in therapeutic perspectives.

PS1-51 / #419

Theme: *1.9 - Basic sciences in NMD: Others*

Fibromyalgia and hyperCKemia: electromyographic aspects, muscle biopsy and exercise related biochemical correlates

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Background: Fibromyalgia is a complex, chronic non-inflammatory syndrome pain that affects at least 2 to 5% of the general population. It's a serious social problem in developed countries in the recent years; individuals with fibromyalgia fell incapable of performing the majority of activities of daily living and experience a loss in quality of life comparable to that found in other chronic diseases. Although the etiology and pathogenesis of this condition are still unknown, this disease appearing probably multi-factorial. It's characterised by chronic widespread pain, with painful spots known as tender points. In this disease, myalgia occurs at rest, during and after exercise. Its clinical manifestations often overlap with debilitating symptoms such as morning stiffness, fatigue, exercise-intollerance, non-restorative sleep, pain, memory problems, difficulty concentrating, trouble falling asleep, and muscle spasm. In some cases, patients show idiopathic hyperCKemia, which is a clinical conditions defined by elevation of serum creatine kinase of undetermined origin. Patients and Methods: The aim of the study has been to perform a multimetric skeletal muscle assessment in a cohort of 20 patients with fibromyalgia (15 females and 5 males, age ranging from 35–45), including serum creatine kinase, the electromyographic and muscle biopsy aspects and the muscle deconditioning phenomenon by the analysis of blood lactate kinetics and oxidative stress parameters during an incremental workload testing on bicycle ergometer.Results and conclusions: of the 20 patients examined, electromyography and muscle biopsy showed aspecific mild idiopatic signs in all the patients. We found mild increase of CK levels (range values 250–300 U/L), and anticipated lactate threshold was observed in 12 patients and abnormal oxidative stress parameters in 15 patients, this suggesting involvement of oxidative metabolism in these patients.The results indicated that among patients affected by Fibromyalgia and mild increased of CK there are evidences of mild myopathic signs and oxidative stress occurrence. Whether or not these results are related to secondary no specific alterations of oxidative metabolism or, alternatively, are expression of an underlying pathogenic mechanisms, more or less directly related to mitochondrial involvement, is matter of further studies.

PS1-52 / #64

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Effect of Sildenafil on skeletal and cardiac muscle function in Becker muscular dystrophy; a randomised, double blind, placebo-controlled crossover clinical trial

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Background: patients with muscular dystrophy due to dystrophin deficiency (Becker and Duchenne muscular dystrophies, BMD and DMD) also lack neuronal nitric oxide synthase (nNOS). nNOS mediates physiological sympatholysis that ensures adequate blood supply in working muscle. In mice lacking dystrophin, restoration of nNOS effects by treatment with a phosphodiesterase 5 inhibitor (Sildenafil), improves skeletal and cardiac muscle performances. Sildenafil also improves blood flow in patients with BMD. We therefore hypothesised that Sildenafil can improve blood flow, maximal work capacity and heart function in patients with BMD.

Methods: we used a randomised, double-blind, placebo-controlled crossover design with two 4-week periods of treatment, separated by a 2-week wash-out. Part one of the study focused on skeletal muscle function with assessment of blood flow in the brachial artery during maximal handgrip exercise (primary outcome measure), 6 minutes walk test, maximal oxidative capacity and life quality scale. Part two of the study evaluated cardiac function with MRI of the heart at rest and during maximal handgrip exercise. Resting end-diastolic volume and exercise-induced cardiac output were primary outcomes. Expression of nNOS and PDE5 in muscle was tested with western blotting in 5 patients.

Findings: Sixteen persons completed all skeletal muscle evaluations and 13 cardiac MRI investigations. Two were excluded from cardiac MRI because of claustrophobia and one because the heart was transplanted.

Sildenafil had no effect on any of the primary or secondary outcome parameters. No serious adverse effects were recorded. PDE5 and nNOS were significantly down-regulated in all patient biopsies.

Interpretation; despite positive evidence from animal models of dystrophinopathy and physiological findings in patients with BMD, this double-blind, placebo-controlled clinical study showed no evidence of Sildenafil on blood flow, maximal work capacity and heart function in adults with BMD. This discrepancy may be explained by significant down-regulation of PDE5 in muscle.

PS1-53 / #75

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Post-exercise protein supplements improve muscle protein balance in Muscular Dystrophies

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Context: Post-exercise protein supplementation has been shown to increase muscle protein build-up in healthy individuals. In patients with muscular dystrophies, it is known that aerobic exercise improves muscle function, but the effect of exercise on muscle protein metabolism is unknown.

Objective: To investigate 1) muscle protein balance before, duing, and after exercise and 2) to investigate the effect of post-exercise protein-carbohydrate supplementation on muscle protein balance in patients with muscular dystrophies.

Methods: In a controlled, crossover, intervention study we included 17 ambulatory patients with myotonic dystrophy type 1, Becker muscular dystrophy, facioscapulohumeral muscular dystrophy or limb girdle muscular dystrophies type 2I, 7 women and 10 men, age range from 18 to 52 years, and 8 healthy controls matched for gender, age, BMI and physical activity.

Muscle protein synthesis and breakdown were measured across an exercising leg using tracer dilution methodology on two occasions; with and without post-exercise protein-carbohydrate supplementation.

Results: In patients, muscle protein breakdown increased in the recovery period (11 ± 1 μmol phe/min) versus pre-exercise (8 ±1 μmol phe/min, $P=0.02$). In contrast, protein-carbohydrate supplementation after exercise abolished post-exercise muscle protein breakdown, and increased fractional synthesis rate (FSR) of muscle protein (0.07 to 0.11 % per hour, $P=0.05$). In line with this, exercise and protein activated the protein synthesis signaling cascade in more subjects (75 %) compared with exercise alone (55 %).

Conclusion: Post-exercise protein supplementation reduces muscle protein breakdown after exercise and enhances skeletal muscle fractional synthesis rate in

patients with muscular dystrophies. The findings suggest that post-exercise protein supplementation could be an important add-on to exercise training therapy in muscular dystrophies, and warrants long-term studies of post-exercise protein supplementation in these conditions.

PS1-54 / #82

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Identification of components of the metabolic syndrome in patients with Duchenne/Becker muscular dystrophy

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Background: Duchenne/Becker muscular dystrophy (DMD/BMD) is caused by mutations in the DMD gene that code for dystrophin, a sarcolemmal cytoskeletal protein. DMD gene mutations that result in complete loss of dystrophin interrupt their translation, giving rise to DMD. Patients with DMD/BMD develop obesity, insulin resistance (IR) and hyperinsulinemia, which are hallmarks of the metabolic syndrome (MS). MS is associated with type 2 diabetes and adverse cardiovascular disease.

Objective: To identify components of the MS in patients with DMD/BMD.

Methods: We studied 78 patients with molecular diagnosis of DMD/BMD. We evaluated components of the metabolic syndrome such as, IR (using HOMA-IR), obesity (waist circumference), triglycerides, fasting glucose and HDL-C in serum in three groups of age (<6y $n=9$, 6y to <16y $n=54$, and ≥16y $n=15$). Also, patients were categorized into one of the three groups: Group I (patients with central obesity who fulfill 0 or 1 criterion for MS, $n=57$), Group II (patients with central obesity who fulfill 2 criteria for MS, $n=14$) and Group III (patients with central obesity who fulfill 3 or 4 criteria for MS, $n=7$).

Results: Patients with DMD/BMD presented components of the MS, and their values were higher in older patients (≥16y). We did not observe difference in age among groups I, II and III. Also, 73%, 18% and 9% of patients were identified in the groups I, II and III respectively. One hundred percent of patients from group III, had IR, hyperglycemia, hyperinsulinemia and hypertriglyceridemia, 85.7% had obesity and 28.6% had reduced HDL cholesterol. These percentages were lower in groups I and II.

Conclusion: Patients with DMD/BMD present components of the MS from early age (<6y). Patients may present more than 3 criteria for MS. Presence of these components generates novel challenges to create scientific bases for the treating physician so as design therapies and better surveillance strategies for dystrophy and to prevent other pathologies.

PS1-55 / #90

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Influence of a two-year steroid treatment on body composition as measured by Dual X-Ray Absorptiometry in boys with Duchenne Muscular Dystrophy

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Introduction: Steroids are nowadays routinely used as a long-term treatment in Duchenne muscular dystrophy (DMD).

Materiel: and methods: Their effects on body composition were assessed using Dual X-Ray absorptiometry. The study followed over two years 29 genetically confirmed DMD patients: 21 in the steroid-treated group and 8 in the steroid-naïve group. Results : After two years of steroid treatment, the lean tissue mass values increased significantly ($P<0.0001$), the percentage of body fat mass remained practically constant ($p=0.94$) in comparison with the initial visit. In the steroid-naïve patients, there were no significant

increases in the lean tissue mass but deterioration in body composition confirmed by a significant increases in the percentage of body fat mass. Besides, significant negative correlations were found between the percentage of body fat mass and the MFM total score ($R = -0.79$, $n = 76$, $P < 0.0001$).

Discussion: A 2-year steroid treatment improves significantly body composition of boys with DMD through a significant increase in lean tissue mass. We suggest that a thorough check of body composition should be carried out before steroid treatment discontinuation in case of overweight gain.

Keywords: Duchenne muscular dystrophy, steroids, body composition.

PS1-56 / #94

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Functional performance and muscle gene expression in dystrophic mdx mouse in relation to age and exercise: defects in mechanical-metabolic coupling

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In Duchenne muscular dystrophy (DMD) the absence of dystrophin causes a complex pathogenetic cascade also involving chronic inflammation and metabolic distress. In line with the view of a defective mechano-transduction occurring in dystrophin-deficient muscles, the mild phenotype of the mdx mouse is aggravated by protocols of forced treadmill exercise. However, the molecular mechanisms underlying exercise susceptibility is still unresolved. We investigated the outcome of 4 (T4, 8 weeks of age) and 12 (T12; 16 weeks of age) weeks of either treadmill exercise or cage-based activity on both functional performance and muscle gene expression in mdx and C57BL/10 (wt) mice. Weakness and fatigability, typical features of DMD patients, were aggravated in exercised dystrophic mdx mice, without overt sign of adaptation. Quantitive real-time PCR analysis in gastrocnemius muscle showed that the basal expression of the exercise-sensitive genes peroxisome-proliferator receptorγ coactivator 1α (PGC-1α) and sirtuin-1 was higher in mdx vs. wt mice at both ages. Exercise increased PGC-1α expression in wt, while in mdx mice the T12 exercise down-regulated PGC-1α, sirtuin-1 and PPARγ. Sexteen-week-old mdx mice showed a basal over-expression of slow-phenotype genes i.e. MHC-I and SERCA2; the T12 exercise contrasted this adaptation as well as the high expression of follistatin and myogenin, being ineffective in wt mice. Damage-related genes, such as NADPH-oxidase, TGFβ, TNFα and cSRC tyrosine kinase, were over-expressed in mdx muscle in all conditions. In parallel the anti-inflammatory adiponectin and the autophagy marker BNIP3 were down-regulated in T12 exercised mdx muscle. Then a chronic exercise with minor adaptive effects in wt muscle, markedly contrasts compensatory changes in the benign mdx phenotype, leading to a disequilibrium between protective and damaging signals. The results pave the way for setting appropriate physical therapy in DMD patients and for better addressing pharmacological intervention in translational research (Supported by Duchenne Parent Project NL).

PS1-57 / #118

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Serum profiling identifies novel dystromiRs and cardiomyopathy-related miRNA biomarkers in GRMD dogs and DMD patients

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Duchenne Muscular Dystrophy (DMD) is a fatal, X-linked neuromuscular disease that affects 1 boy in 3500-5000. The Golden Retriever Muscular Dystrophy (GRMD) dog is the best clinically relevant DMD animal model. In the present study we used a high-thoughput miRNA sequencing screening for identification of candidate serum miRNA biomarkers in GRMD dogs. We confirmed the dysregulation of the previously described dystromiRs, miR-1, miR-133 miR-206 and miR-378, and identified a new candidate dystromiR, miR-95. We identified two other classes of dysregulated serum miRNAs in muscular dystrophy. The first are miRNAs belonging to the largest known miRNA cluster that resides in the imprinting DLK1-DIO3 genomic region. The second are miRNAs associated with cardiac disease, including miR-208a, miR-208b, and miR-499. No simple correlation was identified between serum levels of cardiac miRNAs and cardiac functional parameters in GRMD dogs. Finally we have confirmed a dysregulation of miR-95, miR-208a, miR-208b and miR-499 in a small cohort of DMD patients. Given the strong interspecies conservation of miRNAs, and our preliminary data in DMD patients, these newly identified dysregulated miRNAs in GRMD dogs are strong candidate biomarkers for DMD patients.

PS1-58 / #140

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Successful use of out-of-frame exon 2 skipping induces *in vivo* IRES-driven expression of a highly functional N-truncated dystrophin isoform: promising approach for treating other 5´ dystrophin mutations

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Most mutations that truncate the reading frame of the DMD gene result in loss of dystrophin expression and lead to the most common childhood muscle disease, the severe and progressive Duchenne muscular dystrophy. However, frame-truncating mutations within the first five exons of the DMD gene unexpectedly result in milder dystrophinopathy. We have previously shown that amelioration of disease severity result from the expression of a highly functional N-truncated dystrophin beginning in exon 6 of the DMD thanks to the usage of an internal ribosome entry site (IRES) within exon 5 that is glucocorticoid-inducible. *In vitro* studies with bicistronic reporter assays demonstrate translation at levels approximately 60% of the well-known viral IRES (eMCV), suggesting a relatively strong activity. Activity in humans was confirmed in patient muscle tissues using ribosome profiling and mass-spectrometric peptide sequencing. Despite lacking half of the actin binding domain 1, the resultant N-truncated dystrophin protein produced from this IRES is highly functional, raising the possibility of the therapeutic use of this isoform. Here we demonstrate that the use of a novel out-of-frame exon-skipping approach to generate a truncated reading frame upstream of the IRES, leads to synthesis of a

functional N-truncated isoform in both patient-derived cell lines and in a new DMD mouse model (Dup2). We clearly demonstrate that expression of the N-truncated isoform following intramuscular injection does in fact result in complete correction to wild-type levels (as judged by Evans blue dye uptake, hindlimb grip strength, tibialis anterior specific force, and force correction after eccentric contraction) of the physiologic and most pathologic features seen in the Dup2 mouse. This data support the idea that this novel therapeutic approach could be beneficial for the 5% of dystrophinopathy patients with mutations within the 5' exons of DMD.

PS1-59 / #148

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Axial Muscle Involvment and Disease Progression in Dmd

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Introduction: Although Duchenne Muscular Dystrophy (DMD) is well characterized in terms of its genetics and pathophysiology there is some variability in the disease spectrum. Varied rates of progression can be recognized. Genetics has also been implicated to explain such differences.

Different functional motor outcome measures are currently used to study, understand and characterize the difference among phenotypes. Many of these clinical evaluations (CE) are also critical tools to assess the efficacy of new treatments.

Methods: 529 clinical evaluations (CE) were performed on 87 dmd steroid treated patients able to walk with independently. Each patient had at least 2 evaluations. CE included MMT (35 muscle groups), time to walk 10 m , Gowers time, time to climb 4 steps and upper limb Vignos scale. Two groups were separated according to the neck flexor muscle strength (> or < 3 using the MRC modified scale) (Groups A and B). Comparisons were made among different variables and age at loss of ambulation. Kaplan Meyer and Hazard Plot methods were used for statistical analysis.

Results: Groups A: 35 patients (mean age 10.97 years old) B: 52 patients (9,81 years old). 20% of patients lost the ability to walk in group A and 40% in Group B ($p < 0.004$). Walking velocity was higher for group A (1.55 m/sec) than for Group B (1.26 m/sec) ($p < 0.0001$). Global muscle score was similar for both groups. Main differences favoring those with better neck flexors were also found in trunk and hip flexion.

Discussion / Conclusions: Gait as a function is increasingly used as an outcome measure in clinical trials.

Neck and /or trunk muscle strength is not consistently (usually) evaluated in DMD. Axial muscles play an important role in preserving functions (as the gait) and its better conservation could lead to prolonged ambulation.

These muscle groups should be included and analyzed in the new protocols to dissect the different outcomes and trial results in DMD.

PS1-60 / #157

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Dystrophin-deficient pigs provide new insights into the hierarchy of physiological derangements of dystrophic muscle

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Duchenne muscular dystrophy (DMD) is caused by mutations in the X-linked dystrophin (DMD) gene. The absence of dystrophin protein leads to progressive muscle weakness and wasting, disability and death. Existing animal models have been instrumental to understand the pathophysiology of DMD, but have limitations related to the type of mutation, the clinical phenotype, and the predictive value for molecular therapies. To establish a tailored large animal model of DMD, we deleted DMD exon 52 in male pig cells by gene targeting and generated offspring by nuclear transfer. DMD pigs exhibit absence of dystrophin in skeletal muscles, increased serum creatine kinase levels, progressive dystrophic changes of skeletal muscles, impaired mobility, muscle weakness, and a maximum life span of 3 months due to respiratory impairment. These findings render the DMD mutant pig a promising animal model for testing targeted genetic treatment approaches, such as exon skipping by antisense oligonucleotides (AONs) which partially restored in-frame dystrophin gene expression in our cell culture models. Regarding the recent disappointing functional outcome of DMD patients treated with AONs in human clinical trials, a valid animal model will be warranted.

PS1-61 / #166

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Becker muscular dystrophy severity is linked to the structure of truncated dystrophins

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In-frame exon deletions of the DMD gene produce internally truncated dystrophin that mostly leads to Becker muscular dystrophy, a milder allelic disorder of Duchenne muscular dystrophy. We hypothesise that differences in the retained structure and function of these internally truncated dystrophins may account for the clinical heterogeneity observed in Becker patients. To address this question, we collected the ages of dilated cardiomyopathy onset and wheelchair dependency for Becker patients bearing the four most prevalent in-frame exon deletions, delta45-47, delta45-48, delta45-49 and delta45-51. We show that patients with delta45-47 have a risk of developing dilated cardiomyopathy 9 years earlier than patients with delta45-48. Similarly, patients with delta45-47 and delta45-49 are at risk for being wheelchair-dependent 10 and 13 years earlier than patients with delta45-48. Molecular homology modelling reveals that at the deletion site of the internally truncated dystrophins, deletions of exons 45-48 and 45-51 preserve a structure similar to a wild type repeat of the central rod domain (hybrid repeat) whereas deletions of exons 45-47 and 45-49 lead to an unrelated structure (fractional repeat). Biophysical and biochemical studies indicate that the truncated proteins expressed in a fragment of dystrophin corresponding to repeats 16 to 21 are properly folded in alpha-helices, and their stability is not dramatically impacted. However, refolding rates are strongly correlated to the nature of the protein repeats, with significantly lower refolding rates observed for proteins with fractional repeats compared to wild type and the proteins with hybrid repeats. It is the first experimental evidence that clinical heterogeneity in Becker patients can be analysed in view of the structure of the truncated dystrophin. Disease progression appears to be strongly correlated with the structure at the deletion site, with a predicted milder cardiac and motor phenotype for a deletion leading to a hybrid repeat (delta45-48) compared to deletions leading to fractional repeats (delta 45-47 and 45-49). This difference is critically important for better predicting the clinical outcomes of the ongoing clinical trials in Duchenne patients that aim to restore the DMD reading frame and convert them into Becker-like patients.

PS1-62 / #182

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Sex effect on the efficacy of valproic acid therapy in mdx mice

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Background: Histone deacetylase (HDAC) inhibitors such as trichostatin A and valproic acid are potential therapy of muscular dystrophy. These drugs have the ability to recruit myoblasts and promote muscle regeneration by fusion with destructive muscle fibers.

Materials and Methods: Twenty two adult mdx mice, aged from 18 to 20 weeks, 11 male and 11 female, were divided in two groups. Six male and female mice received diluted Depakine® solution at the concentration of 1:400 (v/v) in the drinking water. The other five male and female mice received blank drinking water as the control. All the animals received blood test for serum creatine kinase (CK) level and valproic acid concentration after a three-month therapy.

Results: The changes of serum CK levels, defined as ($CK_{after\ therapy}/CK_{before\ therapy}$) x (100%) and valproic acid concentration were heterogeneous in all the animals. The changes of CK levels were 13.6 ± 15.6% in the treated males, 42.1 ± 66.2% in the treated females, 486.8 ± 254.6% in the untreated males and 344.3 ± 212.4% in the untreated females. There is statistical significance between the treated and untreated mice. The treated male mdx mice had more prominent decreased CK level after valproic acid therapy as compared with the females, but there is no statistical significance. The serum valproic acid concentrations in the male and female mice were (79.9 ± 38.1 vs. 28.6 ± 26.3 μg/ml, $P<0.05$).

Conclusions: Although the female mice had lower serum valproic acid concentration and less changes of serum CK, valproic acid had more prominent effect on the improvement of CK in the female mice after correcting the serum concentration. Sex effect on the efficacy of valproic acid therapy may be concerned in the treatment of muscular dystrophy.

PS1-63 / #187

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Muscular diseases diagnosis in West Africa: experience from four countries

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Neuromuscular diseases exit worldwide. Few cases have been reported from sub Sahara Africa especially in West Africa. African practitioners gathered in West African network for myopathies are trying to organize caring since 2009.

Aims: Report on this experience through epidemiologic and diagnostic aspects and point out futures steps.

Methods: Patients are recruited during muscular diseases consultations from 2009 through 2013. Each patient was clinically examined. Consistent cases with neuromuscular problems went for more investigations. Blood was collected for creatin phosphate kinase (CK), DNA was extracted and appropriate gene mutational research was carried out.

Results: In Benin, Burkina Faso, Niger and Guinea Conakry, over 25, 20, 17 and 14 patients that were seen for neuromuscular diseases, 20, 15, 7 and 2 respectively had myopathic history. Confirmation rate was respectively 10/25, 4/15, 2/7 and 0/2 for patients from Benin, Burkina Faso, Niger and Guinea. Findings were Duchene muscular Disease, gamma sarcoglycanopathy, spinal muscular atrophy and Becker myotony. .

Conclusion: Duchene muscular Disease is the most frequent myopathy in West Africa. Diagnosis could be improved by access to muscular biopsy and appropriate analysis especially western blot essay.

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PS1-64 / #193

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Splice site strength and density of ESE and ESS motifs determine splicing pattern in the cases with splice site mutations in the dystrophin gene

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Duchenne/Becker muscular dystrophy (DMD/BMD) is caused by mutations in the dystrophin gene. Splice site mutations account for 5% of mutations, which result in exon-skipping or cryptic splice site activation. Splicing patterns are critical for assessing clinical phenotype, however cis-elements determining the splicing pattern are still unclear. The aim of this study was to clarify the splicing patterns in the cases with splice site mutations, and identify factors determining the splicing pattern. 19 cases (11 DMD and 8 BMD cases) with single-nucleotide changes in splice sites were included. Splicing patterns were analyzed using mRNA from lymphocytes. Furthermore, the effect of cis-elements including splice site strength, exonic splicing enhancer (ESE) motifs and exonic splicing silencer (ESS) motifs were investigated. Among six mutations in splice acceptor sites, two induced exon-skipping and three induced cryptic splice site activation, and one induced both exon-skipping and cryptic splice site activation. Shapiro score of splice acceptor site was higher and the density of ESE motifs was lower in cases with exon-skipping than in cases without exon-skipping. Among 13 mutations in splice donor sites, eight induced exon-skipping and four induced cryptic splice site activation, and one induced both exon-skipping and cryptic splice site activation. Including all cases, the density of ESS was significantly higher in cases with exon-skipping than in cases without exon-skipping ($p < 0.05$). Our findings suggest that splicing pattern is determined by splice site strength, ESE and ESS.

PS1-65 / #196

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Expanded clinical spectrum of dystrophin copy number variants

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Cognitive impairment associated with dystrophinopathies is rarely reported in the absence of muscle weakness. We report a cohort of patients with unexpected dystrophin copy number variants (CNVs) identified on chromosomal microarray performed for developmental delay or intellectual disability. 20 subjects with a dystrophin CNV were identified from 12 062 microarrays performed. Six subjects had DMD or a contiguous gene syndrome. Five subjects were unavailable for review. Nine probands (seven male), aged 0-9 years had a CNV in the dystrophin gene with an atypical phenotype. The CNVs were confirmed by multiplex ligand-dependent probe amplification in seven. Muscle weakness was absent or minimal, and creatine kinase normal or mildly elevated. Muscle biopsy was normal in three and dystrophic in one. Dystrophin CNVs were identified in 11 relatives, including three asymptomatic adult males. Ultimately, mild

Becker muscular dystrophy was diagnosed in three families. No muscle abnormalities were identified in the remaining families. Four male probands had isolated cognitive impairment. Microarray testing expands the phenotypic spectrum associated with dystrophin mutations, including isolated cognitive impairment and asymptomatic individuals. Further study is required to understand the apparent absence of muscle pathology in these patients, and the relationship of the dystrophin CNV to the cognitive impairment.

PS1-66 / #217

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Development of a novel approach using TALE nucleases to correct duplications in the dystrophin gene

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Background: Duchenne Muscular Dystrophy (DMD), caused by mutations in the DMD gene, is the most common inherited muscular dystrophy. DMD patients suffer progressive weakening of muscles, and ultimately heart and respiratory failure, leading to premature death. Aims: Here we propose a genome editing method to remove duplications in the DMD gene (cause of ~10% of DMD cases). Transcription activator-like effector nucleases (TALENs) insert a DNA double strand break (DSB) in a specified region of the genome. By targeting TALENs to duplicated intronic regions of dystrophin, we aim to produce two DSBs flanking one copy of the duplicated region, leaving the cell to repair the DSBs by non-homologous end joining, removing the duplication. Methods or Patients or Materials: TALENs have been introduced by transfection as well as integrated into a viral vector for delivery into HEK293 cells and patient-derived fibroblasts. Results and conclusion: We have identified a TALEN that targets with high efficiency the DMD gene. Current work is aimed at establishing a viral expression system and determining the efficiency in repair of duplications in DMD-derived cell lines.

PS1-67 / #227

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Morphologic and morphometric analysis of muscle degeneration in DMD: evolution in patients aged from 1 to 10 years

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Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy resulting from mutations in the X-linked dystrophin gene. Lack of dystrophin leads to progressive muscle degeneration that is responsible for death around the third decade of life.

We performed a morphologic and morphometric analysis of muscle biopsies in 40 DMD patients aged 1 to 10 years. We considered the following parameters: fibrotic tissue, necrotic fibers, regenerating fibers, hypercontracted fibers, internal nuclei, fiber size variability and inflammatory reaction. This allowed us to record the natural morphological evolution of muscle alteration in DMD. Some of the studied parameters led to interesting considerations. Around 6 years of age the amount of connective tissue increases rapidly from 18,5% to 29,7%. At the same age we also observed the peak in the number of necrotic fibers. Percentage of regenerating fibers remained stable throughout the years independently on degree of muscle alteration These data allowed us to both clarify the morphological evolution of the muscular alteration and to define the turning point corresponding to when the fibrotic degeneration exponentially increases.

A great number of therapeutic trials in DMD patients have been done worldwide and new ones are ongoing. The morphological endpoints are the restoration of dystrophin and the reduction of muscle alteration in muscle biopsies taken during and/or after the treatment. Because the efficacy of the treatment also depends on the degree of the muscle alteration at start, it is possible to speculate that when the connective tissue affects a large percentage of muscle fibers,

muscle tissue restoration can be very difficult. For this reason, when the starting age of a clinical trial is decided, it is important to know that the connective tissue rapidly increases at age 6 years. Moreover, establishing the morphologic natural history of the disease evolution is useful to figure out if a particular treatment has any effect.

PS1-68 / #240

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

MiR-21 and miR-29 play opposing roles in the progression of fibrosis in Duchenne muscular dystrophy

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Fibrosis, characterized by excessive deposition of collagen and other extracellular matrix proteins that progressively replace muscle fibers, represents the endpoint of most severe muscle diseases. MicroRNAs have been recently implicated in regulation of pro- and anti-fibrotic genes. In several fibrotic conditions, persistent miR-21 overexpression inhibits tissue repair and contributes to tissue fibrosis, and downregulation of miR-29 correlates with increased expression of genes for collagens and other extracellular matrix proteins. To investigate the roles of miR-21 and miR-29 in muscle fibrosis in Duchenne muscle dystrophy (DMD), we evaluated their expression in muscle biopsies from 14 patients, and in muscle-derived fibroblasts and myoblasts, in comparison to control biopsies and cells.

In muscle biopsies from DMD patients, miR-21 expression was significantly increased, and correlated directly with patient age and with COL1A1 and COL6A1 transcript levels. MiR-21 expression was also significantly increased in DMD fibroblasts, more so after TGF-beta 1 treatment. The expression of miR-21 target genes PTEN and SPRY-1 was significantly reduced; while COL1A1 and COL6A1 transcript levels and soluble collagen production were significantly increased.

MiR-29a and miR-29c expression were significantly reduced in DMD muscle and myoblasts, together with significantly increased expression of the miR-29 target genes: COL3A1, FBN1 and YY1.

MiR-21 silencing in DMD fibroblasts restored expression levels of PTEN and SPRY-1 and significantly reduced collagen I and VI expression; while miR-29 mimicking in DMD myoblasts significantly decreased the expression of miR-29 target genes. The fibrinolytic system PAI-1/plasmin was also investigated as it is involved in miR-21 regulation and remodelling of the extracellular matrix in damaged skeletal muscle. In DMD fibroblasts PAI-1 expression was increased and plasmin activity reduced, both promoting the profibrotic microenvironment. MiR-21 silencing in DMD fibroblasts induced a significant reduction of PAI-1 production and a significant increase of plasmin activity.

Overall our findings indicate that miR-21 and miR-29 play opposing roles in DMD fibrosis and suggest that pharmacological modulation of their expression may have therapeutic potential for fibrosis treatment.

PS1-69 / #275

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Loss of ambulation in the Cooperative International Neuromuscular Research Group (CINRG) Duchenne Muscular Dystrophy (DMD) cohort is synergistically influenced by glucocorticoid corticosteroid treatment and candidate genetic polymorphisms.

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Despite a uniform biochemical defect in DMD, age at loss of ambulation (LoA) varies by more than a decade. Glucocorticoid corticosteroid (GC) treatment is a major determinant of such variability, but its long-term effect, and differences between several prescribed regimens, are incompletely characterized. Recently, single nucleotide polymorphisms (SNPs) in SPP1 (osteopontin) and LTBP4 (latent TGF-beta binding protein) have been reported to influence LoA in DMD. Here we analyze effects of GCs and candidate SNPs on LoA in DMD.

We studied 332 patients enrolled at 20 worldwide Centers participating in the CINRG Duchenne Natural History Study (McDonald et al, 2013). LoA was defined as continuous wheelchair use. Patients were grouped by GC treatment before LoA and GC regimen. We defined "GC-treatment" as lasting at least 1 year. Two known modifier and 3 functionally related SNPs were genotyped: rs28357094 (SPP1, dominant model), rs10880 (LTBP4, recessive), rs2616262 (IBSP, integrin-binding sialoprotein, recessive), rs15705 (BMP2, bone morphogenetic protein 2, dominant), and rs4522809 (TGFBR2, TGF-beta receptor 2, recessive). Median LoA was compared by Kaplan-Meier plots and log-rank test (age as time variable). SNP effects concurrent with GC treatment were tested by Cox regression analysis, with GC treatment as a time-varying covariate. To identify SNP x GC treatment interaction, Cox regression analysis by genotype was performed in GC-treated only.

LoA in GC-treated patients (73.5% of total) was 2.5 years of age later than untreated (12.5 vs. 10.0 y, $P<0.0001$). Prednisone/prednisolone (PRED) were prescribed to 50.4% of GC-treated patients, while 32.8% received deflazacort (DFZ), and 15.2% switched between these two drugs while ambulatory. Patients treated with DFZ had a later LoA than PRED (13.5 vs. 11.1 years, log-rank $p=0.0005$), although other confounding factors were not explored. Age at start of treatment was similar.

In Cox regression analysis, no SNPs were significantly associated with LoA when adjusting for GC treatment as a time-varying covariate. The SPP1rs28357094 G allele was associated with earlier LoA in GC-treated patients only (12.0 vs. 14.0 years, $p=0.04$).Lack of validation of the LTBP4 effect might be due to limited power for recessive models.

In conclusion, our data support long-term effect of GCs in DMD, highlighting the relevance of adjusting for GC treatment in discovery and validation of genetic modifiers.

PS1-70 / #279

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Muscular Dystrophy, antioxidants and metabolites: efficacy, mechanisms and optimal drugs

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Oxidative stress, and the generation of excessive reactive oxygen species, has long been considered a mediator of human disease. An important role for oxidative stress is proposed in Duchenne Muscular Dystrophy (DMD), a fatal inherited muscle wasting disease. Subsequently the use of antioxidants has been investigated as a potential therapy for DMD. Preclinical trials with antioxidants in the mdx mouse model of DMD have shown some benefits, although the few clinical trials in DMD boys have had limited success. Research in our laboratory has focused on understanding the efficacy and mechanisms of action of antioxidants in dystrophic muscles of mdx mice. We have linked increased protein thiol oxidation to dystropathology, and have shown that precursors to the thiol antioxidants cysteine and glutathione, such as N-acetylcysteine (NAC) and l-2-oxothiazolidine-4-carboxylate (OTC), are highly effective in decreasing protein thiol oxidation and protecting mdx muscle from damage. However, we conclude that the beneficial effects of these cysteine-based drugs may not be due to direct reduction of oxidative stress *in vivo*, but instead be indirectly mediated through other mechanisms. Specifically, we have shown that systemic OTC (and possibly NAC) may reduce the severity of dystropathology by increasing metabolism in the liver of the amino acid taurine, which is delivered to the muscle via the blood. Taurine is essential for muscle function, and we have shown that dystrophic mdx muscle is deficient in taurine, especially in severely

effected muscles. The administration of taurine improves the function of mdx muscle, and we suggest this is indirectly linked to decreasing protein thiol oxidation. Our research continues to explore the role of oxidative stress and protein thiol oxidation in dystrophic muscle, to identify the best drug to reduce severity of dystropathology.

PS1-71 / #285

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Forced exercise induces weakness in the mdx mouse

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Even though the mdx mouse is the most commonly used mouse model of Duchenne muscular dystrophy, the slow deterioration of muscle weakness is a significant obstacle to its use in translation studies.

Objective: To evaluate the effect of forced exercise regimen on mdx mouse muscle strength in order to facilitate and shorten pre-clinical drug screening experiments.

Methods: Muscle strength was compared in two parallel studies in the mdx mice performed at our laboratory at the same time. In the first study, WT and mdx mice were subjected to treadmill running, twice a week for 30 minutes at the velocity of 12 m/min in a 40 week experiment from the age of 8 weeks ($n=6$). In a parallel study no forced treadmill exercise was employed in mdx mice and controls ($n=10$). All mice were weighted once a week, and muscle strength was measured using a grip strength meter.

Results: The average strength of non-exercised mdx mice at 48 weeks of age was 9.03 ± 0.205 gr force/gr bodyweight, similar to that observed in non-exercised wild-type mice at the same age (8.56 ± 0.212 gr force/gr bodyweight). Treadmill forced exercised mdx mice at that age had significantly reduced strength compared to forced exercised wild-type mice (7.45 ± 0.56 vs. 10.06 ± 0.912 gr force/gr bodyweight $p<0.05$) and ANOVA test showed a statistically significant difference in strength between these two groups throughout the study $p<0.008$.)

Conclusions: Twice weekly forced exercise is associated with significantly reduced strength as of 28 weeks of age compared to WT mice. Such weakness is not observed in sedentary mdx mice. Our findings provide additional support of cumulative muscle weakness with forced exercise in mdx mice. Furthermore, using forced exercise may shorten study duration in the mdx model translational studies.

PS1-72 / #298

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Utrophin modulators to treat Duchenne muscular dystrophy (DMD): Future clinical trial plans for SMT C1100

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Utrophin modulation i.e. the re-programming of utrophin transcription such that utrophin RNA and protein is continually expressed in mature fibres is expected to be a disease modifying treatment for Duchenne muscular dystrophy (DMD). SMT C1100 is a small molecule utrophin modulator demonstrating significant benefit on the muscular dystrophy in the dystrophin deficient mdx mouse. These data led to the nomination of SMT C1100 as the candidate for evaluation in DMD clinical trials.

In 2012 we reported that SMT C1100 successfully completed a Phase 1 healthy volunteer trial in which an oral paediatric formulation was deemed safe and well tolerated with plasma levels well above those determined to be effective to modulate utrophin levels in cells and animals.

The first DMD patient trials of SMT C1100 have started with a safety and dose finding Phase 1b study in DMD boys. We aim to present the data from study. We will provide our proposed plans for the Phase 2 proof of concept trial based on current data to date.

PS1-73 / #319

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Antisense oligonucleotide-mediated knockdown of TGF-?/myostatin type I receptor as a potential therapy for Duchenne and other muscular dystrophies

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Skeletal muscle fibrosis and impaired muscle regeneration are major contributors to progressive decline of muscle function in Duchenne Muscular Dystrophy (DMD) and other types of muscle dystrophies. TGF-β/myostatin signaling is directly involved in DMD pathology and inhibition of these signaling cascades has been shown to improve regeneration and reduce fibrosis in the dystrophic muscle. We ,therefore, have developed an efficient method to selectively inhibit the function of type I myostatin/TGF-β receptor Tgfbr1(ALK5) based on antisense oligonucleotide (AON)-mediated exon skipping.

Our results show that myostatin/TGF-β type I receptor ALK5 can be efficiently downregulated . AON-mediated exon skipping in ALK5 receptor resulted in increased myogenic differentiation of C2C12 myoblasts. In addition, efficient AON-mediated knockdown of ALK5 was achieved *in vivo* after intramuscular injection in mdx mice, a DMD mouse model. ALK5 AON treatment resulted in an increase in myogenic gene expression, a decrease in Collagen expression andother downstream target genes of TGF-β.

To summarize, our experiments suggest that this novel strategy of AON-mediated targeting of myostatin/TGF-β receptor may provide a therapy to selectively inhibit myostatin and TGF-β signaling and improve muscle quality and function. Further studies will investigate the effect of AON-mediated knockdown of type I receptors on muscle growth , muscle regeneration and fibrosis after the short- and long-term ALK5 AON treatment in DMD and other myopathic mouse models.

PS1-74 / #333

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

DMD/BMD patient registry in Japan: Remudy

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We report current status of a national patient registry in Japan: Remudy. Clinical trials for new therapeutic strategies are currently being planned for Duchenne and Becker muscular dystrophies (DMD/BMD); however, many challenges exist in the planning and conducting a clinical trial for rare diseases. The epidemiological data, the total number of patients, natural history, and clinical outcome measures are mostly unclear. An adequate number of patients is needed to achieve significant results in clinical trials.

To solve these problems, a patient registry, especially of rare diseases such as a DMD/BMD, is an important infrastructure worldwide. Both in and out of Europe, TREAT-NMD alliance, a clinical research network for neuromuscular disorders, has developed a global database for dystrophinopathy patients. In 2009, we have developed a national registry of Japanese DMD/BMD patients: Remudy in collaboration with the Japanese national muscular dystrophy research groups, 27 traditional muscular dystrophy wards and hospitals belonging to the National Hospital Organization, the Japanese Muscular Dystrophy Association, and finally the TREAT-NMD alliance. The database includes clinical and molecular genetic data as well as all items required for the TREAT-NMD global patient registry. As of December 2013, 1,226 patients were registered in this database. The data has been annually renewed by registrant self reports. Japanese Remudy DMD/BMD registry supplied the epidemiological data for feasibility studies, provided timely

information to registrants about upcoming two independent clinical trials, and accelerated the effective recruitment of eligible patients as expected. This registry data is also providing more detailed knowledge about natural history, epidemiology, and clinical care.

In recent years, drug development has become dramatically globalized, and global clinical trials (GCTs) are being conducted in our country, Japan, as well. It is appropriate, particularly with regard to orphan diseases, to include Japan in GCTs to increase evidence for evaluation, because it would be difficult to conduct such large-scale trials solely within one country. GCTs enable the synchronization of clinical drug development in Japan with those in other countries, minimizing drug approval delays. Now, the patient registries are recognized as a powerful tool to accelerate the clinical research of the various rare diseases.

PS1-75 / #338

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

New orally available compounds which modulate utrophin expression for the therapy of Duchenne muscular dystrophy (DMD)

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DMD is a devastating X-linked muscle-wasting disease caused by lack of the cytoskeletal protein dystrophin. By pharmacologically modulating the dystrophin-related protein utrophin, we aim to develop a therapy applicable to all DMD patients by targeting the primary defect and restoring sarcolemmal stability. In partnership with Summit plc we previously developed SMT C1100; a small molecule utrophin modulator that reduced dystrophic symptoms in the mdx mouse. As a potential first-in-class molecule, SMT C1100 recently successfully completed a Phase 1 trial and DMD patients are currently being dosed in an ongoing Phase 1b trial.

The successful clinical progression to-date provides crucial proof-of-concept for the strategy we developed. We are now developing next generation utrophin modulators using an improved drug screening assay based on immortalised myoblasts from the utrophin luciferase knock-in mouse. This enables us to screen utrophin in its genomic context, better mimicking the *in vivo* situation and enabling identification of compounds which modulate utrophin through regulatory pathways outside of the 8.4 kb promoter fragment used in our previous screen. Screening a filtered subset (7000 molecules) from our 25,000-member compound collection, selected to maximise drug-like properties and the hit rate, has identified at least four structural classes which significantly increase utrophin in mouse and human DMD myoblasts. The compounds exhibit favourable solubility, stability, oral absorption and are well tolerated in the mouse. Structure-analogue relationship studies are well underway to allow us to improve compound effectiveness and optimise drug-like properties.

PS1-76 / #354

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Assessing T cell-mediated immune response to dystrophin in the natural history of Duchenne muscular dystrophy.

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Background: The pre-symptomatic induction of inflammatory cascades and invasion of muscle by immune cells in dystrophin deficient muscle contributes to the pathology of Duchenne muscular dystrophy (DMD). Traditional and RNA-based gene therapy approaches for DMD are progressing through clinical development. A study on viral delivered mini-dystrophin, has shown evidence that dystrophin epitopes expressed in revertant fibres can elicit T cell production in untreated patients which may accelerate a post-treatment immune response.

Methods: To extend this observation, we are recruiting a minimum of 30 ambulant and 30 non-

ambulant DMD boys with exon skippable deletions. We are performing annual ELISPOT IFN-gamma assays on DMD patients recruited into a longitudinal natural history study and disease control. ELISPOT assays on all patients are performed with a full-length dystrophin peptide set whilst patients with exon 51 or 53 skippable deletions are also assessed with peptides corresponding to unique epitopes generated by exon skipping. This allows us to assess pre-existing immunological responses to dystrophin epitopes in patients prior to inclusion in clinical trials, as well as the likelihood of a post-exon skipping immune response to newly-generated dystrophin protein.

Results: Here we present the results from patients recruited during the first year; we correlated data to factors such as age, ambulation status, steroid regime and DMD deletion. Four of 27 (~15%) DMD individuals were found to have a relatively low-level pre-existing immune response to dystrophin. All 4 positive individuals were treated with prednisone (2 on 10:10 days intermittent and 2 on a daily regimen). We have mapped positive responses to specific dystrophin epitopes using further epitope mapping. The positive epitopes are located before, and/or after the patients' deletion.

Conclusion: Our data support to the statement that ~20% of steroid treated DMD individuals have a pre-existing T cell-mediated immune response to dystrophin. Although our responses are relatively low-level, this information should be considered as a useful immunological baseline before undertaking clinical trials and future DMD studies. Our study on disease controls will also help us to understand how specific to DMD this phenomenon is.

PS1-77 / #357

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Extracellular Adenosine-triphosphate (e-ATP) and purinergic signalling in inflammatory pathogenesis of dystrophin-deficient skeletal muscle

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The inflammatory response is an important pathological component of Duchenne Muscular Dystrophy (DMD) and thus an attractive target for pharmacological therapy.

Extracellular Adenosine-5'-Triphosphate (eATP) is a "danger" associated molecule which plays a crucial role in the priming of immune response and which directly regulates calcium homeostasis in muscle cells.

Primary muscle cells express various eATP-purinergic receptors (P2X) in physiological conditions. Significant up-regulation of P2X7 is present in skeletal muscle from mdx mice as well as from DMD patients. Exposure of dystrophin-negative myoblasts to eATP triggers increase in cytosolic Ca++ and release of IL1b, suggesting that muscle cells can actively participate in the inflammatory process through purinergic signalling.

To evaluate the role of eATP in the *in vivo* inflammatory response and progression of the degenerative process associated to dystrophinopathy, we analyzed the consequences of P2X7 pharmacological inhibition on mdx muscle function and morphology and on molecular markers of innate and adaptive immune response. We treated mdx mice with periodate-oxidized ATP (oATP), a compound that irreversibly antagonizes P2X receptors and ameliorates the phenotype of animal models with different inflammatory diseases.

Pharmacological inhibition of P2X receptors improved muscular function and morphology, enhancing myofiber regeneration. The beneficial effect exerted by purinergic blockade was associated with i) a reduction of the number and area of the inflammatory infiltrates; ii) a decrease of Il1 and Il6 muscle protein levels, iii) a decrease of muscle infiltrating CD3+ T cells with iv) a parallel 2 fold increase of FOXP3 protein levels, a transcription factor marker of regulatory T (Treg) cells. These results indicate that purinergic antagonism affected the T cell pool composition in dystrophic muscles and increased the subset of proregenerative immune cells.

Finally, oATP inhibitory effect on innate and adaptive immunity translated into a decrease of the expression of TGFβ and CTGF, two key molecular players of the link between inflammation and fibrotic replacement.

In conclusion, purinergic antagonism leads to a functional and histological improvement of the dystrophic process bound to dystrophin deficiency. This effect is mediated by a double effect on the inflammatory response, the down-regulation of the innate inflammasome pathway and the induction of Treg cell population.

PS1-78 / #359

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Restoration of mdx mice neuromuscular junctions as a mark of success of bone marrow stem cells therapy

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Duchenne muscular dystrophy (DMD) is a progressive degenerative disease caused by a mutation in the dystrophin gene, which encodes the protein dystrophin. Mdx mice are a model of DMD. Muscles of mdx mice are characterized by a high level of death of striated muscle fibers (SMFs) and accordingly by a high level of regeneration. As a result most fibers in striated muscle of mdx mice have centrally located nuclei. Neuromuscular junctions (NMJs) in mdx mouse are also altered. Acetylcholine receptors (AChRs) mdx mice distributed as small islands instead of branches. One way of treatment the mdx mice dystrophy is the replacement of mutant bone marrow (BM) for BM of wild type. Our early investigations shown that the number of dystrophin-positive SMFs in transplanted lethal irradiated mdx mice wasn't increased that is consistent with another investigations. Here we transplanted wild type BM cells to mdx mice after non-lethal X-ray irradiation. Male, 2 month old mdxmice (gift of Prof. T.A. Partridge, UK) were irradiated by X-ray in dose 3 Gy. BM cells were harvested from C57BL/6 mice (Rappolovo animal farm, St. Petersburg). 24 h after irradiation, mdx mice were injected intravenously by BM cells. The study of m. quadriceps femoris and diaphragm was made at 2, 4, 6 month after transplantation. Values are means ± S.E.M. We observed the increasing part of dystrophin-positive SMFs in m. quadriceps femoris mdx mice up to 27.6±6.7% at 6 month after transplantation and in diaphragm of mdx mice up to 12.0±3.2% in 4 month after transplantation, which was accompanied by accumulation of SMFs without central nuclei. We observed also decreasing of SMFs death in m. quadriceps femoris mdx mice. In turn it is considered that achievement of therapeutic effect possibly at synthesis restoration dystrophin not less, than in 20% of SMFs. The part of diaphragm NMJs with clusters of AChRs distributed as continuous branches was increased up to 49.1±2.1% and part of NMJs with AChRs clusters as islands was decreased up to 48.2±2.3% after 6 month transplantation. Recovery of structure of NMJs was accompanied by recovery of the resting potential of the diaphragm end-plate membrane. Transplantation of wild type BM stem cells after non-lethal X-ray irradiation restores NMJs structure and function and a dystrophin synthesis. This study was supported by Research Project #1.38.231.2014 of St. Petersburg State University, the Russian Foundation for Basic Research (#13-04-00973, #14-04-32205).

PS1-79 / #367

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Increased constitutive calcium entry via TRPC and TPV2 channels and decreased SERCA2a in mouse dystrophic cardiomyocytes

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Abnormal high cytosolic calcium concentration ($[Ca^{2+}]_c$) is involved in the progressive degeneration

of muscle fibers in Duchenne muscular dystrophy (DMD). In this context, several studies describes an altered Ca^{2+} influx from extracellular medium in both-DMD human muscle cells and mdx mouse muscle cells, an animal model for DMD. Several members of transient receptor potential channels family (TRPs), such as TRPC1, TRPC3 and TRPV2 are involved in altered intracellular Ca^{2+} homeostasis in dystrophic muscle cells (1). TRP proteins assemble into multimeric channel complexes (i.e. TRPC1/4) that participate in store operated Ca^{2+} entry (SOCE), mediated by STIM1/Orai1 proteins and the depletion of intracellular Ca^{2+} stores, or in non-SOCE, gated by PLC/DAG pathway.

Fura-2 loaded wild-type (WT) and mdx mouse cardiomyocytes were incubated with TPEN, a Ca^{2+} SR chelator, to mime Ca^{2+} store depletion without affecting SERCA pump activity. Subsequent SOCE activation was determined by quenching fura-2 with Mn^{2+}. Our data showed an increase of cation influx after cell stimulation with TPEN in WT cardiomyocytes that is reduced by YM-58483, a potent inhibitor of SOC and Orai1 channels, but not by red ruthenium (RR), an inhibitor of TRPV2. TPEN-induced SOCE were not different in WT and mdx cardiomyocytes. In contrast, we observed a constitutive Ca^{2+} influx only in resting mdx cardiomyocytes. This constitutive influx is attenuated by both inhibitors YM-58483 and RR pretreatment.

Since the expression of TPRC1 is enhanced in mdx skeletal muscle and cardiomyocytes cells (2,3), we performed western blotting experiments to examine changes in the expression of TRPC4 in mdx cardiomyocytes. Our results showed an increase in the level of expression of TRPC4 in mdx cardiomyocytes. On the contrary, the SERCA2a was decreased in mdx cardiomyocytes.

These findings suggest that a constitutive entry of cation is observed in dystrophic cardiomyocytes. The pharmacology and the protein expression profile suggest TRPC1/4 participate in this constitutive Ca^{2+} influx independent of SOC together with TRPV2. Ca^{2+} entry through TRPC1/4 together with decrease of Ca^{2+} reuptake by SERCA2a, could lead the activation and translocation to the plasma membrane of TRPV2, which may explain the abnormal ($[Ca^{2+}]^c$) in mdx muscle cells.

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PS1-80 / #371

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

SERCA2a is involved in the stabilization of plasma membrane calcium channels in human skeletal myotubes

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A disruption in the dystrophin-glycoprotein complex is hypothesized to promote direct Ca^{2+} influx and/or abnormal cytosolic Ca^{2+} homeostasis, leading to increased myofibers necrosis. Forced expression of sarcoplasmic reticulum Ca^{2+} ATPase (SERCA1 or SERCA2a) was reported to reverse a defect in Ca^{2+} reuptake and rescue the dystrophic phenotype in a mouse model (Goonasekera et al., (2011) J Clin Invest 121: 1044-1052). Here we demonstrated strong down-regulation of both SERCA1 and SERCA2a together with up-regulation of TRPC4 channel in tibialis and gastrocnemicus of mdx mice by WB analysis. We hypothesized that being able to control Ca^{2+} reuptake is critical to the regulation of Ca^{2+} influx multiprotein signaling complex activity.

The aim of this study was to analyze the effect of SERCA2a forced expression on plasma membrane (PM) channels functioning in human myotubes.

Human skeletal myoblasts were induced to differentiate into myotubes for 10 days. Marked SERCA2a up-regulation was observed in the differentiating myotubes together with appearance of the marquers of contractile skeletal muscle, such as LTCC, RyR, a-actinin and fast myosin heavy chain. No up-regulation of SERCA1 was observed at this stage.

Ca^{2+} transients were recorded in FURA2-loaded human myotubes transduced with adenovirus encoding either SERCA2a and GFP or beta-galactosidase and GFP. Transduced cells were identified by GFP fluorescence. Serotonin (5-HT, 10μM) induced a long-lasting increase of intracellular Ca^{2+} in FURA-2 charged

differentiating myotubes supported by SR Ca^{2+} release and extracellular Ca^{2+} influx, as it was demonstrated by changing Ca^{2+} concentration in extracellular medium. SERCA2a robust induction by gene transfer in differentiating myotubes results in 1) modified 5HT-induced transient from steady-state to oscillatory mode and 2) suppression of extracellular Ca^{2+} influx following agonist stimulation. Transduction of cells with control virus AdGFP has no effect on Ca^{2+} transient. Taken together these data demonstrated that SERCA2a induction during myotube formation is associated with the appearance of key contractile markers, such as LTCC, RyR and contractile filaments. Furthermore, SERCA2a increases the frequency of agonist-induced transient and completely suppresses extracellular Ca^{2+} influx.

This study provides evidence for the first time that PM channels in skeletal myotubes are potentially controlled by SR Ca^{2+} contain and the velocity of Ca^{2+} re-uptake.

PS1-81 / #378

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Assessment of Upper Limb function in DMD patients:12 month changes

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As a result of an international effort, a new tool, the Performance of Upper Limb (PUL) has recently designed to assess upper limb function in DMD boys. The purpose of the PUL is to assess changes that occurs in motor performance of the upper limb over time from when a boy is still ambulant to the time he loses all arm function when non-ambulant.

The test has proved to be reliable, suitable for multicentric studies with excellent inter and intra-observer reliability.

Cross -sectional results in 322 DMD patients (mean age 12.7; range 4.1-35.1) showed a progressive deterioration of scores with age, with early involvement of the proximal muscles that was more obvious after the age of 10 years. Even the oldest and weakest DMD patients were still able to perform some of the distal items , suggesting that the scale is capable of measuring small distal movements (lifting small weights, tracing a diagram) that are important as they relate to activities of daily living such as using a mobile or using a computer mouse.

The aim of the present study was to assess the PUL in 12 month changes in the same cohort.

Results: The results showed some variability I the 12 month changes. Further analysis in ongoing to establish the effect of age at baseline, steroids, and functional level (ambulant vs non ambulant) in this cohort.

Conclusion: The PUL Scale demonstrated to be a useful tool for upper limb motor disease assessment in DMD ambulant and non-ambulant patients, both for evaluation in clinical trials and for therapy follow-up.

PS1-82 / #385

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Diapocynin, a putative NADPH oxidase inhibitor, ameliorates the phenotype of a mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe X-linked muscular disease that causes premature death and for which no cure exists. We have shown previously that *in vitro* treatment of dystrophic myotubes and excised muscles with diapocynin, a dimer of the classically used NADPH oxidase inhibitor apocynin, ameliorated several molecular events involved in DMD pathogenesis, of which ROS production, phospholipase A_2 activity, Ca^{2+} influx and sarcolemmal integrity.

Here, we report on the *in vivo* effects of diapocynin and apocynin in mdx^{5Cv} dystrophic mice, a model of DMD. Apocynin (50 mg/kg/day) and diapocynin (10 and 100 mg/kg/day) were given orally to mdx^{5Cv} mouse pups, first via the lactating mothers from post-natal day 14 to 28 and subsequently directly to the weaned pups till post-natal day 35 ± 1 or 60 ± 3. Diapocynin but not apocynin enhanced spontaneous locomotor activity, rescued voluntary wheel running capabilities, and ameliorated diaphragm structure of dystrophic mice. Diapocynin and apocynin were equally potent at increasing the resistance to fatigue of triceps surae muscles exposed to repeated isometric contractions in situ and at preserving sarcolemmal integrity as evidenced by Evans blue dye uptake. Although apocynin and diapocynin had beneficial effects in dystrophic mice, diapocynin was superior in improving locomotion. Our findings suggest that diapocynin holds therapeutic potential for DMD.

PS1-83 / #395

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Design of a confirmatory phase 3, multicenter, randomized, double-blind, placebo-controlled study of ataluren in patients with nonsense mutation Duchenne muscular dystrophy

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Introduction: In ~13% of patients, Duchenne muscular dystrophy (DMD) is caused by a nonsense mutation (nm) in the dystrophin gene. Ataluren is an investigational oral drug designed to promote ribosomal read-through of premature stop codons in mRNA, leading to production of full-length, functional protein. We describe an ongoing, confirmatory, phase 3, placebo-controlled study designed to assess the efficacy and safety of ataluren 40 mg/kg/day in boys with nmDMD. The design of this study reflects lessons learned from prior studies and targets a study population to best show a treatment effect over 48 weeks.

Methods: Key study entry criteria require that patients are male with a nonsense mutation in the dystrophin gene, 7–16 years of age, receiving a stable dose of corticosteroids, and have a screening 6-minute walk distance (6MWD) ≥150 m but below the protocol-specified %-predicted threshold. Overall, 220 patients will be randomized in a 1:1 ratio to placebo or ataluren. The primary endpoint is change in 6MWD over 48 weeks. Secondary efficacy measures include timed function tests, quality of life, North Star Ambulatory Assessment, and patient/parent-reported disease-related symptoms and activities of daily living.

Results: In a retrospective subgroup analysis of patients in the phase 2b trial of ataluren in nmDMD who met the current study criteria, the difference between ataluren 40 mg/kg/day (administered as 10, 10, 20 mg/kg; $n=30$) vs placebo ($n=31$) in mean change in 6MWD over 48 weeks was approximately 50 m.

Conclusions: This study is designed to confirm the treatment effect of ataluren seen in the phase 2b ataluren trial and is anticipated to be one of the largest trials conducted in DMD.

PS1-84 / #401

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Preclinical Safety Profile of Srp-4045, A Potential Phosphorodiamidate Morpholino Oligomer Treatment for Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a recessive X-linked form of muscular dystrophy caused by mutations in the dystrophin gene. Dystrophin provides structural stability to the dystroglycan complex within the muscle cell membrane, and mutations that cause an out-of-frame reading shift which results in a truncated non-functional dystrophin product.

SRP-4045 is a phosphorodiamidate morpholino oligomer (PMO) currently in preclinical development that is intended as a treatment for patients with DMD that have mutations amenable to skipping exon 45. SRP-4045 binds to exon 45 of the dystrophin pre-messenger RNA in a sequence-specific fashion, promotes exon 45 skipping, and thereby restores a correct reading frame.

A safety pharmacology study in non-human primates featuring single intravenous doses of SRP-4045 up to 320 mg/kg did not produce abnormal cardiovascular, respiratory, hemodynamic, body temperature, or neurological parameters, and a 12 week repeat dose toxicology study in non-human primates determined a no observed adverse effect level at the maximum feasible dose of 320 mg/kg (mean C_{max} and AUC_{0-t} values of 1,490,000ng/mL and 1,930,000ng.hr/mL, respectively). SRP-4045 was found to be negative in the bacterial reverse mutation assay up to 5000 mg SRP-4045/plate, chromosomal aberration assay up to 500µg SRP-4045/mL, and was negative for inducing micronucleated polychromatic erythrocytes in the bone marrow of mice following single intravenous doses up to 2000 mg/kg.

Ongoing safety assessment in the mouse is investigating weekly SRP-4045 intravenous doses up to 960mg/kg for 12 weeks. Preclinical safety data support that SRP-4045 is well tolerated and has a similar safety profile as another PMO, eteplirsen, currently in development for DMD amenable to skipping of exon 51.

✶PF4

PS1-85 / #404

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

A Novel First in Human Study Design to Establish Tolerability of a Target Dose to Treat Patients with Duchenne Muscular Dystrophy (DMD) Amenable to Exon 53 Skipping

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Objective: To establish tolerability and exploratory efficacy of 30 mg/kg/wk dose for the long-term (48 weeks) treatment of SRP-4053 targeted to skip exon 53.

Background: DMD is an X-linked myopathy caused by the inability to produce the dystrophin protein. SRP-4053 is an investigational therapy designed to enable functional dystrophin production in boys who are amenable to exon 53-skipping therapy.

Methods: A 2-Part 4-site first in human (FIH) study design planned in 3 EU countries: Part 1 consists of double-blind, placebo-controlled, 3escalating doses (4, 10, and 20 mg/kg) for 2 weeks each followed by up to 5 weeks of 30 mg/kg/wk in 12 boys randomized in 2:1 (SPR-4053:Placebo). Dose escalation through the 30mg/kg dose will be based on safety data review (individual site level). A DSMB review of aggregate safety data will occur when the 12th enrolled patient

completes 2 weeks of the 30 mg/kg, and will sanction transitioning the study into Part 2. Part 2 is an open-label design where the 4 placebo-treated patients in Part 1 and 12 treatment naïve patients will receive 30 mg/kg/wk ($N=24$ treated patients). An untreated control arm consisting of eligibility criteria-matched DMD patients not amenable to exon 53 skipping ($N=24$) will also be enrolled.

Patients 6 to 15 years old with stable walking ability on the 6MWT (250 meters or more), a total NSAA score more than 17, or Gowers' time less than 7 seconds will be eligible. Stable corticosteroid dose, cardiac, pulmonary, and other safety criteria are required.

Assessments will include 6MWT, NSAA, PFT, upper and lower body muscle strength and performance, leg muscle MRI and MRS, full PK profiles, and detailed dystrophin restoration profile including percentage of positive-dystrophin fibers in pre- and on-treatment muscle biopsies.

Discussion: This FIH study was designed in 2 parts to allow for dose escalation to the target dose in a placebo-controlled double-blind fashion before expanding the treatment to naïve patients, and therefore reducing the time needed to achieve long term efficacy and safety data collection.

PS1-86 / #405

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Phase 2b study of ataluren in nonsense mutation Duchenne muscular dystrophy: results across disease spectrum based on %-predicted 6MWD categories

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Introduction: In ~13% of patients, Duchenne muscular dystrophy (DMD) is caused by a nonsense mutation (nm) in the dystrophin gene. Ataluren is an investigational oral drug designed to promote ribosomal read-through of premature stop codons in mRNA, leading to production of full-length, functional protein. In a phase 2b, international, multicenter, randomized, double-blind, placebo-controlled trial (Study 007), ataluren 40 mg/kg/day was shown to slow the decline in 6-minute walk distance (6MWD) compared with placebo in ambulatory boys aged ≥5 years with nmDMD. Here, we describe findings from a post-hoc analysis designed to assess the changes in 6MWD with ataluren compared with placebo over 48 weeks across baseline %-predicted 6MWD categories.

Methods: A total of 174 patients were enrolled at 376 sites in 11 countries. Mean [range] age was 8 [5-20] years and median [range] height was 123 [99–173] cm. Patients received ataluren 80 mg/kg/day ($n=60$), ataluren 40 mg/kg/day ($n=57$) or placebo ($n=57$) for 48 weeks. For the post-hoc analysis, absolute 6MWD data were converted to %-predicted 6MWD using a previously published age- and height-based equation (Geiger et al. J Pediatr 2007;150:395–9) to adjust for maturational differences. Using these values, patients were categorized accordingly to %-predicted 6MWD at baseline: >70%, 50–70% or <50%.

Results: Over 48 weeks, in patients with baseline %-predicted 6MWD >70%, the mean difference between ataluren 40 mg/kg/day (administered as 10, 10, 20 mg/kg; $n=18$) vs placebo ($n=20$) in mean change in 6MWD was approximately 20 m; In patients in the early decline phase of ambulation (50–70% of predicted 6MWD at baseline), the mean difference between ataluren ($n=22$) and placebo ($n=23$) was 47 m; in those in the late decline phase of ambulation (<50% of predicted 6MWD at baseline), the mean difference between ataluren ($n=15$) and placebo ($n=12$) was 41 m. See figure.

Conclusions: In this post-hoc analysis, the decrease in 6MWD over 48 weeks was less in patients receiving ataluren 40 mg/kg/day than in those given placebo in all stages of disease, however the effect of ataluren was most evident in patients in the decline phase of ambulation (<70% of predicted at baseline).

★PF4

PS1-87 / #407

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Pulmonary Function and Safety Results at Week 120 of Exon-Skipping Drug Eteplirsen from the Phase 2b Study in Patients with Duchenne Muscular Dystrophy (DMD)

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Objective: To establish long-term efficacy of eteplirsen treatment targeted to skip exon 51.

Background: DMD is a rare, degenerative, genetic disease that results in progressive muscle loss and premature death affecting 1 in 3500 male births. DMD is caused by the inability to produce the dystrophin protein. There are no approved drugs available to treat DMD. Eteplirsen is an investigational therapy designed to enable functional dystrophin production in boys who are amenable to exon 51?skipping therapy.

Methods: 12 boys with median age of 9.7 years were randomized 1:1:1 to 30mg/kg, 50mg/kg, or placebo. Upon completion of a 24-week double-blind, placebo-controlled phase, all patients were enrolled in an open-label extension and the placebo-treated patients initiated eteplirsen treatment. FVC, FVC% predicted, MEP, and MIP (pulmonary function tests or PFT) were assessed every 12 to 24weeks, while safety assessment was continuous. For all 12 patients changes at Week 120 were examined from Week 1, and from last assessment pre-eteplirsen administration (Week 1 for 8 patients and Week 24 for 4 patients). One-sample *t*-test was used for statistical analysis.

Results: Reported here are the results for all 12 patients, including two patients who became non?ambulate by Week 24. After 120 weeks of treatment and at median age of 12 years, FVC change from pre-eteplirsen administration, and MEP change from Week 1 showed a statistically significant increase of 0.2 liters ($p \leq 0.02$) and 10.2 cm H_2O ($p \leq 0.04$), respectively. Changes in all other PFT measures were not significant, with most showing a slight quantitative increase from pre-treatment. No deaths, discontinuations due to AEs, treatment-related SAEs, or clinically significant abnormal laboratory, ECG, or ECHO findings were reported.

Conclusions: After 120 weeks of treatment, eteplirsen demonstrated preservation effect on the 4 PFT measures, contrary to a steady decline expected in DMD patients of this age. Eteplirsen dosed for up to 120weeks exhibited an unremarkable safety profile and was well tolerated.

PS1-88 / #409

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Phase 2b study of ataluren (PTC124®) in nonsense mutation Duchenne muscular dystrophy – results of a clinical efficacy robustness analysis

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Introduction: In ~13% of patients, Duchenne muscular dystrophy (DMD) is caused by a nonsense mutation (nm) in the dystrophin gene. Ataluren is an investigational oral drug designed to promote ribosomal read-through of premature stop codons in mRNA, leading to production of full-length, functional protein. In a 174-patient, phase 2b, international, multicenter, randomized, double-blind, placebo-controlled, 48-week trial (Study 007), ataluren 40 mg/kg/day (administered as 10, 10, 20 mg/kg) was shown to slow the decline in 6-minute walking distance (6MWD) compared to placebo in ambulatory males aged ≥5 yrs with nmDMD. In a corrected ITT (cITT) analysis, the treatment effect approached statistical significance in the primary 6MWD analysis, and multiple secondary endpoints of physical function showed positive trends. Larger effects were seen in a subgroup of patients in the ambulatory decline phase. We describe analyses demonstrating the internal consistency aspect of robustness of 6MWD and key secondary timed function test (TFT) endpoint results.

Methods: To evaluate the robustness and internal consistency of the positive results in the 6MWT and TFTs, sensitivity analyses were performed. Monte

Carlo simulations repeated the endpoints analyses 1000 times with a random 10% of the patients in each treatment arm removed each time. In another sensitivity analysis, the endpoint analyses were repeated after removing one or more of the extreme results from each treatment arm. Both methods were applied to the overall Study 007 population and in the decline phase subgroup.

Results: Monte Carlo analysis by randomly deleting 10% of the data in each treatment arm supports a mean change of >30 m in 6MWD with ataluren 40 mg/kg/day in the overall cITT population and >45 m in the decline phase subgroup compared to placebo, supporting the primary analyses. Application of this method to the TFT data also supported the trend towards better performance with ataluren 40 mg/kg/day than placebo in both populations. Best/worst deletion analysis found differences between ataluren and placebo of approximately 30 and 50 m in the overall and decline phase groups, respectively, indicating that the Study 007 results were not distorted by outliers.

Conclusion: These analyses substantiate the robustness of the 6MWD and TFT results for ataluren 40 mg/kg/day in patients with nmDMD seen in the Phase 2b 007 study.

PS1-89 / #431

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Preclinical evaluation of tamoxifen and other selective estrogen receptor modulators in mdx5Cv dystrophic mice

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We are investigating the effects of selective estrogen receptor modulators (SERMs) in mdx^{5Cv} dystrophic mice, a model for Duchenne muscular dystrophy (DMD). SERMs display either pro-estrogenic or anti-estrogenic activities in a tissue-dependent manner. Tamoxifen (TAM),a leading SERM, has been used for over 30 years to treat estrogen-sensitive breast cancer in both women and men and has been reported to be also well tolerated in pre-pubertal boys.

In 2013, we published that oral treatment of mdx^{5Cv} mice from 3 weeks of age for 15 months with TAM at a dose of 10mg/kg/day caused remarkable improvements of muscle force and of the structures of diaphragm and heart. TAM and its metabolites were present in nanomolar concentrations in plasma and muscles, suggesting signalling through high affinity targets, likely the estrogen receptors alpha and beta that were several-fold more abundant in dystrophic muscle than in normal ones (Dorchies et al., Am J Pathol, 2013;182(2):485–504).

Next, we tested TAM in adult mdx^{5Cv} mice in order to investigate its efficacy in the low-intensity chronic stage of the disease, which resembles most closely the DMD condition. TAM at doses as low as 0.1mg/kg/day improved motor performance of active mice (evaluated with a grid hanging test) and enhanced the contractile characteristics of the triceps surae, a large muscle group of the lower leg (judged using isometric contraction assays in sedated mice).

We are currently testing other SERMs (all at 3mg/kg/day), of which the Chlorinated TAM analogues clomiphene and toremifene, the 3-hydroxylated TAM derivative droloxifene, as well as raloxifene (RAL), a second generation SERM unrelated to TAM, and the estradiol-based pure anti-estrogen fulvestrant (Faslodex). Overall, the ranked efficacy was as follows: TAM > toremifene > clomiphene > droloxifene ≈ RAL > Faslodex.

TAM-derived SERMs exist as stereoisomers with distinct biological activities. The Z and E isomers of TAM and of clomiphene are currently being purified and will be evaluated in mdx^{5Cv} mice, as well as other TAM analogues (ospemifene, panomifene), RAL analogues (arzoxifene, bazedoxifene, pipendoxifene, trioxifene), and third generation SERMs unrelated to TAM and RAL (ormeloxifene, lasofoxifene), several of which were developed to treat hormonal disorders in men.

Our data suggest that TAM and other SERMs with pro-estrogenic activities on muscle might be beneficial for DMD and maybe also for other muscular dystrophies.

PS1-90 / #471

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Effects of High Dose Gamma Irradiation on the EOM and Limb Muscles from a Mouse Model of Muscular Dystrophy

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Sparing of the extraocular muscles (EOM) in Duchenne (DMD) and other muscular dystrophies is well known; however, the mechanism for this sparing has been unclear. Our recent work showed that the EOM contain an enriched population of myogenic precursor cells, the EECD34 cells, compared to limb skeletal muscles. These EECD34 cells are maintained in the EOM of mouse models of DMD and down-regulated in limb muscle. We propose that these cells maintain normal EOM morphology throughout the life span of individuals with DMD due to greater proliferative potential and/or greater resistance to injury. For example, when the EECD34 cells were isolated from limb and EOM, the EECD34 cells derived from EOM had significantly greater proliferation rates. In addition, 18Gy gamma irradiation of the EOM and limb muscles of mdx:utrophin+/– mice resulted in decreased limb muscle size over time and a decreased density of Pax7-positive cells. In contrast, in EOM a dystrophic phenotype developed, but by 2 months post-irradiation normal morphology was restored. This suggests the presence of an irradiation-resistant myogenic precursor population in the EOM. To test this hypothesis, the EOM and one limb muscle were subjected to two doses gamma irradiation at 18Gy separated by one month and muscle morphology and myogenic precursor populations were assessed.

All research was approved by the Institutional Review Board at the University of Minnesota. Adult mdx:utrophin +/– mice were anesthetized and subjected to two bouts of 18Gy gamma irradiation given 1 month apart. At 7, 14, 30, 60 and 180 days, the animals were euthanized and the muscles removed for morphometric analyses.

Two bouts of 18Gy gamma irradiation resulted in significant reduction in limb muscle cross-sectional areas that correlated with a significant depletion of the Pax7-positive myogenic precursor cells. In contrast, the EOM showed no change in mean myofiber cross-sectional areas at any post-irradiation time point nor did central nucleation appear. Interestingly and paradoxically, the Pax7 population showed a decreased density as post-irradiation time increased.

This suggests that there is at least one myogenic precursor cell population in the EOM that is resistant to 18Gy irradiation and is able, in the long term, to maintain EOM morphology over time. Ongoing studies are focused on defining the distinct populations of myogenic precursor cells in EOM as well as defining the cellular environment in which they reside

PS1-91 / #483

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Succesful pilot trial of L-arginine and metformin in Duchenne's muscular dystrophy

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Duchenne muscular dystrophy (DMD) is the most common inherited muscle disorder leading to relentless muscle wasting and premature death in affected children. The only currently available symptomatic treatment for DMD consists of corticosteroids, resulting in modest beneficial effects but relevant side-effects. In DMD dystrophin expression is lost disrupting the normal cytoskeletal structure. While most research has focused on the structural consequences, e.g. destabilization of the dystrophin associated glycoprotein complex resulting in muscles fibres that are more sensitive to mechanical damage and thus degenerate. In contrast, we approach the metabolic consequences of dystrophin loss which is associated with a severe reduction of neuronal nitric oxide (NO) synthase (nNOS). NO stimulates the up-regulation of nuclear genes involved in mitochondrial biogenesis and ATP generation. Therefore, NO precursors (as the amino acids L-arginine and L-citrulline) and the biguanide antidiabetic drug metformin (indirect nNOS activator) could serve as treatment for DMD. We were

the first using this approach in human DMD patients, and performed an investigator initiated sixteen week pilot study in five ambulant DMD patients with L-arginine and metformin. Biomarker analysis in muscle biopsies of these patients show increased cGMP and nitrotyrosin concentration after treatment and reduced markers of oxidative stress. We observed an improved lipid metabolism using indirect calorimetry, improved functional abilities (motor function measurement (MFM) score), and prolonged walking distances in the 2 min walking distance. MFM scores improved more than the reported mean improvements after onset of (standard) steroid treatment in DMD. Furthermore, quantitative muscle magnet resonance imaging (MRI) indicated that our treatment slows progression of muscle degeneration in DMD. These results must of course be confirmed in a placebo controlled randomized clinical trial (RCT) with a larger group of patients. In case of positive results a more effective and safer symptomatic treatment will be available in DMD and might also lead to the development of even more potent drugs.

PS1-92 / #485

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Comparison of Deflazacort and Prednisone in Duchenne Muscular Dystrophy

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Objective: Duchenne muscular dystrophy (DMD) is a degenerative disease that usually becomes clinically detectable in childhood as progressive proximal weakness. No cure is yet available for DMD, but the use of steroids improves muscle strength and function. This study has been carried out to select the best steroid for the management of DMD.

Materials & Methods: This study is a single-blind, randomized clinical trial with a sample volume of 34 DMD patients. Half of these patients were treated with deflazacort (0.9 mg/kg daily) and the other half with prednisone (0.75 mg/kg daily) for a period of 18 months. The motor function score and excess body weight were registered one year after the start and also at the end of the study and compared between the two groups.

Results: Deflazacort was more effective in the improvement of motor function after one year, but there was no significant difference between the two drugs at the end of the study (18 months after start). Weight gain after one year and at the end of the study was higher in prednisone group and steroid treatment with deflazacort appears to cause fewer side effects than prednisone regarding weight gain.

Conclusion: Deflazacort seems to be more effective than prednisone in the improvement of motor function causing fewer side effects, particularly weight gain. This medication may be important for the improvement of motor function and could be used as the best steroidal treatment for Duchenne muscular dystrophy.

✶PF4

PS1-93 / #495

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Drisapersen treatment for Duchenne muscular dystrophy (DMD): results of a 96-week follow-up of an open-label extension study following two placebo-controlled trials

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Background: Drisapersen (DRIS) is a 2'-O-methyl-phosphorothioate oligonucleotide designed to skip exon 51 in the dystrophin pre-mRNA of subjects with Duchenne muscular dystrophy (DMD). We report results of an open-label extension study (DMD114349) following two 48-week placebo-controlled trials (DMD114117 and DMD114044). Both safety data (datacut June 2013) and efficacy data (datacut October 2013) are reported. In the feeder studies, a treatment benefit favouring DRIS on 6-minute walk distance (6MWD) was shown after 48 weeks of treatment (DMD114044, 10.3 meters; DMD114117, 35 meters).

Objectives: The study aim was to assess the long-term safety, tolerability and efficacy of 6mg/kg/wk

DRIS by subcutaneous injection; the efficacy endpoint was the 6MWD.

Methods: DMD subjects completing feeder studies (inclusion: ≥5 years; ambulant; steroid-treated; rise from the floor ≤7 sec [DMD114117 only]; and a dystrophin mutation correctable by exon 51 skipping) were eligible.

Results: Safety analysis (n=186) demonstrated that injection-site reactions, renal events and thrombocytopenia were the most prominent findings. At 48 weeks of DMD114349, subjects who received DRIS (n=69) showed a clinically meaningful difference in 6MWD compared with those in the placebo/delayed-treatment arm (n=44; mean [95% CI] change, -66.8 [-96.6, -36.9] and -112.9 [-152.0, -73.8] meters for a total of 96 and 48 weeks of DRIS, respectively; mean difference, +46 meters). Subjects previously enrolled in DMD114044 had a 49-meter difference between DRIS (n=52) and placebo/delayed treatment (n=31) from original baseline. Those previously enrolled in DMD114117 had a 52-meter difference from original baseline between DRIS (n=17) and placebo/delayed treatment (n=13) in favour of DRIS; decline was only 5 meters in the DRIS arm.

Discussion/Conclusions: The long-term safety of DRIS appears to be similar to that seen in other clinical trials, with the exception of the occurrence of thrombocytopenia. A total of 96 weeks of DRIS treatment resulted in a clinically meaningful difference from placebo/delayed DRIS of 46 meters. This extension study suggests maintenance of benefit in a feeder study population with less severe disease, and a clinically meaningful benefit taking longer to emerge in a feeder study population that is, on average, more severely affected.

Study support: GSK

PS1-94 / #502

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Extracellular microRNAs: identity and function in relation to muscle regeneration and dystrophic pathology

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Extracellular (ec) microRNAs (miRNA) are promising, non-invasive biomarkers for a variety of pathological conditions, including the progressive wasting disorder Duchenne muscular dystrophy (DMD). miR-1, miR-133a, miR-133b and miR-206 are muscle-specific miRNAs (myomiRs) that regulate myogenic differentiation. MyomiRs are highly abundant in serum of DMD patients and dystrophic animal models and hence have been investigated for their capacity to monitor disease progression and the outcome of therapeutic interventions. Previously, we showed that ec myomiRs exhibit dynamic patterns of expression over time in mdx mice and showed restoration to wild-type levels after exon-skipping therapy. The mechanism by which small RNAs are released into the circulation is still unclear, however it has previously been assumed that increased membrane permeability in dystrophic muscle allows for random release of miRNAs.

Conversely, we observed an asymmetry in expression patterns of myomiRs between the musculature and the circulation in mdx mice. This observation suggests a controlled, miRNA-specific release mechanism with ec miRNAs acting paracrine regulators of muscle homoestasis. Hypothesising that myomiRs provide information about the physiology of the muscles from which they originate; we monitored serum myomiR levels for two weeks in mdx mice following one acute bout of exercise. The results suggest an immediate (acute degenerative response) and a delayed release (regeneration) of myomiRs by the musculature. Consonantly, modelling of miRNA release by differentiating murine muscle cells showed that myomiRs were preferentially secreted at later stages of

differentiation. Additionally, we found dynamic changes of ec myomiRs in juvenile mdx mice. Levels were found to be highest in neonatal and one week old animals and then decreased steadily until week four, thereby demonstrating that ec myomiRs can be elevated under physiological conditions in addition to pathophysiological conditions (i.e. dystrophinopathy). Furthermore, studies separating extracellular vesicles, lipoprotein and protein complexes by ultracentrifugation and size exclusion chromatography demonstrated that myomiRs are transported in serum by different means than non-muscle specific miRNAs.

In conclusion, our results suggest that ec miRNA release is a highly regulated and profoundly physiological process that is likely to be of direct relevance to muscle homeostasis both in development and regeneration.

PS1-95 / #514

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Neuromuscular synaptic dysfunction in Duchenne muscular dystrophy mouse models

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Duchenne muscular dystrophy is an X-linked myopathy caused by the loss of dystrophin. Dystrophin is present intracellularly at the sarcolemma and connects actin to the dystrophin-associated glycoprotein complex. Interestingly, the protein is found enriched at the postsynaptic membrane of the neuromuscular junction (NMJ), but its function at that location is largely unknown. Utrophin, a dystrophin homologue, is also concentrated at the NMJ. We studied possible functional roles of dystrophin at NMJs in mdx mice (which lack dystrophin) and mdx-Xist(deltaHS) female mice (which express defined low dystrophin levels as a consequence of non-random X-inactivation) and control strains (C57BL/10ScSnJ and Xist(deltaHS) mice). In addition, mdx-utrn+/- and mdx-utrn-/- mice were used to investigate possible roles of utrophin. The Duchenne mouse models showed muscle weakness when tested *in vivo*, compared to wild type, with mdx-utrn-/- mice being the weakest. In mdx-Xist(deltaHS) mice, muscle strength was partially restored. Repetitive nerve stimulation electromyography in the several Duchenne mouse models showed a decrease in initial compound muscle action potential amplitude and a trend towards decrement upon repetitive nerve stimulation, suggesting mild NMJ dysfunction. Reduced synaptic strength at NMJs was further suggested from increased d-tubocurarine sensitivity in ex vivo muscle contraction experiments. Miniature endplate potentials (MEPPs) and endplate potential (EPPs) were measured in micro-electrode studies. Duchenne mouse model NMJs showed significantly smaller MEPP amplitudes compared to wild type, while having similar EPP amplitudes. Consequently, calculated quantal content (i.e. the number of acetylcholine quanta released per nerve stimulus) was found considerably increased. High rate nerve stimulation induced a more exaggerated EPP rundown. NMJ morphology study revealed fragmented acetylcholine receptor area, being severest in mdx-utrn-/- mice. Many of these *in vitro* electrophysiological and morphological features were also seen at NMJs of mdx-Xist(deltaHS) mice. Thus, low levels of dystrophin in these mice seem able to restore muscle strength to some extent but do not correct NMJ deviations. Overall, our results indicate mild NMJ dysfunction in the several Duchenne mouse models, which may contribute to onset or progression of the overall muscle weakness. It will be of interest to study possible beneficial effects of drugs specifically acting at the NMJ.

PS1-96 / #525

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Duchenne muscular dystrophy (DMD): human IgG improves the performance of mdx mice in voluntary wheel running

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Objective: Duchenne muscular dystrophy (DMD) is an x-chromosomal, progressive myopathy due to a complete loss of the dystrophin protein. Although research is continuously progressing, finding a curative treatment is still a challenge. The current standard therapy with oral prednisolone prevents inflammatory downstream effects of muscle degeneration but is associated with considerable side-effects. Therefore, immunomodulation by immunoglobulin G (IgG) could be a suitable alternative for treatment.

Methods: Three weeks old mdx mice received i.p. human IgG at a dose of 2g/kg or an equal amount of human albumin as a control every four weeks (total of two injections over 8 weeks; $n = 10$ each group). After one day of rest, both groups were placed in separate cages equipped with a voluntarily accessible running wheel. The detection of several observer independent parameters like maximum velocity, distance and number of runs was performed fully computerized 24-hours per day. Body weight and grip strength were determined once a week. After 8 weeks, animals were sacrificed and blood, diaphragm and lower limb muscles were removed for quantitative PCR, histological analysis and ex vivo muscle contraction tests.

Results: Human IgG compared to sham treatment improved the running parameters such as distance and cumulative run time of mdx mice. In keeping with this, ex vivo muscle contraction assessment of IgG treated animals revealed a significantly improved muscle fatigability.

Real-time PCR demonstrated in skeletal muscle a reduced production of relevant inflammatory mediators including TGF-β, CCL-2 and osteopontin after IgG compared to albumin injection. Upon IgG, myopathic changes including central nuclei index were significantly reduced in lower limb muscles. Human IgG was evidenced by ELISA in serum and by immunohistochemistry in skeletal muscle. Serum-CK, bodyweight and grip-strength of the forelimbs remained unchanged.

Conclusions: 1) Our results support the validity of the computerized running wheel system for evaluation of treatment studies in mdx mice. 2) Human IgG is well tolerated in mice over a time period of eight weeks. 3) Human IgG treatment improved the running performance and reduced myopathic changes as well as muscle inflammation. Taken together, modulating the immune response with IgG might be a potential treatment option for DMD.

PS1-97 / #527

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

A Trial of Sildenafil treatment for patient with Duchenne muscular dystrophy

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Background: Sildenafil, Phosphodiesterase 5Ainhibitor, is expected to become an effective therapeutic agent for Duchenne muscular dystrophy (DMD) because of improvement of microcirculation abnormality.

Aim: To evaluate the effectiveness of Sildenafil for DMD.

Subjects and methods: Four DMD patients (Case A-D) were recruited. Their age ranged from 7–17 years old. Case C was ambulatory and others were chair-bound. All preserved respiratory function. Case B had undergone spine surgery. Sildenafil was administered with gradual increase from 0.25mg/kg to 1.0mg/kg for 2weeks. We evaluated manual muscle test (MMT), vital capacity, ejection fraction of heart ultrasonography, plasma BNP, serum CK, circulating CD34-positive (CD34+) cells, activity evaluated by watch-type activity measure, and subjective change of disease condition before (pre), at the end of treatment (post), and 2weeks ~ 2months after treatment (after).

Results: All participants completed the trial. Case D complained of transient hot flash after starting Sildenafil. There was no significant change in MMT, vital

capacity, ejection fraction and BNP. Serum levels of CK in all participants increased at the end of treatment and decreased after treatment in 3 cases (Case A CK(IU/l): pre,4928; post,5575; after,4446?case B: 1635; 1851; 2022?case C:11404; 24638; 12434?case D: 3836; 7581; 4989) . Levels of circulating CD34+ cells were fluctuated during trial in each case (case A CD34+ cells (counts/µl) : pre,3.8; post,10.96; after,5.04, case B: 1.2; 1.5; 1.32, case C: 2.12; 2.95; 2.53, case D: 7.01; 5.3; 3.34). Daily activities in each participant were invariable. There was no subjective improvement of motor function.

Conclusion: There was no objective and subjective improvement in short term administration of Sildenafil. However, there was no adverse side effect. Examinations of adaptation of Sildenafil administration and an effective examination method are necessary.

PS1-98 / #538

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Development of Rimeporide, a NHE-1 inhibitor, for patients with Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is a rapidly progressive form of muscular dystrophy that affects approximately 1 in 3500 male births worldwide. It is caused by a mutation in a gene which encodes the dystrophin. Absence of dystrophin causes relentlessly progressive skeletal muscle degeneration, progressive respiratory muscle weakness and cardiac failure. Cardiomyopathy is the leading cause of death in Duchenne patients.

A number of cellular mechanisms are involved in DMD. A significantly higher influx of Ca^{2+}, partly triggered via intracellular Na^+ concentrations, has been observed in DMD cells versus healthy muscle cells. This mechanism is likely to be of importance in several aspects of DMD pathophysiology including cardiomyopathy.

The sodium-hydrogen exchanger (NHE-1) is a key membrane transporter regulating the intracellular pH, Na^+ concentration and catalyzing the electroneutral counter transport of Na^+ and H^+ through the plasma membrane. The NHE-1 isoform present on muscle fibers, is activated rapidly in response to various stimuli, causing a cascade of events leading to significant increase in intracellular [Na^+] and driving the Na^+/Ca^{2+} exchanger (NCX) into reverse mode hence, triggering an intracellular [Ca^{2+}] overload.

Blocking NHE-1 receptors was shown to decrease intracellular Na^+ and Ca^{2+} overload and represents a new therapeutic avenue in DMD. Rimeporide is a safe, potent and selective NHE-1 inhibitor which has been developed until phase 1 for treatment of chronic heart failure and which showed remarkable beneficial effect on cardiac outcomes (including survival) in 2 animal models. Rimeporide has the potential to address skeletal muscular degeneration, inflammation processes and cardiomyopathy in DMD.

Rimeporide represents an innovative way to tackle Duchenne pathogenesis. EspeRare aims to accelerate the pace of the development of this new therapeutic opportunity in Duchenne patients by leveraging on the extensive data package that includes pharmacology, toxicology, manufacturing and human safety data.

EspeRare is currently executing preclinical studies in DMD models with the goal to provide convincing validation of the therapeutic potential of Rimeporide in DMD. This should allow to initiate the clinical development of Rimeporide in patients early in 2015 to hopefully show that this drug prevents the devastating consequences of Duchenne relatedmuscledamage.

✶PF2

PS1-99 / #551

Theme: *2.1 - Muscle diseases of genetic origin: Dystrophinopathy*

Comparison of rAAV6 and rAAV9 transduction in canines via jugular vein infusion

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Targeting the heart and diaphragm is critical for Duchenne muscular dystrophy (DMD) therapies.

Recent reports suggested that pre-existing neutralizing antibodies (NAb) in humans and canine models could limit the efficiency of systemically delivered recombinant adeno-associated virus serotype 6 (rAAV6)-mediated gene transfer. In this study, we compared the transduction of rAAV6, rAAV8 and rAAV9 in C2C12 cells and by direct intramuscular and systemic injections in mice and in canine striated tissues using a muscle specific CK8 promoter driven human placental alkaline phosphates reporter gene (CK8-hPLAP). Of these serotypes, rAAV6 showed the highest transduction efficiency. In contrast to studies by others, we found that only 2/47 naïve canine serum samples exhibited detectable neutralizing activity against rAAV6. These results suggest that canine source, breed or housing may lead to marked differences among dogs in separate colonies to neutralize specific subtypes of AAV. Jugular vein administration of rAAV6 and rAAV9 in the dog showed cardiac gene transfer with sub-optimal doses of rAAV6-CMV-hPLAP and rAAV6-CK8-hPLAP, with transgene expression in regions throughout the heart of rAAV6-injected dogs, while the same dose resulted in minimal expression in the heart with rAAV9-CK8-hPLAP. Further comparisons with the addition of empty capsid decoys did not enhance rAAV transduction in canines, likely due to low NAb titers. Our data suggest that rAAV6 may be the more efficient serotype for myocardial gene transfer in canines compared to rAAV9.

PS1-100 / #55

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Autosomal Recessive Limb Girdle Muscular Dystrophy: Prospective study and characterization of 280 cases by Immunohistochemistry and Immunoblotting

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Background: ARLGMD is a common disorder in Southern India due to high consanguinity. Objective: To study pattern of ARLGMD in India. Materials and methods: Prospective study of 300 cases seen between February 2010-October 2012. All suspected cases of ARLGMD attending the NMD clinic underwent phenotypic characterisation, muscle Immunohistochemistry (IHC) and Immunoblotting (IB). Results: 280 cases biopsied, 226 had IHC and 176 of these IB. 54 patients excluded. Consanguinity-45.2%. 200 confirmed to have specific ARLGMD by IHC and/or IB. Commonest form: LGMD 2B(82/246–33.33%). Mean age of onset-21.17±6.32(8–41years). Mean duration-7.24±5.86(1–36 years). Mean CK-7966.7±6029.6 IU/L(131–24037). All demonstrated dysferlin deficiency on IHC/IB. Second commonest-LGMD 2I(51/246–20.73%). Mean age of onset-12.64 ± 7.17(1–29 years). Mean duration--8.54±6.50(1–27 years) Mean CK-4059.56±3210.8(211 - 14667 IU/L). α-DG deficiency by IHC-9, IB-51. Third commonest LGMD 2C-F (35/246 -14.23%). All confirmed by IHC/IB. Mean age of onset-5.89±3.45(1–20 years). Mean duration-4.56 ±2.85(1–12years). Mean CK-8688.31±6113.86 IU/L(684–23577). Fourth group-LGMD 2A (25/246–10.16%). Age of onset-15.52±11.18(2–41 years). Mean duration-9.84 ± 9.33(2–37 years). Mean CK-2742.58±2144.96 IU/L(286–9018). All confirmed by IB. Fifth group-LGMD 2G (8/246–3.25%). Mean age of onset- 12.38±11.35(5 to 40 years).Mean duration-8.50±6.87(2 - 23 years). CK-718–9253 IU/L(mean±SD;2574.4 ±2847.52). Proximo-distal form with muscle atrophy, calf hypertrophy, foot drop. The course was slow in majority. All confirmed on IB. Last group-LGMD 2J- 2 cases. Confirmed on IHC. Conclusion: Our study confirms that LGMD 2B is the most common form of ARLGMD among our cohort. Further LGMD2I and 2G has a wider existence and may be among the common ARLGMD's in Indian population.

PS1-101 / #77

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

LAMA2-related muscular dystrophy-frequency and phenotype

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Introduction: LAMA2-related muscular dystrophy is an autosomal recessive disease typically presenting as a severe, early-onset congenital muscular dystrophy (LAMA2-CMD). However, milder cases with a limb-girdle-type muscular dystrophy (LAMA2-LGMD) have been described. In this study, we assessed the frequency and the phenotype of LAMA2 muscular dystrophy at our hospital.

Methods: The study design was cross-sectional. A total of 14 patients with CMD under age 18 years are followed at the Department of Pediatrics, of whom 6 were diagnosed with the LAMA2 subtype. A total of 113 patients with LGMD over age 18 years, are followed at the Neuromuscular Clinic, of whom 3 patients were diagnosed with the LAMA2 subtype. The clinical findings, MRI of brain, muscle pathology findings and expression of merosin in muscle tissue assessed by western blot were studied.

Results: The 3 LAMA2-LGMD patients had late-onset, slowly progressive muscular dystrophy, with onset at age 1, 10, and 56 years. They are still ambulatory at age 34, 58, and 70 years.

The 6 children diagnosed with LAMA2-CMD had marked hypotonia and muscle weakness at birth. Four of 6 have contractures, 5/6 are wheelchair dependent. One is respirator-dependent.

Brain MRI showed white matter lesions characteristic for LAMA2-related muscular dystrophy in all 9 patients.

On western blot, 6 patients had total loss of merosin, 2 had 50% merosin loss, and one had normal levels.

Molecular genetic testing demonstrated 17 pathogenic mutations in the LAMA2 gene, of which, 6 were novel.

Conclusion: Our findings show that muscular dystrophy due to LAMA2 gene mutations is not so rare as previously believed in cohorts of CMD and LGMD. Our study demonstrates a wide clinical spectrum, but generally milder phenotype than suggested by most other reports on LAMA2 myopathy. This indicates that milder cases are reported less or under-diagnosed.

PS1-102 / #83

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Beta-sarcoglycanopathy: any longer an "orphan" disease? The Family Group of Beta-sarcoglycanopathy NPO was born in 2013.

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Limb girdle muscular dystrophy (LGMD) constitutes a family of rare genetic disease characterized by progressive weakness of pelvic or shoulder girdle musculature due to impairment of the dystrophin-associated protein complex (DPC) components. The sarcoglycanopathies are a common cause of LGMDs, accounting for 3–18%, with a high percentage of severe cases. In particular, LGMD2E is a recessive autosomal disease caused by mutation in the gene, located on chromosome 4q12, encoding the beta-sarcoglycan, a major component of the DPC. Age of onset is between 2 years and the mid-teenage years. The clinical presentation includes progressive limb weakness (mainly of proximal muscles). Cardiac involvement occurs in 20% of the cases.

LGMD2E is classified as a "neglected" disease by EU, underlying a substantial absence of dedicated scientific research; no specific treatments are known and patients receive only physical therapy to prevent worsening of muscle contractures.

Of interest, sarcoglycanopathies should be cured by gene therapy since the sarcoglycans genes are relatively short and with few exons, making them suitable for adenovirus-based therapy. Actually, a phase II clinical trial for gene therapy of alpha-sarcoglycanopathy is ongoing in USA. Another possible approach relies on autologous transplantation of stem cells, such as mesangioblasts.

In 2013 was established the volunteer organization named Family Group of Beta-sarcoglycanopathy Onlus (GFB Onlus www.lgmd2e.org) to: a) contact the highest number of patients affected by LGMD and their families b) collect all data and information available on LGMD2E c) stimulate both basic and clinical research. Moreover, we would like to promote scientific research specific on LGMD2E, create collaborations with scientists to organize both informative and scientific meetings and, eventually, support researchers interested to study this disease. On April 19th 2013 the Gfb Onlus held its First National Conference in Milan.

PS1-103 / #105

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Limb-girdle muscular dystrophies in the Czech Republic

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Limb-girdle muscular dystrophy (LGMD) is defined as a muscular dystrophy with predominantly proximal distribution of muscle weakness. It includes a number of disorders with heterogeneous etiology. In this study, we determined the frequency of LGMD subtypes within a cohort of Czech LGMD2 patients using mutational analysis of the CAPN3, FKRP, SGCA, and ANO5 genes. Mutations of the CAPN3 gene are the most common cause of LGMD2 and in the set of 218 Czech probands with suspicion of LGMD2, mutations in this gene were identified in 71 patients. Totally, we detected 37 different mutations of which 12 have been described only in Czech LGMD2A patients. The mutation c.550delA is the most frequent among our LGMD2A probands and was detected in 47.1% of CAPN3 mutant alleles. The frequency of particular forms of LGMD2 was 32.6% for LGMD2A (71 probands), 4.1% for LGMD2I (9 probands), 2.8% for LGMD2D (6 probands), and 1.4% for LGMD2L (3 probands). We compared the occurrence of particular forms of LGMD2 in the Czech Republic with published studies from other European populations. Further, we present the first results of utilization of a new approach established in the Czech Republic for diagnostic practice of neuromuscular diseases: sequence capture and targeted resequencing. Using this approach, we identified two patients with mutations in the DYSF gene and one patient with mutations in the SGCB gene.

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✶PF4

PS1-104 / #113

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Tricyclo-DNA: highly promising antisense oligonucleotides for exon-skipping approaches in Duchenne Muscular Dystrophy

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Antisense oligonucleotide (AON)-mediated splice switching technology have recently moved forward in clinical applications, in particular for restoring the DMD mRNA open reading frame and the production of functional dystrophin in skeletal muscles of patients with Duchenne muscular dystrophy (DMD). While most popular naked AONs used for exon skipping, such as 2'-O-methylated RNA with phosphorothioate backbone (2OMe) or phosphorodiamidate morpholino oligomers (PMO) have demonstrated encouraging results, both chemistries failed to restore dystrophin to significant levels in heart or diaphragm, and latest reports point up that overall therapeutic efficacy was still limited if exists. To overcome these limitations, we investigated the therapeutic potential of a new class of conformationally constrained DNA analogues: the tricyclo-DNAs (Tc-DNA). In this study, we show that TcDNA AONs are capable of spontaneously forming nanoparticles in solution, which represent an attractive feature for systemic delivery. These properties led to widespread restoration of dystrophin in the mdx mouse following systemic injections of Tc-DNA targeting the donor splice site of the dystrophin exon 23. Remarkably, TcDNA treatment also leads to restoration of dystrophin in the cardiac muscle and detection of exon skipping in the CNS. Importantly, we demonstrated for the first time physiological improvement of the respiratory function and correction of behavioural features linked to the cognitive deficiency associated with the lack of dystrophin in the CNS. Finally, clinical benefit was also demonstrated in the severe mdx/utr- (dKO) mouse model exhibiting most of the clinical signs observed in DMD patients.

PS1-105 / #119

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Executive function and visual memory computerized testing in myotonic dystrophies

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Background: Patients with myotonic dystrophy type 1 (DM1) and 2 (DM2) show typical cognitive deficits with a pronounced impairment in executive and visual-spatial domains. There is still some controversy regarding differences of these neuropsychological functions in DM1 and DM2.

Aim: To compare executive function and visual memory ability in patient with DM1 and DM2 using computerized battery of cognitive tests.

Method: Study comprised 30 DM1 patients and 27 DM2 patients matched for gender (74% vs. 73% females, $p > 0.05$), and of similar age (49 ± 7 vs. 53 ± 10 years, $p < 0.05$) and disease duration (20 ± 8 vs. 16 ± 12

years, $p > 0.05$). Patients underwent Cambridge Neuropsychological Test Automated Battery (CANTAB), including following tests: Pattern Recognition Memory (PRM) and Spatial Recognition Memory (SRM) for visual memory; Spatial Working Memory (SWM), Stockings of Cambridge (SOC), Spatial Span (SSP) and Intra/Extradimensional Set Shift (IED) for executive functions. Results <1SD of healthy population were considered abnormal.

Results: DM1 patients had lower percentage of correct responses on both PRM and SRM (80±13 vs. 87±10 and 72±13 vs. 80±9, respectively, $p < 0.05$). Abnormal results on these tests were found in 20% of DM1 vs. 15% of DM2 patients and 30% of DM1 vs. 11% of DM2 patients, respectively ($p > 0.05$). Patients with DM1 also had worse achievement in frontal lobe tests: number of errors on SWM (56±15 vs. 36±18, $p < 0.01$), number of correct tasks on SOC (5.4±1.7 vs. 6.7±2.3, $p < 0.05$), and the longest sequence on SSP (4.4±1.1 vs. 5.5±0.9, $p < 0.01$). Frequency of DM1 vs. DM2 patients with abnormal results on SWM were 33% vs. 12% ($p > 0.05$), on SOC 73% vs. 50% ($p > 0.05$), and on SSP 53% vs. 7% ($p < 0.01$). Number of completed levels on IED was similar in DM1 and DM2 patients (7.8±1.0 vs. 7.4±1.6, $p > 0.05$), and 60% vs. 63% of patients were below norm ($p > 0.05$).

Conclusion: Executive dysfunction was more pronounced than visual memory impairment in myotonic dystrophies. Frontal and temporal lobe functions were more impaired in DM1, while frontal-striatal dysfunction was similar in both DM1 and DM2.

PS1-106 / #121

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Transcranial sonography in patients with myotonic dystrophy type 2

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Background: Transcranial sonography (TCS) is useful for differential diagnosis of distinct neurodegenerative and affective disorders. Recently, we published a paper about TCS in myotonic dystrophy type 1 (DM1) that offered a new insight into structural changes of several cerebral areas in these patients.

Aim: To analyze TCS findings in patients with DM2.

Method: This cross-sectional study comprised 30 DM2 patients and 30 matched healthy controls (HCs) (73% females, 52±10 years old). Echogenicity of the brainstem raphe (BR) and substantia nigra (SN), as well as the width of the third ventricle (DTV) were assessed by TCS. We also used Hamilton Depression Rating Scale, Fatigue Severity Scale and Daytime Sleepiness Scale.

Results: BR hypoechogenicity was more common in DM2 patients than in HCs (50% vs. 10%, $p < 0.01$). BR hypoechogenicity was present in 60% of patients with vs. 40% of patients without depressiveness ($p > 0.05$), in 67% of patients with vs. 39% without fatigue ($p > 0.05$), and in 86% of patients with vs. 39% without excessive daytime sleepiness ($p < 0.05$). Hypoechogenicity of SN was similarly frequent in DM2 and HCs (10% vs. 7%, $p > 0.05$), while hyperechogenicity was more common in DM2 (23% vs. 0%, $p < 0.05$). DTV was similar in DM2 patients and HCs (5.5 ± 1.6 vs. 5.1 ± 0.9 mm, $p > 0.05$).

Conclusion: TCS revealed the high percentage of BR hypoechogenicity in DM2, especially associated with excessive daytime sleepiness. Increased frequency of SN hyperechogenicity was also observed. Further studies on a larger number of patients in comparison to other imaging techniques and histopathology will be of major importance.

PS1-107 / #122

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Assessment of pain in myotonic dystrophies

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Background: It was not until recently that pain was identified as a significant symptom in noninflammatory neuromuscular diseases, including myotonic dystrophies. In these disorders pain even may be the most disabling symptom with significant influence on quality of life (QoL).

Aim: To assess pain locations, intensity and interference in patients with myotonic dystrophy type 1 (DM1) and 2 (DM2).

Method: Study comprised 45 DM1 patients and 33 DM2 patients matched for gender (60% vs. 73% females, $p>0.05$) and age (54±11 vs. 51±9 years, $p>0.05$), while disease duration was longer in DM1 (21±10 vs. 16±13 years, $p<0.05$). Modified Brief Pain Inventory (BPI) was used to investigate pain characteristics, while SF-36 and Individualized Neuromuscular QoL questionnaire (INQoL) were used for QoL assessment.

Results: In the last four weeks pain other than everyday minor pain was reported in 51% of DM1 and 36% of DM2 patients ($p>0.05$). In DM1 pain was mostly located in lower legs (42%), lower back (31%) and knees (29%). The most common pain locations in DM2 were: lower legs (46%), upper legs (36%), lower back (33%) and head (21%). Mean pain intensity was similar in both groups of patients (3.5±2.4 in DM1 vs. 2.9±2.0 in DM2, $p>0.05$), as well as mean pain interference (3.9±2.9 vs. 3.6±2.3, $p>0.05$). In patients with DM1 the most severe pain interference was in walking ability and general activity domains, while in DM2 it was related with enjoyment in life and mood. Significant percentage of patients used pain killers (53% of DM1 and 42% of DM2 patients, $p>0.05$) with mean pain relief of 62±35% vs. 68±35%, respectively ($p>0.05$). QoL measured with both SF-36 and INQoL was in correlation with pain intensity and interference in both groups ($p<0.01$).

Conclusions: Similar degree of pain intensity and of overall pain interference was observed in DM1 and DM2 patients. Pain had exceptional impact on QoL in both forms of myotonic dystrophy.

PS1-108 / #135

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Oculopharyngeal Muscular Dystrophy in Population of the Czech Republic

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Oculopharyngeal muscular dystrophy is an autosomal dominant muscular dystrophy due to amplification of triplet GCG in the polyadenylate binding protein nuclear 1 gene (PABPN1) located on chromosome 14q11.2-q13. The first symptom can be even in fourth or sixth decade wasting of facial muscles, further symptoms are characterized by dysphagia, progressive ptosis of the upper eyelids and later weakness of limb-girdle muscles and anal or urethral sphincter can be also involved. Prevalence of mutation is very variable in different geographic regions (the highest was estimated in Bukchara Jews in Izrael 1:600 and in Quebec 1:1000 and in opposite very low in France 1:200.000. This mutation is very stabile and shows no anticipation phenomenon. We have correlated the phenotype and genotype of 36 patients from five undependent families and we found no correlation between the beginning of symptoms, the way of progression, severity of phenotype and number of triplet GCG repetitions.Prevalence of OPMD in Czech Republic can be only estimated (35/10 mil it is 1:285 700) and carriers of mutation event. premutation are substantionally more common, because the majority of young relatives of probands with genetic risk 50% refused the presymptomatic testing.

PS1-109 / #158

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Cardiac arrhythmias in patients with laminopathies

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Purpose: Laminopathies are a wide group of diseases associated with mutations in genes encoding nuclear membrane proteins. Emery-Dreifuss Muscular Dystrophy (EDMD) is the most prevalent neuromuscular phenotype in this group. There are two main types of EDMD: depending on emerinopathy (EMD gene; EDMD1) and on laminopathy (LMNA gene, EDMD2). Cardiac involvement, including systolic dysfunction and arrhythmia is common in laminopathic patients (pts). The aim of this study includes analysis of clinical presentation, observation of cardiac arrhythmias and assessment of sudden cardiac death risk in pts with two types of EDMD.

Methods: 34 pts with genetically confirmed EDMD: 24 EDMD1 and 10 EDMD2 (5 males, 5 females) were prospectively observed. All pts had done serial conventional electrocardiography, 24-hours Holter monitoring and echocardiography. In pts with pacemaker implanted data from device were stored.

Results: The mean follow-up was 67.6 +/- 22.1 months. Mean age at the start of follow-up was 29.6 +/- 11.4. 11/34 of pts (32.3%) had symptomatic heart failure; New York Heart Association (NYHA) class II/III/IV (6/3/2), respectively. 20/34 of pts (58.8%) had implanted pacemaker due to bradycardia. In 24/34 of pts (71%) advanced atrio-ventricular block was diagnosed. 16/24 of pts (66.6%) presented supraventricular arrhythmias (SVA), while 5/34 of pts (14.7%) had complex ventricular arrhythmias (VA). There was a trend for significant difference between EDMD1 and EDMD2 pts in terms of SVA (20.0 vs. 58.3 %, $p = 0.09$). During follow-up in 4/20 (20%) patients with pacemaker atrial standstill (absence of atrial electrical and mechanical activity) was found; therefore atrial pacing was no more effective. 5/34 of pts (14.7%) suffered of ischemic stroke, despite anticoagulation. 3/34 of pts (8.8%) died suddenly during follow-up.

Conclusions: Conduction defects requiring pacemaker implantation and early supraventricular arrhythmias are frequently present in pts with laminopathies. Atrial arrhythmias seem to be more common in pts with EDMD1 than EDMD2. Atrial standstill isquite frequent. There is a high risk of sudden death and thrombo-embolic complication despite treatment.

PS1-110 / #169

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Mutation in LMNA gene presenting as an overlapping syndrome with cardiomyopathy and muscle fibre type disproportion (FTD)

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Lamins are structural components of the nuclear inner membrane committed to the maintenance of nuclear shape and structure, with also functional role in transcriptional regulation and heterochromatin organization. Mutations in the lamin A/C protein cause laminopathies, a very heterogeneous group of hereditary disorders including recessive form of axonal neuropathy (CMT2B1) , Emery-Dreifuss muscular dystrophy (EDMD) , limb-girdle muscular dystrophy (LGMD), a dilated cardiomyopathy with conduction defect, different form of lipodystrophy and progeria syndromes.

In this work we provide a clinical picture of a family with an autosomal dominant mutation (heterozygous mutation c.80C>T; pT27I) in the LMNA gene. Three patients (2 men and 1 female, age range 33–58 years, presenting LGMD phenotype with onset in early thirties, cardiac conduction defect and/or dilated cardiomyopathy and cardiac conduction defect, were studied. Three further family members died for sudden cardiac death before diagnosis was available.

Interesting, muscle biopsies performed in 2 cases showed changes consistent with fibre type disproportion (FTD). In particular type 1 muscle fibres were consistently smaller compared to type 2 fibres (more than 50% of average diameter) .

To our knowledge this is the first report of FTD as the distinctive finding in muscle biopsy of patients with LMNA mutations. For this reason, we recommend to include molecular testing for LMNA in the differential diagnosis of myopathies with FTD in consideration of life threatening events.

PS1-111 / #171

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

The mildest end of the dystroglycanopathy phenotypic spectrum: from asymptomatic hyperckaemia to limb girdle muscular dystrophy

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Dystroglycanopathy refers to a heterogeneous group of disorders associated with aberrant glycosylation of α-dystroglycan (aDG). To date a molecular defect of more than 10 different proteins implicated in the glycosylation of α-dystroglycan have been shown to cause a dystroglycanopathy phenotype. The group encompass a striking variability of clinical manifestations ranging from severe congenital muscular dystrophy with brain and eye abnormalities often resulting in early infantile death, such as Muscle-eye-brain (MEB) and Walker-Warburg (WWS) syndromes, to milder cases presenting in adult life with limb girdle muscular dystrophy (LGMD) without central nervous system or eye involvement.

In this work we describe clinical, genetic, morphological and muscle MRI features of a small group of patients with proven genetic defect of aDG, presenting at the mildest end of the clinical spectrum.

8 patients (age range 12-40) were either diagnosed as asymptomatic hyperckaemia (4 patients) or early LGMD (3 patients). One child presented with elevated CK and mild intellectual impairment.

Mutations in the following genes were identified: FKRP (4 patients), POMT2 (2 patients), POMT1 (1 patient) and FKTN (1 patient). Muscle biopsy showed mild to moderate signs of necrosis, degeneration-regeneration and inflammation. Immunolabelling of glycosilated aDG was significantly reduced in all.

Our data reinforce the notion that defect of aDG can result in a very mild phenotype of muscle involvement from subclinical hyperckaemia of children to adult onset LGMD, stressing the importance of aDG testing in routine histological analysis.

PS1-112 / #179

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Exploring mitochondrial dysfunction in CAPN3 related myopathy

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Limb-girdle muscular dystrophy type 2A (LGMD2A) is the most frequent form of recessive LGMD worldwide and it is caused by a defect of calpain-3 gene. Calpain-3 is a muscle specific, calcium dependent, multi-substrate cysteine protease. The exact

pathomechanism underlying muscle damage due to calpain-3 deficiency, remains largely unclear. Animal model (CAPN3 KO mice) exhibits morphological and biochemical evidence of mitochondrial abnormalities in muscle, including irregular distribution of mitochondria and reduced *in vivo* mitochondrial ATP production. These findings have never been reproduced nor quantified in patients' tissue.

In this work we investigated pathological effects of calpain-3 mutations in myoblasts from LGMD2A patients, in terms of a putative mitochondrial dysfunction. In particular we examined the activity and amount of respiratory chain (RC) enzymes, cellular ATP level and ROS production.

Routine histochemical stains for oxidative metabolism in muscle biopsies revealed the typical aspect of subsarcolemmal accumulation of mitochondria. Measurement of RC enzymes revealed reduction of complex I and IV in one case and of complex III in another case, whereas the immunodetection pattern of the RC complexes was within normal values.

Luminometric measurement of ATP in patient's cultured myoblasts showed a specific reduction of ATP content compared to control cells in the presence of 2-deoxyglucose and pyruvate, a condition that supports only mitochondrial ATP synthesis.

We also observed a statistically significant increase of ROS production in patients fibroblasts after a short term H2O2 treatment.

Taken together these data support evidence for a secondary mitochondrial damage with energy production defect and increased oxidative stress also in human calpainopathy.

PS1-113 / #210

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

The relationship of calpain 3 and titin in the M-band

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Several published studies strongly implied the existence of functional relationship between calpain 3 and titin proteins. Calpain 3, the cysteine protease mutated in Limb Girdle Muscular Dystrophy type 2A (LGMD2A), has at least 2 different binding sites on titin, which is also one of calpain 3 substrates. Moreover, Tibial Muscular Dystrophy (TMD) and LGMD2J, both caused by the mutations in the last exons of the titin gene, are characterised by secondary reduction of calpain 3 expression. We also showed that the last domains of titin are not present in the TMD/LGMD2J muscle.

Here, we tested several calpain 3 properties *in vivo* and *in vitro* conditions to better clarify the relationship between calpain 3 and titin in the M-band of the sarcomere, as well as its possible implication in the pathogenesis of LGMD2A and LGMD2J. An *in vitro* analysis validated the C-terminal part of the titin protein as a proteolytic substrate for calpain 3. Moreover, we demonstrated that TMD/LGMD2J mutations induce disruption of these cleavages *in vitro* while calpain 3 is still able to interact with titin. Interestingly and unexpectedly, we showed that the disappearance of the M-band titin seen *in vivo* as a consequence of TMD/LGMD2J mutations is still observed in the absence of calpain 3, indicating that this event is not caused by a calpain 3 proteolytic cleavage.

PS1-114 / #212

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

AAV-mediated transfer for ANO5-linked diseases

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Dominant mutations in the ANO5 gene were identified associated with gnathodiaphyseal dysplasia (GDD), a disorder of the bones. Recently, loss-of-function mutations in ANO5 were identified as the cause of an autosomal recessive form of LGMD (LGMD2L) and a distal non-dysferlin Miyoshi muscular dystrophy (MMD3). As a first step towards testing therapeutic approaches for these diseases, we constructed an Ano5 knockout mouse model. This model was validated and characterized at the molecular and protein level. Histological and phenotypical characterization of the muscle level is ongoing. In the

mean time, we defined the most prevalent ANO5 isoform present in human skeletal muscle and constructed an AAV vector encoding this form. This AAV vector was injected intramuscularly and intravenously into WT mice at different doses during different period of time to initially determine whether overexpression of ANO5 in mouse muscle could be deleterious. No adverse effects were seen histologically either in muscles or in heart after 3 months of expression. This AAV vector will now be injected in our KO mouse model to thereby determine if ANO5 could be a therapeutic tool for ANO5-linked diseases in human.

PS1-115 / #213

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Sarcoglycanopathy in Iran

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Sarcoglycanopathies are a group of autosomal recessive limb girdle muscular dystrophies. So far there is no comprehensive documented study about sarcoglycanopathies from Iran. This study is a retrospective analysis of case series. We reviewed the results of 1400 muscle biopsies of patients referred to our center from different parts of the country during 2009 to 2014. The patients with clinical and histopathological diagnosis of limb girdle muscular dystrophy and immunohistochemical diagnosis of sarcoglycanopathy were selected. 54 patients were diagnosed as sarcoglycanopathy, 52 with Iranian nationality, one from Iraq and one from Azerbaijan. We collected clinical and paraclinical data from them and their affected family members. We also searched for the patients who had genetic confirmation. 92 patients were found among the 54 affected families. Male to female ratio was 44/48. Geographical distribution indicated that 21 out of 52 of the affected Iranian families were coming from the southeast provinces of Iran. There was one adapted child with no family data and only 3 patients were not born from consanguineous parents. Mean age at the time of diagnosis was 10.5 years (range 2–21) and mean age at the onset of symptoms was 5.5 years (range 1–11). Only 2 patients show cardiac abnormality. The highest documented serum Creatine Kinase level was 26000 U/L. The most common IHC finding was complete loss of both alpha and gamma sarcoglycans. In only 2 patients isolated loss of alpha sarcoglycan was seen and 7 patients had isolated loss of gamma sarcoglycan. Molecular analysis was done in only 10 patients. 5 patients had mutations in beta sarcoglycan gene, and 4 patients had abnormal finding in gamma sarcoglycan gene. In 1 patient no mutation was found in alpha, beta or gamma sarcoglycan genes. We recommend that sarcoglycanopathy should be considered as an important differential diagnosis in a child born to consanguineous parents who presents with proximal muscle weakness, calf hypertrophy, elevated CK level, and myopathic pattern on EMG, specially ifthey have positive family history. Also based on our study sarcoglycanopathy in girls with Duchenne-like phenotype is more likely the diagnosis than manifesting carrier of dystrophinopathy in Iran. More genetic analysis of our patients is needed to show the prevalence of different sarcoglycanopathies in Iran and the middle-east region.

PS1-116 / #216

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Properties of mutant R388P of A-type lamins responsible for severe myopathy and lipodystrophy in patient skin fibroblasts and in HeLa cells

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A-type lamins (LA and LC) encoded by LMNA gene are nuclear proteins that belong to the intermediate filament proteins family, involved in nuclear structure and gene expression. Hundreds of LMNA mutations are responsible for diverse human diseases, including muscular dystrophies, lipodystrophies, and premature ageing syndromes. Here we report the identification of a novel heterozygous de novo LMNA substitution R388P in a young female. The clinical phenotype was mixed, including a muscular deficit revealed very early (12 month old) together with cardiac rhythm defects and an abnormal repartition of adipose tissue which started with puberty.

Primary skin fibroblasts issued from the patient were cultured ex-vivo. LA/C proteins were detected at similar levels in control and patient fibroblasts. Immunofluorescence analysis revealed in a subpopulation (~4% of the cells) an abnormal nuclear distribution of A-type lamins, with a honeycomb-like pattern. In comparison to proliferating control cells at the same passage number (12–14), patient fibroblasts showed signs of senescence with reduced proliferative capacity and decreased lamin B1 expression.

Further investigation of Lamin A R388P properties was done by overexpressing it with a FLAG tag in HeLa cells. Biochemical analysis revealed the increased soluble properties upon 0.5% Triton extraction of the mutant LA versus wild-type LA. Accordingly, FLAG-LA R388P did not integrate properly at the nuclear envelope, but accumulated in the nucleoplasm either diffusely (~80 % of the cells) or into ring like structures (~20 % of the cells).

Our data suggest that the lamin A mutant R388P would change the assembly properties of A-type lamins, and that depending on its expression level and cellular context would lead to abnormal A-type lamins intranuclear distribution and/or defects in cellular functions as proliferation.

PS1-117 / #218

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Muscle pathology meets “next-gen sequencing data haystack”: establishing the first diagnosis of DNAJB6-related myopathy in Austria

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We report on a 52-years old female patient with a 20 years history of progressive proximal muscle weakness and exercise-induced muscle pain. EMG revealed a chronic myopathic patterns. CK-levels were moderately elevated (400 U/L). The family history was uninformative. Comprehensive and detailed clinical investigations were performed.

A gastrocnemius muscle-biopsy revealed mixed myopathic/neurogenic changes apart from numerous muscle fibers with sarcoplasmic core-like inclusions, which consisted of vacuolar structures with lysosomal inclusions. Genetic testing of SEPN1 (suspecting a core myopathy) and of SCN4A (vacuolar inclusions) was negative. Consequently a whole-exome sequencing approach was performed in order to identify the causative gene mutation.

Exome capture and next-generation sequencing of the patient’s DNA revealed a pathogenic missense

mutation (c.279C>G) within the DNAJB6 gene, which had been previously described to be causatively involved in autosomal dominant limb-girdle muscular dystrophy type 1E / myopathy (OMIM #603511). The mutation has been shown by others to cause the exchange of a highly conserved phenylalanine residue (p.Phe93Leu) in the co-chaperone DNAJB6, thereby introducing a dominant-negative effect, resulting in protein aggregation due to compromised chaperone function. Based on two published articles reporting clinical symptoms and muscle biopsy findings of patients with the same mutation in 5 Finnish and 3 Japanese families, the convincing diagnosis of a DNAJB6-related myopathy in our patient could be reached.

As typical for sequencing projects involving single patient's exomes, more than 50 rare or unknown variants within genes causatively associated with neuromuscular disorders were identified in the patient's exome, which obviously would severely complicate a data analysis strategy based on genetic data only. This case report therefore demonstrates that detailed clinical investigations in conjunction with a comprehensive analysis of a muscle biopsy are indispensible prerequisites to guide the interpretation of state-of-the-art genome-wide molecular diagnostic assays, in order to reach a final conclusive genetic diagnosis.

PS1-118 / #221

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Determination of the best expression cassette for an AAV-mediated gene transfer – The case of a muscle specific protease, Calpain 3

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Limb-Girdle Muscular Dystrophy type 2A (LGMD2A) is caused by mutations in the CAPN3 gene encoding the Calpain 3 (C3) protein. This skeletal muscle disease affects predominantly the proximal limb muscles. Previously, we demonstrated the potential of adeno-associated virus (AAV)-mediated transfer of the human CAPN3 gene to correct the pathological signs in a C3 deficient mouse model after intramuscular delivery. However, the systemic administration was associated with a cardiac toxicity related to the proteolytic activity of the C3 protease. This toxicity was prevented by constructing vectors with a skeletal muscle restricted expression using miR-regulation and specific promoters (Bartoli et al., 2006, MolTher; Roudaut et al., 2013, Circulation). However, in these studies, the level of expressed protein is still too low to fully restore the phenotype of deficient mice. To improve the efficiency of expression, a number of optimization of the cassette at the level of the promoter, transgene and regulatory elements have been performed and are currently been evaluated *in vivo*. Results will be presented.

PS1-119 / #222

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Hepatic AAV-mediated gene transfer to reduce immune responses against alpha-sarcoglycan

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Alpha-Sarcoglycanopathy (Limb-Girdle Muscular Dystrophy type 2D, LGMD2D) is a recessive muscular disorder caused by deficiency in α-sarcoglycan (SGCA), a transmembrane protein part of the dystrophin-associated complex. We previously reported efficient systemic AAV-mediated transfer of SGCA in Sgca deficient mice (Sgca-null mice), resulting in correction at the biochemical, histological and functional levels. Whereas delivery of the AAV vector by the systemic route was efficient, a specific humoral immune response directed against α-sarcoglycan was observed after intramuscular injection, leading to disappearance of transgene expression in muscle fibers, production of antibodies against the transgene and presence of CD8 T lymphocytes around the transduced fibers. We hypothesized that transduction of the liver could play a crucial role in the absence of rejection of the transgene product after systemic injection. To validate our hypothesis, Sgca-null mice injected intramuscularly with an AAV6-SGCA vector inducing an immune response were concomitantly injected in liver with an AAV9-SGCA. We observed that the liver transduction reduced significantly the immunogenic features induced by the intramuscular injection.

Indeed, an improvement of the stability of the transduced fibers, a decrease of antibodies directed against the SGCA protein and a reduction of CD8 T lymphocyte infiltrates around the transduced fibers were detected. In parallel, the liver injection did not change the humoral immune response against the capsid. In conclusion, we propose that AAV liver transduction plays an essential role in the tolerance of the SGCA transgene product after systemic administration.

PS1-120 / #224

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Serum MYOM3 fragments as new biomarker for the follow-up of LGMD2D therapeutic treatment

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Muscular dystrophies are devastating neuromuscular diseases for which no treatment exist up to now. Currently, the diagnosis process involves the measurement of serum creatine kinase (CK) levels, which are released from the muscle following myofiber tearing. However, the serum level of this biomarker is highly sensible to physical exercises or other conditions that cannot be controlled. It is commonly agreed that CK level is insufficient for evaluation of disease progression or the efficiency of therapeutic treatment. The advent of neuromuscular disorders clinical trials needs valuable biomarkers to follow up the short and long time effects of treatments. Recently, by using a mass-spectrometry bottom-up approach, we identified the specific presence of Myomesin 3 (MYOM3) fragments in the serum of patient with Duchenne Muscular Dystrophy (DMD). The same MYOM3 fragments were also found in sera of DMD animal models: mdx mice and GRMD dog.

To define whether the presence of serum MYOM3 is a specific feature of DMD patients linked to the dystrophin deficiency or a biomarker that can be used for other neuromuscular disorders, we analyzed sera from 3 available patients with α-sarcoglycanopathy (LGMD2D) and from mice model deficient in α-sarcoglycan (Sgca-null). In all studied sera, Western blot analysis demonstrated the presence of MYOM3 fragments of the same size as in DMD patients.

Sgca-null mice model permitted us to study the evolution of the MYOM3 fragments during gene therapy treatment. For this purpose, Sgca-null mice received intravenous injection of two different doses of an AAV8 coding for human α-sarcoglycan. Interestingly, the level of the MYOM3 fragments in sera of Sgca-null mice decreased with the increase in gene transfer efficiency which was associated with reconstitution of the muscle morphology and improvement of physical strength. This effect was already visible 14 days after the AAV injection and persisted at least 56 days post-injection. This experiment also demonstrated a tight negative correlation between the restoration of muscle force and the level of serum MYOM3 fragments after the treatment, while no correlation was seen between CK level and muscle force. Myomesin3 could provide a new important monitoring tool of neuromuscular disorders treatment.

PS1-121 / #225

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Limb Girdle Muscular Dystrophy 2I: Characterization of a new mouse model and testing AAV gene transfer

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Dystroglycanopathies constitute a group of genetic diseases caused by defective glycosylation of alpha-dystroglycan (aDG), a membrane glycoprotein involved in the cell/matrix anchoring of muscle fibers. The aDG glycosylation, a very complex process, requires many proteins whose functions are not fully elucidated. In particular, mutations in the FKRP gene encoding Fukutin related protein, lead to hypoglycosylation of aDG, resulting in different forms of dystroglycanopathies, among which Limb Girdle Muscular Dystrophy type 2I (LGMD2I).

We generated a knock-in mouse model of LGMD2I, carrying the most frequent mutation (L276I) encountered in LGMD2I patients. Molecular characterization of this mouse model showed that the introduction of the mutation did not alter the expression of FKRP. However, the protein appears to have altered function since abnormal glycosylation of aDG was observed. Histologically, the muscles of this model show a dystrophic pattern starting from 6 months of age, consisting both in the presence of central nuclei and in fiber size variability. Interestingly, functional muscle impairment can be observed as early as 2 months of age by a decrease of the muscle resistance to eccentric mechanical stress.

To evaluate gene transfer as a therapeutic approach, we cloned the FKRP cDNA in an AAV vector under the transcriptional control of the desmin muscle promoter. The recombinant AAV2/9 vector was injected intramuscularly or intravenously in the mouse model. Expression of the FKRP transgene was obtained, both at RNA and protein levels. In systemic conditions, a histological rescue was observed by the decrease of central nuclei. The AAV vector also improved the muscle function, since it conferred a better resistance to eccentric stress to the injected muscles.

PS1-122 / #281

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Clinical characterization, measure of disability and mutational spectrum in a Chilean cohort of patients with dysferlinopathy

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Thirty-one Chilean patients with dysferlinopathy were investigated for a genotype-phenotype correlation, and measuring the degree of motor, respiratory and cardiac impairment. Dysferlinopathy was diagnosed based on clinical and biopsy findings, and confirmed by DYSF sequencing. Assessment included Motor Function Measure (MFM), Modified Ranking Scale (MRS), serum CK, baseline spirometry, echocardiogram and electrophysiology. Eight mutations were recurrently identified, four of which (c.5979dupA; c.2858dupT; c.2779delG and c.4390G>T) accounted for 77.4% of the total. Four patients showed only one mutation despite complete DYSF sequencing. Mean onset age was 20.48 years. Symptoms initiated in the legs, distally (22/31) or proximally (9/31), and progressed later on to the upper limbs. Mean serum CK level was 58 (±36) times above normal values. MFM showed greater impairment in the standing and transfers (D1) and axial/proximal (D3) subscores, with relative sparing of distal motor function (D2). Disease duration has a significant correlation with total MFM score and all subscores, but not with onset age, clinical phenotype or CK levels. A significant negative correlation was found between MFM and MRS, and between percentage forced vital capacity (%FVC) and both, disease duration (r=-0.6, p=0.004), and onset age (r= 0.46, p=0.04), but not with ejection fraction. Repetitive nerve stimulation and single fibre electromyography assessment was normal in all patients assessed.Recurrence of some mutations suggests a founder effect in Chilean population. Correlation between MFM and MRS scores and %FVC with disease duration may be useful for the follow-up of patients with dysferlinopathy in further studies.FONDECYT#1110159 ANILLO ACT1121 (Conicyt, Chile).

PS1-123 / #304

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Molecular mechanisms and therapeutic approaches for sarcoglycanopathies

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Sarcoglycanopathies are recessive muscular dystrophies (LGMD2D, E, C, F) caused by mutations in genes coding for α, β, γ or Δ-sarcoglycans. Those transmembrane proteins are associated in a complex interacting with dystrophin to protect muscle fibers against mechanical stress due to contraction. Loss of membrane expression of one sarcoglycan can cause the absence of the entire complex at the membrane. Sixty-six% of the mutations found in patients are missense mutations; some of them being highly prevalent like R77C, the most frequent mutation in α-sarcoglycanopathy.

We previously demonstrated that mutated sarcoglycans are retained in the endoplasmic reticulum by the quality control (ERQC), and that it is possible to rescue the mutated protein by used specific inhibitor of a key ERCQ enzyme: α-mannosidase I, such as kifunensine. *In vitro* analysis in transfected human cells showed that 7 mutated sarcoglycans are sensitive to the action of kifunensine. In addition, *in vivo* efficacy was also demonstrated on an α R77C animal model. The generationand validation of a stable cell line will enable us to perform high-throughput screening and dissection of the underlying molecular pathway will be presented.

PS1-124 / #320

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

A simple questionnaire for screening patients with myotonic dystrophy type 1

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Background: Myotonic dystrophy type 1 (DM1) is a multi-systemic disease and many patients visit various departments before they receive diagnosis of DM1. A part of patients were diagnosed as DM1 after peripartum or perioperative troubles. Objectives: Development of a useful screener for DM1 to avoid such troubles and to introduce multidisciplinary approach smoothly. Results: As a first step, we evaluated the sensitivity and specificity of physical examination and questionnaire in a small number of patients. In this step we found that well designed questionnaire is comparable to physical examination. Then we planned to assess the sensitivity and specificity of self-questionnaire with nine items (cataract: C, whistle: W, dysphagia: D, head-lift: H, sit-up: S, uncapping: U, drop foot: F, myotonia: M and family history of myopathy). Total 347 subjects including 95 DM1, 121 healthy controls (HC) and 131 disease controls (DC) cooperated with this study. Participants answered all questions to their own discretion. Family history was excluded from statistical analysis since many HC were family members of DM1. The sensitivities were quite variable among items and exceeded 70% in only three items (S, F, M) in mild DM1 cases requiring no assistance in daily living. The specificities of all items exceeded 80% in HC and surpassed 95% in three items (H, U, M). The specificities were variable in DC and exceeded 75% in four items (C, H, U, M) in mild cases. Analyses using receive operating characteristic (ROC) curve revealed that combination of H+U+M had the largest values of area of under the curve. The sensitivity of this combination was 100% in mild DM1. The specificity was 98.3% in HC and 69.2% in mild DC. The combination of H+S+U+M had also quite high values. Conclusions: Our study showed that self-questionnaires with a few items enable us to identify DM1 effectively. We hope that disseminating this simple screening will facilitate detection of mild cases and early introduction of multidisciplinary care.

PS1-125 / #348

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

New perspectives in LGMD-2D therapy: small molecules "to cure" the mutated alpha-sarcoglycan

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The key pathogenetic event of an extremely heterogeneous group of genetic diseases, collectively called Unfolded Protein Diseases (UPD), is the presence of gene mutations that cause either unfolding or misfolding of a coded protein. This usually leads to loss of function because of either aggregation or premature disposal of the mutant by the cell's quality control system. Type 2D Limb Girdle Muscular Dystrophy (LGMD-2D) is a genetic disease of striated muscles showing traits of UPDs: i.e. normal level of the mutated transcript but almost undetectable level of the coded product, alpha-sarcoglycan. Evidence demonstrates that alpha-sarcoglycan mutants are substrate of the ER associated protein degradation (ERAD) terminating in proteasomal destruction (Gastaldello et al., 2008).As no effective therapies are available for this neglected disorder, our efforts aim to identify a possible cure by counteracting protein removal. We utilized two different pharmacological approaches either "to save" the mutated protein by preventing its degradation or "to repair" its folding and improve trafficking. To prevent degradation of alpha-sarcoglycan mutants via drug treatments, it is mandatory to identify the key components of its degradative route, a quality control pathway so far unexplored. To this end, we studied the second most frequently reported mutation in LGMD-2D (V247M), taking advantage of a cell model expressing only alpha-sarcoglycan. By disclosing the ERAD pathway, which is led by two E3 ligases, HRD1 and RFP2, we identified new potential druggable targets. Notably, we demonstrated that the pharmacological inhibition of HRD1 rescues V247-alpha-sarcoglycan both in the cell model and in myotubes derived from a LGMD-2D patient carrying the L31P/V247M mutations. This represents the first evidence that the activity of E3 ligases is eligible for drug interventions to treat sarcoglycanopathy (Bianchini et al., submitted). Regarding the protein repair strategy, we are testing several small molecules that, by assisting folding, promote maturation and trafficking of mutants. Our data demonstrate that drug treatments not only preserve alpha-sarcoglycan mutants from degradation but also permit their proper localization. Altogether our results constitute the proof of principle for the development of innovative pharmacological therapies for the cure of rare muscle diseases.

✶PF1

PS1-126 / #356

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Full-Length Dysferlin Expression Driven by Engineered Human Dystrophic Blood-Derived CD133+ Stem Cells

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The protein dysferlin is abundantly expressed in skeletal and cardiac muscles, where its main function is membrane repair. Mutations in the dysferlin gene

are involved in two autosomal recessive muscular dystrophies: Miyoshi myopathy and limb-girdle muscular dystrophy type 2B. Development of effective therapies remains a great challenge. Strategies to repair the dysferlin gene by skipping mutated exons may be suitable only for a subset of mutations, while cell and gene therapy can be extended to all mutations. Herein, we show for the first time the *in vitro* production of full-length dysferlin mediated by a lentiviral vector in blood-derived CD133+ stem cells isolated from patients with Miyoshi myopathy. Transplantation of engineered blood-derived CD133+ stem cells into scid/blAJ mice resulted in sufficient dysferlin expression to correct functional deficits in skeletal muscle membrane repair. Multi-exon skipping of blood-derived CD133+ stem cells isolated from the same patients led to partial dysferlin reconstitution *in vitro*, but failed to ameliorate the dystrophic phenotype *in vivo*. Our data suggest that lentivirus-mediated delivery of full-length dysferlin in stem cells isolated from Miyoshi myopathy patients is a feasible strategy to develop novel therapeutic approaches for treatment of dysferlinopathies.

PS1-127 / #369

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

A wide screening of the ANO5 gene by Next Generation Sequencing and Sanger sequencing confirms the clinical and genetic heterogeneity of LGMD2L and the incomplete penetrance

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Limb-girdle muscular dystrophies type 2L (LGMD2L) is one of the most common LGMDs in North Europe, caused by recessive mutations in the ANO5 gene. We performed a genetic analysis on this gene in a group of 400 dystrophic patients, using both the Sanger sequencing technology and the Next Generation Sequencing approach.

As first step, we screened the most common mutations located in exons 5 and 20 in 150 unrelated patients, selected from our LGMD cohort for their clinical presentation. This screening revealed eight patients with the recurrent mutations c.191 dupA and c.2272 C>T. Half of them are from central or northern Europe, confirming that these mutations are rare in the rest of the Europe because of a founder effect.

Subsequently, by Sanger sequencing, we sequenced the whole coding region of the ANO5 gene in all the patients already screened for exons 5 and 20. An additional 250 patients were analyzed by next generation sequencing after a custom enrichment.

In this way, we identified both known and novel mutations, never described in literature, spanning fifteen different exons of the ANO5 gene. This suggests that, at least in Italy, the simple screening of one or two recurrent mutations is not effective: a targeted next generation analysis investigating, at the same time, a high number of genes involved in neuromuscular disorders, including the ANO5 gene, represents a useful innovative approach. Our data confirm that only a small number of dystrophic patients have mutations in the ANO5 gene and show the absence of a stringent genotype-phenotype correlation. In particular, as suggested by further evidence in literature and the gender skewing, we found in the same family both affected and unaffected members with the same mutations, suggesting a low penetrance.

PS1-128 / #377

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Common and rare variants in genes related to limb girdle muscular dystrophies: questioning the disease-causing effect of previously reported genetic variants

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Limb girdle muscular dystrophies (LGMDs) are a heterogeneous group of neuromuscular disorders, characterized by an initial weakness and wasting of the proximal limb muscles not due to dystrophin gene mutations. Hundreds of variants in 21 autosomal genes have been described as being causative, but the associations with these diseases are based on their absence in a limited number of controls or on weak studies of familial co-segregation. Until recently, the correct interpretation of the disease-causing effect of variants has been hampered by the limited statistics of the genetic variations in the general population.

To clarify the meaning of low-frequency variants, we have selected all the point mutations so far described as causative from the Leiden Open Variation Database (LOVD) and the Human Gene Mutation Database (HGMD). We calculated their frequency in the whole exome data from the new NHLBI GO Exome Sequencing Project (ESP) and in our cohort of patients analyzed by Next Generation Sequencing (NGS). Moreover, we used several bioinformatic tools to predict the effect of all LGMD-associated missense variants on the protein.

In the ESP, we have identified about 3% of the variants previously associated with autosomal dominant inheritance and about 12% of those associated with recessive inheritance. Moreover, a number of variants (about 20–25%) were predicted in silico to be not damaging. Finally, for specific forms of LGMDs, the putative disease alleles are much more frequent than the calculated disease prevalence.

We hypothesize that a number of disease-associated variants could be non-pathogenic or, alternatively, that they are pathogenic, but not fully penetrant. This suggests that a more complex view of the genetic mechanisms of muscular dystrophies has to be considered. A non-biased testing of all variants in patients will be necessary in the future both for therapeutic approaches and genetic counseling.

PS1-129 / #413

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Natural history and peculiar aspects in LGMD2B

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Limb girdle Muscular Dystrophy 2B (LGMD2B) is an autosomal recessive dominant disorder caused by mutations in DYSF gene, encoding the protein dysferlin. Since now few therapeutic approached have been proposed for this form of LGMD, such as the use of corticosteroid therapy or the administration of antisense oligonucleotides to promote exon-skipping.

However the possibility to demonstrate the effectiveness of these approaches strongly relies on a clear and detailed knowledge of the natural history of these forms. Since now only small samples of patients have been described. The aim of the study is to give a detailed characterization of clinical and molecular aspects as far as natural history of a large sample of LGMD2B patients. We studied a sample of 80 Italian patients belonging to 61 families affected with molecularly proven LGMD2B. All patients underwent to a detailed molecular and clinical characterization comprehensive of neurological, cardiac and respiratory evaluations. In order to trace the natural history we evaluated muscular strength through the Medical Research Council (MRC) scale and through functional evaluations such as the Motor Function Measure (MFM) Scale and the 6 Minute Walking Test (6MWT). We found 62 different mutations in DYSF gene. Mean follow-up was 16.6 ± 10.5 years. At onset (mean age 24.3 ± 11.3 years) the majority of patients complained weakness (45/65) and over years 30% of patient lose independent ambulation (mean age of 43.1 ± 11.3 years). Tendon retractions were relatively frequent (21/50) and creatine kinase levels were moderately increased (4899 ± 5318 U/L). Muscle biopsy showed a moderate/severe increase of connective tissue in 26/40 samples and inflammatory signs in 15/47 probands. Muscle magnetic resonance, performed in 13 patients, showed both anterior and posterior compartment involvement. Cardiac and respiratory involvement was uncommon (respectively 4% and 14%). Functional evaluation (MRC, MFM) as far as 6MWT showed a minimal decline among years. Overall collection of these detailed clinical and functional data is important in order to define LGMD natural history. Considering the slow progression of the disease more sensible functional measures are needed, muscle MRI could be one of them.

PS1-130 / #446

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Emery-Dreifuss muscular dystrophy with LMNA mutation characterized by progressive cardiac conduction abnormality: a case report

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Emery-Dreifuss muscular dystrophy (EDMD) is characterized by muscular atrophy, contracture of certain joints, and cardiac conduction defects. Here we report a 39-year-old male with severe lordosis and contracture of elbows and heels. His severe lordosis made himself difficult to take supine position at the age of five. After he gave up to walk himself at 12, he crawled with wheelchair positioning his abdomen on the seat to move. He started to use nasal ventilator at 32. Periodical cardiac examination revealed that cardiac conduction block occurred at 32, and gradually progressed. QRS pauses resulted from complete atrioventricular block were increased in number and duration. Consequently, artificial cardiac pacemaker was implanted to him at 33. The histological and genomic examination with his left pectoralis major muscle collected among the operation of cardiac pacemaker implantation revealed a heterozygous c.1357C>T; p. R453W substitution of LMNA. Respiratory failure progressed and atrial fibrillation occurred at 35, recovered with adjustment of the condition of nasal

ventilation. It is important not to hesitate implantation of cardiac pacemaker to the patients suspected EDMD with clinical features, especially progressive cardiac conduction block. Conversely, myological examination is requisite for the patients with progressive cardiac conduction block, coexisting weakness or joint contracture.

PS1-131 / #484

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

Caveolin 3 and lamin A/C: a common physiological way?

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The LMNA gene encodes lamins A and C, mainly present at the nuclear envelope. Mutations in the LMNA gene cause diseases called laminopathies forming an extensive phenotypic spectrum, including presentations with tissue-specific and with extremely severe presentations of premature aging syndrome. Some clinical presentations, close to laminopathies, but varying, for example by severity or age of onset, are classified as atypical laminopathies. Digenism cases involving the LMNA gene and genes encoding functionally related proteins have already been reported.

During this project, in a cohort of patients with atypical laminopathies, we identified several probable cases of digenism with a mutation both in the LMNA gene and an hypomorphic mutation in the gene CAV3. The latter encodes a cytoplasmic membrane protein, caveolin 3, involved in various pathologies including skeletal muscle. Our work aims to confirm a situation of digenism given mutational data obtained. To do this, we hypothesized a combined deleterious effect of LMNA mutations and CAV3 via an indirect influence on the myostatin-SMAD way.

Unexpectedly, we show an interaction, never reported previously, between lamin A and caveolin 3. This probably occurring in a perinuclear subcellular compartment.

PS1-132 / #498

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

W2710X filamin C knock-in mice: a physiological model for filamin C-related myofibrillar myopathies

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Mutations of the human filamin C gene (FLNC) on chromosome 7q32 cause an autosomal dominant form of myofibrillar myopathy, which is morphologically characterized by the presence of filamin C-, desmin- and Xin-positive pathological protein aggregates. We generated W2710X-filamin C knock-in mice, which harbour the most frequently encountered human pathogenic filamin C mutation. The analysis of two months old heterozygous and homozygous animals revealed that the expression of the W2710X filamin C mutant leads to the occurrence of filamin C, Xin, Xirp-2, aciculin and myotilin positive protein aggregates in skeletal and cardiac muscle tissues. This accumulation is accompanied by an increased cellular stress response (Hsp27, Hsp70) as well as an increased autophagic build-up (BAG3). However, this protein aggregation pathology is not yet associated with detectable myopathological alterations in these young animals. The latter findings indicate that the presence of protein aggregates precedes the development of progressive muscle damage. Our W2710X-filamin C knock-in mice mirror essential aspects of the human protein aggregation pathology. We will report on the results of the ongoing clinical phenotyping, ultrastructural and biochemical analyses as well as the immortalization of myoblasts derived from heterozygous and homozygous W2710X-filamin C knock-in mice.

PS1-133 / #537

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

A platform dedicated to the immortalization of human myoblasts isolated from patients with various neuromuscular disorders

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With increasing advances in gene therapy these last years, and the orientation towards tailored therapies adapted to each mutation (i.e. personalized medicine), the use of patient cells becomes essential to assess therapeutic strategies. Human myoblasts derived from muscle biopsies represent an ideal *in vitro* model to adapt therapeutic strategies in neuromuscular disorders, but their limited proliferative capacity restricts their use.

In recent years we have developed an innovative strategy to immortalize human myoblasts derived from muscle biopsies of dystrophic patients. By transduction of telomerase (hTERT) and cyclin-dependent kinase 4 (cdk4) into patient primary myoblasts, we have generated *in vitro* cellular tools for a wide range of neuromuscular diseases, including DMD, LGMD2B, OPMD and FSHD. We now have 70 human immortalized myoblast lines from 22 different neuromuscular disorders. Control cell lines from newborn to old subjects were also generated.

To confirm that immortalized cell lines maintain their original behavior, we have analyzed the potential of these cells to differentiate and to regenerate *in vivo* after their injection into the TA muscles of immunodeficient mice. Transcriptomic analyses confirmed that the process of immortalization does not modify the myogenic status of the cells compared to the primary culture.

Since access to muscle biopsies may present a challenge for some muscle diseases, we have also developed an alternative cellular model based on the myogenic conversion of skin fibroblasts. Fibroblasts can be isolated easily from the skin of patients, immortalized by transduction of hTERT, and then converted by transduction of inducible MyoD.

These cell lines from various neuromuscular disorders are accessible to the scientific community on a collaborative basis. Recently, we have developed new control cell lines for which patient consent specifically permits their use by private enterprise. The platform of immortalization of the Myology institute is also open to new requests of immortalization.

PS1-134 / #550

Theme: *2.2 - Muscle diseases of genetic origin: Muscle dystrophies (non dystrophinopathy)*

An International Web-based Registry for Dysferlinopathy Involving Participation of Patients and their Doctors

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The International Dysferlinopathy Registry is available in seven languages and is open to all patients worldwide affected with a dysferlinopathy: Limb Girdle Muscular Dystrophy type-2B (LGMD2B), Miyoshi Myopathy or other clinical presentations related to mutations in the dysferlin gene.

To initiate their registration, patients sign their online consent on www.dysferlinregistry.org, provide their personal details and contact information for

doctor(s) involved in the diagnosis and follow-up of their disease. While the registry curator contacts the medical doctor(s) to obtain some genetic and biological data, patients are invited to connect to their personal user account in order to complete an online secure medical questionnaire. The patients' registration is validated after they have completed their medical questionnaire and once the registry curator has confirmed their eligibility (i.e. at least one pathogenic mutation identified in the dysferlin gene).

Registrants will receive information relevant to their condition, such as whether they might be suitable for certain clinical trials or research studies as well as about better ways of caring for patients with a dysferlinopathy once those ways are identified. Data collected will help researchers to be better equipped for finding therapies for this disease, to understand how many people worldwide are affected by this rare condition and what the precise genetic defects are, and will help to support other activities to improve patient care, such as assessment and dissemination of standards of care. In addition, third parties can request anonymised medical data from the registry and - subject to approval - use the obtained information for research, study/trial feasibility or planning, or patient recruitment into clinical studies/trials.

To facilitate the combined use of phenotypic data, biosamples and -omics data for rare disease research, the Registry will work with the EU project RD-Connect to implement a Global Unique Identifier for patients and implement standardized coding systems and ontologies.

PS2-135 / #117

Theme: *2.3 - Muscle diseases of genetic origin: Congenital muscular dystrophy*

Satellite cells from Largemyd and Lama2dy2j/J: Are they different in their myogenic potential?

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The role of muscle satellite cells (SC) in muscle regeneration in the dystrophic process is of upmost importance for the identification of potential therapeutic targets. We recently showed that two different severely affected mouse models for muscular dystrophies, the Large^{myd} and Lama2^{dy2j}/J mice, have a similar pattern of degeneration but with differences in the expression of genes involved in the regeneration cascade. Therefore, they constitute interesting models to study the mechanism of activation and action of SC in the degeneration caused by different gene mutations.

To better understand the characteristics of SC from both models, muscle derived cells were obtained through enzymatic dissociation and plated (PP1). Preplating technique was applied after 24h (PP2) and 144h (PP6). The different populations were characterized by flow cytometry, using markers for myogenic and mesenchymal populations (CD29, 90, 105, 133, 73, 56, 44, 13, 31, 45, Sca1, CXCR4) and compared with the profile presented by myoblasts C2C12 and bone-marrow mesenchymal stem cells.

In the normal muscle, both PP1 and PP2 populations showed similar phenotypic characteristics, shifting to the myogenic phenotype of C2C12 cells. On the other hand, the population of cells with much delayed adhesion ability (PP6) presented a mixed pattern of myogenic and mesenchymal stem cell's characteristics.

In dystrophic muscles, we could identify differences in the constitution of the initial pool of PP1 populations of muscle cells. The Lama2^{dy2j}/J PP1muscle line was more directed to the myogenic phenotype, while the Large^{myd} PP1 population was positive to less myogenic markers. The PP2 populations of both strains were similar, with a lower expression of muscle markers, suggesting that the pre-plating technique is negatively selecting the cells with a higher myogenic potential.

These observations are corroborating our previous results of gene expression, suggesting that the mutation present in Large^{myd} mouse is associated to a worst regenerative potential of satellite cells, than the one present in the Lama2^{dy2j}/J model. FAPESP-CEPID, CNPQ-INCT, FINEP, CAPES-COFECUB.

⋆PF2

PS2-136 / #192

Theme: *2.3 - Muscle diseases of genetic origin: Congenital muscular dystrophy*

Impaired viability of muscle precursor cells in muscular dystrophy with glycosylation defects and amelioration of its severe phenotype by limited gene expression

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A group of muscular dystrophies, dystroglycanopathy, is caused by abnormalities in post-translational modifications of dystroglycan (DG). To better understand the pathophysiological roles of DG modification and to establish effective treatment for dystroglycanopathy, we generated 2 distinct conditional knockout (cKO) mice for fukutin, the first dystroglycanopathy gene identified for Fukuyama congenital muscular dystrophy. The first dystroglycanopathy model-myofiber-selective fukutin-cKO (MCK-fukutin-cKO) mice-showed mild muscular dystrophy. Forced exercise experiments in presymptomatic MCK-fukutin-cKO mice revealed that myofiber membrane fragility triggered disease manifestation. The second dystroglycanopathy model-muscle precursor cell (MPC)-selective cKO (Myf5-fukutin-cKO) mice-exhibited more severe phenotypes of muscular dystrophy. Using an isolated MPC culture system, we demonstrated that defects in the fukutin-dependent modification of DG lead to impairment of MPC proliferation, differentiation, and muscle regeneration. These results suggest that impaired MPC viability contributes to the pathology of dystroglycanopathy. Since our data suggested that frequent cycles of myofiber degeneration/regeneration accelerate substantial and/or functional loss of MPC, we expected that protection from disease-triggering myofiber degeneration provides therapeutic effects even in mouse models with MPC defects; therefore, we restored fukutin expression in myofibers. Adeno-associated virus (AAV)-mediated rescue of fukutin expression that was limited in myofibers successfully ameliorated the severe pathology even after disease progression. In addition, compared to other gene therapy studies, considerably low AAV titers were associated with therapeutic effects. Our findings indicated that fukutin-deficient dystroglycanopathy is a regeneration-defective disorder, and gene therapy is a feasible treatment for the wide range of dystroglycanopathy even after disease progression.

PS2-137 / #268

Theme: *2.3 - Muscle diseases of genetic origin: Congenital muscular dystrophy*

Allele-specific silencing of a common dominant-negative COL6A3 mutation using siRNAs alleviates the phenotype of a cellular model of Ullrich congenital muscular dystrophy

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Congenital muscular dystrophy type Ullrich (UCMD) is a severe progressive neuromuscular disorder of early childhood onset, presenting with generalized muscle weakness, distal joint hypermobility, progressive joint contractures, and respiratory failure as main features. At present, there are no pharmacological treatment options available for children affected with this disease. In the laboratory we aim at exploring targeted RNAi and antisense approaches as potential therapies for UCMD. Dominant and recessive mutations in the three genes coding for collagen type VI (COL6A1, COL6A2, COL6A3) underlie UCMD, with dominant-negative mutations accounting for the majority of cases. Achieving allele-specific silencing of the mutant collagen VI transcript would convert this dominant-negative state into a clinically asymptomatic haploinsufficient state. We have designed a series of siRNA oligos to target a mutant mRNA transcript lacking the exon 16 of the Collagen VI α3 gene (COL6A3). We tested this series of siRNA

in four UCMD-derived dermal fibroblast cells lines. Transcript analysis by semi-quantitative and quantitative RT-PCR identified at least two siRNA oligos of high allele-specific knockdown potential, as they significantly reduced the expression of the mutant transcript, without affecting the expression of the normal allele. In HEK293T cells these siRNA oligos selectively suppressed protein expression from a reporter construct carrying the mutation, but not from a wild-type construct. Furthermore, we found that treating UCMD fibroblasts with these siRNA oligos considerably improved the quantity and quality of the collagen VI extracellular matrix in cell culture, as visualized and quantified by confocal microscopy of immunostained collagen VI. Our current study serves as a proof-of-principle and establishes RNAi as a promising molecular approach for treating UCMD caused by dominant-negative mutations.

✶PF2

PS2-138 / #346

Theme: *2.3 - Muscle diseases of genetic origin: Congenital muscular dystrophy*

Novel collagen VI chains in zebrafish skeletal muscle

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Collagen VI (COLVI) is a heterotrimeric protein, ubiquitously expressed in connective tissues. COLVI-plays multiple biological roles in the maintenance of structural integrity, cellular adhesion, migration and survival. COLVI trimers are formed by the assembly of 2 "short" and 1 "long" chain. To date, six COLVI chains are recognized in mammalians with 2 short (alpha1–2(VI)) and 3 long (alpha3–6(VI)) chains. Little is known regarding the possible assembly of the newly characterized alpha4–6(VI) polypeptides with the short chains, and a putative functional compensation between the different long chains. In humans, deficiency in alpha1–3(VI) due to mutations in the COL6A1–3 genes causes a heterogeneous group of neuromuscular disorders collectively termed COLVI-myopathies. We identified 2 orthologs of the alpha4–6(VI) chains in zebrafish, a relevant model of neuromuscular disorders. In light of their stronger homology with the mammalian alpha4(VI) chain, we named the genes encoding the novel chains col6a4a and col6a4b. Throughout zebrafish development, the col6a4b gene presents a unique kinetics of expression. To further unveil the roles of the corresponding proteins, we created COLVI deficient zebrafish embryos using morpholinos that block splicing of col6a2, col6a4a and col6a4b, thereby creating premature termination codons. As expected, the targeted transcripts levels were drastically reduced, likely due to degradation by the nonsense mediated RNA decay. All morphant embryos presented macroscopic and morphologic phenotypes that overall resulted in functional muscle defects: altered muscle structure detected by birefringence analysis and impaired motility upon touch-evoked test. These alterations were confirmed at the ultrastructural level by electron microscopy. Nevertheless, some phenotypical specificity was uncovered between the col6a4a and col6a4b morphants, strongly suggesting specific functions.

In conclusion, col6a2 deficient embryos recapitulate the severe end of the COLVI-myopathy phenotypical spectrum, thereby confirming the importance of col6 genes in muscle development. Importantly, the phenotypes associated with alpha4a(VI) and alpha4b(VI) deficiency may provide important clues for the phenotypes that may be associated with mutations in the corresponding human genes.

PS2-139 / #360

Theme: *2.3 - Muscle diseases of genetic origin: Congenital muscular dystrophy*

A novel mutation in COL6A1 caused recessive Bethlem myopathy.

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In our study we characterized a Russian origin girl with Bethlem myopathy. She was floppy infant in neonatal period, but started to walk independently at 12 months. She had gait disturbances and diffuse weakness more proximal than distal. To the age 13 she developed contractures in the shoulder, elbow and ankle joints but it was noted a laxity in the metacarpophalangeal joints. In addition to the clinical features she had hyperlordosis of the lumbar spine, scoliosis, scapula winging, valgus foot deformities. Skin abnormalities like hypertrichosis, hyperkeratosis, keloid scar on her thigh (after a biopsy of muscle tissue) were preserved. She applies Gower's maneuvers in her daily activities, and cannot rise alone from the floor and from the chair, walks with assistance. Muscle MRI revealed "tiger" pattern in her thigh muscles. One previously unreported missense variant was identified in this girl by direct DNA sequencing analysis of Collagen, type VI, alpha genes (COL6A1, COL6A2, COL6A3). The analysis was done in Department of Human Genetics (The University of Utah, USA). Sequence revealed one unreported heterozygous missense variant in Exon 3 of the COL6A1 gene with nucleotide change c.380C>A, and protein change p.Thr127Asn. The parents and the elder sister were also examined on this rare variant, but they were negative. This variant has not been previously observed in the published literature (Lampe and Bushby 2005) or mutation databases, and is of unknown pathogenicity. The COL6A1 p.Thr127Asn missense variant occurs in the single N1 vWF domain of the alfa-1(VI) collagen. An analogous COL6A1 p.Lys121Arg missense mutation in the N1 vWF domain has been reported once, and cosegregates with an autosomal dominant Bethlem myopathy phenotype in large four generation family (Scacheri et al. 2002). Given the evolutionary conservation of the p.Thr127 residue and its proximity to the p.Lys121 mutation, it is likely that the p.117Asn variant may have similar deterious effect on alfa-1(VI) collagen level or function.

PS2-140 / #509

Theme: *2.3 - Muscle diseases of genetic origin: Congenital muscular dystrophy*

Body composition and energy expenditure in duchenne muscolar distrophy: longitudinal study

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Background: Duchenne muscolar distrophy (DMD) is characterised by decreased fat-free mass (FFM) and increased fat mass (FM). Skeletal muscle metabolism is the major determinant of the resting energy expenditure (REE). A reduction of REE, according to the severe muscle loss, is hypothetical in DMD subjects but in the literature there are few and conflicting data regarding this relationship.

Objective: to provide longitudinal data about the natural evolution of body composition and REE in DMD and to investigate their relationship.

Methods: at baseline we studied 11 subjects with DMD median age 11 years (IQR: 9–13). They were divided in normal-weight and obese according to Italian BMI growth norms table. Only five patients were assessed at follow-up after 12 months. Body composition (FFM, FM, FFMI) was measured using DEXA; REE was assessed by indirect calorimetry; dietary energy intake was also investigated.

Results: at baseline in obese subjects mean FM% was significantly greater than in normal-weight (51.2; IQR:50.2–58.1vs39.1; IQR:29.7–46.4, $p=0.014$). Also the FFM was greater in obeses. The REE values were smaller in normal weight subjects (1325.5;IQR:1006–1467.5vs1633;IQR:1402–1683 kcal/day) but similar when adjusted for kg/FFM (50.5; IQR:48.1–57vs51.1; IQR:48.8–53.2). The primary longitudinal outcomes show a mean weight gain of

3 kg and a mean FM% increase; even the mean FFM significantly increase (26.2; IQR:20.4–31.6, kg $p=0.043$). REE and REE/FFM mean values decreased. The caloric intake was stable respect to basal observation.

Discussion: in the obese patients FM was greater but also FFM values. This higher value of FFM in obese may be due to the difference in mean age between groups beside possible genetic determinants of body size. The REE was significantly lower than the value obtained from the literature in healthy children of the same age and it was significantly lower in the normal-weight children than in the obese subjects. The REE/FFM, nevertheless, was similar between the two groups, due to the higher values of FFM in the obese subjects. At the follow-up the significant increase of FFM is probably due to the influence of growth and of sexual hormones. Moreover we can suppose that the absence of significant changes in REE was secondary to the too short follow-up.

Conclusion: DMD patients suffer from progressive weight gain and increase fat mass but in young boys the hormonal pattern probably influence FFM and its decrease may be detected later.

PS2-141 / #549

Theme: *2.3 - Muscle diseases of genetic origin: Congenital muscular dystrophy*

Inflammation in patients with Duchenne muscular dystrophy / Becker (DMD / BMD) with different nutritional status

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DMD/BMD are diseases that cause muscle chronic inflammation.Fibrotic and adipose tissue replacement exist and probably cause metabolic disturbances as obesity.Obesity presents inflammation that is associated with the risk of insulin resistance(IR).Our group found that27.3% patients with DMD/BMD have RI despite having malnutrition.This suggests that the RI could be associated with the state of inflammation of the disease.The objective is to study if the inflammation is related with the nutritional status,measuring the concentration of circulating markers of inflammation inDMD/BMD patients with different nutritional status and determine if an association between the inflammation and severity of patients exists.

Patients ($N=66$) with clinical and molecular diagnosis ofDMD/BMD between4–17 years old were studied.The Vignos Scale was used to assess the muscle strength.The cytokines pro-inflammatory (TNFalpha,IL6,CRP) and the creatine-kinase(CK) in serum were quantified by chemiluminescent immunometric assay.Weight and height were recorded to calculate body mass index(BMI),the body composition was measure by absorptiometry dual-energy X-ray to know the total fat percentage.Patients were classified in 3 groups according to their BMI:malnourished(BMI percentile<5), normal (BMI percentile>5<85) and overweight/obese(BMI percentile ≥85) according to the Centers for Disease Control and Prevention.Data was analyzed using SPSS,confidence intervals were 95% with an alpha value($p<0.05$).

The nutritional status of a patient doesn't affect the inflammation level.The older and more affected patients present lower concentrations of TNFa,CK,and higher CRP concentration and total mass fat;in comparison with younger patients which presents higher concentrations of TNFa,CK and lower CRP concentration and total mass fat.Therefore the pro-inflammatory cytokines(TNFa,IL-6) follow the same pattern,due their CK concentration levels are minor in the older patients,which also present higher muscular damage.A positive correlation exists between the age of a patient,muscular damage,fat percentage,and CRP concentration.A negative association between the muscular damage and the pro-inflammatory cytokines and the CK exists.

The degree of inflammation isn't related with their nutrional status.Patients with minor muscular damage present a major concentration of pro-inflammation molecules and CK.Patients with major muscular damage have minor concentration of pro-inflammation molecules and CK

PS2-142 / #60

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Aerobic training in patients with congenital myopathy

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Introduction: Congenital myopathies (CM) are clinically and genetically heterogeneous disorders characterized by skeletal muscle weakness. While most patients experience muscle weakness and fatigue, progression of symptoms is rare. There are no specific treatments for CM. Other studies have indicated that endurance training can safely improve oxidative capacity, strength and endurance in patients with muscular dystrophies. However, is exercise safe and beneficial in conditions with aberrations in genes encoding sarcomeric structures and related proteins, as in CM? We investigated this in a cohort of patients with CM.

Methods: 19 patients with CM are randomised to training ($n=13$) or no training ($n=6$). At present, four subjects have completed the program.

Patients perform home-based pulse-monitored exercise on a bike ergometer for 30 minutes, three times weekly, for 10 weeks. Training is performed at 70% of their maximal oxygen uptake. Plasma CK and myoglobin is assessed before, during and after training as markers of muscle damage. Efficacy was evaluated by maximal oxygen uptake during cycling and a number of functional tests before and after training. Patients who did not train underwent the same tests 10 weeks apart.

Results: Training resulted in improvements in maximal oxygen uptake ($19\pm6\%$; $p=0.009$) and maximal workload ($24\pm3\%$; $p=0.02$) in the three patients who have completed the program. This was accompanied by constant levels of CK and myoglobin and no reports of adverse effects. No changes in maximal oxygen uptake were seen in the control subject.

Conclusion: The preliminary results indicate that endurance training is safe and can improve fitness effectively in patients with CM. The findings so far, therefore, suggest that moderate-intensity training should be recommended as treatment for patients with CM.

PS2-143 / #65

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Endurance training in patients with collagen VI-related myopathy

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Introduction: Mutations in genes encoding the alpha-chains of Collagen VI, can lead to muscular dystrophy with a wide clinical spectrum of phenotypes. Currently, treatment of collagen VI myopathy is limited to general medical care, focused on handling contractures and respiratory function. Previous studies show, that endurance training safely improves oxidative capacity and muscle function in some myopathies. Collagen VI is located and functions in the extracellular matrix surrounding myocytes. The unique pathogenesis of collagen VI myopathy makes investigating responses to training particularly interesting in this patient group, since the environment in tissue surrounding muscles may counteract a training effect. In support of such a notion, creatine kinase (CK) levels are generally high in these conditions.

Methods: In 5 patients with collagen VI myopathy, we studied the effect of a 10-week home-based, pulse-watch monitored, moderate-intensity cycle training program. The training program was flanked by two test days, where patients underwent a maximal exercise test, three functional muscle tests and dynamometry. Plasma CK was assessed before, during, and after the program as a marker of muscle damage. Primary outcome measures were maximal oxygen uptake (VO_{2max}) and workload (W_{max}) in the maximal exercise test.

Results: So far, 2 subjects have completed training. Results from all 5 training patients, plus 2 non-training control patients will be included at the time of the conference. Training improved VO_{2max} from 30.8 to 36.4 ml $O_2*min^{-1}*kg^{-1}$ and W_{max} from 133 to 183 Watts. Improvements in oxidative capacity were accompanied by stable CK levels, and no reports of adverse effects.

Discussion: These preliminary results indicate that training is well-tolerated and improves oxidative capacity, in patients with a mild collagen VI myopathy phenotype.

✶PF3

PS2-144 / #70

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Muscle histopathology in nebulin-related nemaline myopathy: ultrastrastructural findings correlated to disease severity and genotype.

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Nemaline myopathy (NM) is a rare congenital myopathy characterised by hypotonia, muscle weakness, and often skeletal deformities with the presence of nemaline bodies (rods) in the muscle biopsy. The nebulin(NEB) gene is the most commonly mutated and is thought to account for approximately 50% of genetically diagnosed cases of NM. We undertook a detailed muscle morphological analysis of 15 NEB-mutated NM patients with different clinical forms to define muscle pathological patterns and correlate them with clinical course and genotype. Three groups were identified according to clinical severity. Group 1 ($n=5$) comprises severe/lethal NM and biopsy in the first days of life. Group 2 ($n=5$) includes intermediate NM and biopsy in infancy. Group 3 ($n=5$) comprises typical/mild NM and biopsy in childhood or early adult life. Biopsies underwent histoenzymological, immunohistochemical and ultrastructural analysis. Fibre type distribution patterns, rod characteristics, distribution and localization were investigated. Contractile performance was studied in muscle fibre preparations isolated from seven muscle biopsies from each of the three groups. G1 showed significant myofibrillar dissociation and smallness with scattered globular rods in one third of fibres; there was no type 1 predominance. G2 presented milder sarcomeric dissociation, dispersed or clustered nemaline bodies, and type 1 predominance/uniformity. In contrast, G3 had well-delimited clusters of subsarcolemmal elongated rods and type 1 uniformity without sarcomeric alterations. In accordance with the clinical and mor-

phological data, functional studies revealed markedly low forces in muscle bundles from G1 and a better contractile performance in muscle bundles from biopsies of patients from G2, and G3. In conclusion NEB-mutated NM patients present heterogeneous morphological features. It is difficult to establish firm genotype phenotype correlation. Interestingly, there was a correlation between clinical severity on the one hand and the degree of sarcomeric dissociation and contractility efficiency on the other. By contrast the percentage of fibres occupied by rods, as well as the quantity and the sub sarcolemmal position of rods, appears to inversely correlate with severity. Based on our observations, we propose myofibrillar dissociation and changes in contractility as an important cause of muscle weakness in NEB-mutated NM patients.

PS2-145 / #99

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Adaptive role of mitochondrial changes in congenital central core myopathy

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Background: Increasing number of mitochondria in skeletal striated muscle fibers plays a role in adapting to specific non-mitochondrial disorder. In particular, it was shown that the severity and progress duration of congenital central core myopathy depend on the phenomenon of abnormal increase of the mitochondria number presence in muscle biopsies. Therefore, careful analysis of mitochondrial morphometric characteristics at the ultrastructural level in muscle tissue of these patients is necessary.

Objective: Electron microscopy assay of muscle tissue mitochondria in patients with congenital central core myopathy.

Methods: striated muscle biopsies of 21 patients with congenital central core myopathy in age from 4 to 42 years were analyzed. Material fixed in glutaraldehyde and osmium tetroxide was embedded in epoxy resin mixture and then ultrathin sections were analyzed in the electron microscope.

Results and Discussion: significant negative correlation among the severity of disease (points score), the number (correlation coefficient - 0.3) and size (correlation coefficient - 0.47) of mitochondria was determined. The same ratio was established in the correlation calculation between the severity of disease and relative volumes of mitochondria compared with myofibrillar volume (correlation coefficient - 0.32). Same ratio is determined between mitochondrial ultrastructural parameters and lactate-pyruvate levels in plasma.

Conclusion: received morphometric data confirm assumption about compensatory value of mitochondrial fraction relative volume increase in muscle tissue in the central core myopathy. Apparently in this case lactate - pyruvate concentrations play important role in triggering a mitochondria compensatory proliferation.

PS2-146 / #129

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Water-soluble fullerene improves skeletal muscle regeneration

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Purpose: Muscular dystrophy is a hereditary disease, which cause severe muscle weakness and atrophy in clinically, and skeletal muscle degeneration and necrosis in pathology. In recent years, the importance of involvement of oxidative stress by acute and chronic inflammation was revealed in the process of muscle degeneration. Therefore, reducing oxidative stress is considered as one of the new treatment strategy in muscular dystrophy. Fullerene is an allotrope of carbon the same as diamond and water-soluble type has been reported that it can detoxify and absorb free radical.

To evaluate the effectiveness of water-soluble fullerene, we chronologically evaluate the histology in rat tibial muscles during a cycle of regeneration induced by cardiotoxin injection. Materials and Methods: Tibial muscles of Wistar rats (8 weeks old) were injected with cardiotoxin with fullerene or without fullerene. We used 4 types of fullerene with different number of hydroxyl groups. The injected muscles were removed and stained H&E on 1, 3, 5, 7, 14, and 28 days after the injection.

Muscle extract were prepared and western-blotting was performed to evaluate expression of muscle proteins, such as syntrophin, and nNOS.

Results: Average diameter of regenerate muscles 28 days after injection, fullerene group was bigger than those of cardiotoxin group. Futhermore, fullerene with low number of hydroxyl groups is more effective for muscle regeneration. Muscle protein expression in co-administered fullerene group, Syntrophin and nNOS was observed in the earlier stage by Western blot than those of cardiotoxin group. During the experiment, there is no significant and critical change was observed.

Conclusion: We revealed that Fullerene has a function to promote the regeneration of skeletal Muscle in this experiment. We believe fullerene and can be applied in the treatment of muscular dystrophy.

PS2-147 / #133

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Myosinopathies: Identification of MYH2, MYH3 and MYH8 cases by clinical, MR Imaging, histopathological and Electron microscopic studies

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Background: Hereditary myosin myopathies are a new group of diseases with onset at birth, early childhood or in adult life. Aim: To describe clinical, muscle MRI, histopathological and Electron microscopy(EM) findings in Myosinopathies. Materials and methods: Five cases from 3 families evaluated in last one year. Results: Family1:A 32 years lady had progressive asymmetrical foot drop for 6 years, pelvic girdle weakness for 4 years, left hand extensors and hand grip weakness of 2 years. CK-178(20–170). Her son aged 6 years was born with contractures which is progressive. Has prominent muscles with no weakness. CK was 2712IU. Biopsy in both: Rimmed vacuoles with mild variation in diameter with irregular and dabbled mass of eosinophilic material in many fibers, staining intensely green on MGT and remained unstained on SDH and NADH-Tr, intensely stained on ATPase reaction involving both fiber types . Family2: 50 year old lady was born with clenched fists. Was always a weak child. By 19 years developed progressive pelvic girdle weakness. Father had severe talipes equinovarus and clenched fists since birth. Lived till 72years. Elder brother and sister have clenched fists. Son aged 29 years was a floppy child. Has nuchal contracture. Has motor disabilities since childhood with hypermobile joints. Family3: 23 year old lady had delayed acquisition of all milestones. Born with foot and hand deformities. Able to walk from 7 years of age. From infancy unable to open mouth fully. Chews and swallows food slowly. Seizures for 6 months.Has low set ears, micrognathia, mal occlusion of teeth, jaw trismus, hand and foot pseudocamptodactyly, ulnar deviation of wrists, right foot equinovalgus deformity. Contractures at all major joints. Grade 4 muscle weakness. Conclusion: First report from India on MYH2, MYH3 and MYH8 types of myosinopathies diagnosed by clinical, histopathological and EM findings.

PS2-148 / #168

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Congenital titinopathies: an expanding spectrum

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Core myopathies (CM), the main non-dystrophic muscle disease in childhood, remain genetically unexplained in many cases. Heart disease is not considered part of the typical CM spectrum. Childhood-onset dilated cardiomyopathy (DCM) has been reported in two families with minicores and homozygous TTN mutations. TTN encodes the giant protein titin, which plays a key role in skeletal and heart muscles. Relatively few mutations (mostly heterozygous and associated with adult-onset cardiac or skeletal muscle disease) have been reported for this huge gene, whose complexity precluded full screening before NGS. Thus, the prevalence and spectrum of titinopathies is probably underestimated and TTN importance in CM and/or pediatric heart conditions is incompletely understood.

To clarify the association of CM with heart disease and to characterize its molecular basis, we analyzed 23 families with congenital CM and primary heart disease using TTN M-line targeted sequencing followed in selected patients by whole-exome sequencing and functional studies.

We identified 7 novel homozygous or compound heterozygous TTN mutations (5 in the M-line, 5 truncating) in 5 patients. All heterozygous parents were healthy. Phenotype analysis disclosed 4 novel phenotypes previously unreported or non-associated with TTN, including several congenital cardiopathies. The first antenatal-onset titinopathy presented with multiminicore disease (MmD), arthrogryposis, left ventricular non-compaction and ventricular septal defect. Molecular, ex vivo and *in vitro* studies showed that this severe phenotype was due to the first-reported absence of a functional titin kinase domain in humans. Other novel phenotypes combine MmD with auricular and/or ventricular septal defects, Rigid Spine, Emery-Dreifuss (EDMD) phenotype and/or adult-onset DCM. The histological pattern was consistent, associating minicores, abundant central nuclei and structural lesions. Conversely, the spectrum of severity and of heart abnormalities was large.

In conclusion, we demonstrate that TTN M-line truncating mutations are typically recessive, manifesting only when associated with a second TTN mutation, and represent a significant cause of MmD with heart disease. Our results represent the first series of recessive titinopathies, expand TTN mutational and phenotypic spectrum, establish TTN as a candidate gene in arthrogryposis, EDMD and multiple pediatric heart defects and suggest titin kinase implication in cardiac morphogenesis.

PS2-149 / #201

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Altered myosin cross-bridge cycling kinetics is associated with a paradoxical gain of muscle function *in vivo* in a mouse model of nemaline myopathy

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Nemaline myopathy (NM) is a genetically and clinically highly heterogeneous congenital myopathy and is characterized by muscle weakness and the presence of rod shaped structures in skeletal muscle fibres. The first disease causing mutation (Met9Arg) was identified in the gene encoding α-tropomyosin$_{slow}$ gene (TPM3) [1]. Met9Arg mutation in the TPM3 gene causes mild form of NM in humans and is associated with a late-onset of muscle weakness. Considering the conflicting findings of the previous studies on the transgenic (Tg) mice carrying the TPM3^{Met9Arg} mutation [2, 3], we investigated carefully the effect of the Met9Arg mutation in 8–9 month-old Tg(TPM3)^{Met9Arg} mice on muscle function using a multiscale methodological approach including skinned muscle fibers analysis (maximal force, calcium sensitivity and k^{tr}) and *in vivo* investigations by magnetic resonance imaging and 31-phosphorus magnetic resonance spectroscopy. While *in vitro* maximal force production was reduced by 15% in Tg(TPM3)^{Met9Arg} mice as compared to controls, *in vivo* measurements revealed an improved mechanical performance (+30%) in the transgenic mice as compared to the former. The reduced *in vitro* muscle function might be related to an alteration of cross-bridges cycling kinetics and a decrease in force per cross-bridge. *In vivo* muscle improvement was not associated with any changes in either muscle volume or energy metabolism. Our findings indicate that TPM3(Met9Arg) mutation leads to a mild muscle weakness *in vitro* related to an alteration at the cross-bridges level and a paradoxical gain of muscle function *in vivo*. These results clearly point out that *in vitro* alterations do not necessarily translate into similar changes *in vivo*.

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✶PF2

PS2-150 / #203

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Multimodal MRI and 31P-MRS investigations of the ACTA1(Asp286Gly) mouse model of nemaline myopathy provide evidence of impaired *in vivo* muscle function, altered muscle structure and disturbed energy metabolism

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Nemaline myopathy (NM), the most common non-dystrophic congenital disease of skeletal muscle, can be caused by mutations in the skeletal muscle α-actin

gene (ACTA1) (~25% of all NM cases and up to 50% of severe forms of NM). Muscle function of the recently generated transgenic mouse model carrying the human Asp286Gly mutation in the ACTA1 gene (Tg(ACTA1)^{Asp286Gly}) has been mainly investigated *in vitro* (1). Therefore, we aimed at providing a comprehensive picture of the *in vivo* hindlimb muscle function of Tg(ACTA1)^{Asp286Gly} mice by combining strictly non-invasive investigations. Skeletal muscle anatomy (hindlimb muscles, intramuscular fat volumes) and microstructure were studied using multimodal magnetic resonance imaging (Dixon, T_2, Diffusion Tensor Imaging (DTI)). Energy metabolism was studied using 31-phosphorus Magnetic Resonance Spectroscopy (^{31}P-MRS). Skeletal muscle contractile performance was investigated while applying a force-frequency protocol (1–150 Hz) and a fatigue protocol (6 min-1.7 Hz). Tg(ACTA1)^{Asp286Gly} mice showed a mild muscle weakness as illustrated by the reduction of both absolute (30%) and specific (15%) maximal force production. Dixon MRI did not show discernable fatty infiltration in Tg(ACTA1)^{Asp286Gly} mice indicating that this mouse model does not reproduce human MRI findings. Increased T_2 values were observed in Tg(ACTA1)^{Asp286Gly} mice and might reflect the occurrence of muscle degeneration/regeneration process. Interestingly, T_2 values were linearly related to muscle weakness. DTI experiments indicated lower λ_2 and λ_3 values in Tg(ACTA1)^{Asp286Gly} mice, which might be associated to muscle atrophy and/or the presence of histological anomalies. Finally ^{31}P-MRS investigations illustrated an increased anaerobic energy cost of contraction in Tg(ACTA1)^{Asp286Gly} mice which might be ascribed to contractile and non-contractile processes. Overall, we provide a unique set of information about the anatomic, metabolic and functional consequences of the Asp286Gly mutation that might be considered as relevant biomarkers for monitoring the severity and/or the progression of NM and for assessing the efficacy of potential therapeutic interventions.

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PS2-151 / #223

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

De novo mutation in RYR1. Case report

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Central core disease ,also known as minicore or multicore myopathy, is one of the most common congenital myopathies. In most cases it caused by mutations in the skeletal muscle ryanodine receptor gene (RYR1) that encodes the Ca^{2+} release channel of the sarcoplasmic reticulum. This channel plays an essential role in maintnance of Ca^{2+} homeostasis and on excitation-contraction coupling in skeletal muscle cells.

Central core disease exhibits an autosomal dominant pattern of inheritance, but also cases with autosomal recessive inheritance and sporadic cases due to de novo mutations were reported.

Clinical phenotype varies from mild to severe. The most common (about 75% of all cases) is the classic form which presents in the first year of life. Affected infants are hypotonic, some children have associated abnormalities such as dislocation hips or arthrogryposis. Extremity weakness may be static or slowly progressive and most patients are ambulant into adulthood. Weakness of the axial musculature often results in kyphoscoliosis and spinal rigidity that can results in significant restriction of lung capacity and are commonly associated with progressive respiratory insufficiency. Adult onset minicore disease is seen in minority cases in association with the rigid spine syndrome. There is also a high risk of malignant hyperthermia, tha's why can't use volatile inhalational anesthetic agent and the muscle relaxant succinylcholine.

We would like to present two case reports of patient with de novo mutation in RYR1 gene. These patients were the first in the Czech republic diagnosed by the

novel method of sequence capture and targeted resequencing.

Case report: Two girls diagnosed in age of 3 and 6 years., both with negative family history. Both girls have progressive hypotonia and muscle weakness and also progressive kyphoscoliosis without respiratory insuficiency. Girls were hospitalized at the Depatment of Paediatric Neurology at University Hospital Brno (at age of 6 month resp. 3 years) where they underwent lot of testing including also magnetic resonance (original diagnose of youger girl was post hypoxic encephalopathy bcs of pre and prinatal risks), muscle biopsy (older girl) and genetic testing which confirmed diagnose congenital myopathy caused by de novo mutation in RYR1

✶PF1

PS2-152 / #292

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Reducing dynamin 2 rescues myotubular myopathy in mice

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Centronuclear myopathies (CNM) are congenital disorders associated with muscle weakness and abnormally located nuclei in skeletal muscle. An autosomal dominant form of CNM results from mutations in the gene encoding dynamin 2 (DNM2), and loss-of-function mutations in the gene encoding myotubularin (MTM1) result in X-linked centronuclear myopathy (XLCNM, also called myotubular myopathy), which promotes severe neonatal hypotonia and early death. Currently, no effective treatments exist for XLCNM.

We and others showed that overexpression of wild-type DNM2 in skeletal muscle cause a CNM-like phenotype. We thus hypothesized myotubularin and dynamin 2 function in a common pathway, where either MTM1 loss-of-function or DNM2 gain-of-function lead to the CNM phenotype. To test this hypothesis, we reduced the expression of DNM2 in $Mtm1^{-/y}$ mice that reproduce a CNM phenotype with a progressive myopathy leading to death by 6–12 weeks. $Mtm1^{-/y}Dnm2^{+/-}$ mice survived up to 2 years. Classical CNM histological features including fiber atrophy and nuclei mispositioning were prevented or strongly delayed and reduced, and muscle strength was increased. Downregulation of Dnm2 selectively in skeletal muscle during embryogenesis or in young mice after onset of the disease showed that the rescue is cell autonomous and that downregulation of Dnm2 can stop and potentially revert the progression of the phenotype.

In conclusion, we identified MTM1 and DNM2 are in a common pathway regulating muscle organization and force. We introduce the original concept of 'cross-therapy' where one form of the disease (XLCNM, MTM1) can be rescued by decreasing expression of another gene mutated in CNM (DNM2 in ADCNM). While DNM2 is a key mechanoenzyme for important cellular processes, its reduction is strongly beneficial for centronuclear myopathy and represents a novel potential therapeutic approach.

PS2-153 / #299

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Adult-onset autosomal dominant centronuclear myopathy due to BIN1 mutations

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Objective: Centronuclear myopathies (CNM) are congenital muscle disorders characterized by type I myofiber predominance and increased number of muscle fibres with nuclear centralization. The severe neonatal X-linked form is due to mutations in MTM1, autosomal recessive CNM with neonatal or childhood onset results from mutations in BIN1 (amphiphysin 2), and dominant cases were previously associated to mutations in dynamin 2 (DNM2). Our aim was to determine the genetic basis and physiopathology of patients with mild dominant CNM without mutations in DNM2. Methods: We sequenced BIN1 in patients with adult-onset CNM diagnosed by histological and ultrastructural analyses. The molecular diagnosis was complemented by functional investigations. Results: We characterized a homogeneous cohort of 9 patients from 5 families with adult-onset CNM without any facial involvement, including 3 sporadic cases and 2 families with dominant disease inheritance. All patients had similar histological and ultrastructural features involving type I fiber predominance and hypotrophy, as well as prominent nuclear centralization and clustering. We identified heterozygous BIN1 mutations in all patients. Two mutations in the N-terminal amphipathic helix strongly decreased the membrane-deforming properties of amphiphysin 2 and three no-stop mutations resulted in a stable protein containing 52 supernumerary amino acids. Immunolabeling experiments revealed abnormal central accumulation of dynamin 2 and caveolin-3, and general membrane alterations of the triad, the sarcolemma, and the basal lamina as potential pathological mechanisms. Interpretation: We identified BIN1 as a novel gene for dominant adult-onset centronuclear myopathy. Our data provide the evidence that specific BIN1 mutations can cause either recessive or dominant CNM with different severity and molecular mechanisms.

PS2-154 / #331

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Selenoprotein N and oxidative stress regulate myogenesis and muscle stem cell differentiation

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Background: Inherited defects of Selenoprotein N (SelN, encoded by SEPN1) cause SEPN1-related myopathy (SEPN1-RM), a congenital disorder typified by severe weakness and wasting of neck and trunk muscles leading to respiratory failure. Previously we showed that, in human muscle cells devoid of SelN, there is an increase in intracellular oxidant activity and protein carbonylation, suggesting that SelN plays a key role in antioxidant protection. It has also been shown that sepn1-/- mice have a reduced rate of muscle repair and a depletion of the muscle stem cell (satellite cell, SC) population after injury when compared to wild-type mice. The underlying mechanisms are unknown.

Objectives: To clarify a potential role of SelN and/or the oxidative stress associated to its deficiency in regulating muscle regenerative capacity and myogenesis, and to identify the underlying mechanisms.

Results: Quantification of SEPN1 expression and ROS levels in different populations of muscle cells stem cells from sepn1-/- mice demonstrated a direct correlation between SelN expression, ROS content and degree of stemness. Oxidant activity and SEPN1 expression were significantly higher in PICs and SCA+ cells than in the more committed fibroblasts and satellite cells. Furthermore, cells from SEPN1-RM patients devoid of SelN, muscles from the

sepn1-/- mice and C2C12 myoblasts knocked-down for SEPN1 showed perturbations in the level and subcellular localisation of myogenic and cell cycle exit factors. Congruently, we observed an accelerated initiation of cell differentiation in C2C12 knocked-down for SEPN1. We further showed that this is, at least in part, due to the role of SelN in regulating redox homeostasis, including specific modulation of expression of antioxidant genes.

Conclusions: These results show that SelN has an important role in maintaining the balance between self-renewal and differentiation of the muscle stem cells, thus representing a novel link between the intracellular and tissue redox environment and myogenesis. We show that these mechanisms can target preferentially specific stem cell populations, less differentiated subpopulations of muscle stem cells having the highest ROS production and SEPN1 expression. Thus, different types of muscle stem cells are maintained in different redox states, suggesting that close control of redox homeostasis is important for maintenance of their stemness and self-renewal.

PS2-155 / #351

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Amyoplasia : Muscle MRI findings and comparison with motor function

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Amyoplasia, the most frequent form of arthrogryposis multiplex congenita, presents with diminished muscle mass, a characteristic position of the limbs at birth, and muscle replacement by fibro-adipose tissue. We questioned whether there is a common pattern of muscle involvement in amyoplasia and whether there is a correlation between fibro-adipose infiltration of muscles and motor function. Twelve patients with a clinical diagnosis of amyoplasia underwent T1 weighted muscle MRI of the upper and lower limbs. Eight patients were community or household ambulators, four were non ambulators. Fibro-adipose infiltration of muscles was evaluated using Mercuri score, and muscle strength using the MMRC score. All patients showed muscle replacement by fibro-adipose tissue in the lower limbs. The severity of fibro-adipose infiltration was heterogeneous for a given muscle among patients, and markedly asymmetric especially in the calves. Adductor muscles of the lower limbs were significantly less infiltrated ($P<0,01$). Muscle strength and muscle replacement by fibro-adipose tissue were not significantly different between ambulators and non ambulators. In conclusion there is a characteristic pattern of muscle involvement in our patients with significantly less fibro-adipose infiltration of the adductor muscles of the lower limbs. We could not find a difference in muscle strength and muscle infiltration by fibro-adipose tissue between ambulators and non ambulators.

PS2-156 / #353

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Novel ECEL1 mutations and associated distal arthrogryposis phenotypes

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Endothelin-converting enzyme like 1 (ECEL1) is a member of the neutral endopeptidase (neprilysine, NEP) family with a critical role in intramuscular axon

branching of motor neurons during development. Recently we identified ECEL1 mutations in an autosomal recessive form of distal arthrogryposis with a recognizable phenotype including camptodactily, absent flexion of the knee, ptosis, tongue atrophy and muscle atrophy and fatty infiltration especially of the biceps femoris. Most of ECEL1 mutations are splice site or non-sense mutations and predicted to yield truncated proteins and mRNA decay. The pathophysiological mechanism of false sense mutations is less well known. Our *in vitro* studies show that all missense mutations we have identified so far do not modify protein expression and its subcellular localisation. Review of the clinical data of all our patients furthermore confirms that ECEL1 mutations yield a homogeneous phenotype but expands the spectrum of severity (muscle atrophy, limb pterygia).

PS2-157 / #370

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Inhibition of the ubiquitin proteasome system rescues defective SERCA1 protein causing Chianina cattle pseudomyotonia

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A missense mutation (Arg164His) in ATP2A1 gene, encoding SERCA1 protein, causes Chianina cattle congenital pseudomyotonia (PMT), a muscle disorder clinically characterized by an impairment of muscle relaxation induced by exercise. Skeletal muscles of PMT affected cattle are characterized by a selective reduction in SERCA1 levels in sarcoplasmic reticulum (SR) membranes. SERCA1 plays a key role in the Ca^{2+} homeostasis in skeletal muscle fibres, being responsible for pumping Ca^{2+} from cytosol back into SR lumen, to initiate relaxation.

Here we provide evidence that the ubiquitin proteasome system (UPS) is involved in the reduced density of mutated SERCA1: this event deprives muscle fibers of a critical protein and explains the pathological phenotype. By using a heterologous cellular model (HEK-293) overexpressing R164H SERCA1 mutant we demonstrated that the protein undergoes ubiquitination and that the treatment with MG132, a well-known inhibitor of UPS, rescues the expression level and membrane localization of the mutant SERCA1. Cells co-transfected with the Ca^{2+} sensitive probe aequorin, have shown that the rescued SERCA1 mutant exhibits the same ability of wild-type pump to maintain Ca^{2+} homeostasis within cells. The role of the UPS in physiopathological mechanism underling cattle PMT was definitively demonstrated by incubation with MG132 of adult skeletal muscle fibres from a PMT subject, that leads to the selective accumulation of fully functional, although mutated, SERCA1 in SR membranes.

These data show that the Arg164His mutation generates a protein most likely corrupted in proper folding but not in catalytic activity. Rescue of mutated SERCA1 to SR membrane can re-establish resting cytosolic Ca^{2+} concentration and prevent the appearance of pathological signs of cattle PMT.

The relevance for human pathology of these experiments is bound to similarities of cattle PMT with human Brody disease. Our results provide new insight into the pathogenesis of congenital pseudomyotonia as well as Brody myopathy and suggest possible molecular intervention to treat the human disease (Bianchini et al., submitted).

PS2-158 / #388

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Successful cardiac transplantation in a young boy with congenital myopathy caused by ACTA 1 gene mutation.

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Background: Nemaline myopathy caused by mutations in the ACTA 1 gene is a rare muscular disorder with a broad spectrum of phenotypes. Cardiac involvement in ACTA 1 related myopathy is very unusual. So far there is only one case report describing acute heart failure due to dilated cardiomyopathy in childhood. Our case report is the first example of successful cardiac transplantation in such a patient.

Case report: We present a 9-year boy who at the age of 6 years after a viral infection developed acute heart failure due to dilated cardiomyopathy. At presentation he showed facial muscle weakness and generalised muscle hypotonia with mild muscle weakness. Since infancy he had been followed up for mild motor development delay with no specific diagnosis. The heart failure was firstly treated conservatively but failed to prevent further deterioration so that heart transplantation became inevitable. Prior to transplantation a biventricular assist device was implanted and the boy spent three months on it. In the meantime cardiac and skeletal muscle biopsy was performed. The cardiac muscle biopsy showed unspecific changes of chronic cardiomyopathy. The skeletal muscle biopsy revealed cytoplasmatic bodies with labelling for alpha actinin. Cardiac transplantation was successful and the boy was discharged home. Further analysis was undertaken to determine the cause of his skeletal and cardiac myopathy. The lamin, desmin, FHL1, BAG3, alphaB crystalline, myotilin and cofilin 2 genes were tested with normal results. Finally, 2 years after the heart transplantation, a de-novo novel mutation c.175G>A in ACTA1 gene was found.

Conclusion: Our case is an example of congenital ACTA1 associated myopathy where the diagnosis was only made following cardiac failure and successful transplantation at the age of 6 years. The signs of congenital myopathy in this case were very subtle and had not prompted diagnostic referral.. Our case shows the difficulties in the diagnosis of rare forms of congenital myopathies, where in some cases finding the causal gene abnormality can takes many years. Based on this case report we would recommend looking for signs of congenital myopathy in any unclear cases of acute heart failure in childhood and on the other hand screening for early signs of cardiac failure in congenital myopathy caused by ACTA1 gene mutation.

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PS2-159 / #399

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Identification by NGS of a novel MYH7 mutation in an Italian family affected by distal myopathy with multi-minicores

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Mutations in the slow beta myosin heavy chain (MYH7) gene are an increasingly recognized cause of familial cardiomyopathy and a wide spectrum of myopathies ranging from Laing distal myopathy to hyaline body myopathy. Recently several authors reported mutations in MYH7 in multiminicore disease. It has been postulated that the resulting phenotype depends on the location of the mutation in the MYH7 gene.

Here we report a clinical, molecular, muscle MRI and histological characterization of an Italian

Family affected by distal myopathy with multiminicores histological findings, harboring a novel mutation in MYH7 gene that was identified by a targeted next generation sequencing (NGS) approach that include 93 disease genes.

The two affected members, a 45 years old mother and her 14 years old daughter, shared a common clinical phenotype dominated by weakness of upper and lower extremities and neck flexors muscles.

CK was in the normal range in both patients and no signs of cardiac involvement were reported.

Muscle biopsy performed in the mother at age 44 showed prominent myopathic features with marked predominance of type 1 fibre and multi-minicores in nearly 30% of fibres.

Muscle MRI detected, in both patients, a significant fatty replacement of tibialis anterior and extensors in the anterolateral compartment of the leg. In addition the mother displayed involvement of both gastrocnemii and initial changes on thigh level with an atypical asymmetric distribution.

NGS of theMYH7gene revealed a novel heterozygous p.A1603P (c.4807G>C) missense change: the mutation segregated to the two patients and was not reported in the MYH7 locus-specific database (http://www.gen2phen.org/gene/myh7/).

The mutation site lies within the myosin rod, which is classically associated with myopathy, and it was well conserved among different species. In silico analysis predicted that the mutation may affect protein function, with scores of 0.986 (Polyphen2). No other relevant mutation was detected in the remaining genes.

In conclusion, our data expands the variations panel of MYH7 gene and emphasize its causative role in a wide spectrum of myopathic conditions, including multi-minicores myopathy.

PS2-160 / #414

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Tubular aggregate myopathy is caused by mutations in the calcium sensor STIM1

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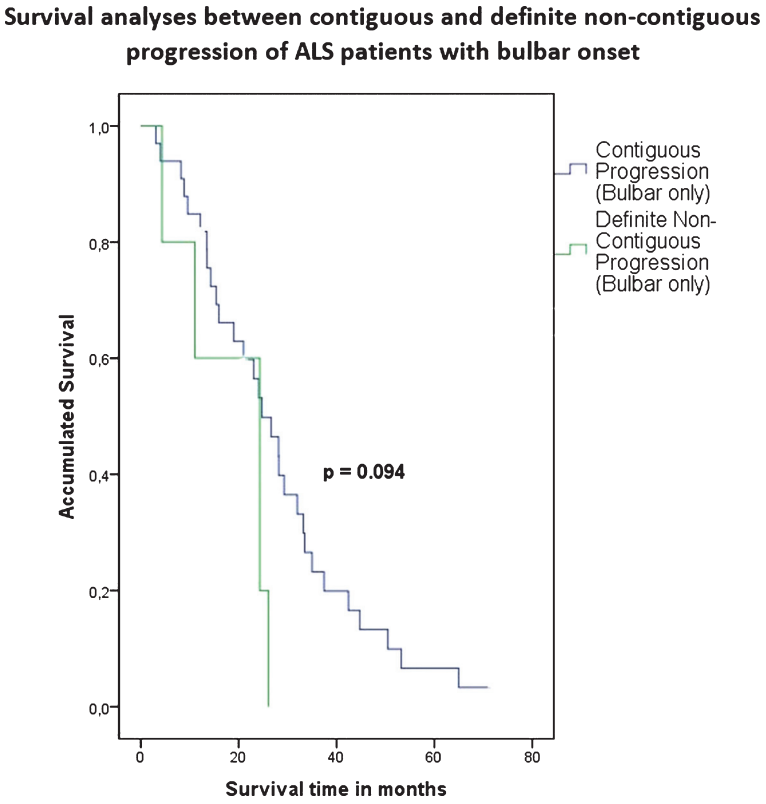
Calcium (Ca^{2+}) is a physiological key factor and plays a pivotal role in a plethora of cellular functions. In skeletal muscle, calcium triggers contraction and acts as a second messenger controlling growth and differentiation. In order to ensure normal muscle function, calcium storage in the sarcoplasmic reticulum and release to the cytoplasm need to be tightly regulated.

STIM1 (Stromal Interaction Molecule 1) is the main calcium sensor in the sarcoplasmic reticulum. It contains an intraluminal region with two EF hands and a SAM domain, a single transmembrane domain, and a cytosolic part interacting with other proteins.



The EF hands sense and bind calcium within the sarcoplasmic reticulum. Upon calcium store depletion, STIM1 unfolds, oligomerizes and activates calcium release-activated calcium channels (CRAC) as ORAI1 to trigger extracellular calcium entry. This STIM1-dependant mechanism of calcium store refill is known as store-operated calcium entry (SOCE).

Using high-throughput sequencing, we have identified STIM1 mutations as the genetic cause of tubular aggregate myopathy (TAM). This progressive muscle disorder can involve muscle weakness, cramps and pain. Biopsies from TAM patients typically show prominent aggregates consisting of densely packed membrane tubules. These aggregates also appear as secondary features in a variety of inherited and acquired muscle disorders and strikingly accumulate in normal muscle with age.

All 10 STIM1 mutations identified to date (including 6 unpublished) affect highly conserved amino acids in the EF hands. In order to assess their impact on the biological function of STIM1, we transfected C2C12 myoblasts with STIM1-YFP constructs. In contrast to wild-type STIM1, all mutants were constitutively oligomerized, indicating that calcium sensing was impaired. To further investigate the pathomechanism underlying the disorder, we measured the response to SOCE in myoblasts from TAM patients. Compared to controls, TAM myoblasts displayed increased calcium levels under basal condition. Addition of calcium to the medium induced a massive intracellular calcium increase in TAM myoblasts, while only moderate calcium influx was observed in control myolasts.

These results demonstrate that the STIM1 mutations impair calcium sensing and induce constitutive STIM1 clustering and activation of the calcium channels, resulting in aberrant calcium homeostasis in muscle cells from TAM patients.

PS2-161 / #416

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Molecular characterization of canine models for centronuclear myopathies

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Spontaneous muscle diseases can arise in dogs. The molecular characterization of such animals is important for 1) providing potential counseling to breeders, 2) validating a large animal model to study further the physiopathology and 3) for pre-clinical trials. Centronuclear myopathies (CNM) are mainly due to mutations in either the phosphatase myotubularin (MTM1, myotubular myopathy), the membrane curvature sensing protein amphiphysin 2 (BIN1) and the large GTPase dynamin 2 (DNM2). Through collaborations with Vets, we identified two breeds with mutations in CNM genes. A non-conservative missense mutation p.N155K in the PH-GRAM domain of MTM1 is the cause of a myopathy in several male Labrador retrievers. The mutant protein was strongly decreased in affected muscle and sequestered in proteasomes upon exogenous expression in cultured cells, suggesting protein instability. Moreover, we found the inherited myopathy of Great Danes (IMGD) is due to a splice mutation in the muscle specific exon of BIN1, and lead to a strong decrease in the protein levels. In both cases, the mutations segregate with the disease and are absent in unaffected dogs from the same breed and from other breeds. Both models are affected with progressive muscle weakness and atrophy, and display on the muscle biopsy a high number of centrally located nuclei. These models share altered triads shape and defect in the localization of triad markers. Large plasma membrane invaginations are also seen in the affected Great Danes. These histopathological signs are very similar to what is noted in patients with MTM1 or BIN1 mutations respectively. We conclude these canine models are faithful to the human conditions and should prove valuable for further studies and therapeutic development.

PS2-162 / #430

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Congenital myopathies and neonatal bone fractures

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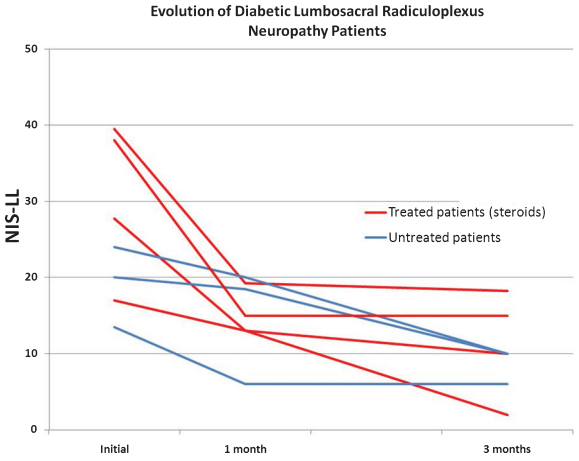
Children with reduced mobility in utero are at increased risk of osteopenia and pathological fractures, as a secondary effect of severe fetal muscular inactivity. Immobilization reduces cortical thickness, with the subsequent development of fragile bones, fractures and fetal bone osteopenia. Neonatal Spinal Muscular atrophy has been described with bone fractures as well as some congenital myopathies such as nemaline myopathy, severe congenital RYR1-associated myopathy and myotubular myopathy with MTM1 mutations. There are few reports in the literature that focus on neonatal fractures in severe congenital myopathies. Our aim is to describe four patients with congenital multiple long bone fractures and congenital myopathies and review from literature all the reported cases describing newborn fractures in severe hypotonic patients in order to define the major etiological possibilities when approaching a patient with severe hypotonia and neonatal bone fractures at birth. Results: Two patients harbored a mutation in ACTA1 gene; both died soon after birth one of them a girl with distal arthrogryposis as main clinical presentation. A third patient living only 8 month was a boy with a previous brother diagnosed of myotubular myopathy and a mutation in MTM1 gene. The last patient is still alive at the age of 7 years. He presented at birth with severe hypotonia, ophthalmoplegia, multiple fracture in femur and humerus, requiring early gastrostomy and tracheostomy. No mutation in MTM1 was found and a search for RYR1 mutation is still carried on.

We found only 9 articles describing congenital myopathies associated with neonatal fractures. ACTA1 mutation was found in 11 patients, MTM1 in only 1, RYR1 mutation in 4 cases and KLHL40 in 10 newborns. Surprisingly we found no reports describing fractures in the newborn affected by congenital muscular dystrophy patients, nor in congenital myotonic dystrophy. Prader Willi syndrome as an important cause of hypotonia at birth was also reviewed and no reported neonatal fractures were found.

Conclusion: There are clearly 4 etiologies associated with neonatal fractures and congenital myopathies: ACTA1; RYR1; MTM1 and KLHL40 genes. These are probably those who we must search in these patients with priority. It seems that there are other factors probably related to the specific genetic background to explain the manifestation of fractures other than the fetal inactivity itself.



PS2-163 / #436

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Expanding the clinicopathological and genetic spectrum of RYR1 related Congenital Myopathies with cores and minicores: an Italian population study

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Mutations in RYR1 gene, encoding the skeletal muscle ryanodine receptor, are a well-known cause of central cores myopathy and multiminicores myopathies.

In this work we collected clinical, histopathological and muscle MRI feature of 71 patients presenting with congenital myopathies with cores and minicores and mutations in RYR1 gene.

28 males and 43 females between 2 and 72 years were included . 36 patients had a positive family history.

66 mutations, generally missense, were identified, of which 27 have never been described previously. Most of the mutation were in single heterozygosity, however 6 compound heterozygous and 2 homozygous cases were recognised.

Clinical onset was congenital or during childhood in the vast majority of patients, nonetheless 12 patients presented first symptoms later in adult age.

Clinical phenotype was highly heterogeneous ranging from asymptomatic or paucisymptomatic hyperckemia (13 patients) to severe muscle weakness (31 patients) and skeletal deformity with loss of ambulation (3 patients). Facial weakness was also common (20 patients) but ophthalmoparesis was present in only 3 cases. Foot deformity and contractures were frequently reported (28 patients) as well as kyphoscoliosis (27 patients). 12 patients presented restrictive respiratory insufficiency, requiring non-invasive ventilation in 2 cases.

Muscle biopsies were revaluated in 59 patients, all showing central cores and multiminicores in a variable percentage of fibres (from 9 to 100%). Additional features included central nuclei (36 cases), fibre type predominance (41 cases) and nemaline bodies (1 case).

Muscle MRI was available in 37 patients and showed, in 14 patients, at the tight level the typical pattern of muscle involvement previously reported in association with RYR1 mutations.

Our survey add to the clinical and genetic heterogeneity of RYR1 related myopathies and confirm the high frequency of mutations in RYR1 gene in patients with congenital myopathies.

PS2-164 / #450

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Congenital muscle disease with Rigid Spine, joint hyperlaxity and skin abnormalities: a novel phenotype and locus

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Background: Definition of congenital myopathies is mainly based on histological hallmarks and on identification of the responsible genes. However, there is a significant number of patients with infantile myopathies whose biopsies show patterns previously unreported or difficult to classify, and whose genetic bases remain unknown.

Objectives: To define the phenotype and the molecular defects in a large consanguineous family presenting with a previously unreported form of infantile myopathy.

Results: We studied a French family with two consanguineous branches totalizing 4 affected children. All the patients presented with neonatal hypotonia, delayed motor development with limited or no independent ambulation and generalized muscle weakness involving predominantly neck and trunk muscles. This axial involvement lead to head lag, Rigid Spine, scoliosis and severe respiratory failure, which caused death of one patient at 18 months and required assisted ventilation in the others. Interestingly, limb joints showed absence of contractures and a variable degree of hyperlaxity associated with a skin phenotype (follicular hyperkeratosis, dry skin, hyperextensibility) reminiscent of collagenopathies, but linkage to the COL6 loci was excluded. Both parental couples were healthy.

Histologically, 4 biopsies from 3 patients showed a previously-unreported pattern, combining lesions typical of several congenital myopathies (cap lesions, minicores and centrally-located nuclei) with mild dystrophic findings (endomysial fibrosis and fatty replacement, regenerating fibers) compatible with a congenital muscular dystrophy.

Whole-genome linkage studies (Human Panel V, 6056 SNPs) excluded linkage to known genes and identified linkage to a new locus of 2,6Mb on chromosome 15 (Merlin Analyses, LOD Score >3,8). This locus includes 32 positional candidate genes, none of which has been previously associated with muscle conditions. Validation of a potential pathogenic change in one of these genes is in progress.

Conclusions: We report a novel congenital muscle condition clinically close to collagenopathies but associated with a unique histological pattern, stressing the phenotypical overlap between different forms of congenital myopathies and congenital muscular dystrophies. Molecular studies confirm that this represents a new entity, thus contributing to enlarge the spectrum of congenital muscle conditions and to shed further light on the complexity of their classification.

PS2-165 / #454

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

STIM1 mutations and muscle imaging in four Italian Tubular aggregate myopathy patients

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Background: Tubular aggregate myopathy (TAM) is a genetically heterogeneous disease characterized by the presence of tubular aggregates as the pathological hallmark on muscle biopsy.

Objectives: Aim of our study was to characterize the phenotype, the molecular genetic background and the involvement on muscle imaging of four TAM patients.

Methods: TAM patients underwent direct sequencing of the recently identified STIM1 gene and muscle imaging through magnetic resonance.

Results: The four patients displayed different clinical features: two were unrelated and showed a phenotype compatible with a congenital myopathy, with childhood onset and no significant myalgia/cramps, while the other two patients were siblings and had a later onset with a clinical diagnosis of limb-girdle muscular dystrophy. In the latter patients, myalgia was reported as an accompanying symptom only in the advanced disease course. All of the patients harbored mutations in the STIM1 gene, two of which were novel. Lower limb muscle imaging displayed a common pattern of involvement, although the extent and severity of lesions varied.

Discussion: despite the different phenotypes (age at onset, presence/absence of myalgias, overall severity) all the four analyzed patients presented mutations in STIM1, and muscle imaging findings were rather homogeneous among them.

Conclusion: Mutations in STIM1 are a very common cause of TAM. Muscle imaging can provide clues for the diagnosis of this disease.

PS2-166 / #459

Theme: *2.4 - Muscle diseases of genetic origin: Congenital myopathies / myopathies with prominent muscle contractures*

Clinical heterogeneity in adult forms of FHL1 related myopathies. The "Institut de myologie" experience

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Background: FHL1 mutations are responsible for reducing body myopathy (RBM), a rare condition characterized by progressive muscle weakness and the presence of intracytoplasmic aggregates. The age at onset ranges from early onset in infancy, through childhood and in some cases adult age. FHL1 mutations are also associated with allelic disorders including Emery-Dreifuss like muscular dystrophy (EDMD), hypertrophic cardiomyopathy (HCM), X-linked myopathy with postural muscle atrophy and generalized hypertrophy (X-MPMA) and X-linked scapuloperoneal myopathy (X-SM).

Objectives: To report clinical, muscle imaging, histological and genetic features found in a series of 10 adult patients carrying FHL1 gene mutations, followed at the "Institute de Myologie", Pitié-Salpétrière Hospital, Paris.

Methods: Medical reports of the 10 patients were retrospectively reviewed.

Results: Ten adult patients (5 M, 5 F) belonging to 8 families were available for this study. Pseudodominant mode of inheritance was suggested in 4 families, X-linked in 3 and sporadic in one. They were investigated at an age ranging from 22 to 52 years old. Three had EDMD, 2 X-SM, 1 X-MPMA, 1 HCM while 2 patients had atypical features and 1 subject was still asymptomatic. When symptomatic, female patients had all a striking asymmetric muscle involvement. Five patients had hypertrophic cardiomyopathy

requiring heart transplantation in one patient at 18 years old. Two patients required night time ventilation due to diaphragm paralysis. Histologically, only 3 patients among the 8 probands had reducing bodies (RBs) at muscle biopsy, while 3 had nonspecific pattern, 1 had neuropathic pattern and one muscle biopsy was considered as normal.

Discussion and conclusion: In contrast to infantile forms of FHL1 related myopathy that usually show RBs at muscle biopsy and lead to early death, adult forms had wider clinical and histological presentations. Moreover, FHL1 gene screening should be considered in those undiagnosed patients suffering from myopathies withdiaphragm paralysis and/or HCM where X-linked inheritance is not excluded even in the absence of RBs at muscle pathology.

PS2-167 / #76

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

Induction of autophagy reduces aggregates in a cellular model of desminopathy

No authors listed for this abstract. Please contact us to add a list of authors.

Myofibrillar myopathies (MFMs) are characterized by a severe myopathy, present a progressive muscle weakness, and cardiomyopathy generally associated in 15 to 30 % of the cases. Cardiomyopathy may develop before the onset of skeletal myopathy, or simultaneously. The disease is usually transmitted by autosomal dominant inheritance but sporadic cases are frequent, with generally an adult onset of muscle symptoms. MFMs are characterized by disturbed sarcomeric Z-line and abnormal aggregation of proteins predominantly involved in the structure or maintenance of the Z-line. MFMs are mainly caused by mutations in the desmin, alpha B-crystallin, myotilin, ZASP, filamin C or BAG3 genes.

We are studying mutations in the desmin gene, coding for the specific muscular intermediate filament, to understand how muscular cells react when abnormal desmin is synthetized and accumulate as aggregates. We have analyzed the kinetic of accumulation of desmin aggregates following transient transfection in murine myoblasts and found an exponential growth following a latency period of 20 h, then reaching a plateau.

We next tested the possibility to reduce aggregation by modulating cell signaling pathways related to the cytoskeletal networks. Among 30 kinases or GTPases, we found that PAK1 (p21-activated kinase) and PKC (protein kinase C) are efficiently reducing the percentage of myoblasts with aggregates during the early latency period of aggregation, and are also efficiently inducing autophagy.

We then analyzed pharmacological compounds that could help cells to reduce their aggregate contents: anti-aggregate, anti-oxydant, and autophagy-inducing products. We found that tocopherols and PP242, a powerfull inducer of autophagy in muscular cells, were efficiently inhibiting aggregation, without showing toxicity for these cells.

In conclusion, we have found cell signaling pathways, antioxydant treatments and inducers of autophagy that could pave the way to pharmacological treatments of MFMs.

PS2-168 / #78

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

Desmin, mechanics and myofibrillar myopathies

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The cytoskeleton plays a central role in transmitting and generating mechanical forces through cells. It is composed of 3 interconnected networks, actin, microtubules and intermediate filaments (IF). Desmin belongs to the type III IF, specifically expressed in muscles and is essential to maintain the integrity and functioning of muscles. More than sixty mutations have been identified in its gene, leading to rare diseases belonging to MyoFibrillar Myopathy group (MFM). These pathologies are mainly characterized by aggregates formation in muscle tissue, associated with misorganizations of the contractile apparatus. Moreover patients progressively develop muscle weakness. Currently, pathophysiology and molecular defects of MFMs remain largely unknown, and no treatments are available.

The aim of our study is to clarify whether desmin mutations implicated in MFMs plays a role at early

stage of expression, by impairing the properties of myoblasts. First we have studied the formation of desmin aggregates in living myoblasts over-expressing for 24h wild-type (WT) or different mutant desmins. We show that each mutant has a specific impact on the desmin network organization. Second we have performed mechanical measurements on C2C12 cells, focusing on the E413K mutant, which induces a large desmin network disorganization associated with important aggregation. We have compared the mechanical properties of WT-cells, C2C12 over-expressing desmin-WT-GFP and C2C12 expressing mutated desmin E413K-GFP. Visco-elastic properties of cells have been evaluated at the cortical and the whole-cells scaleby using optical tweezers and a single-cell rheometer. The 3 cells types share the same visco-elastic behaviour. Finally, we have investigated the impact of mutated desmin on myoblasts contractility, and demonstrate that E413K-mutation significantly decreases cell contraction abilities specifically for cells with desmin aggregates, while aggregates of WT-desmin do no induce the same effect.

PS2-169 / #79

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

N-acetyl-L-cysteine prevents stress-induced desmin aggregation in cellular models of desminopathy

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Mutations within the human desmin gene are responsible for a subcategory of myofibrillar myopathies called desminopathies. However a single inherited mutation can produce different phenotypes within a family, suggesting that environmental factors influence disease states. Although several mouse models have been used to investigate organ-specific desminopathies, a more general mechanistic perspective is required to advance our knowledge toward patient treatment. To improve our understanding of disease pathology, we have developed cell models to observe desmin behaviour in myoblasts upon formation of cytoplasmic desmin aggregates, within an isogenic background. We cloned the wildtype and 3 mutant desmin cDNAs using a Tet-On Advanced® expression system in C2C12 cells. Mutations were selected based on positioning within desmin and capacity to form aggregates in transient experiments, as follows: S46Y (head domain; low aggregation), D399Y (central rod domain; high aggregation), and S460I (tail domain; moderate aggregation). Introduction of these proteins into a C2C12 background permitted us to compare between desmin variants as well as to determine the role of external stress on aggregation. 3 different types of stress, likely encountered during muscle activity, were introduced to the cell models-heat shock, redox-associated (H2O2 and CdCl2), and mechanical (stretching) stresses-after which aggregation was measured. Cells containing variant D399Y were more sensitive to stress, leading to marked cytoplasmic perinuclear aggregations. We then evaluated the capacity of biochemical compounds to prevent this aggregation, applying dexamethasone (an inducer of heat shock proteins), N-acetyl-L-cysteine (NAC) or fisetin (2 antioxidants) before stress induction. Interestingly, NAC pre-treatment prevented D399Y aggregation during most stress. NAC has recently been described as a promising antioxidant in myopathies linked to selenoprotein N or ryanodin receptor defects. Our findings indicate that this drug warrants further study in animal models to speed its potential development as a therapy for D399Y-linked desminopathies.

PS2-170 / #104

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

The German patient registry for protein aggregate myopathies

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Protein aggregate myopathies (PAM) are a genetically and clinically heterogeneous group of neuromuscular and cardiac disorders which manifest from early childhood to later adulthood. The estimated prevalence of PAM is 2–4:100,000. Patients suffer from progressive muscle weakness and / or cardiac impairment resulting in disability and reduced life expectancy. No causative or ameliorating treatment is currently available but new therapeutic strategies are investigated. The early and accurate diagnosis can improve patients' quality of life and life expectancy.

A German (and in a second step internationally harmonized) patient registry for PAM is being established to overcome the bottlenecks of data fragmentation, lack of harmonization and suitable infrastructures and lack of trial readiness for these diseases.

We aim to include preferably all German PAM patients across the entire age range based on histopathological findings and molecular genetic diagnosis. A harmonized dataset including clinical data, muscle biopsy reports and genetic data is recorded using a dual patient and professional online report system (www.pam-register.de). National legislation, data protection laws and ethical recommendations are strictly followed in this process. Approval of the project by the Munich University Ethics Board is awaited for early 2014.

The registry will start operating in mid 2014 and aims at collecting high-quality, curated and regularly updated longitudinal data about the majority of German PAM patients in the mid- and long-term. In addition, the registry may help to address research questions like prevalence, natural history, disease characterization and genotype-phenotype correlations.

The PAM registry will provide the unique opportunity not only to effectively recruit German patients for future international clinical trials and to support the translation of innovative therapies "from bench to bedside", but also to improve patient care by assessing standards of diagnosis and care.

✶PF1

PS2-171 / #106

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

The pathogenesis of desminopathies: lessons from R350P desmin knock-in mice

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Mutations of the human desmin gene on chromosome 2q35 cause autosomal dominant, autosomal recessive, and sporadic myopathies and/or cardiomyopathies. We generated heterozygous and homozygous R350P desmin knock-in mice, which harbour the most frequently occuring human desmin point mutation. These mice develop a progressive desmin-positive protein aggregate myopathy and cardiomyopathy, which recapitulate central aspects of human autosomal dominant and autosomal recessive desminopathies. The expression of mutant R350P desmin in striated muscle leads to different morphological pattern of pathology in fast and slow contracting muscles, and reduced elasticity of skeletal muscle fiber bundles. Cardiac pathology comprises increased susceptibility to cardiac arrhythmias and/or

conduction defects as well as true cardiomyopathy. We demonstrate that the R350P desmin mutant i) inflicts an increased turn-over of desmin protein , ii) induces proteasomal activity and autophagic built-up, iii) leads to an abnormal subcellular localization of desmin's direct binding partners synemin, syncoilin and plectin, and iv) elicits age-related protein aggregation pathology. It is a matter of debate if, and to what extent, these protein aggregates contribute to the progressive muscle fiber damage in desminopathies. Our experiments reveal that marked desmin-positive protein aggregate pathology can be present without concomitant myopathological alterations. We provide a novel pathogenetic concept in which the expression of a point-mutated desmin induces a vicious circle with an increased turn-over of desmin, thereby affecting desmin's direct binding partners. The subsequent changes in the structural and functional organization of the extrasarcomeric cytoskeleton are likely the molecular basis for the increased vulnerability of muscle fibers.

✶PF4

PS2-172 / #116

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

Chemical chaperone ameliorates pathological protein aggregation in plectin-deficient muscle

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The multifunctional cytolinker protein plectin is essential for muscle fiber integrity and myofiber cytoarchitecture. The most common disease caused by plectin mutations, epidermolysis bullosa simplex with muscular dystrophy (EBS-MD), is characterized by skin blistering and muscular dystrophy. Muscle biopsies from EBS-MD patients and plectin knockout mice display pathological desmin-positive protein aggregation and degenerative changes of the myofibrillar apparatus, which are the morphological hallmarks of myofibrillar myopathies (MFM). We developed immortalized murine myoblasts to examine the pathogenesis of plectinopathies at the molecular and single cell level. Plectin-deficient myotubes mirrored the pathological features of MFM myofibers, including the presence of desmin-positive protein aggregates and disarrangement of the myofibrillar apparatus. Using this cell model, we demonstrated that plectin deficiency leads to increased intermediate filament network and sarcomere dynamics, marked upregulation of heat shock proteins, and reduced myotube resilience following mechanical stretch. Currently, no specific therapy is available for plectin-related muscular dystrophy and other forms of MFMs. We assessed the therapeutic potential of chemical chaperones on skeletal muscle structure and function in our cell and animal models for EBS-MD. Treatment with 4-PBA resulted in remarkable amelioration of the pathological phenotypes in plectin-deficient myotubes; desmin protein aggregate load was drastically reduced, sarcomeric structures were stabilized, and resilience against mechanical strain was increased. Most strikingly, *in vivo* 4-PBA treatment of plectin-deficient mice led to a significant reduction of desmin aggregate formation as well as to a functional muscle improvement. Beyond novel insight into the molecular pathogenesis of plectin-related muscular dystrophy, our data may help to pave the way to novel treatment strategies for EBS-MD and other forms of MFM.

PS2-173 / #220

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

Differential proteomic analysis of abnormal intramyoplasmic aggregates in myotilinopathy

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Myotilinopathy is a subtype of myofibrillar myopathies (MFM) caused by mutations in MYOT, the gene encoding the sarcomeric Z-disc protein myotilin. The formation of intramyoplasmic protein aggregates is a histopathological hallmark of MFM and seems to play an important role in pathogenesis. We applied a highly sensitive proteomic approach to decipher the composition of protein aggregates in myotilinopathy.

Skeletal muscle sections from 15 MFM patients with four different MYOT mutations were included in this study. Aggregate samples from abnormal muscle fibers and intraindividual controls from normally looking fibers were collected by laser microdissection and analyzed by a label-free mass spectrometry approach for identification and relative quantification of proteins. A total of 1913 different proteins were identified. 442 proteins were detected in at least five samples and 79 of these showed a statistically significant over-representation in aggregates samples compared to controls. The proteomic profiles were similar for the different MYOT mutations with a high abundance of myotilin and additional Z-disc and Z-disc associated proteins. We also identified sarcolemmal proteins, proteins that regulate actin dynamics and myofibrillar organization, chaperones and proteins involved in protein degradation. The results of our proteomic analysis could be validated by immunofluorescence studies.

The proteomic profile in myotilinopathy indicates that Z-disc and Z-disc-associated proteins constitute the most abundant group of proteins that accumulate in aggregates. An over-representation of several further proteins in deposits suggests that these proteins, especially components of protein quality control and protein degradation, are also disease-relevant. In conclusion, our proteomic data provide important new insights into the composition of pathological aggregates in myotilinopathy and expand our knowledge about proteins that seem to be involved in pathogenesis.

PS2-174 / #259

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

The “sphinx with dropped feet”: a peculiar phenotype associated with heterozygous MYH7 mutation and core myopathy

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Background: mutations in MYH7 gene are described in patients with distal myopathy. Objective: Describe a peculiar clinical phenotype in a patient from a family carrying a dominant mutation of MYH7 gene and core myopathy. Methods: physical examination, EMG study, whole-body MRI, muscle biopsy findings and genetic testing were performed in the index case. Results: He presented at the age of 7 years with minimal walking difficulties which became disabling by the age of 11 years due to severe cervical hyperlordosis. Several members of the family had stepping gait consistent with a dominant inheritance and some of them were labeled as Charcot-Marie-Tooth disease but gene PMP22 was negative. The sister showed also cervico-axial deficit and had a cardiac arrest during spinal surgery. At 19 years, our patient had a striking rigid hyperextension of the neck along with anterior leg atrophy and foot drop. No major respiratory or cardiac abnormalities were found. EMG showed diffuse myogenic changes and ruled out a peripheral neuropathy. Whole body MRI T1-TSE sequences showed severe enhancement of tibialis anterior muscle; moderate involvement of longus colli, dorsal erector spinae, psoas, and flexors of the toes. Muscle biopsy showed marked type 1 fibre predominance. Oxidative staining revealed the presence of well delimited cores localized in subsarcolemmal regions in numerous fibres; the latter pattern corresponded to 'Eccentric Cores Disease' (ECD). Cores were only observed in Type 1 fibres. Ultrastructural analysis demonstrated the presence of core lesions, devoid of mitochondria, spanning along more than sixty sarcomeres (full extent of the muscle section). No mutations were identified in RYR1, ACTA1 and LMNA genes. MYH7 testing revealed a non-sense mutation in the tail domain of the MYH7 gene at the heterozygous state. This same mutation was also identified in his sister. Discussion: this is the second family reported to have eccentric cores and MYH7 mutations. Both families share distal and trunk involvement Conclusion: MYH7 dominant mutations may present with a core myopathy associated with axial involvement, in addition to distal myopathy. Our study broadens the spectrum of clinical, radiologic and morphological findings associated with MYH7-related disorders.

PS2-175 / #327

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

Distal myosin heavy chain-7 (thumb) myopathy due to the novel transition c.5566G>A with heterogeneous cardiac involvement

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Myosin-heavy-chain (MYH7)-myopathy manifests clinically with a distal, scapuloperoneal, limb-girdle (proximal), or axial distribution and may involve the respiratory muscles. In the majority of the cases the heart is affected, ranging from relaxation impairment to dilative cardiomyopathy with ventricular arrhythmias. Progression of cardiac involvement and earlier onset in successive generations has not been reported in MYH7-myopathy. In a five-generation family MYH7-myopathy manifested with late-onset, distal>proximal myopathy and variable degree of cardiac involvement. The index patient developed myopathy from age 49y with anginal chest pain. Her mother presented with a similar phenotype but had only developed myocardial relaxation impairment. The daughter of the index patient had no overt myopathy but presented with left ventricular hypertrabeculation / noncompaction and required an ICD because of ventricular arrhythmias since age 37y. Her daughter was diagnosed with dilated cardiomyopathy at infancy, also without overt skeletal muscle disease. MYH7-myopathy in the presented family was due to the novel mutation c.1566G>A in the MYH7 gene. There is cardiac involvement in MYH7-myopathy, and cardiac affection in MYH7-myopathy is highly variable between the generations ranging from relaxation abnormality to noncompaction, ventricular arrhythmias, and dilated cardiomyopathy. Cardiac disease in MYH7-myopathy seems to progress with successive generations.

PS2-176 / #435

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

A case of surplus protein myopathy: clinical and pathological characterization

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Myopathies characterized by aggregates of particular proteins are defined "surplus protein myopathies". We describe the case of a 44-years-old Caucasian male, who came to our attention because of a ten months history of exercise-induced muscle pain and early fatigue in daily activities with exercise intolerance. His family history was inconsistent for neuromuscular diseases. The neurological examination revealed a moderate proximal muscle weakness at lower limb (score 4 at MRC scale), with Gower's sign. Serum creatine kinase resulted repeatedly increased (1000–1500 U/L, n.v. <200). Inflammation markers, immunologic testing, anti-acetylcholine receptor antibodies and thyroid hormones were within the normal range. Needle electromyography recorded a myopathic pattern. Cardiac evaluation was normal. The assessment of acid α-glucosidase activity on dried blood spot resulted in normal range. The morphological study of quadriceps femoris showed several fibers with clear vacuoles in sections stained with haematoxylin and eosin and Gomori-modified trichrome. Vacuoles had an oval shape or lobulated border. Some vacuoles contained fine amorphous material. Vacuoles did not stain with acid phosphatase, but some of them showed a rim of PAS positive material. Ultrastructural analysis revealed the presence of highly electrondense inclusions, mostly polygonal with rectangular or quadrangular shape, and, sometimes, irregular contour. They were located among myofibrils and were often associated with glycogen particles.Theclinical picture and the pathological features observed in our case really resemble the muscle disease previously described as a new surplus protein myopathy, called SERCA1 and calsequestrin storage myopathy.

PS2-177 / #438

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

Fhl1 W122S knock-in mice manifest late–onset mild myopathy

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FHL1 is a member of the four-and-a half-LIM (FHL) domain protein family and it is highly expressed in adult human skeletal muscle. It participates in sarcomere assembly, muscle growth and differentiation, and in the biomechanical stress responses. Mutations in FHL1 gene have been associated with different myopathies: reducing body myopathy, scapuloperoneal (SP) myopathy, X-linked myopathy with postural muscle atrophy, rigid spine syndrome (RSS), and Emery-Dreifuss muscular dystrophy. A recently described FHL1-null mouse model showed an age-dependent myopathy. The generation of knock-in and transgenic models of FHL1 mutations will further elucidate FHL1 function.In 2008, we identified a missense mutation in the second LIM domain of FHL1 (c.365 G>C, p.W122S) in a family with SP myopathy. We have generated a knock-in mouse model harboring the c.365 G>C Fhl1 mutation and have investigated the effects of this mutation at 3 stages of the disease (3–5 months, 7–10 months, and >12 months) in hemizygous males and heterozygous females mice. Our results showed that the survival was comparable in mutant and wild-type animals. Adult hemizygous

males mice showed a mild progressive myopathy with reduced forelimb strength and exercise capacity. Western blot analysis showed absence of FHL1 protein in muscle of hemizygous males at advanced stages.In conclusion, adult hemizygous males mice showed a slowly progressive phenotype similar to humans patients with late-onset muscle weakness. Mice did not show the typical cytoplasmic inclusions (reducing bodies) detected in human muscle biopsy, suggesting that loss of FHL1 function is involved in the development of the phenotype. This animal model may help to elucidate the role of FHL1 in skeletal muscle and the pathomechanism of FHL1 mutations in X-linked dominant SP myopathy.

PS2-178 / #444

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

Recessive desmin deficiency myopathy with fatigability: Clinical features and response to salbutamol treatment

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Objective: We aimed at describing the clinical and genetic findings in two cousins with recessive fatigable desminopathy and the response salbutamol treatment in one of them.

Methods: We performed clinical, pathological and genetic investigations in both patients. The surviving patient was treated with 6 mg/day salbutamol P.O. Efficacy of the treatment was assessed by the changes from baseline to 3 months in muscle strength (MRC score), six-minute walking test (6MWT), forced vital capacity (FVC) and patient's history.

Results: Both patients presented with infantile onset weakness with fatigability. Neurological examination revealed generalized weakness and muscle wasting, myopathic face, and symmetrical ptosis with ophthalmoparesis. The older patient developed cardiomyopathy at the age of fifteen and died at the age of seventeen due to respiratory and cardiac complications. Repetitive nerve stimulation revealed decremental response over 10% at 2–3 Hz. The 6MWT baseline was 380 m and salbutamol treatment 3 months lead to an increase of 44 m. The patient reported improvement in her movements, but no change in muscle strength was noted. By whole exome sequencing, a homozygous truncating DES mutation c.345dup (p.N116Qfs*2) in exon 1 was found which was assessed to be disease causing. Immunostaining did not detect any desmin in the muscle biopsy, consistent with a desmin-null phenotype. The mRNA level was found to be 0.33% of normal by quantitative PCR, verifying the null phenotype.

Conclusions: Desmin deficiency resulting from null mutation caused an infantile onset myopathy with profound fatigability and cardiomyopathy in our patients. Salbutamol treatment could be beneficial in reducing the fatigability and may lead to a better quality of life in other patients with a similar phenotype.

PS2-179 / #448

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

Characterization of ZASP-skeletal muscle actin interaction and its role in myofibrillar myopathy

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Background: Myofibrillar myopathies (MFM) are defined by skeletal muscle Z-disc disruption. Zaspopathy, a prototype MFM, is caused by A147T and A165V mutations in the sZM domain in the Z-disc alternatively spliced PDZ motif protein (ZASP). The functions of sZM are not yet known.

Objective: Characterize functional interactions of sZM, which may play a role in the structural integrity of the Z-disc

Methods: Proteins interacting with sZM were identified by a yeast two-hybrid (Y2H) screen of a human skeletal muscle cDNA library using sZM_{132aa} wild type (WT) or A165V as bait. Interactions were validated by pairwise Y2H, co-immunoprecipitation (co-IP), slot blot overlay, and co-sedimentation assay. Distribution of ZASP and F-actin was examined by immunostaining in patient vastus muscle.

Results: The most frequent prey clones encoded the 131 C-terminal amino acids of skeletal muscle actin ($ACTA1_{247–377}$). Pairwise Y2H assays using different

ZASP regions as bait against ACTA1 validated sZM$_{132aa}$ as an actin-binding region of ZASP. Deletion analysis of ACTA1 showed that the amino acids 304-325 of skeletal alpha-actin are essential for binding to sZM$_{132aa}$. ZASP-skeletal actin interaction was validated by co-IP of these two proteins from mouse muscle as well as transfected nonmuscle cell lysates. Presence of mutations did not affect this interaction. ZASP-actin interaction was abolished by deletion of sZM from ZASP. WT and mutant ZASP bound to F-actin with micromolar affinity. ZASP and F-actin accumulations were observed in patient muscle fibers.

Discussion: We show that ZASP binds to F-actin, which is important to the Z-disc structure in postnatal skeletal muscle. ZASP-actin interaction is preserved in zaspopathy. F-actin accumulation in patient skeletal muscle as well as in mice overexpressing mutant ZASP (separate submission) indicates disruptive effects of mutant ZASP on actin filaments. Other MFM gene products are known to bind F-actin or actin-binding proteins. Together with our finding of ZASP-actin interaction, it is likely that the molecular pathways leading to skeletal muscle Z-disc disruption are shared by these myopathies and altered F-actin dynamics may emerge as a unifying disease mechanism in this group of disorders.

Conclusion: ZASP is a novel actin-binding protein in skeletal muscle. The sZM domain that is mutated in zaspopathy is important for this interaction. ZASP-actin interaction expands the role of ZASP and defines the mechanism in MFM.

✶PF2

PS2-180 / #451

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

ZASP mutations in actin-binding domain cause disruption of skeletal muscle actin filaments in myofibrillar myopathy

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Background: Myofibrillar myopathies (MFM) are characterized by skeletal muscle Z-disc disruption. The core of Z-discs consists of actin filaments (F-actin) from adjacent sarcomeres crosslinked by alpha-actinin. Zaspopathy, a prototype MFM, is caused by dominant A147T and A165V mutations in the sZM domain of the Z-disc alternatively spliced PDZ motif protein (ZASP). We identified sZM as an actin-binding domain of ZASP (separate submission). ZASP binds to alpha-actinin via the PDZ domain.

Objective: To explore the effects of the sZM mutations on F-actin in skeletal muscle.

Methods: Wild type (WT) and mutant ZASP-GFP versions of muscle-specific isoforms were expressed in C2C12 cells and in mouse tibialis anterior (TA) muscles of opposite limbs by intramuscular injection. Muscle fiber morphology was assessed by immunofluorescence and electron microscopy. GST-ZASP fusions were used for *in vitro* studies.

Results: All WT and mutant ZASP-GFP isoforms localized to F-actin in muscle cells. Zaspopathy mutations in postnatal long ZASP (delta10) but not other isoforms consistently caused F-actin disruption. The F-actin organization was normal in cells expressing WT proteins. The architecture of the Z-disc and F-actin was preserved in mouse TA muscle fibers expressing WT-delta10-GFP protein. In contrast, there was a loss of alpha-actinin-2 in muscle fibers expressing mutant protein as early as one week after the electroporation. By 4 weeks, phalloidin staining showed focal F-actin accumulations that co-localized with A165V-delta10-GFP in the sarcoplasm. WT and mutant GST-ZASP pulled down myotilin from mouse muscle lysates. Immunostaining of the eletroporated muscle fibers showed a loss of myotilin from Z-discs and accumulation of myotilin and A165V-delta10-GFP in the sarcoplasm. Ultrastructural studies of the muscle expressing mutant protein confirmed discrete disruption of the Z-discs and adjacent F-actin reminiscent of the human disease. *In vitro* studies showed that ZASP mutants disassembled actinin-crosslinked F-actin, the core structure of skeletal muscle Z-disc.

Conclusions: The isoform-specific effects of mutations on F-actin suggest a molecular mechanism for zaspopathy and highlight the essential role of the delta10 isoform in Z-disc structure. Our results show that the sZM mutations have deleterious effects on actinin-crosslinked F-actin, the core structure of Z-discs in skeletal muscle, and support a dominant gain-of-function disease mechanism in zaspopathy.

PS2-181 / #489

Theme: *2.5 - Muscle diseases of genetic origin: Distal myopathy / Myofibrillar Myopathies*

Distinct distal myopathy phenotype caused by common MATR3 gene mutation in a new family

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Late onset autosomal vocal cord and pharyngeal distal myopathy (VCPDM) is a very rare distal myopathy reported so far in few families only. VCPDM is a slowly progressing disease, with initial weakness in ankle dorsiflexion and foot drop combined with bulbar symptoms dysphonia and dysphagia. In all families that have been reported at the moment VCPDM is caused by a missense mutation S85C in exon 2 of the MATR3 gene. Here we report a family with European ancestry that carries the identical MATR3 mutation, but unlike the other families with the mutation, our patients do not suffer from prominent bulbar symptoms. Instead some patients show a respiratory failure. Atrophy of small hand muscles without particular finger extension weakness is distinct from Welander distal myopathy. Muscle MRI's show that the posterior calf muscles of the lower legs are more affected than the anterior, although earlier descriptions indicated a greater involvement of the anterior extensors. Besides marked rimmed vacuolar muscle pathology immunohistochemical stainings reveal a considerable reduction of matrin 3 labelling in the central part of the nuclei. Together with the expanded clinical features and muscle MRI findings, nuclear matrin 3 reduction by immunohistochemistry is a useful tool for diagnostic purposes. Distal myopathy caused by MATR3 mutation might be more common than recognized and our findings will increase the diagnostic accuracy of the disease.

PS2-182 / #47

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Genetic Study of Brugada Syndrome in Tunisian population

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Brugada syndrome (BS) is an autosomal dominantly inherited form of ventricular fibrillation in normal structural heart, characterized by ST-segment elevation in the right pericordial leads V1-V3 and right bundle-branch block on surface electrocardiogram, in conjunction of high incidence of sudden death secondary to ventricular tachyarrhythmia. Mutations have been identified in the gene that encodes the alpha subunit of sodium channel (SCN5A). Most of them were missense type, that alter the splice-sites or shift the reading frame by deletion / insertion or substitution of nucleotides which results an in-frame stop codon. Others genes in relation with BS were described, until today more than ten genes were cited and stratified in many subsets of BS. SNP and haplotypes were reported in SCN5A but their roles were not clear yet. In our population, genetic studies for SB were limited and the last work was a screening for all SCN5A exons by fluorescent genotyping technique in nine families recruited from department of cardiology of CHU Sfax, but no significant results was found. The aim of our study is to identify the genetic causes of familial BS and determine the genetic profile of BS in Tunisian population. Eighteen families were recruited from departments of cardiology from Sousse and Sfax, genomic DNA extraction for all members of families will be performed by the phenol-chloroform method. Genetic analysis for the whole SCN5A gene will be carried out by polymerase chain reaction

(PCR) followed by high resolution melting analysis (HRM) and direct sequencing. Sequences will be compared with the reference genomic and cDNA sequences of SCN5A using BLASTN / ENSEMBL. In where necessary, searching for eventual mutations in others genes will be recommended for better understanding of the genetic form of BS in Tunisian patients and their families. As primary results, interpretation of pedigrees and family genetics investigations were in favor of a molecular basis for this pathology, which will be confirmed by molecular biotechnology techniques. Our research will allow us to characterize mutations of BS study population, to improve the genetic counseling and the therapeutic management.

PS2-183 / #51

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Unusual manifestation in myotonic dystrophy type 1 mimicking bilateral internuclear ophthalmoplegia

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Myotonic dystrophy type 1 (DM1), the most common form of muscular dystrophy in adults, is caused by the expansion of an unstable CTG repeat in the myotonin protein kinase (DMPK) gene on chromosome 19q13.3. DM1 may rarely be associated with external ophthalmoplegia. We report a patient with DM1 mimicking bilateral internuclear ophthalmoplegia.

A 49-year-old man presented with dysarthria and limb weakness. At age 30 years, he noted a dysarthria and arm weakness. These symptoms have progressed slowly, and he had leg weakness since 4 years ago. He had a characteristic facial appearance with frontal baldness, ptosis, and long lean face. On neurological examination, he had percussion myotonia of the thenar eminence. The extremities showed symmetrical muscular wasting and weakness, with depressed deep tendon reflexes. There is an apparent limitation of ocular motility that simulates a bilateral internuclear ophthalmoplegia. But, he did not complain diplopia. The muscle biopsy showed nonspecific myopathic changes, but the ragged-red fibers, rimmed vacuole, and centronuclear findings were negative. Anti-acetylcholine receptor antibodies were not detected. We performed genetic testing for DM1 and PABPN1 gene and was found to have allele in the DMPK gene locus with 650 CTG repeat expansions.

The relative sparing of extraocular muscles is well known in myotonic dystrophy. This case provides additional evidence of extraocular muscle involvement in DM1. DM1 may be considered as another possible cause of pseudo-internuclear ophthalmoplegia.

PS2-184 / #563

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Understanding the pathogenic mechanisms underlying X-linked Charcot Marie Tooth neuropathy (CMTX6) caused by the R158H PDK3 mutation

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Hereditary motor and sensory disorders of the peripheral nerve form one of the most common groups of human genetic diseases collectively called Charcot-Marie-Tooth (CMT) neuropathy. CMT is a clinically and genetically heterogeneous disorder and X-linked CMT (CMTX) accounts for 15-20% of CMT cases. Clinically, CMT is characterised by progressive distal wasting, weakness and sensory loss, which begins in the lower limbs. The 'dying back' of the nerve (axonal degeneration) occurs in a length dependent manner and is a pathological hallmark of the disease. We recently mapped a new locus for X-linked dominant CMT to chromosome Xp22.11 (CMTX6).

Using a combination of linkage information and whole exome sequencing identified a causative missense mutation c.G473A (p.R158H) in the pyruvate dehydrogenase kinase isoenzyme 3 (PDK3) gene (Kennerson, Yiu et al 2013). PDK3 is one of the four isoenzymes regulating the pyruvate dehydrogenase complex (PDC) by reversible phosphorylation and is a nuclear-coded protein located in the mitochondrial matrix. PDC catalyzes the oxidative decarboxylation of pyruvate to acetyl CoA and is a key enzyme linking glycolysis to the energy-producing Krebs cycle and lipogenic pathways. We previously reported that the R158H mutation confers enzyme hyperactivity and binds with stronger affinity than the wild-type to the inner-lipoyl (L2) domain of the E2p chain of PDC (Kennerson, Yiu et al 2013). Based on our initial biochemical findings, we have hypothesised the enzyme overactivity caused by the R158H PDK3 mutation will lead to hyperphosphorylation of pyruvate dehydrogenase thereby locking PDC in a predominantly inactive state. We have assessed the phosphorylation status of pyruvate dehydrogenase (PD) in patient fibroblasts harbouring the R158H mutation and shown hyper-phosphorylation of the PD at selected Serine residues. The downstream effects of the hyperphosphorylation are currently under investigation. These findings may suggest a reduced pyruvate flux is leading to secondary downstream effects that are the underlying pathogenic cause of peripheral neuropathy. Our results highlight an important causative link between peripheral nerve degeneration and an essential bioenergetic or biosynthetic pathway required for the maintenance of peripheral nerves.

✶PF3

PS2-185 / #92

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Cardiac involvement in myotonic dystrophy: a nationwide cohort study

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Smaller studies of patients with myotonic dystrophy (DM) suggest an increased risk of cardiac disease, particularly conduction disorders and arrhythmias. However, the magnitude of this issue remains unclear.

We identified a nationwide cohort of 1146 DM patients (period 1977-2011) using the National Patient Registry (NPR) and a subcohort of 485 patients who had undergone genetic testing for DM1. Information on incident cardiac disease was obtained from the NPR. We estimated standardized incidence ratios (SIRs) of cardiac disease in DM patients compared with the background population, overall and according to selected diagnostic subgroups of cardiac disease (cardiomyopathy, heart failure, conduction disorders, arrhythmias, and device implantation).

In the DM cohort, SIR for any cardiac disease was 3.42 (95% confidence interval [CI] 3.01–3.86); for a cardiac disease belonging to the selected diagnostic subgroups 6.91 (95% CI 5.93–8.01) and for other cardiac disease 2.59 (95% CI 2.03–3.25). For a cardiac disease belonging to the selected subgroups the risk was particularly high in the first year after DM diagnosis (SIR 15.4 [95% CI 10.9–21.3]) but remained significantly elevated in subsequent years (SIR 6.07 [95% CI 5.11–7.16]). Moreover, the risk was higher in young cohort members (<40 years old: SIR 18.2 [95% CI 12.6–25.5]) compared with older (e.g. 60–79 years old: SIR 3.99 [95% CI 2.98–5.23]) but with the risk remaining significantly increased in all age-categories. Results were similar in separate analyses of the genetically confirmed DM1 patients.

DM is strongly associated with cardiac disease. The risk is pronounced in the young and remains elevated throughout life, stressing the importance of lifelong cardiac follow-up from time of DM diagnosis.

PS2-186 / #98

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

The ultrasonographic evaluation of diaphragm in myotonic dystrophy

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[Purpose] Myotonic dystrophy (DM) patients have a respiratory failure because of respiratory muscle weakness. We evaluate the function of the diaphragm by using the ultrasonography. [Method] We examined the diaphragm in 18 DM patients (male 11, female 7, mean age 50.7±9.6) and 18 normal controls (male 12, female 6, mean age 53.6±6.8) by the ultrasonography from the right intercostal view, the left intercostal view and the right posterior view. We measured the thickness of the diaphragm at the end of inspiration and the expiration and calculate the changing ratio = (inspiration - respiration) / inspiration. We also calculate the velocity of the diaphragm's movement. [Result] The median of the thickness (mm) observed from the right intercostal view in the normal respiration is normal controls: 1.75, DM patients: 1.5 ($p<0.05$) at the expiration, and normal controls: 2.2, DM patients: 1.7 ($p<0.01$) at the inspiration. The changing ratio of the thickness in the deep respiration is normal controls: 1.1, DM patients: 0.35 ($p<0.01$) observed from the right intercostal view and normal controls: 1.2, DM patients: 0.5 ($p<0.01$) observed from the right posterior view. The velocity of the diaphragm's movement at the sniffing in the DM patients is significantly lower than in normal controls observed from the right intercostals view and the right posterior view. [Conclusion] Recently, some articles have reported a detail method of evaluation by using the ultrasonography, and these are useful methods to evaluate the function of the diaphragm in DM patients.

PS2-187 / #173

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Lower Urinary Tract and Bowel Dysfunction in Patients with Myotonic Dystrophy

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Background and aims. Myotonic dystrophy is a dominantly inherited disorder with multisystem clinical features. In CzechRepublic (Central Europe), the type 2 (MD2) is more frequent than type 1 (MD1). The aim of the study was to evaluate symptoms of lower urinary tract and bowel dysfunction and compare the data with healthy population.

Patients and methods. Eighty six patients from the Czech Myotonic disorders registry ReaDy (24 with MD1 and 62 suffering from MD2) and 28 healthy volunteers participated- were evaluated and compared using questionnaires focused on lower urinary tract and bowel dysfunction. (International Consultation on Incontinence Modular Questionnaire- ICIQ: ICIQ-FLUTS- urinary females, ICIQ-MLUTS- urinary males, ICIQ-B- bowel symptoms). Mean age of patients with myotonic dystrophy was 49 years (18–74 years, 67% females), in control population 42 years (25–82 years, 79% females). Mean duration of the disease was 18 years (2–48 years), all patients, except four, were ambulatory.

Results. Some problems with continence were found in 42% of patients with MD in opposite to 18% of control group ($p=0.0242$). Stress incontinence was present even in 51% of persons with MD (14% in control group, $p=0.0008$). Also questionnaire focused on bowel functions discovered the significant symptoms of anal incontinence.Among patients with MD 56% had problems with the control (leakage) of liquid stool. This problem had only 14% people from control group ($p=0.0001$). 15% of MD patients were never

able to control watery stool leaking and 29% were not able to control accidental loss of solid stool (4% in control group, $p=0.0039$). 23%of people with MD had unpredictable stool leakages, this symptom was not present in control group ($p=0.0031$).

Conclusion. The study confirmed frequent and important problems with urinary and anal incontinence among patients with myotonic dystrophy and reflects weakness of the pelvic floor muscles. These complaints have significant impact on quality of life of patients suffering from myotonic dystrophy.

PS2-188 / #177

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

MBNL1 autoregulates its function by binding to the 5′UTR of MBNL1 pre-mRNA and mRNA

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Muscleblind 1 (MBNL1) is an RNA-binding protein with a crucial role in alternative splicing regulation, mRNA stability and cellular localization. MBNL1 recognizes its RNA targets using four zinc-finger domains arranged in two tandems. During tissue differentiation process, MBNL1 expression level increases, while functional down-regulation of its activity is a key contributor to the pathomechanism of a dominantly inherited muscle disorder, myotonic dystrophy (DM). DM results either from a CTG repeat expansion in the 3'UTR of DMPK or CCTG multiplication in the first intron of ZNF9. Once transcribed, expanded CUG and CCUG repeats fold into stable RNA hairpin structures that accumulate in the nucleus in the form of ribonuclear foci. Toxic RNA sequester MBNL1, which leads to profound changes in pre-mRNA alternative splicing reminiscent of the embryonic splicing pattern. The RNA deep sequencing of UV cross-linking and immunoprecipitation (CLIP) products revealed several new MBNL1 targets, including the 5'-most region of exon 1 of MBNL1 encoding both 5'UTR and N-terminal part of the protein. This indicated a possible autoregulative function of MBNL1. We hypothesized that MBNL1, by binding to exon 1 of pre-mRNA, might regulate the efficiency of its inclusion into mRNA, which could lead to production of either full length or non-fully functional protein without two zinc fingers. We also hypothesized that the interaction of MBNL1 with 5'UTR of its mature mRNA could exert a translational effect. Our results indicate that MBNL1 indeed autoregulates the exclusion of exon 1 in various mouse and human cell lines and tissues, as well as mediates a positive autoregulative effect on translation.

PS2-189 / #232

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Epidemiology of myotonic dystrophy in Bashkortostan (Russian Federation)

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Myotonic dystrophy type 1 (DM1, 19ql3.2-ql3.3) is a severe progressive hereditary disorder characterized by multisystem involvement, early disability and mortality. We present the study on the epidemiological characteristics of DM1 in our population. Information about the patients was collected from various sources, including information from regional neurologists, archival data of genetic counseling and information gathered from on-site (outreach) surveys. The patients were examined by a neurologist and neurogeneticist, and then their medical records were entered into the registry. Some of the patients underwent direct mutation analysis. The estimated prevalence is for January 1, 2014. The registry contains information about 265 residents living on the territory of the republic at the present time who suffer from DM1 (140 men, 125 women), 64 deceased patients and 143 probable mutation carriers. So the prevalence of DM1 in the Republic of Bashkortostan amounts to 6.53 / 10^5 of population and is characterized by uneven ethnic and territorial distribution. According to ethnicity, Bashkirs (49.06%) who are the indigenous inhabitants of the republic predominated among the diagnosed patients, the prevalence of the disease among them

being 11.09 / 10^5 (1:13878), followed by Tatars (24.15%) - 5.46 / 10^5 (1:25402) and Russians (17.45%) - 3.35 / 10^5 (1:40290). There were discovered five places where the families suffering from DM1 come from. Migration and urbanization have a significant effect on the spread of the disease in the republic. Cases of DM type 2 were not registered in the republic. The obtained data and the results received by using mathematical modeling exceed those of the previous studies. High figures are due to the founder effect and result from the improved diagnosis of the disease in the region. The created register optimizes the management of patients with DM1 and can be used in clinical trials.

PS2-190 / #242

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Functional characterization of recessive ClC-1 mutations causing myotonia congenita

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Myotonia congenita (MC) is an inherited disease characterized by impaired muscle relaxation after voluntary contraction, resulting in muscle stiffness. It is caused by loss-of-function mutations of the muscle ClC-1 chloride channel. In the present study, we describe the clinical phenotype and functional study of three new missense mutations found in patients affected by recessive MC. Two brothers were found to carry the T82A mutation in compound heterozygosis with the known G190S, one patient carries the R453W together with G190S, and one patient is homozygous for G270V mutation. The mutations were introduced in recombinant hClC-1 and expressed in tSA cells for patch-clamp studies of chloride current properties. The G270V and G190S mutations induce a dramatic shift of activation voltage-dependence toward more positive potentials, resulting in nearly zero chloride current within physiological voltage range. Diversely, chloride currents generated by T82A and R453W mutants show density, kinetics and voltage dependence similar to WT currents. We thus performed studies of cotransfection in order to reproduce the heterozygosis found in patients. The T82A/G190S cotransfection produced chloride currents about half of those generated by WT, which theoretically should be insufficient to induce myotonia. In conclusion, the G270V mutation determines a drastic reduction of chloride currents that can explain the myotonia in the homozygous patient. The T82A and R453W mutations produce chloride currents similar to WT, questioning their guilty in MC. However, the results suggest that G190S does not exert dominant-negative effect on T82A. Further studies are thus warranted to complete our understanding of the effects of T82A and R453W mutations on chloride currents, in order to draw a correlation with the clinical phenotype. Supported by Telethon-Italy (GGP10101) and Association Française contre les Myopathies (#15020).

PS2-191 / #271

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Bacteraemic Pneumococcal Pneumonia

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Streptococcus pneumoniae is the major bacterial cause of pneumonia, meningitis and otitis media, and continues to be associated with significant morbidity and mortality in individuals both in the developed and developing world. Management of these infections is potentially complicated by the emergence of resistance of this pathogen to many of the commonly used first- line antimicrobial agents. A number of significant risk factors exist that predispose to the occurrence of pneumococcal pneumonia, including lifestyle factors, such as exposure to cigarette smoke, as well as underlying medical conditions, such as HIV infection. Several of these predisposing factors also enhance the risk of bacteraemia. The initial step in the pathogenesis of pneumococcal infections is the occurrence of nasopharyngeal colonization, which may be followed by invasive disease. The pneumococcus has

a myriad of virulence factors that contribute to these processes, including a poly- saccharide capsule, various cell surface structures, toxins and adhesins, and the microorganism is also an effective producer of biofilm. Antibacterial re- sistance is emerging in this microorganism and affects all the various classes of drugs, including the b-lactams, the macrolides and the fluoroquinolones. Even multidrug resistance is occurring. Pharmacokinetic/pharmacodynamic parameters allow us to understand the relationship between the presence of antibacterial resistance in the pneumococcus and the outcome of pneumo- coccal infections treated with the different antibacterial classes. Furthermore, these parameters also allow us to predict which antibacterials are most likely to be effective in the management of pneumococcal infections and the correct dosages to use. Most guidelines for the management of community-acquired pneumonia recommend the use of either a b-lactam/macrolide combination or fluoroquinolone monotherapy for the empirical therapy of more severe hospitalized cases with pneumonia, including the subset of cases with pneu- mococcal bacteraemia. There are a number of adjunctive therapies that have been studied for use in combination with standard antibacterial therapy, in an attempt to decrease the high mortality, of which macrolides in particular, corticosteroids and cyclic adenosine monophosphate-elevating agents appear potentially most useful.

PS2-192 / #330

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Predominantly myalgic phenotype caused by the p.A1156T mutation in SCN4A gene

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The mutations in sodium channel gene (SCN4A) have been linked to a group of disorders termed paramyotonia congenita and periodic paralyses. To date over 50 dominant mutations have been reported. The SCN4A mutations result in variable phenotypes depending on the type and location of the mutations. The p.A1156T mutation in SCN4A gene was originally reported in a family of Finnish origin with incomplete penetrance. The phenotype consisted of features of both hyperkalemic periodic paralyses (HyperPP) and paramyotonia congenita. We examined 16 Finnish patients from eight different families with p. A1156T mutation. The age of onset of symptoms varied considerably from four to 45 years of age. The main clinical manifestations were quite constant in our patients, although the severity of the symptoms varied to some extent. The typical clinical features were exercise and cold induced muscle cramps, muscle stiffness and myalgia. Several patients also experienced muscle weakness or fatigue during and shortly after exercise, although, on clinical examination the muscle strength was normal in all but one patient. Periodic paralysis was not diagnosed in any of them. Myotonic discharges were detected in most, but not all, electromyogram studies; increased insertion activity was apparent in some of them. However, clinical myotonia or typical paramyotonia was not evident on clinical examination. The unspecific symptoms of myalgia and exercise intolerance with normal clinical findings in our patients make the diagnosis of sodium channel disorders challenging and the symptoms may be confused with other similar myalgic syndromes including fibromyalgia.

PS2-193 / #372

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Quantitative EMG analysis in myotonic dystrophy type 1 (DM1) and type 2 (DM2): comparative study

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Myotonic dystrophy (DM) is the most common dystrophy in adults, characterized by an autosomal dominant progressive disease with multisystemic involvement due to RNA toxicity.

Two genetically distinct forms of DM (DM1 and DM2) are identified, caused by dynamic mutations in the DMPK and ZNF9 genes, respectively. DM1 and DM2 share many clinical features such as myopathy, myotonia, cataracts, cardiac and endocrine abnormalities. Despite phenotype similarities there are significant clinical differences between these two disorders. The clinical picture of DM2 is much more variable than that of DM1 and requires further characterisation.

The aim of our study was to analyze and compare the quantitative EMG in 33 DM1 and 30 DM2 genetically verified patients.

EMG tests were recorded from biceps brachii (BB), rectus femoris (RF), first interosseus dorsal (FID) and tibial anterior (TA) using concentric needle electrodes. Parameters of single motor unit action potentials (MUAPs), the incidence of outliers during voluntary muscle activation and density of maximal muscle contraction were estimated. Spontaneous activity at rest was analyzed.

Mean values of amplitude and size index (SI) of MUAPs recorded in TA and RF muscle, mean duration in TA and mean value of SI in BB muscles were significantly higher in DM2 than in DM1. High amplitude potentials were registered in 50% of RF and TA muscles in DM2 cases whereas in DM1 only in 25.5% of TA and in 18.1% of RF muscles, respectively.

We conclude that EMG is a helpful test in the initial differentiating diagnosis of myotonic dystrophies.

A high amplitude of MUAPs in lower limb muscles in patients without the full range of common DM2 clinical features can be an additional criterium for genetic DM2 testing.

The presence of MUAPs with a high amplitude in lower limb muscles could be explained by fibres hypertrophy but further studies are needed in this area.

PS2-194 / #376

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Effects of acetazolamide on sarcolemma ionic conductances and excitability properties of skeletal muscle fibers as a possible therapeutic mechanism in disorders of skeletal muscle excitability

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Acetazolamide (ACTZ) is a carbonic anhydrase inhibitor empirically used in therapy for a variety of disorders of skeletal muscle membrane excitability including myotonia and periodic paralysis. One possible mechanism accounting for membrane electrical stabilization by ACTZ likely consists in the opening of skeletal muscle calcium-activated potassium channels (Tricarico et al., Ann Neurol 2000). More recently, it was shown that the drug is able to negatively shift the open probability voltage dependence of the muscle hClC-1 chloride channel heterologously expressed in HEK cells, which may favor membrane voltage stabilization (Desaphy et al., Exp. Neurol 2013). Here, we examined the effects of ACTZ on the resting chloride conductance (gCl), sustained by the ClC-1 channel, and on the excitability parameters in rodent skeletal muscle fibers using the two-intracellular microelectrode technique in current-clamp mode. In Extensor Digitorum Longus muscle (EDL) fibers of adult rats, 100 μM ACTZ significantly increased the resting gCl from 2785 ± 56 μS/cm^2 ($n=10$) to 3141 ± 60 μS/cm^2 ($n=10$, $P<0.001$ by Student's t test). The resting potassium conductance was slightly but not significantly increased by ACTZ. In EDL muscle of 20-months old mice, the gCl was low (2220 ± 10 μS/cm^2, $n=10$) in control conditions, as expected from the known effect of aging, but increased to 2760 ± 40 μS/cm^2 ($n=11$) after *in vitro* application of 100 μM ACTZ. In addition, the muscle fibers of aged mice showed an increased latency of the single action potential and increased maximal number of elicitable action potentials (N-spikes), indicating a condition of hyperexcitability recalling a myotonic condition. Application of 100 μM ACTZ markedly reduced the latency of the action potential by 33% and the N-spikes by 39%. In conclusion, ACTZ is able to increase the gCl in muscle fibers of adult rats and aged mice, and to counteract the sarcolemma hyper-excitability of aged mouse muscles, suggesting that activation of ClC-1 channels is likely involved in the therapeutic action of ACTZ in disorders of skeletal muscle excitability. Supported by Telethon-Italy (GGP10101) and Association Française contre les Myopathies (#15020).

PS2-195 / #383

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Functional and pharmacological characterization of a new hNav1.4 sodium channel mutation causing myotonia permanens

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Mutations in the SCN4A gene encoding the skeletal muscle Nav1.4 sodium channel are responsible for paramyotonia congenita or sodium channel myotonia, a group of muscle diseases characterized by impaired muscle relaxation after voluntary contraction, resulting in muscle stiffness. The sodium channel blocker mexiletine has received orphan drug designation in nondystrophic myotonia. We now report the case of a young Algerian girl showing a severe phenotype, including a pronounced eyelid and extraocular myotonia, lid-lag phenomenon, tongue myotonia, generalized muscle hypertrophy and slowness of movements. No episode of muscle weakness was reported. She obtained some improvement with mexiletine treatment, but her father asked for mexiletine withdrawal because of side effects. Treatment was shifted to another sodium channel blocker, flecainide, which was previously shown to exert antimyotonic activity in patients resistant to mexiletine (Desaphy et al., Eur J Clin Pharmacol 2013). Both the patient and her father claimed great improvement of stiffness with flecainide. A new point mutation was identified in codon 1158 of SCN4A, where a leucine substitutes for a proline in the intracellular S4-S5 linker of domain III. The P1158L mutation was introduced in recombinant hNav1.4 and expressed in tsA201 cells for patch-clamp studies. Compared to wild-type, the mutant channel showed a slower rate of inactivation and a ~6-mV positive shift in voltage dependence of fast inactivation. Voltage dependences of activation and slow inactivation were not altered. The P1158L currents were less sensitive to mexiletine compared to WT; The half-maximum inhibitory concentration for tonic block (holding potential of -120 mV, stimulation frequency of 0.1 Hz) increased from 295 in WT to 479 μM in P1158L. In a myotonic-like condition (-90 mV, 50 Hz), inhibition of P1158L currents by 10 and 30 μM mexiletine was significantly reduced compared to WT. Conversely, the sensitivity to flecainide was not altered by P1158L. In conclusion, the symptoms presented by the patient resemble a condition of myotonia permanens, a severe sodium channel myotonia subgroup. The impairment of sodium channel fast inactivation caused by the P1158L mutation likely accounts for the symptoms. The reduced sensitivity of P1158L to mexiletine may have contributed to the unsatisfactory response of patient. Supported by Telethon-Italy (GGP10101) and Association Française contre les Myopathies (#15020).

PS2-196 / #387

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Clinical and molecular analysis of twenty five Algerian patients with myotonic dystrophy

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Introduction Myotonic dystrophy (MD1) is the most common form of adult muscular dystrophy with autosomal dominant transmission. It is an inherited disease in which there is an abnormal expansion of CTG trinucleotide repeat at 19q13.3. It is manifested as a chronic progressive multisystem disorder. The aim of these study, was to report the clinical and molecular analysis of 25 Algerians patients (MD1). Patients and methods Our study was realized in the service of neurology of the CHU of Constantine.

Since January 2001 to July 2006. The patients were selected according to the following criteria: Beginning and prevalence of the muscular attack at the distal level with facial participation, myotonie shown by the EMG, attack of other Bodies (eye, heart...), with an abnormal amplification of tri nucleotide CTG, repeated more than 50 times in gene MDPK. The complementary investigations comprised: a biological systematic assessment of CPK, LDH, T3, T4, TSH, PTH, FSH, LH, Testosterone, Phosphocalcic assessment and glycaemia), the electromyogram (EMG) was carried out at all the case index as well as the cardiac assessment, the ophthalmologic examination (lamp with slit) was carried out among 15 patients, the genetic study carried out at the laboratory of genetic Dgc of the CHRU of Strasbourg by analysis of the number of repetition CTG of gene of the MD kinase by technique of TP-PCR (ref Warner et al.. Med genet. 1996, 33: 1022–1026). Results Our 25 DM1 patients were from 11 unrelated Algerian families, mean age 38, 33 years (with the extreme ones going from 15 - 70 years), with 14 males and 11 females All the patients presented a myotonie, the calvicie among 11 patients, the distal muscular weakness was observed among 20 patients with facial participation among 23 patients, the cataract was observed among 11 patients and the cardiac attack at 07 patients. The genetic study confirmed Dgc of MD1 in all the cases by the description of amplifications of tri nucleotide CTG of gene of the MD kinase. Conclusion Our results approach the data of the literatures observed in the other countries of the world

PS2-197 / #408

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Direction of progression of motor impairment in DM1 patients; relation to CTG expansion

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The progression of impairments in DM1 is generally considered to proceed from distal to proximal muscle groups. This assumption has shaped assessment tools for the disorder. Reported relations between CTG expansion and muscular strength vary substantially; lack of awareness of early impairments in trunk may account for some of this variability.

31 adult-onset DNA confirmed DM1 patients (Age 21–61; 18 male 13 female) included. All able to walk independently and within normal IQ range.

Expansion size reassessment was done by Southern Blot analysis of lymphocytes. Muscular strength assessed by 1–5 MRC scaling in 2 distal extremities muscle groups (DE-mg): Ankle-dflx and wrist-ext. 6 Proximal extremities muscle groups (PE-mg): Shoulder ab, hip-flx, Elbow-flx, elbow-ext, knee-ext, knee-flx and 3 muscle groups in truncus (T-mg): back-ext, abdominal-flx and neck-flx.

T-mg was most affected in all disease-duration groups (<7 yrs, 8-12 yrs, 13-24yrs, >25 yrs) DE-mg was the second most affected in all duration groups and PE-mg was least affected in all duration groups.

T-mg strength was highly related to CTG expansion (r=–61 p< 01); PE-mg strength was less closely related (r=–53 P<.01) DE-mg was not significantly related to CTG. One-way ANOVA test between 3 CTG groups (1:<1kb, 2:1kb-2,3kb, 3:>2,3b) found significant difference in T-mg (F=5,99 p .008); No significant group differences were found for PE-mg (F = 3,22 ns) and DE-mg (F = 1,53 ns).

The current study finds no support for the assumption of a Distal-to-Proximal progression of motor impairment in DM1. The CTG expansion size is particularly related to strength in trunk muscle groups.

PS2-198 / #411

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

DM1 and gender; how CTG expansion affect men and women differently

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Gender differences among individuals with DM1 are well recognized in terms of germline instability and transmission severity; less is known about gender differences in the clinical manifestation of CTG expansion size.

Reported relations between CTG expansion and clinical manifestation vary substantially in the DM1 literature; lack of awareness of gender differences may account for some of this variability.

31 adult-onset DNA confirmed DM1 patients (Age 21-61; 18 male 13 female) included. All able to walk independently and within normal IQ range. Males and females matched for age of first symptom (?20,4 yrs; sd 12.9 .? 19,2 yrs, sd 9,3). Expansion size (kb. CTG) differed, but not significantly (?1,42, ?2,32; T 1,6 ns)

CTG expansion studied in relationship to:, Strength in Trunk (Neck, Back, Abdomen - T-mg) Proximal Extremities (Hip, Knee, Shoulder, elbow - PE-mg) Distal Extremities (Hand, foot - DE-mg) Pain (P), Mobility index (M), 6min Walk-test (6-W), Fatigue after 6-W (F), Timed up and go (TUG) Activity index (A) and Disease-duration for each sex separately.

Males and females differed with respect to variables associated with CTG size (*: $p<.05$, **$p<.01$) Only Disease duration was sig. correlated to CTG expansion in both sexes (?r.66*, ? r .65*)

In ?, CTG expansion was related to: PE-mg (r -.61*) .In ? CTG expansion was related to: T-mg (r -.82**), P (r .71*) DE-mg (r -.69*.), 6-W (r -.67*.), F (r .67*.), TUG (r .66*), and A (r -.62*.)

Findings suggest gender-specific DM1 progression; if substantiated this could improve prognosis and intervention for DM1 patients. Gender differences in clinical manifestation may also be relevant for understanding the pathophysiology of the DMPH mutation, including the Parental-Gender effect in transmission of DM1.

PS2-199 / #417

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Cis-elements regulating expression of muscleblind in Drosophila embryos

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The phylogenetically conserved family of Muscleblind proteins are RNA-binding factors involved in a variety of gene expression processes including alternative splicing regulation, RNA stability and subcellular localization, and miRNA biogenesis, which typically contribute to cell-type specific differentiation. In humans, sequestration of Muscleblind-like proteins MBNL1 and MBNL2 has been implicated in degenerative disorders, particularly expansion diseases such as myotonic dystrophy type 1 and 2. Drosophila muscleblind was previously shown to be expressed in embryonic somatic and visceral muscle subtypes, and in the central nervous system, and to depend on Mef2 for transcriptional activation. Genomic approaches have pointed out candidate gene promoters and tissue-specific enhancers, but experimental confirmation of their regulatory roles was lacking. In our study, luciferase reporter assays in S2 cells confirmed that regions P1 (515 bp) and P2 (573 bp), involving the beginning of exon 1 and exon 2, respectively, were able to initiate RNA transcription. Similarly, transgenic Drosophila embryos carrying enhancer reporter constructs supported the existence of two regulatory regions which control embryonic expression of muscleblind in the central nerve cord (NE, neural enhancer; 830 bp) and somatic (skeletal) musculature (ME, muscle enhancer; 3.3 kb). Both NE and ME were able to boost expression from the Hsp70 heterologous promoter. In S2 cell assays most of the ME enhancer activation could be further narrowed down to a 1200 bp subregion (ME.3), which contains predicted binding sites for the Mef2 transcription factor. The present study constitutes the first characterization of muscleblind enhancers and will contribute to a deeper understanding of the transcriptional regulation of the gene.

PS2-200 / #421

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

White matter changes and cognitive decline in DM1

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Background: Steinert's Disease (DM1) the most common muscular dystrophy in adults, is character-

ized by multisystem involvement including functional/morphological brain abnormalities to different extents. To date, however, brain imaging and neuropsychological data correlation studies have given contrasting results on that. Objective.To perform an overall neurological, neuropsychological and brain imaging characterization of a sample of DM1 patients in order to contribute to clarify the relationship between structural alterations in specific brain regions and related cognitive tasks.Methods. 40subjects with established clinical-genetic diagnosis, underwent a complete neurological assessment, including psychological interview and neuropsychological evaluation. Main caregiver underwent patient's Quality-of-life interview too. A selected subgroup of 15 patients underwent brain MRI investigation.Results. Brain imaging revealed involvement of the white matter in frontal (53%), parietal (27%) and temporal (73%) lobes. Moreover, we found reduced scores in neuropsychological tests for frontal functions (61%) and visuospatial impairments (66%); interestingly, verbal abilities were rather preserved (80%). Behaviour was characterized by mixed mood conditions (anxiety, depression, apathy) and by variable sets of pathological personality traits, even though without fulfilling diagnostic criteria for major psychiatric disorder according to DSM-IV. Patient's and main caregiver's reports showed internal discrepancies (63%), with patients tending to denial of physical and behavioural aspects of their condition, as well. Statistical analysis showed significant relationships between reduced spatial memory performances and parietal lobe white matter changes (Fisher-Exact-Test $p<0.05$).Discussion. White matter lesions can be common in DM1 patients, even if independently from muscle impairment and disease-duration. Our study indicates that CNS involvement in DM1 is an heterogeneous condition characterized by variable cognitive/psychopathological dysfunctions; in any case, it could be a prominent feature in DM1, frequently associated with increased burden of the disease.Conclusions. Brain imaging can be helpful to understand cognitive impairment in DM1 and should be properly investigated since the early phases of illness

PS2-201 / #447

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Myotonic dystrophy type 2 coexisting with myasthenia gravis, pilomatricoma and lipoma in a family: a case report

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Motonic dystrophy type 2 (DM2), myasthenia gravis (MG) and pilomatricoma are rare disorders and their coexistence in the same family is unusual.

The family case we present concerns a 53-year-old father and his 24-year-old son, both with genetically confirmed DM2. DM2 was first diagnosed in the son, who complained of painful muscle cramps. Moreover, a cutaneus node on his skin was histologically verified as pilomatricoma. Subsequently, a genetic DM2 testing was performed in the father, who was previously diagnosed with myasthenia gravis at the age of 34. At that time, he presented apokamnosis of bulbar and face muscles. MG was confirmed by a repetitive nerve stimulation test and elevated serum anti-acetylocholine receptor antibodies. The thymectomy revealed thymic hyperplasia. At present, on neurological examination,the fathershows a mild weakness and apocamnosis of both orbicular muscle of eye and proximal muscle of lower legs. The systemic disorders like cataracts, hypoacusis, endocrine disturbances (hypergonadotropic hypogonadism, Hashimoto disease) and monoclonal protein in serum were detected. The abdominal echography revealed adrenal adenoma. Moreover, the lipoma on the back was diagnosed. As the symptoms of myasthenia were very mild, pharmacological treatment with pyridostigmine bromide was needed only sporadically. In both patients, neither active or percussion nor myotonic discharges on EMG examination were observed.

An increased risk of a rare skin tumor- pilomatricoma is well documented in DM1. Also, there are a few cases reported of DM1 accompanied with myasthenia gravis and lipoma, however, this combination of disorders is very rare in DM2. According to our best knowledge, there are only two cases reported of

DM2 coexisting with MG and none DM2 accompanied with lipoma or pilomatricoma.

This report offers a contribution to a better characterization of possible associations of several rare disorders coexisting in the same family.

PS2-202 / #465

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Muscle channelopathies: clinical and genetic features in a large cohort of Italian patients

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Background: Skeletal muscle channelopathies represent a heterogeneous group of rare diseases, including non-dystrophic myotonia and periodic paralysis. To date 4 genes are involved in their pathogenesis: CLCN1, SCN4A, CACNA1S and KCNJ2. However muscle channelopathies are associated with a great inter- and intrafamilal phenotypic variability, making challenging genotype-phenotype correlations. To this purpose studies on large populations of patients are needed.

Objective: Aim of our study was to report clinical and molecular features of a wide cohort of Italian patients affected by skeletal muscle channelopathies.

Methods: We included patients referred to our neuromuscular service with a clinical diagnosis of periodic paralysis or nondystrophic myotonia and molecular characterization through genetic analysis. CLCN1, SCN4A and KCNJ2 genes were fully sequenced. Mutations in CACNA1S gene were searched only in exons known to be involved in periodic paralysis (exons 4, 11, 21, 26, 27 and 30).

Results: We included 301 patients, among which 195 (64.3%) patients mutated in CLCN1 gene, 74 (24.6%) in SCN4A, 28 (9.3%) in CACNA1S and 4 (1.3%) in KCNJ2. We found 22 novel mutations, among which 8 in CLCN1 gene, 13 in SCN4A and 1 in CACNA1S. All the mutations detected in SCN4A, CACNA1S and KCNJ2 genes were missense, except for an unreported 9-nucleotide deletion in SCN4A. On the other hand mutations in CLCN1 gene were missense in 131/195 (67.2%) patients, whereas remaining cases showed nonsense, splice site or deletion mutations. CLCN1 mutated patients showed simple heterozygous mutations in 99 (50.7%) cases and compound heterozygous or homozygous mutations in 96 (49.3%). Among patients mutated in SCN4A we observed 28 (37.8%) cases with paramyotonia congenita, 24 (32.4%) with sodium-channel myotonia, and 17 (23%) with hypo/hyperkaliemic periodic paralysis. Five (6.8%) patients mutated in SCN4A showed a clinical phenotype not clearly classified in one of the three categories.

Conclusions: Our study confirms clinical and genetic heterogeneity of muscle skeletal channelopathies, although a relatively small number of mutations is responsible for most of the cases, as already suggested in the literature.

PS2-203 / #470

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Higher risk of developing malignancies in myotonic dystrophy patients.

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Background: Myotonic Dystrophy type 1 (DM1) is an autosomal dominant multisystem disorder which affects muscle, eye, brain, heart and endocrine tissues. It has been published recently an association with higher cancer risk.

Objectives: To describe the incidence rate of cancer in the cohort of DM1 patients from the Basque region of Gipuzkoa and to compare it with the incidence rates of the Basque population cancer registry.

Methods: Data from patients were retrospectively obtained from the medical records of the Gipuzkoa

historical DM1 cohort (1985-2013). DM1 diagnosis was perfomed based on molecular testing. The exposure time for cancer was the age at cancer diagnosis and the age at last visit or death for patients without cancer. We calculated standardized incidence ratios (SIRs) by dividing the observed numbers of cancer by expected numbers for all cancers combined and stratified by sex. Expected numbers were obtained by multiplying the specific age and sex incidence rate from de Basque population cancer registry to the person-years observed in the study cohort. We estimated 95% confidence intervals using the Poisson distribution. Mean repeat length was compared for patients with and without cancer by Student t-test. All statistical tests were considered significant if $p < 0.05$. Statistical analysis was performed using STATA-SE 12 (StataCorp LP, College Station, TX)

Results: A total of 424 DM1 patients were included in this study, 214 women (50.5%) and 210 men. Mean repeat expansion size was 684 (SD 535). During 18796 person-years of follow-up, 62 patients were diagnosed with cancer, compared with 30.29 expected cases. We calculate a two-fold increase in cancer risk as compared with the general population of our region (SIR = 2.05, 95% CI = 1.31 to 3.30). The risk increase was bigger for women (SIR = 3.33, 95% CI = 1.84 to 9.45) than for men (SIR = 1.45, 95%CI = 0.80 to 2.65). The small number of cancers prevented us for a further risk analysis by cancer site. Repeat expansion size was not associated with cancer occurrence in our patients.

Conclusion: In the DM1 patients from our region we have found a higher risk for developing malignancies, especially in women and this risk is not associated with the length of the expansion.

✶PF2

PS2-204 / #497

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Digenic expression of sodium and chloride channel mutations in patients with non dystrophic myotonia

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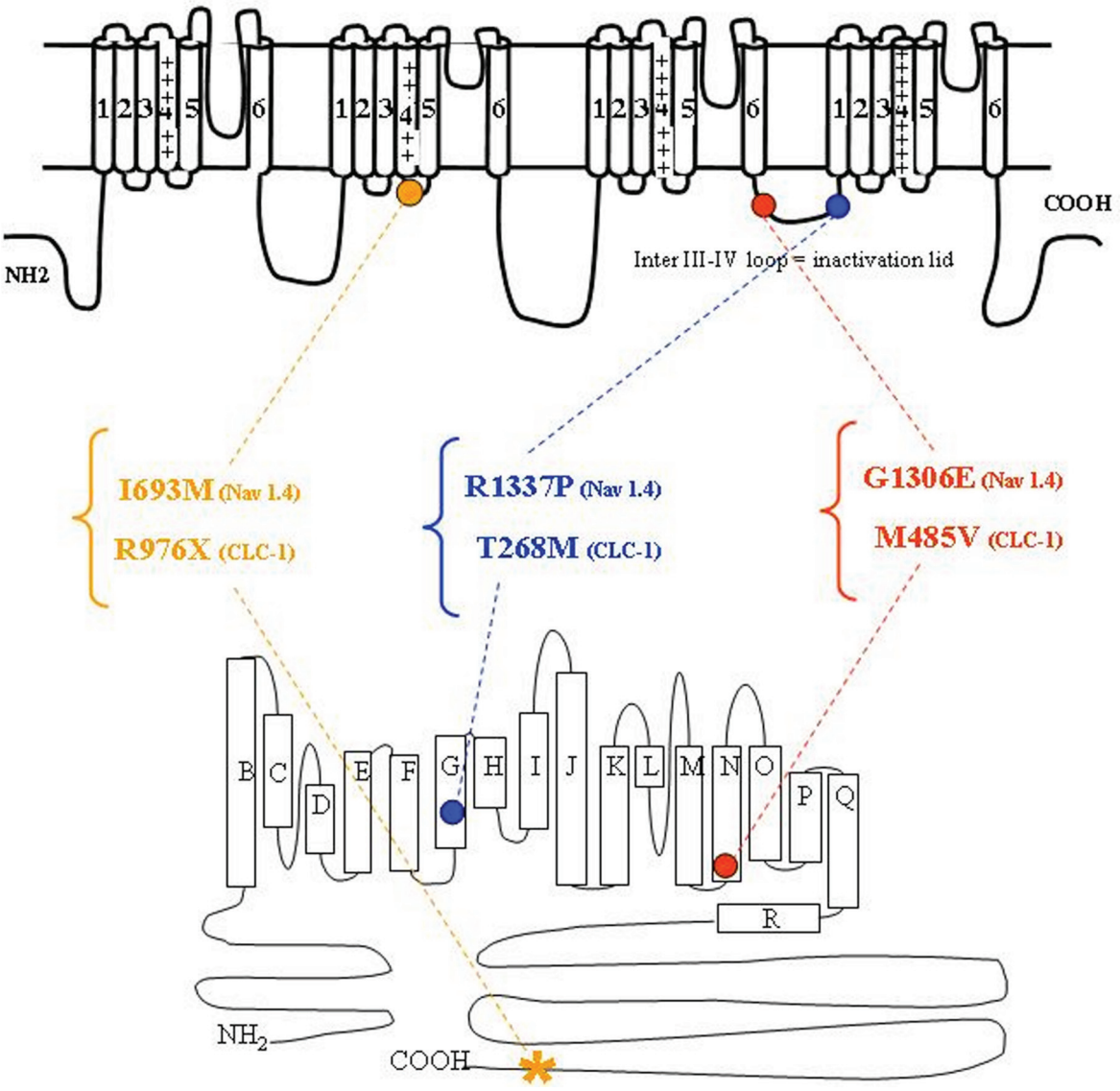
Nondystrophic myotonias (NDM) are characterized by muscle stiffness triggered by voluntary movement. They are caused by mutations in the chloride channel gene (CLCN1) in myotonia congenita (MC), or in the sodium channel gene (SCN4A) in paramyotonia congenita (PC) and sodium channel myotonias (SCM). Clinical and electromyographic (EMG) phenotypes of these different disorders have been delineated. At EMG, repeated short exercise test (SET) discriminate between SCN4A-linked PC (stable decrease of CMAP amplitude after exercise, type I profile), CLCN1-linked MC (transient decrease of CMAP amplitude after exercise, type II profile), and SCN4A-linked SCM (no change, type III profile). We report the clinical and electrophysiological observations from three patients with mutations in both chloride and sodium channels. Patient 1, a 26-year-old man, had permanent myotonia in face and limbs. He reported moderate aggravation at cold and mild warm-up phenomenon. He had heterozygous SCN4A G1306E mutation, compatible with the diagnosis of SCM subtype myotonia permanens. Patient 2, a 13-year-old child, experienced stiffness ofmovements,especiallymarked in legs,or after long exercise, with a mild warm-up phenomenon, withoutcold-sensitivity. He had a new heterozygous SCN4A R1337P mutation, compatible with the diagnosis of SCM. However, in both patients, SET showed a type II pattern, not explained by SCN4A mutations,

Figure. Location of digenic SCN4A and CLCN1 mutations on Nav1.4 and CLC-1 proteins: patient 1 (red); patient 2 (blue); patient 3 (orange).



prompting us to sequence CLCN1. Patient 1 had a recessive CLCN1 M485V mutation, patient 2 had a recessive or semi-dominant CLCN1 T268M mutation. Patient 3, a 25-year-old man, showed cold-triggered facial myotonia, marked blocks in hands andlegs, and a slight intermittent strabismus. He reported a clear warm-up phenomenon. SET showed a type III pattern. He had a new heterozygous SCN4A I693M mutation, compatible with the diagnosis of SCM. However, the warm-up phenomenon and the marked leg blocks prompted us to sequence CLCN1 gene, which revealed a heterozygous recessive R976X mutation. The presence of an additional CLCN1 recessive mutation seems to modulate the clinical and electrophysiological expression of the SCN4A dominant mutation in those 3 patients. It is worth screening both genes in NDM whenphenotypic elementsare not fully explained by mutations found in the first gene. A careful combined clinical and electrophysiological investigation is needed in NMD patients, thus enabling an appropriate molecular genetics analysis and mutation interpretation.

PS2-205 / #524

Theme: *2.6 - Muscle diseases of genetic origin: Myotonic disorders / Muscle channelopathies and related*

Cortical response during myotonia in myotonic dystrophy: an fMRI study

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Myotonic dystrophy (MD) is a common adulthood muscular dystrophy, characterized by muscle wasting, myotonia and multisystemic manifestations. The precise pathomechanism of myotonia is unclear. This study explores cortical involvement during grip myotonia.

Brain blood oxygen level dependent (BOLD) signal was monitored during "grip task" in MD patients. Eight patients with apparent grip myotonia ("myotonia group") were compared to 8 patients without apparent myotonia ("control group"). Furthermore, BOLD signals were compared in both groups before and after a warm-up procedure.

Myotonia group: significantly higher activation was found during post-grip myotonic condition compared to post-grip non-myotonic condition (due to warm-up) in the supplementary motor area (SMA) and in the dorsal anterior cingulate cortex (dACC) -anterior midcingulate cortex (aMCC). Similar difference in activation pattern was detected between the post-grip myotonic and non-myotonic conditions when comparing the "myotonia group" to the "control group".

Our results implicate that myotonia originated from skeletal muscle triggers secondary brain activity of areas responsible for motor control: supposedly due to muscle relaxation initiation and error detection.

PS2-206 / #101

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

A rare case of combined congenital pathology: facioscapulohumeral muscular dystrophy (FSHD) and cystic fibrosis

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Combination of two progressive independent genetic conditions is extremely rare in children as it usually leads to early pregnancy interruption or its severe complications. We present a rare case of FSHD diagnostics in a 6,5 years old girl that was previously diagnosed and treated with cystic fibrosis (CF).

The child was born after phisiological pregnancy, in time, by normal spontaneous delivery to healthy unrelated parents that deny any teratogenic influences. After birth she had weak suction and bilateral facial

nerve palsy. At 3 months according to the positive neonatal screening she was diagnosed with cystic fibrosis (CF) (F508del) and began symptomatic treatment in pulmonary department. At 2 years she passed endovascular occlusion of congenital intratrial septum defect. Then parents noticed the beginning thorax deformation, growing hyperlordosis, equinus and 'wing-scapula' deformation, proximal limb muscle and facial muscle weakness and excessive fatiguability, rhinolalia. The girl passed adenectomy with minimum improvement. Her creatine phosphokinase was 1131 U/l, needle EMG demonstrated myopathy. After genetic and neurology consultation other CF mutation (E92K) was found, but quadriceps muscle biopsy was normal. Clinically she was diagnosed as having infantile form of FSHD. For molecular-genetic assessment she was referred to 'Institut Myology' (Paris) where mosaic deletion 4q35 (shortening of D4Z4 region 2+/- 1 repetition) was identified, that proved the diagnosis of FSHD. The rehabilitation of the patient was complemented with special orthosis and corset wearing, adopted wheelchairs and regular neurological, orthopedic, cardiological, surdologal and speech therapist assessment and treatment.

The case demonstrates that one progressive genetic disease may mask for a long time symptoms of another unrelated genetic condition. Neglect of neuromuscular disease in this case could lead to misunderstanding of symptoms and inadequate help to a child.

✶PF1

PS2-207 / #197

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

MRI validation of a transcriptional cascade propagation model in FSHD muscular dystrophy

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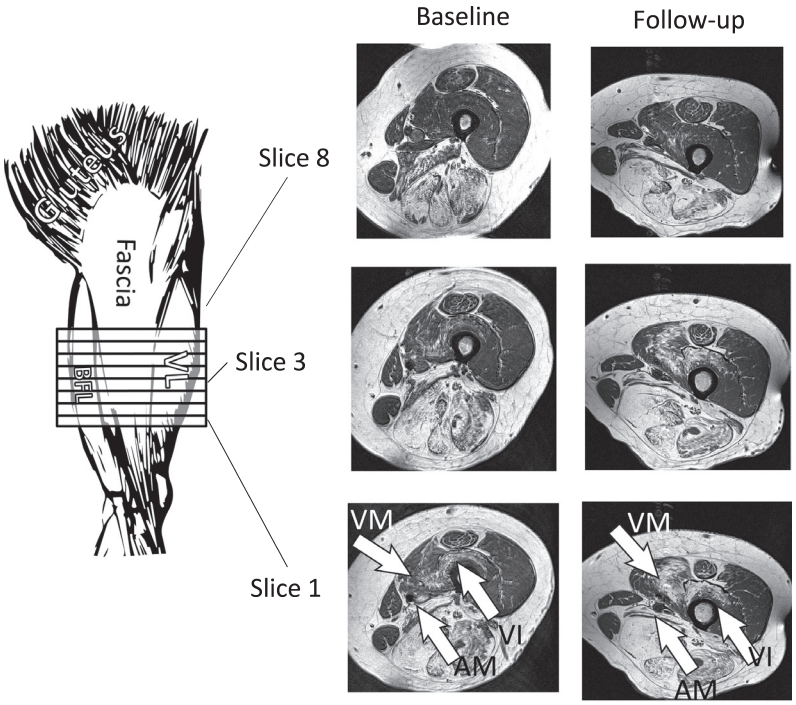
Facioscapulohumeral muscular dystrophy (FSHD) is associated with contractions of the D4Z4 repeat array, leading to inadequate repression of DUX4, a pro-



tein toxic to muscle cells[1]. However, also non-affected first degree siblings may show DUX4 expression[2]. Furthermore, recent biopsy studies found that DUX4 is expressed in only 1/1000 FSHD myoblasts[3]. These findings demonstrate that other mechanisms, apart from DUX4 expression, must be involved in FSHD muscle pathology. Tassin ea.[4] presented cellular evidence for a model of "dynamic propagation and initiation of a transcriptional cascade" to explain how such a rare occurring protein could cause a myopathy. In this model DUX4 proteins are yielded after the DUX4 gene is activated in one or few myonuclei. These proteins diffuse into the cytoplasm towards neighboring nuclei where they activate target genes, causing expansion into a transcriptional cascade of deregulation (oxidative stress, inflammation, fatty infiltration, atrophy).

The aim of this study was to discover patterns that could validate this propagation model in quantitative MRI data of 36 genetically proven patients with FSHD scanned in a clinical 3 Tesla MRI-system.

We identified a quasi-binary distribution of fatty infiltration in FSHD muscles suggesting a fairly abrupt transition from normal to complete fat infiltration. Moreover, the few muscles with an intermediate muscle fraction showed rapid disease progression. This result is coherent with the clinical observation that the course of FSHD has long periods of stability interrupted by short periods of rapid progression in one muscle or muscle group. In addition we showed that this progressive phase has a decreased PCr/ATP ratio and gradient of fatty infiltration over the length of the muscle (see figure).[5] The transcriptional cascade model matches very well our findings. The model predicts rapid propagation of muscle deregulation towards the fully diseased phase after a stochastic DUX4 gene activation. The model also predicts a gradient of deregulation because of the multinucleated nature of muscle fibers over the length of the muscles as we found in our study reflected by a fatty infiltration gradient. We can add to the model that fatty infiltration starts in the distal part of muscles by an (as yet) unknown trigger causing initiation of DUX4 expression.

[1]Lemmers R Science 2010, [2]Jones T Hum Mol Genet 2012, [3]Snider L PloS Genet 2010, [4]Tassin A J Cell Mol Med 2013, [5]Janssen BH PLoS One 2014

PS2-208 / #206

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Caveolar proteins: putative FSHD biomarkers?

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Facioscapulohumeral muscular dystrophy (FSHD) is linked to chromatin opening and DNA hypomethylation at the D4Z4 repeat array in the 4q35 subtelomeric region. The open chromatin facilitates the expression of the DUX4 gene located in the last D4Z4 unit flanked by a polyA signal. The DUX4 protein is a potent transcription factor that initiates a deregulation cascade affecting many genes and that causes the disease.

We have previously compared the proteome of primary FSHD and control myotubes at day 4 of differentiation (Tassin et al. 2012). FSHD myotubes presented a disturbance of two major caveolar proteins: PTRF (cavin-1) and MURC (cavin-4). Caveolae are membrane nanodomains, considered as a subset of lipid rafts, enriched in cholesterol and sphingolipids. They play a major role in signal transduction for in many biological processes. Caveolae contain the muscle specific caveolin (caveolin-3) and small clusters of GPI-anchor proteins, also increased in FSHD myotubes. The extracellular protein AHNAK, a member of the dysferlin protein complex, is also slightly increased in FSHD myotubes. Caveolin-3, MG53 and dysferlin interact as well as PTRF and MG53 suggesting a link to the plasma membrane repair affected in dysferlinopathies (Cai et al. 2009; Zhu et al. 2011).

To evaluate the FSHD caveolar defect, we investigate some proteins of the membrane repair complex and cavins (PTRF, SDPR, SRBC, MURC) by western-blot and immunofluorescence. We will also study

their putative relocations with a lipid raft tracer: the FITC-conjugated cholera toxin B subunit. We perform these experiments on iC2C12-DUX4 myoblasts (kindly provided by Prof. M. Kyba), human primary or immortalized mosaïc myoblasts (Prof. G. Butler-Browne and Prof. D. Laoudj-Chenivesse) in proliferation or differentiation.

If caveolar dysfunction can be shown in FSHD, deregulation of MURC and PTRF that are essential for skeletal muscle membrane stability could lead to deregulation of many cellular processes. In addition, if a link with DUX4 can be demonstrated, these proteins could be used as additional FSHD biomarkers. Identification of FSHD biomarkers and their validation is essential to assess the therapeutic approach developed by our laboratory i.e. antisense tools targeting the DUX4 mRNA and preventing protein expression.

PS2-209 / #207

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Development of fibrosis and its impact on muscle regeneration in FSHD muscle

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Background: Facioscapulohumeral dystrophy (FSHD) is an inherited disorder characterized by atrophy and muscle weakness progressing in an asymmetric antero-posterior gradient. This inherited disorder affects 1/17000 individuals at birth. After muscle injury, quiescent satellite cells or recruited mesenchymal stem cells are activated and divide to provide myoblasts that repair the damaged muscle fibers. Despite the significant muscle damage, low muscle regeneration is observed in FSHD compared to other muscular dystrophies. The extracellular matrix (ECM) is a major component of the satellite cell niche that can also recruit other muscle progenitor cells after muscle damage.

Objective Our histological analysis of affected FSHD muscles showed an increase of the connective tissue even in areas with histologically normal fibers. We hypothesized that modifications in the ECM composition of FSHD muscles are contributing to the observed low muscle regeneration.

Methods and Results Our preliminary data suggest deregulations of some ECM components in FSHD myoblast cultures and muscle biopsies. As fibroblasts produce most components of the ECM, alterations of resident fibroblasts in FSHD muscles could contribute to the pathology. In order to characterize components of the ECM in FSHD muscles we established fibroblast and myoblast primary cultures from control and FSHD muscle biopsies. The cells were purified by magnetic sorting with superparamagnetic particles that are conjugated to highly specific antibodies against a particular antigen on the fibroblast or myoblast surface. This material will allow us to determine fibrosis is an early event in FSHD and could contribute to the incorrect response of progenitor muscle cells. For this purpose, we will analyze the synthesis and secretion of ECM components (among which enzymes contributing to the ECM remodeling) in the cell cultures we purified.

Conclusion and Discussion A characterization of the ECM components is essential to a better understanding of the regenerative defect present in FSHD and could indicate whether currently developed therapeutic strategies targeting myoblasts should also target fibroblasts.

PS2-210 / #208

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Study of atrophy in facioscapulohumeral muscular dystrophy

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Introduction: Facioscapulohumeral muscular dystrophy (FSHD) is a progressive hereditary muscle disease related to chromatin opening in the 4q35 region which facilitates the expression of the DUX4 (Double Homeobox 4) gene encoding a transcription factor. It plays a major role in the development of FSHD and initiates a cascade of genes deregulation causing muscle atrophy, oxidative stress, defects in muscle differentiation and inflammation.

Several signaling pathways can lead to muscle atrophy including the NF-kB and the PI3K/AKT/FOXO pathway. The latter is involved in the balance between hypertrophy and atrophy in normal muscle. Our laboratory has shown that abnormal expression of DUX4 in FSHD muscle cells induced expression of the atrogenes MuRF1 and atrogin-1 (MAFbx) (skeletal muscle atrophy markers).

Objective: FOXO1 is a transcription activator of the atrogenes and we have identified two putative DUX4 binding sites in the FOXO1 promoter. Our aim is to investigate whether the atrogenes expression we observe in FSHD muscle cells is dependent on FOXO1 transactivation by DUX4.

Methods and results: We have built two reporter vectors containing the luciferase gene under the control of the FOXO1 promoter regions containing either one (pGL3-B) or two (pGL3-A+B) putative DUX4 binding sites. We transfected control or FSHD immortalized myoblasts with each vector and measured the luciferase activity. We found a 3-fold higher luciferase activity in FSHD myoblasts compared to control myoblasts. We also co-transfected C2C12 myoblasts with pGL3-A+B or pGL3-B and with a DUX4expression vector to evaluate the effect of DUX4 on the FOXO1 promoter activity. As negative controls we used in parallel a DUX1 expression vector (a non-pathologic paralog protein) or an empty vector. The FOXO1 promoter (A+B or B sites) were activated by DUX4 in a dose-dependent manner. The inactivation by mutagenesis of the putative DUX4 binding A site in the FOXO1 promoter induced a decrease of the dose-dependent luciferase activity. Mutagenesis of the B site is on-going.

Discussion and conclusion: Our results suggest that the atrogenes expression is probably due to a direct FOXO1 gene activation by DUX4. FOXO1 could therefore constitute a novel DUX4 target and would be a possible druggable target to interfere with FSHD muscle atrophy.

PS2-211 / #239

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Effects of vitamin C, vitamin E, zinc gluconate and selenomethionine supplementation on muscle function and oxidative stress biomarkers in patients with facioscapulohumeral dystrophy: a double-blind randomized controlled clinical trial

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disease characterized by progressive weakness and atrophy of specific skeletal muscles. As growing evidence suggests that oxidative stress may contribute to FSHD pathology, antioxidants that might modulate or delay oxidative insult may be useful in maintaining FSHD muscle function.

Objective: Our primary objective was to test whether oral administration of vitamin C, vitamin E, zinc gluconate and selenomethionine could improve the physical performance of patients with FSHD.

Design: Adult patients with FSHD ($n=53$) were enrolled atMontpellier University Hospital (France) in a randomized, double-blind, placebo-controlled pilot clinical trial. Patients were randomly assigned to receive 500mg vitamin C, 400mg vitamin E, 25mg zinc gluconate and 200µg selenomethionine ($n=26$) or

matching placebo ($n=27$) once a day for 17 weeks. Primary outcomes were changes in the two-minute walking test (2-MWT), maximal voluntary contraction and endurance limit time of the right and left quadriceps (MVC_{QR}, MVC_{QL}, T_{limQR} and T_{limQL}, respectively) after 17 weeks of treatment. Secondary outcomes were changes in the antioxidant status and oxidative stress markers.

Results: Although 2-MWT, MVC_Q and T_{limQ} were all improved in the supplemented group at the end of the treatment compared to baseline, only MVC_Q and T_{limQ} variations were significantly different between groups (MVC_{QR}:$P=0.011$; MVC_{QL}:$P=0.014$; T_{limQR}: $P=0.044$; T_{limQL}: $P=0.006$). Similarly, vitamin C ($P<0.001$), vitamin E ($P<0.001$) and lipid peroxides ($P<0.001$) variations were significantly different between groups.

Conclusions: Antioxidant supplementation has no significant effect on the 2-MWT, but improves MVC_Q and T_{limQ} by enhancing the blood antioxidant status and reducing the lipid peroxides level. This trial was registered at clinicaltrials.gov as number NCT01596803.

✶PF3

PS2-212 / #244

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Facioscapulohumeral muscular dystrophy type 1: quantitative MR imaging and clinical correlation

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Introduction: Facioscapulohumeral muscular dystrophy type 1 (FSHD1) is the third most common inherited muscular dystrophy. Clinical expression is highly variable and disease progression is slow. Development of quantitative biomarkers is essential to assess the progression of the disease for planning future clinical trials. The purpose of this study was to evaluate muscular fatty infiltration with an automated quantitative magnetic muscular (MR) imaging method in a large FSHD1 patient's cohort.

Patients and methods: Thirty-five FSHD1 patients were compared to 22 healthy volunteers. Each patient had a clinical evaluation (manual muscular testing, Ricci score and motor function measure scale). MR images of the legs were acquired for all patients and volunteers. Muscular fatty infiltration was determined by two methods: a semi quantitative visual scale and by quantitative method with an automated segmentation tool in order to calculate the intramuscular fat fraction of thighs.

Results: Using semi quantitative visual scale, the most affected thigh muscles were the semimembranosus, semitendinosus, biceps femoris and adductors. In the lower legs, the most affected muscles were the medial gastrocnemius followed by the tibialis anterior. Three different subgroups of patients were distinguished: a subgroup of patients with a near normal imaging, , a second one with prominent involvement of the posterior compartment of thighs and a selective involvement of gastrocnemius medialis, a third one with a severe diffuse fatty infiltration The intramuscular fat fraction of thighs obtained by quantitative MRI was significantly higher in patients (21.9 ± 20.4%) than in volunteers (3.6 ± 2.8%) ($p<0.0001$). Muscular fatty infiltration evaluated by semi quantitative scale and quantitative method were significantly correlated ($p<0.0001$). Quantitative MRI was more accurate to determine muscular fatty infiltration as we observed a ceiling effect of semiquantitative scale for patients with a severe fatty infiltration. Significant correlations were observed between fat fraction and the following clinical scores: muscular testing, Ricci score and MFM score ($p<0.005$).

Conclusion: Quantitative MRI is a promising tool to assess disease progression in FSHD1 patients.

PS2-213 / #249

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

A mouse model of facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) pathology requires the misexpression and missplicing of the DUX4 (double homeobox 4) gene encoded within the 4q35 D4Z4 macrosatellite repeat array resulting in production of a pathogenic protein, DUX4-FL. The human DUX4 gene, encoding a paired homeobox domain transcription factor, evolved from recent retrotransposition and gene conversion events and is specific to the old world primate lineage. Many of the DUX4 genomic binding sites are similarly primate-specific. In addition, the DUX4-FL protein is highly toxic to somatic cells when exogenously expressed while the DUX4-S protein, produced from an alternatively spliced DUX4 mRNA, is both non-toxic and a dominant negative to DUX4-FL toxicity. Together, these difficult circumstances have hindered the generation of phenotypic FSHD-like mice based on DUX4-fl expression. Here we present the successful generation of DUX4/D4Z4 chimeric and transgenic mice that resemble molecular and physical characteristics of FSHD. DUX4-fl mRNA expression is very low, comparable to that found in human FSHD muscle biopsies. Many, but not all, of the conserved DUX4-FL target genes tested were misregulated in tissues from these mice as well as at least one FSHD misregulated gene not known to be a direct target of DUX4-FL. Mice expressing low levels of the DUX4-fl mRNA showed progressive physical deterioration over time, including exhibiting weight loss, strong to severe ataxia due to abnormal mobility of the hindquarters, falling and hyperexcitation. Although these lines are in the early stages of development and characterization, we believe they may be a valuable tool for testing therapeutics targeting DUX4-fl mRNA, protein, and certain downstream molecules.

PS2-214 / #250

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Regulation of DUX4 expression in facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is linked to both the epigenetic dysregulation of the chromosome 4q35 D4Z4 macrosatellite and the muscle-specific misexpression and missplicing of the DUX4 (double homeobox 4) gene encoded within the D4Z4 repeat array, together resulting in production of the pathogenic DUX4-FL protein. However, this does not account for the tissue specificity of FSHD pathology, which requires stable expression of DUX4-fl mRNA from the D4Z4 array in skeletal muscle. Here we describe the identification of two enhancers, DUX4 Myogenic Enhancer 1 (DME1) and 2 (DME2) which activate DUX4-fl expression in skeletal myocytes, but not fibroblasts. Analysis of the chromatin revealed histone modifications and RNA Polymerase II occupancy consistent with DME1 and DME2 being functional enhancers. Chromosome conformation capture (3C) analysis confirmed association of DME1 and DME2 with the DUX4 promoter *in vivo*. The strong interaction between DME2 and the DUX4 promoter in both FSHD and unaffected primary myocytes was greatly reduced in fibroblasts, suggesting a muscle-specific interaction. Nucleosome occupancy and methylome sequencing (NOME-Seq) analysis indicated that in most FSHD myocytes, both enhancers are associated with nucleosomes, but have hypomethylated DNA, consistent with a permissive transcriptional state, sporadic occupancy, and the observed DUX4 expression in rare myonuclei. Our data support a model in which these myogenic enhancers associate with the DUX4 promoter in skeletal myocytes and activate transcription when epigenetically de-repressed in FSHD, resulting in the pathological misexpression of DUX4-fl.

PS2-215 / #314

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

The French National Registry for Facio-Scapulo-Humeral muscular Dystrophy: one year later.

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The French National Registry for Facio-Scapulo-Humeral muscular Dystrophy (FSHD) has been launched in June 2013 to collect epidemiological data, to promote clinical research, and to develop standards of care for FSHD patients. A dedicated database and website (www.fshd.fr) have been developed to enable online data input. Genetic and clinical data are validated by molecular and clinical curators. Data content has been established according to the feedback of 42 FSHD patients in a preliminary study and in agreement with items of pre-existing American and Italian registries to enable future international registry harmonisation.

The aim of this study is to draw up a first annual report of the inclusions in the French FSHD Registry.

Patients included are FSHD1 patients genetically confirmed, and patients presenting with FSHD phenotype without the typical D4Z4 contraction (FSHD2 and/or FSHD-like patients). All patients must sign an informed consent form. FSHD1 patients can be enrolled by their referring physician (specialized or not) directly via a self-reported form, and/or by their referring specialized neurologist through a clinical evaluation form. FSHD2 and FSHD-like patients can only be enrolled via a dedicated clinical evaluation form filled in by a neurologist. Data collected are related to genetic diagnosis, muscular and extra-muscular involvement, pain and patient care. Once data entered and validated, a unique patient number is generated to allow patient and referring physician to access to the data through the website. Preliminary analysis of clinical and genetic data will be presented.

So far, more than 300 patients have been enrolled in France. 30% have been enrolled via the clinical evaluation form AND the self-reported form. 58% of the patients have been enrolled through the self-reported form only. These results show that 88% of the patients filled the self-reported form. Only 12% have been enrolled via the clinical evaluation form, including 3% of FSHD2/FSHD-like patients who are not allowed to fill in the self-reported form.

The self-reported form is an important time-saving option of inclusion that allows good quality data collection, while clinical evaluation form allows collection of more detailed clinical and genetic data that will be helpful for future clinical research projects.

A lot of work has to be done to include as much FSHD patients as possible. More centres will participate and other methods of implementation will be developed.

PS2-216 / #316

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Toll-like receptors and innate immune system in the pathophysiology of oculopharyngeal muscular dystrophy

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Oculopharyngeal muscular dystrophy (OPMD) is a late-onset muscle disease clinically characterized by progressive eyelid ptosis, dysphagia and limb muscle weakness and by the presence of intranuclear tubulofilamentous inclusions in skeletal muscle.

Although short (GCN)11-17/polyalanine expansions at the N-terminus of the polyadenylate-binding protein nuclear 1 gene (PABPN1) is considered the main cause of both the common dominant and the rarer recessive forms of OPMD, the specific factors that initiate and perpetuate disease progression are not well understood.

PABPN1gene plays a key rolein the regulation of RNA metabolism, by modulating post-transcriptional processes including transcript stability, nuclear export and translation. Here we hypothesized that accumulation of the expanded mutant Pabpn1 protein and the consequent impairment of protein homeostasis might represent an endogenous danger signal able to activate Toll-like receptors (TLRs) and innate immunity, promoting degenerative downstream processes in skeletal muscle. Also, recent findings revealed a negative regulatory role for Pabpn1in let-7 miRNA biogenesis or activity. Since let-7 is involved in activation of innate immune response through TLR4 and TLR7 signalling pathway, we investigated the possible relation among PABPN1mutations, let-7, TLR4 and TLR7 in the muscle tissue of OPMD patients.

The analysis of the mRNA transcript levels of TLR3, TLR4, TLR7 and TLR9 (involved in the recognition of endogenous and exogenous nucleic acid molecules), as well as of the TLR-inducible cytokine interferon beta, showed their upregulation in OPMD muscle samples compared to controls. By immunofluorescence we observed highly positive staining for all the TLRs investigated, particularly for TLR4 and a great proportion of DAPI-positive nucleic acid accumulations scattered in the muscle tissue. TLRs localized mainly on the sarcolemma or diffusely in the cytoplasm of some muscle fibers.

Overall, our findings demonstrated that innate immunity, and in particular TLRs, might contribute to OPMD pathophysiology; this might have important implications for new therapeutic approaches.

PS2-217 / #332

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Back pain and paraspinal muscle involvement in patients with FSHD

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Introduction: Facioscapulohumeral dystrophy (FSHD) is the second most common autosomal dominant muscular dystrophy, and is characterized by progressive asymmetric weakness and atrophy of facial, scapular and proximal arm muscles. The clinical presentation varies, and both leg and abdominal muscle involvement is common. Paraspinal muscle weakness has only been reported rarely. However, paraspinal weakness has not been investigated systematically in patients with FSHD. In this study, involvement of paraspinal muscles in FSHD patients was evaluated using MRI and muscle strength tests, and the prevalence of lower back pain was assessed by questionnaire.

Methods: The Dixon technique of MRI was utilized to perform muscle fat fraction analysis of the paraspinal muscles, specifically the lower cervical, thoracic and lumbar regions. Muscle strength in the neck, lower back and legs was quantified with a hand held dynamometer that measured maximum voluntary isometric contraction. All subjects completed the Low Back Pain Rating scale questionnaire.

Results: So far, 49 patients with FSHD and 29 age-matched controls have been included, and the images of 39 patients and 20 controls have been analyzed. The fat fraction of the paraspinal muscles was significantly higher in the patients than in the controls: cervical fat fraction was 32% in FSHD patients compared to 22% in controls ($p < 0.005$), thoracic was 44% compared to 16% ($p < 0.0001$), and lumbar was 38% compared to 23% ($p < 0.0001$). Increased fat fraction correlated with age ($p < 0.0001$), FSHD severity score ($p < 0.0001$), and inversely with muscle strength in the neck and lower back ($p < 0.005$). The prevalence of lower back pain was significantly higher in the FSHD patients that in the controls ($p < 0.0001$), but it did not correlate with the lumbar fat fraction.

Conclusions: This study indicates that involvement of paraspinal muscles in patients with FSHD is prominent, and should be considered in the management of this condition.

PS2-218 / #390

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Mitochondrial dysfunction reveals defective poly(A) tail regulation of specific mRNAs as a primary defect in oculopharyngeal muscular dystrophy

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Oculopharyngeal muscular dystrophy (OPMD), a late-onset syndrome characterized by progressive degeneration of specific muscles, results from the extension of a polyalanine tract in poly(A) binding protein nuclear 1 (PABPN1). While the roles of PABPN1 in nuclear polyadenylation and in the regulation of alternative poly(A) site choice are established, the molecular mechanisms behind OPMD remain undetermined. Using a Drosophila model of OPMD, we have found that OPMD pathogenesis depends on affected poly(A) tail length regulation of specific mRNAs. We identify a set of mRNAs encoding mitochondrial proteins that are down-regulated during OPMD progression. Reduced levels of these mRNAs correlate with their shortened poly(A) tails. Partial rescue of the levels of these mRNAs when deadenylation (shortening of poly(A) tails) is decreased using a deadenylase mutant improves mitochondrial function and reduces muscle weakness.

Interestingly, the down-regulation of these mRNAs already occurs in the earliest stages of disease progression, indicating that this defect is one of the first molecular defects in OPMD. Importantly, the down-regulation of mRNAs encoding mitochondrial proteins has been validated in the mouse model of OPMD. Moreover, a proteomic approach has also validated the down-regulation of mitochondrial proteins in clinically non-affected muscles of OPMD patients. This is consistent with this defect being one of the primary defects in the disease.

We propose a model where the primary defect in OPMD corresponds to defective poly(A) tail regulation of specific mRNAs encoding mitochondrial proteins, leading to decreased synthesis of mitochondrial proteins and defective mitochondrial activity.

PS2-219 / #513

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Nuclear protein spreading: implication for pathophysiology of neuromuscular diseases

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While transfer of a protein encoded by a single nucleus to nearby nuclei in multinucleated cells has been known for almost 25 years, the biological

consequences for gain-of-function diseases have not been considered. Here, we have investigated nuclear protein spreading and its potential consequences in 2 of the 3 most prevalent neuromuscular diseases,. By performing co-cultures between diseased or control human myoblasts and murine C2C12 myoblasts, we demonstrate that in FacioScapuloHumeral Dystrophy (FSHD), although the transcription of the toxic protein DUX4 occurs in only a limited number of nuclei, the resulting protein diffuses into nearby nuclei within the myotubes, thus spreading aberrant gene expression. In Myotonic Dystrophy type 1 (DM1), we observed that in human-mouse heterokaryons, the expression of a mutated DMPK from a human nuclei titrates splicing factors produced by neighbouring nuclei, inducing the mis-splicing of several pre-mRNAs in murine nuclei. In both cases, the spreading of the pathological phenotypes from one nucleus to another is observed, highlighting an additional mechanism that contributes to the dissemination and worsening of the muscle pathogenesis. These results indicate that nuclear protein spreading may be an important component of pathophysiology of gain of function muscular diseases which should be taken into consideration in the design of new therapeutic approaches.

PS2-220 / #515

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Gene therapy strategy for oculopharyngeal muscular dystrophy (OPMD)

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OPMD is an autosomal dominant inherited, slow progressing, late onset degenerative muscular disorder where a small group of specific muscles (pharyngeal and eyelid) are primarily affected. The genetic mutation is a short GCG triplet expansion in the coding region of the ubiquitous polyA binding protein nuclear 1 (PABPN1) gene. Currently there is no cure for OPMD disease. In a phase I/IIa clinical trial of autologous myoblast transplantation in pharyngeal muscles of OPMD patients, we demonstrated safety, absence of toxicity and a cell dose effect regarding improvement in swallowing (Perie et al Mol Ther 2013). However, the current protocol involves the transplantation of unmodified autologous cells, still carrying the genetic mutation.

We are therefore developing a gene therapy strategy based on (1) RNA interference to silence the expression of the mutant PABPN1 allele and (2) gene replacement based on the redundancy of the genetic code to restore a functional protein, as the mutated form of PABPN1 can not be specifically knockdown. This project is conducted both *in vitro* by lentivirus (LV) transduction of OPMD human myoblast cell lines and *in vivo* in OPMD mice by direct intramuscular injection of self-complementary AAV-based serotype 8 vectors (scAAV8).

Several siRNA with different efficiency were validated, designed as shRNA, cloned as single and triple cassettes in viral LV- and AAV-based vectors and tested (1) in control and OPMD human muscle cells lines by lentiviral transductions and (2) in control and OPMD (A17.1) mice by direct AAV injection in tibilias anterior (TA) muscles. In parallel, a codon-modified sequence of PABPN1 (PABOPT) resistant to RNAi degradation has been synthesized and validated. PABOPT cDNA has been cloned together with our shRNA vectors. A strong knockdown of PABPN1 both at mRNA and protein level was obtained *in vitro* in OPMD patient cells and *in vivo* in WT and A17.1 mice with in parallel a good restoration of PABPN1 protein with the PABOPT sequence. PABPN1 extinction at mRNA and protein level, the effects on cell survival, proliferation and myogenic program will be presented.

PS2-221 / #516

Theme: *2.7 - Muscle diseases of genetic origin: Facioscapulohumeral Muscular Dystrophies / Oculopharyngeal Muscular Dystrophy*

Cellular effectors of the exacerbated fibrosis in affected muscles of oculopharyngeal muscular dystrophy

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OPMD is an autosomal dominant inherited, slow progressing, late onset degenerative muscle disorder where a small group of specific muscles (pharyngeal and eyelid) are primarily affected. Even if the genetic mutation was found 15 years ago - a short triplet expansion in the coding region of the ubiquitous PABPN1 gene - the pathophysiological mechanisms leading to the alterations of the myogenic program in those very specific muscles remains to be determined.

Pharyngeal and eyelid muscles show atypical features, such as hypotrophic muscle fibers surrounded by connective tissue that are usually considered to be pathological in other skeletal muscles (limb muscles). We recently demonstrated that OPMD affected pharyngeal muscles are characterized by an exacerbated fibrosis without any sign of inflammation together with fiber atrophy and an increased number of satellite cells.

This peculiar phenotype lead us to evaluate *in vitro* and *in vivo* the behaviour of primary cell cultures from pharyngeal muscles of control and OPMD patients compared to control limb muscles, in order to decipher the respective role of myoblasts and fibroblasts in this particular muscle.

Using CD56 cell marker, we followed *in vitro* the proliferation rate of both CD56+ and CD56- cell fractions. *In vivo* studies were also performed using a xeno-transplantation model in immunodeficient mice to follow their respective behaviour during muscle regeneration. Our study demonstrated that the CD56- cell fraction of pharyngeal muscle is very different from that of limb muscle with a strikingly high proliferative capacity, inducing a rapid loss of myogenicity of primary cultures. The full characterisation of the role of these cellular effectors will help understanding the molecular and cellular mechanisms underlying the relationship between fibrosis and muscle atrophy in this muscle, essential step to develop new therapeutic strategies.

✶PF4

PS2-222 / #560

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Preliminary clinical efficacy and safety of BMN 701, GILT-tagged recombinant human acid alpha glucosidase (rhGAA) in late onset Pompe disease: Results of an extension study

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BMN 701, a novel chimeric fusion protein of Insulin-like Growth Factor 2 (IGF-2) and acid alpha-glucosidase (GAA), is designed to reduce glycogen storage in striated muscle. In preclinical studies BMN 701 clears glycogen in heart, diaphragm, and skeletal muscles at a lower dose than rhGAA. Recently, preliminary safety and efficacy results of a 24 week Phase 1/2 study in late onset Pompe disease patients treated with BMN 701 every other week (POM-001)

were described. This presentation describes results of an extension study of patients who completed POM-001 (POM-002). Primary objectives included evaluation of safety and antibody response to BMN 701 and IGF1 and IGF2. Other objectives included assessment of pulmonary function, 6MWT, PK, quantitative muscle testing and urinary tetrasaccharide. Twenty-one patients have been treated; the longest exposure is greater than 2 years, including the 24 weeks of treatment in POM-001. In the 20 mg/kg group patients have maintained initial improvement initial improvement in respiratory muscle strength as measured by Maximum Inspiratory and Maximum Expiratory Pressure (absolute mean increase of 5.1 and 11 % predicted respectively), and in endurance as measured by 6MWT (mean increase of 22.3 meters). Hypoglycemia, an anticipated pharmacologic effect, was observed in the majority of patients at the 20 mg/kg dose, was transient and managed by oral and IV caloric intake without sequelae. Two patients discontinued the study and two patients required BMN 701 desensitization regimens due to infusion related reactions. These extension study results support ongoing evaluation of BMN 701 in late onset Pompe patients; a Phase 3 study is anticipated to begin in 2014.

PS2-223 / #12

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Hypoparathyroidism as the First Manifestation of Kearns-Sayre Syndrome: A Case Report

No authors listed for this abstract. Please contact us to add a list of authors.

Kearns-Sayre syndrome is a mitochondrial myopathy, which was first described by Tomas Kearn in 1958. Diagnostic symptoms include retinitis pigmentosa, chronic and progressive external ophthalmoplegia plus one or more of following factors: heart conduction system disorders, cerebellar ataxia, or cerebrospinal fluid (CSF) protein content above 100mg/dL. The nature of this uncommon disease is yet to be clarified. In this paper, we report a case of Kearns-Sayre syndrome. According to the previous records, the first manifestation of Kearns-Sayre syndrome as hypoparathyroidism is uncommon and in this article, we report a case with this problem.

Keywords: Kearns-Sayre; Hypoparathyroidism; Ophthalmoplegia; Mitochondrial Cytopathy

PS2-224 / #66

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Mitochondrial disease and risk of cancer

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Mitochondrial mutations are commonly reported in tumors, but it is unclear whether impaired mitochondrial function is a cause or a consequence of cancer. To elucidate this, we assessed the risk of developing cancer in a nationwide cohort of patients with mitochondrial disease.

By use of results from genetic testing for mitochondrial disease and the Danish Civil Registration System, we constructed a nationwide cohort of 343 patients with a mitochondrial disease caused by either maternally inherited mutations in mitochondrial DNA, de novo mutations in mitochondrial DNA or mutations in nuclear DNA affecting mitochondrial function. Cancer development among cohort members was identified by linkage to the Danish Cancer Registry. Risk of cancer in patients with mitochondrial disease was assessed by means of standardized incidence ratios (SIRs) as the ratio between observed and expected cancers; the latter estimated using population-based cancer rates.

During 7,077 person-years of follow-up, 21 cohort members developed a primary cancer. The corresponding SIRs for any primary cancer was 1.13 (95% CI 0.75-1.70) and for any multiple cancer 1.31 (95 % CI 0.91-1.90), cases=26 (5 cases with more than one cancer). Analyses with stratification according to sex and current age indicated no heterogeneity.

Patients with mitochondrial disease are not at increased risk of developing cancer compared with the general population. This finding suggests that frequent mitochondrial abnormalities found in various cancers are the result and not the cause of cancer.

PS2-225 / #73

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Vacuolation of eyelid levator muscle in early-treated infantile-onset Pompe disease

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Patients with infantile-onset Pompe disease (IOPD) who have been treated from an early age using recombinant human acid alpha-glucosidase often still have ptosis, articulation disorders, and swallowing dysfunction, but the etiologies underlying these symptoms remain unclear. We followed a 7-year-old boy who has been treated since 2.5 months of age. He had good motor development and a post-treatment biopsy from the quadriceps revealed adequate clearance of the accumulated lysosomal glycogen. He walked independently at the age of 15 months. After 71 months of ERT, echocardiography revealed only mild left ventricular hypertrophy. He could walk and run, though with a myopathic gait. However, he had bilateral ptosis, articulation disorders, hypernasality, myopia of -10.0 diopters, and mild macroglossia. His anti-rhGAA IgG antibody titers were low (last assessment at 1:800). We increased his alglucosidase alfa dosage to 40 mg/kg/q2weeks for 7 months, but his ptosis failed to improve.An eyelid levator muscle biopsy revealed prominent vacuolation and interruption of the muscle fibers. Therefore, poor treatment response of the small muscles is likely to explain the residual symptoms in the early-treated IOPD patients.

✶PF2

PS2-226 / #85

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Pompe disease: pathophysiology and novel approaches to therapy

No authors listed for this abstract. Please contact us to add a list of authors.

Pompe disease is a lysosomal storage disorder in which acid alpha-glucosidase (GAA), the enzyme involved in the breakdown of glycogen to glucose, is deficient or absent. The clinical spectrum ranges from fatal hypertrophic cardiomyopathy and skeletal muscle myopathy in infants to relatively attenuated late-onset forms, which manifest as a progressive myopathy usually without cardiac involvement. The currently available therapy, designed to provide the missing enzyme, proved to be very successful in reversing cardiac but not skeletal muscle abnormalities. Although the overall understanding of the disease has progressed, the pathophysiology of muscle damage remains poorly understood. Lysosomal enlargement and rupture has long been considered a mechanism of relentless muscle damage in Pompe disease. In past years, our group gathered abundant evidence that this simple view of the pathology is inadequate. We have shown that the pathological cascade in skeletal muscle involves dysfunctional autophagy, a major lysosome-dependent intracellular degradative pathway. The autophagic process in Pompe skeletal muscle is affected at both the initiation of autophagosomal formation (an increase) and at the termination stage (impaired autophagosomal-lysosomal fusion, a condition known as autophagic block). The dysfunctional autophagy contributes significantly to the pathogenesis of the disease and interferes with delivery of the drug to the lysosomes. Yet another abnormality in the diseased muscle is the accelerated production of large, unrelated to ageing, lipofuscin deposits -a marker of cellular oxidative damage and a sign of mitochondrial dysfunction. Indeed, damaged mitochondria with reduced membrane potential, altered calcium buffering capacity, and decreased oxygen consumption were detected in Pompe muscle cells. Several new therapeutic approaches have been successfully tested in Pompe muscle cell lines and in GAA-KO mice: genetic suppression of autophagy, modulation of

calcium levels, and lysosomal exocytosis/glycogen clearance following the overexpression of transcription factor EB (TFEB) and a closely related but distinct factor E3 (TFE3).

PS2-227 / #141

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

X linked sideroblastic anemia and ataxia (XLSA/A) with mitochondrial myopathy and mental retardation caused by novel mutation of ATP-binding cassette transporter (ABCB7) gene

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Purpose: ATP-binding cassette transporter (ABCB7) gene mutation has been reported only four families with X linked sideroblastic anemia and ataxia (XLSA/A). Previously, we reported two sibling patients with mental retardation, mitochondrial myopathy and sideroblastic anemia in 2010 ICNMD. Their biopsied muscle showed many ragged-red fibers and paracrystalline inclusions. In their mitochondrial DNA (mtDNA), pseudouridine synthase 1 (PUS1) gene and SLC25A38 gene analysis, they have no pathological mutations. To investigate the cause of this disease, we performed additional gene analyses.

Methods: The two sibling patients (patient 1; 49-year-old brother and patient 2; 44-year-old sister) have muscle weakness, mental retardation and sideroblastic anemia from early infancy. Patient 1 had very slight cerebellar ataxia. Total nuclear DNA was isolated from patients' white blood cells. Exome sequencing analysis was performed using the Illmina Inc. Hiseq2000 system.

Results: They have novel mutation in ABCB7 gene. Male patient 1 had very slight cerebellar ataxia, while female patient 2 had no cerebellar ataxia. But both 2 patients had sideroblastic anemia, mitochondrial myopathy and mental retardation. In all patients in previous reports?of XLSA/A, there were no mitochondrial myopathy and mental retardation.

We think that XLSA/A with mitochondrial myopathy and mental retardation in the two siblings may be a new phenotype.

PS2-228 / #144

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Severely impaired diaphragmatic function in Pompe disease visualized by cine-MRI

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Background: Respiratory insufficiency is a serious threat to patients with Pompe disease, a treatable metabolic neuromuscular disorder caused by lysosomal acid alpha-glucosidase deficiency. Pulmonary function is decreased particularly in supine position, suggesting a major role of the diaphragm in the pathophysiology of the respiratory dysfunction. Standard pulmonary function tests provide only indirect information about diaphragmatic function, and they do not supply information about chest mechanics in detail.

Objectives: To explore whether cine-MRI is an appropriate technique to assess the dynamic performance of respiratory muscles - especially the diaphragm - and to compare the MRI data with the results of simultaneously performed pulmonary function testing.

Methods: Ten adult Pompe patients and six healthy, age and sex matched, volunteers participated. We

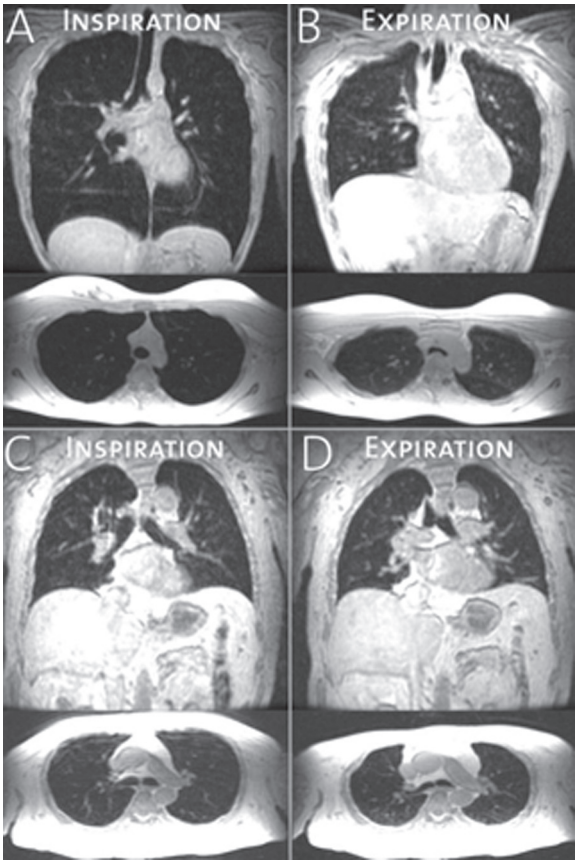

Figure 1. Cine-MRI static scans at end-inspiration and end-expiration in a healthy volunteer (A,B) and a Pompe patient (C,D) clearly show the impaired diaphragmatic displacement in the patient with Pompe disease, indicating severe diaphragmatic dysfunction.

performed two static scans at end-inspiration and end-expiration to evaluate lung anatomy and lung volumes. Three dynamic 3D acquisitions - a sequence of volumetric scans over time - were performed to inspect overall respiratory dynamics. Using manual segmentation of the acquired images, three length ratios were calculated for analyses. Movement in cranio-caudal direction reflects diaphragmatic displacement, while chest wall displacement is established by movement in antero-posterior and left-right directions.

Results: Cine-MRI showed that Pompe patients clearly have a decreased cranio-caudal length ratio compared to healthy volunteers ($p<0.001$), indicating impaired diaphragmatic displacement. This ratio correlated strongly with forced vital capacity in supine position ($r=0.88$), and 'postural drop' ($FVC_{sitting}$ - FVC_{supine}; $r=0.89$). The difference in antero-posterior length ratio was less pronounced ($p=0.04$), while there was no difference in left-right length ratio ($p=0.1$).

Conclusions: Cine-MRI is a promising, non-invasive, technique to assess chest mechanics and to visualize impaired diaphragmatic movement in Pompe patients. It may aid in evaluating efficacy of enzyme replacement therapy and in deciding when to treat.

PS2-229 / #147

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Enzime Replacement Therapy (Ert) In Pompe Disease – New Outcome Measures

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Introduction: Pompe Disease (PD) is is a metabolic recessive disease caused by a deficiency in lysosomal acid α-glucosidase (GAA). Patients with the late-onset form present with progressive proximal weakness in the pelvic and shoulder girdles and a variable progression of respiratory involvement. Outcome measures to evaluate response to ERT are based on muscle strength, respiratory and/or function tests but changes in these parameters take long time to appear. Subjective transient improvement in resistance to fatigue or general wellbeing after each ERT cycle are frequently reported by the patients.

Methods: 7 Patients (ages 29 to 60) underwent Ergometric Stress Tests (EST) 24-48 hs pre infusion and 48-72 hs post infusion assessing parameters such as maximal O2 consumption (VO2), time of exercise, maximum speed, heart rate, anaerobic threshold and blood lactate. A total of 21 non consecutive cycles were analyzed (3 cycles each patient) plus 1 baseline control. FVC, MMT, QMT and 6MWT were assessed at startup and at the end of the study.

Results: Friedman / Conover tests were used for the statistical analysis. VO2 increased in the three post-infusion tests reaching statistical significance in the first two cycles ($p = 0.0025/0.0032$) and a clear tendency in the third (almost as significant as in the two previous) ($p = 0.0636$).

The time of exercise increased significantly in the 3 post infusion EST. The classical parameters such as FVC, MMT and the 6MWT didn't show any changes along the study. All the considered parameters declined by the end of each infusion cycle.

Conclusions: Using this method it's possible to evaluate the effects of the ERT in LOPD patients as soon as 48 hours after the infusion. This tool could also be used with other purposes such as analyzing issues such as dose and infusion regimes without having to wait months to see the results transformed in physical parameters such as increased muscle strength or VC.

PS2-230 / #149

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Benefit of recombinant human acid alpha glucosidase treatment (Myozyme*) in late onset Pompe disease: About six cases with treatment for six months

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Purpose: Since 2007, patients with late onset Pompe disease have been treated by recombinant human acid alpha glucosidase (ERT). We want to evaluate the benefit of this treatment six years later.

Method: Six patients were treated by ERT: 4 men and 2 women. The younger woman was sick since she was 19 years old. When she started treatment, at 35, she used a wheelchair needed non invasive ventilation.

The older woman was 71. The disease began ten years ago. She had an important walking disability, but no respiratory insufficiency.

One of the men is the first patient's brother: he was 50. The disease started when he was 40. He had light walking disability and no respiratory failure.

Another man started his disease when he was 40 but the muscle biopsy was not informative and at this time, the level of alpha glucosidase in leucocytes was normal. A control performed 13 years later reveals the lack of enzyme. When he started the treatment, he needed non invasive ventilation.

The two others patients were 60 and 70 years old when they were diagnosed. The Pompe disease began respectivly 4 and 15 years ago. They had walking disability and all needed non invasive ventilation for sleeping.

Every 6 months, each patient had an evaluation: ECG, breath analysis, gazometry, SF36, tiredness and handicap scale, 6 minutes walking test, MFM test by physiotherapist, Anti GAA antibodies and urinary Glc4.

Results: When they started the treatment, five of six patients used non invasive ventilation but only one (the younger) needed a wheelchair.

During the first three years, five of them were stabilised and improved the 6MWT but only two improved their respiratory function and the three others were stable. One patient had no walking improvement and had a respiratory failure and deceased after three years.

A second patient deceased after four years of treatment.

The other patients saw their pulmonary capacity decreasing more than their walking test.

After 6 years of treatment (20 mg/kg twice a month), no adverse event was observed.

Conclusion: if during the first two years of treatment most of the patients improved their respiratory and walking function, after 6 years of follow up, two patients are deceased and the others have a decreasing of their capacity, but probably more slowly that untreated patients.

✶PF3

PS2-231 / #151

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

A nationwide survey of Danon disease in Japan

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Introduction: Danon disease, an X-linked dominant vacuolar cardiomyopathy and skeletal myopathy, is caused by primary deficiency of lysosome-associated membrane protein-2 (LAMP-2). However, the clinical features and the prevalences of Danon disease have not been well established.

Patients and Methods: We sent questionnaires on Danon disease to 2,617 hospitals in Japan that have departments of neurology, cardiology, or pediatrics. We reviewed clinical histories and muscle specimens provided by hospitals with Danon disease patients. In addition, we performed genetic analyses of the LAMP-2 gene.

Results: We identified 28 Danon disease patients (16 men and 12 women) from 13 families.?Cardiomyopathy and ECG abnormalities were evident in all patients with Danon disease. Hypertrophic cardiomyopathy (HCM) was documented in most men, while dilated cardiomyopathy was more common among women. WPW syndrome was noted at a relatively higher incidence of 38%. Permanent pacemakers were placed in four men and five women. Heart transplantation, the most effective therapy, was performed in only one man. Pathologically, autophagic vacuoles with sarcolemmal features (AVSF). AVSF expressed virtually all sarcolemmal proteins on their vacuolar membranes in all Danon disease patients. All Danon disease patients had LAMP-2 gene mutations. Half of the probands showed de novo mutations.

Conclusion: Danon disease is a very rare muscular disorder and may be primarily caused by lysosomal dysfunctions. Cardiomyopathy is the most important prognostic factor and the main cause of death among Danon disease patients. Danon disease may be overlooked in patients with HCM, since other clinical features including myopathy can be mild, particularly in women.

PS2-232 / #159

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Increased aortic stiffness and blood pressure in adults with Pompe disease

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Background: Pompe disease is an inheritable neuromuscular disorder caused by acid α-glucosidase deficiency leading to glycogen accumulation in various body tissues, in adults predominantly skeletal and smooth muscle fibers. Vascular abnormalities and glycogen accumulation in vascular smooth muscle have been described. The clinical effects of glycogen storage in the vascular wall remain unknown. We studied whether aortic stiffness is increased in patients with Pompe disease.

Methods: Carotid-femoral pulse wave velocity (cfPWV), the gold standard methodology, was used to determine aortic stiffness. Intima media thickness and distensibility of the right common carotid artery were measured using a Duplex scanner. Aortic augmentation index, central pulse pressure, aortic reflexion time and cfPWV were assessed using the SphygmoCor® system.

Results: Eighty-four adult Pompe patients and 179 age- and gender-matched volunteers participated in this cross-sectional case-controlled study. CfPWV was higher in patients than in volunteers (8.8 vs 7.4 m/s, $p < 0.001$). This difference was still present after adjustment for age, gender, mean arterial blood pres-

sure (MAP), heart rate and diabetes mellitus ($p=0.001$), and was shown by subgroup analysis to apply to the 40-59 year age group ($p=0.004$) and 60+ years age group ($p=0.01$), but not to younger age groups ($p=0.99$). Except for a shorter aortic reflexion time ($p=0.02$), indirect indicators of arterial stiffness did not differ between patients and volunteers. Relative to volunteers (20%), more Pompe patients had a history of hypertension (36%, $p=0.005$), and their MAP was higher (100 vs 92 mmHg, $p<0.001$).

Conclusion: This study shows that adult patients with Pompe disease have increased aortic stiffness and blood pressure. Whether this is due to glycogen accumulation requires further investigation.

✶PF4

PS2-233 / #161

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Anti-alglucosidase alfa antibodies and infusion-associated reactions in 73 treated adult Pompe patients

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Background: Enzyme replacement therapy (ERT) with alglucosidase alfa is the registered treatment for Pompe disease. In infants sustained high antibody titers against alglucosidase alfa reduce treatment efficacy. The only randomized controlled trial in 59 adults does not indicate such a relationship, while a recent study in 3 adults does. We studied whether these antibodies have an impact on treatment outcome and infusion-associated reactions (IARs) in a large cohort of adults.

Methods: In thisprospective single-centre cohort study, adult Pompe patients were treated with 20 mg/kg alglucosidase alfa every other week. Antibody titers were determined before start of ERT and during ERT at approximately 6, 12 and 36 months using ELISA. The neutralizing effects of antibodies on enzyme activity was measured in *in vitro* studies. Clinical outcome and IARs were monitored.

Results: 71 of the 73 (97%) patients developed antibodies during follow-up. The median titer peaked at 6 months to 1:1250 declining thereupon to 1:250 at the last measurement. 12 patients (16%) had sustained high antibody titers (≥1:31.250 at at least 2 time points). *In vitro* studies showed that the higher the antibody titer, the more the enzyme activity was inhibited in the medium ($\rho = 0.75$; $P<0.01$) and in the target cells ($\rho = 0.69$; $P=0.01$). Overall, no relationship with clinical outcome was found. 16 patients had high titers (≥1:31250) at any time during follow-up, of whom 7 (44%) experienced IARs; compared to 5 (19%) out of 27 with intermediate titers (≥1:1250 and ≤1:6250) and only 1 (3%) out of 30 with no or low titers (≤1:250) ($P=0.001$).

Conclusion: During ERT almost all adult Pompe patients developed antibodies against alglucosidase alfa. The clinical response to ERT was not significantly correlated with antibody titers. Antibody formation however was related with the risk for IARs. Therefore close monitoring of IARs is indicated in patients with antibodies.

PS2-234 / #164

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Histopathological, biochemical and clinical features in muscle biopsies of late-onset Pompe Disease patients before and after ERT

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Glycogenosis type II is an autosomal recessive lysosomal storage disorder that results from a deficiency in the glucosidase alpha acid (GAA) enzyme. The disease is characterized by progressive accumulation of lysosomal glycogen in various tissues, primarily in cardiac and skeletal muscles.

The histopathological hallmarks in the muscle are fiber vacuolization and autophagy.

GSDII is clinically classified into three forms: infantile, juvenile, and late-onset. Late-onset form usually manifests as slowly progressive myopathy associated with respiratory insufficiency and without cardiac symptoms.

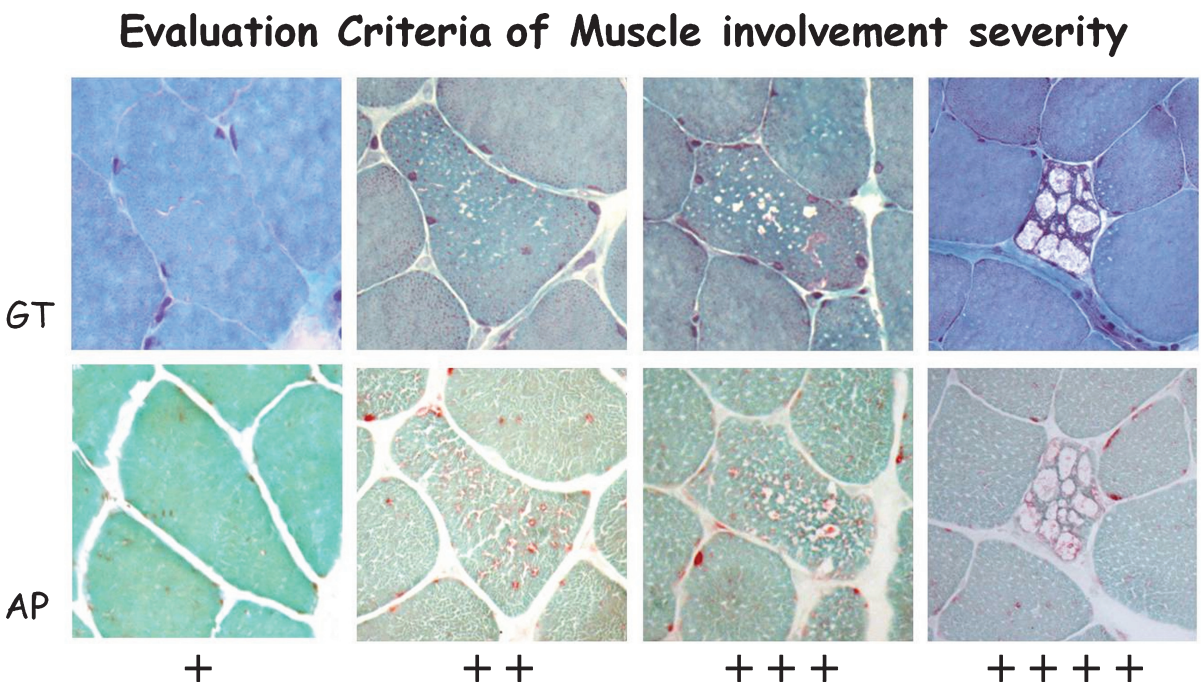
Recombinant human GAA is the only approved enzyme replacement therapy (ERT) available for disease treatment. It is effective in most infantile patients, whereas the improvement is quite variable in adults.

Our project aims at studying muscle biopsies from 14 late-onset patients at molecular, biochemical, and histopathological level in order to evaluate the effects of therapy.

All patients clinically improved or remained stable after ERT.

Evaluation of the following morphological parameters was performed: CSA, number of vacuolated fibers, degree of glycogen accumulation, percentage of vacuolization in type I and type II fibers.

Pre-treatment muscle biopsies showed a histopathological divergent spectrum, ranging from almost normal morphology, with very few scattered vacu-



oles, to severe vacuolar myopathy.

Post-treatment muscle biopsies were morphologically improved in seven patients, worsened in two patients, whereas no significant histopathological modifications were seen in all the other subjects.

We performed immunohistochemical analysis of the autophagic/lysosomal markers: EEA1 (early endosome antigen 1), LC3 (microtubule-associated protein 1 light chain 3), and LAMP2 (lysosome associated membrane protein 2). Our results show a variable binding of the three antibodies in both the first and the second biopsies.

We tested GAA enzymatic activity by a fluorimetric assay in both lymphocytes and muscle tissue from 5 patients before and after ERT, showing a mild increase of enzymatic activity in skeletal muscle. Also GAA expression assessed by immunoblotting slightly increased in a few patients.

In conclusion, this study shows positive effects of ERT in late-onset GSDII patients. A larger cohort of patients will allow to make a correlation between activation/inactivation of the autophagic pathway and morphological results.

PS2-235 / #170

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Mitochondria transfer from Wharton's jelly-derived stromal stem cell: A potential rescue strategy for mitochondrial diseases

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Objectives: Wharton's jelly-derived stromal cell (WSC) retaining property of stem cell has been suggested to possess therapeutic potential for mitochondrial diseases caused by mitochondrial genomic mutations. This study aims to examine whether WJC transfer mitochondria to reverse physiological performance compromised by mitochondrial deficiency.

Methods: WJC was co-cultured with mitochondria DNA deficient ρ^0 cell in the presence of bromodeoxyuridine and absence of pyruvate/uridine. Mitochondrial DNA (mtDNA) content and identification was verified by PCR and sequencing the hypervariant region 2 (HVR2). Oxygen consumption was determined by Clark electrode. Adhesion-independent growth was evaluated by soft agar assay. Cellular motility was examined using Matrigel-coated transwell. Mitochondria-dependent ATP production was investigated in the presence of oligomycin using ATP Assay kit (BioVision).

Results: Mitochondrial DNA depleted (ρ^0) cell following co-culture with WJC (ρ^{+W} cell) survives the selection, whereas neither WJC alone nor ρ^0 cell alone are alive. Respiratory function, anchorage-independent growth and cell motility are totally recued in ρ^{+W} cell and compatible to parental cell, as well as cybrid cell harbouring exogenous mitochondria by artificial fusion. Mitochondrial-dependent ATP production is also strikingly ameliorated in ρ^{+W} cell. The sequence of HVR2 mtDNA of ρ^{+W} cell is identical to WJC, instead of parental cell, suggesting mitochondrial transfer from WJC.

PS2-236 / #175

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

A constitutive knock-out animal model for Glycogen storage disease type III

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Glycogen storage disease type III (GSDIII) is an autosomal recessive disease caused by amylo-1,6- α-glucosidase, 4- α -glucanotransferase (AGL, or glycogen debrancher enzyme, GDE) deficiency.

The disease is characterized by onset in infancy or early childhood, with hepatomegaly, hypoglycemia, hyperlipidemia, short stature, occasional seizures, and growth retardation. Progressive skeletal yopathy, neuropathy, and/or cardiomyopathy become predominant in adults, and cause disability in dults and precocious death. No cure is available.

To create a constitutive knock-out mouse model, we excised Agl gene , coding for the glycogen binding domain. Agl-KO mice were viable but presented higher mortality in adulthood than wild-type mice. Agl-KO mice showed lack of enzyme activity and western blot analysis revealed the complete absence of protein n liver, skeletal muscle, heart and brain.

This animal presented GSD's cardinal features, namely progressive intracytoplasmic glycogen accumulation in liver and skeletal muscle, impaired glycaemic control, decreased muscle and respiratory function, and decreased survival. Agl-KO skeletal muscle showed a significant accumulation of glycogen leading to severe muscle alterations. Glycogen storage accumulation and tissue damage increase with age.

We generated an AGL knockout (KO) mouse model, a critical tool for advancing the GSDIII field.

PS2-237 / #205

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Adult Polyglucosan Body Disease: Clinical and histological heterogeneity of an Italian family

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Introduction: Adult Polyglucosan Body Disease (APBD) is a rare autosomal recessive leukodystrophy due to mutations of glycogen branching enzyme gene (GBE1), leading to accumulation of polyglucosan bodies (PB) in central and peripheral nervous system. The disease mainly affects the Askenazi Jewish descent.

Methods: Three siblings from a non-Jewish Italian family, affected with APBD.

Results: The proband, a 57-years-old man, presented with progressive distal paresthesia at the age of 55 years. A sensory-motor demyelinating neuropathy was diagnosed at nerve conduction study (NCS). Subsequently, gait ataxia and urinary urgency were reported. His sister, now aged 56 years, has been showing a slowly worsening paraparesis since the age of 52 years, complicated by neurogenic bladder in the last months. The youngest affected sister, aged 53 years, had a recent, transitory, episode of orthostatic vomit and mild ataxia. The MRI of all subjects showed diffuse hyperintense infra- and supratentorial white matter abnormalities, with bulbar and spinal cord atrophy. In both sisters NCS was normal, whereas their muscle biopsies only showed non-specific alterations. In the proband, both muscle and nerve biopsies showed PB, which prompted molecular investigation for GBE1. All siblings were compound heterozygous for a previously described mutation (c.1604A>G), and a novel one (c.1064G>A).

Conclusion: We demonstrated that in a large APBD family, common clinical signs occurred together with "atypical" ones (demyelinating neuropathy/transient symptoms) featuring a peculiar intrafamilial variability. Indeed, PB detection at muscle/nerve biopsy correlates with NCS alteration, which makes the integration between peripheral and central nervous system findings necessary for a correct diagnosis.

PS2-238 / #276

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

What factors are associated with the prevalence of sub-sarcolemmal mitochondrial aggregates (SSMA) in paediatric skeletal muscle? Examining the use and limitations of SSMA as a diagnostic muscle biopsy marker

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Paediatric mitochondrial disease (mtD) is clinico-genetically heterogenous. Muscle biopsies often lack classical disease markers like ragged red (RRF) and COX negative fibres. Instead, subsarcolemmal mitochondrial aggregates (SSMA) are considered more prominent and various %SSMA cut-offs (>2%,≤4%) have been proposed as markers of mitochondrial biochemical defects.

Our main objectives were to assess the prevalence of %SSMA in muscle biopsies of patients with mtD and age-matched controls (CTRL) from 0-16 years, examine their relationship to the biopsy age, mtDNA copy number and biopsy group (mtD versus CTRL) by conventional light microscopy (CLM), and develop a novel image analysis (IA) tool for precise quantitation.

We retrospectively audited 54 diagnostic muscle biopsies (mtD=25, CTRL=29) performed at our institution. The mtD group included cases defined genetically, biochemically, and/or pathologically (RRF and/or COX-negative fibres). CTRL group included histologically normal/minimal change biopsies in whom mitochondrial disease was excluded. Biopsies were assessed for fibre type and size, lipid content, capillary density, internal mitochondria and SSMA by CLM and by developing a novel IA tool (Definiens). mtDNA copy number was obtained by real-time PCR in 41 biopsies. Stata was used for data analysis.

The mtD and CTRL groups did not differ significantly in terms of biopsy age, gender, slow predominance, prominent internal mitochondria and tube-feeding. Significant differences were observed for %SSMA; mtD 4.6 versus CTRL 15.5 ($p=0.01$), mtDNA copy number; mtD 27.5 versus CTRL 95.5 ($p=0.001$) and lipid content; mtD 60% versus CTRL 13.8% ($p<0.0001$). Linear regression showed significant association of biopsy group, biopsy age and mtDNA copy number with %SSMA in the unadjusted analysis. With multivariable analysis increasing biopsy age was the only variable significantly associated with increasing %SSMA ($p<0.0001$). Applying the previously suggested 4% SSMA cut-off to our cohort yielded 50% sensitivity and 82.7% specificity (PPV=70.6%, NPV=66.7%) for mtD.

In line with recent observations %SSMA prevalence is significantly lower in mtD and correlates with the mtDNA copy number. Combining CLM and a novel IA technique we demonstrate for the first time that prevalence of %SSMA in paediatric skeletal muscle is age-dependent and increases with age. Therefore continuous age-stratified %SSMA cut-offs may better predict mitochondrial disease in paediatric muscle biopsies.

PS2-239 / #287

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Phosphoglucomutase type 1 (PGM1) deficiency bridges muscle glycogenosis and glycosylation disorders.

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Phosphoglucomutase type 1 deficiency (PGM1) has been described in rare patients presenting with exercise intolerance and rhabdomyolysis. This enzyme which catalyzes the reversible conversion of glucose-6-P to glucose-1-P is encountered in glycogen storage type XIV. Recently, the discovery of mutations in PGM1 gene in patients with CDG syndrome has broadened the phenotype associated with PGM1 deficiency.

We report 3 adults presenting with exercise intolerance and rhabdomyolysis episodes, with onset in adolescence. Among these 3 patients, two had additional features such as cleft palate, facial dysmorphism, hepatic cytolysis. One of them has also mental retardation and behavioural disorder. All patients had elevated CK level at rest (x3N). A standardized forearm exercise test revealed a hyperammonemia with normal lactate increase during exercise in two patients. Muscle biopsy revealed only mild glycogen accumulation without vacuoles. PGM1 activity in muscle was severely reduced (< 1% of residual activity). Genetic analysis showed compound heterozygous PGM1 mutations in these 3 patients Abnormal fractions of transferrin isoforms were detected in blood by transferrin electrofocusing in the three cases.

PGM1 deficiency is a newly recognized inborn error of both glycogenolysis and glycosylation with a broad clinical spectrum ranging from pure exercise intolerance to a more complex phenotype associating high CK levels, cardiomyopathy central nervous system involvement, growth retardation, dysmorphic features, , hepatic or endocrine dysfonction.

Abnormal transferrin isoforms are suggestive of PGM1 deficiency, and should be performed in patients with short exercise intolerance associated with high CK levels or rhabdomyolysis episodes.

PS2-240 / #294

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

"Rolling in the deep": Atypical presentations in late-onset pompe disease – beyond the "limb-girdle dystrophy phenotype"

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Background:Pompe disease (PD) or acid maltase deficiency (glycogen storage disease type II) is a rare autosomal recessive lysosomal glycogen storage disease particularly affecting the myocardium, skeletal muscle and liver. Late-onset Pompe disease (LOPD) is not an uncommon presentation of PD and has a wide clinical variability.

Objectives:To report four cases of LOPD patients with atypical clinical presentation mimicking other clinical entities.

Methods: Case 1, female patient, 15 years old, referred for evaluation of progressive scoliosis without complaints of skeletal muscle weakness; case 2, male patient, 4 years old, presenting with arrested motor milestones and velopharyngeal insufficiency; case 3, female patient, 38 years old, referred for evaluation of "progressive external ophtalmoplegia", presenting with asymmetric eye ptosis; case 4, female patient, 43 years old, referred for evaluation of "prolapsus disci intervertebralis". Enzyme assays for alpha-glucosidase (acid maltase) on filter paper showed decrease activity in all patients, later confirmed in leukocytes

assay and fibroblasts (two patients); mild elevation of Hex4 was seen in one patient. Molecular studies showed that all patients but one harbored at least one mutation associated with the late-onset form of Pompe disease (c.-32-13T>G). All of themnormal or borderlineCK levels.

Discussion/Conclusions:LOPD comprises a continuum of phenotypes ranging from typical progressive limb-girdle myopathy to more severe diaphragmatic weakness as first presentation. Probably because of this wide range of clinical manifestations, many patients are misdiagnosed with more common diseases. Scoliosis is not an uncommon feature of LOPD, although it was never reported before as the main manifestation in a patient with subclinical muscle weakness. Hypotonia with velopharyngeal insufficiency and ptosis have been described in some early-treated classical Pompe patients, but not as the main initial clinical manifestations in LOPD patients Our report reinforces the importance of looking for atypical presentation of LOPD and the practical aspects of using dried-blood spots in the investigation of such patients.

PS2-241 / #295

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Behr`s syndrome is a mitochondrial disease due to autosomal recessive mutations in the C12orf65 gene

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Background: Behr`s syndrome is a classical phenotypic description of childhood-onset optic atrophy combined with various neurological symptoms, including ophthalmoparesis, nystagmus, spastic paraparesis, ataxia, peripheral neuropathy and learning difficulties.

Objective: Here we describe 4 patients with the classical Behr`s syndrome phenotype from 3 unrelated families who carry homozygous nonsense mutations in the C12orf65 gene encoding a protein involved in mitochondrial translation.

Methods: Whole exome sequencing was performed in genomic DNA.

Results: We detected 2 different homozygous C12orf65 nonsense mutations in 4 patients with a homogeneous clinical presentation matching the historical description of Behr`s syndrome. The first symptom in all patients was childhood-onset optic atrophy, followed by spastic paraparesis, distal weakness, motor neuropathy and ophthalmoparesis.

Conclusions: We think that C12orf65 mutations are more frequent than previously suggested and screening of this gene should be considered not only in patients with mitochondrial respiratory chain deficiencies, but also in inherited peripheral neuropathies, spastic paraplegias and ataxias, especially with pre-existing optic atrophy.

✶PF4

PS2-242 / #313

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Long-term neurologic and cardiac correction in the Pompe disease mice by intrathecal gene therapy

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Pompe disease (glycogen storage disease type II) is a lysosomal storage disorder caused by acid-alpha-glucosidase (GAA) deficiency leading to progressive accumulation of glycogen in the heart, muscles, and central nervous system (CNS). The disease manifests as a fatal cardiomyopathy in infantile form. Cardiac correction by enzyme replacement therapy (ERT) has recently prolonged the lifespan of these patients, revealing a new natural history. The emergent neurologic phenotype and the poor correction of skeletal muscles in survivors are currently partly attributed to CNS glycogen storage, uncorrected by ERT. We evaluated a gene therapy strategy using the neurotropic and cardiotropic AAV serotype 9 injected intrathecally (ie in the cerebrospinal fluid) to restore GAA activity in the CNS and the heart.

GAA-KO mice were injected with AAV9-gaa into the cisterna magna at one month. Their neurologic and motor skills were periodically monitored from three to twelve months by hind limb clasping reflex, brainstem auditory evoked potentials, wire-hang test, and accelerating rotarod; cardiac function was assessed by M-mode echocardiography at 12 months. Glycogen content, GAA activity, and disease-related pathology were assessed in the CNS and heart at endpoint. We also used real-time RT-PCR to examine transcriptional markers of cardiomyopathy and denervation atrophy in the heart and muscles respectively.

We demonstrate a significant functional neurologic correction in treated animals from 4 months onward, a neuromuscular improvement from 9 months onward, and a correction of the hypertrophic cardiomyopathy at 12 months. The regions most affected by the disease i.e the brainstem, spinal cord, spinal ganglia, and the left cardiac ventricular wall all show enzymatic, biochemical and histological correction. This unprecedented global and long-term CNS and cardiac cure offer new perspectives for the management of patients.

PS2-243 / #322

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Assessing immune responses to recombinant human GAA (rhGAA) in late-onset Pompe disease (LOPD) patients

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Pompe disease (PD) is an inherited metabolic myopathy caused by a deficiency of the lysosomal enzyme acid α-glucosidase (GAA), resulting in massive accumulation of glycogen in lysosomes and disruption of cellular functions. Although enzyme replacement therapy (ERT) has improved the outcome of PD, development of antibody responses against rhGAA are not uncommon and in some cases prevent therapeutic efficacy. Despite these limitations, the role of the immune responses to rhGAA has not been fully characterized, in particular in the late-onset PD patients (LOPD, adult population).

The aim is to provide a fine characterization of the immune responses against rhGAA in adults undergoing ERT. We compared LOPD patients on ERT who develop immunity to the protein, with subjects who do not; we also included LOPD patients who are not treated and healthy donors.

An ELISA capture assay was used to analyze, in the serum from LOPD patients and healthy donors, the specific anti-GAA Ig subclasses that might be secreted during ERT. In our preliminary results LOPD patients have significant amount of anti-GAA antibodies and IgG4 seem to be one of the most represented IgG subclass, reflecting repeated exposures to the antigen. Response to rhGAA in peripheral blood of LOPD is hard to detect and study. To evaluate specific immune responses, PMBCs from treated and untreated LOPD patients were restimulated *in vitro* with rhGAA

following a protocol established in our laboratory. It consist in a previously induction of dendritic cell maturation and then the reactive T cells are analyzed with an IFN-g ELISpot assay. The preliminary results obtained indicate that with this protocol IFN-g-driven T cell responses to rhGAA are detectable in some of the LOPD subjects tested. Interestingly, even untreated PD patients, who apparently never "seen" rhGAA, have circulating T cells reactive to the protein.

These preliminary studies in LOPD suggest that the rhGAA in PD patients can induces pro-inflammatory T cell responses in addition to antibody responses, likely reflecting the activation of antigen-specific T helper cells. More studies are needed to better defining the subsets of B and T cells involved in these responses and the clinical implications of immunogenicity of rhGAA. Understanding the immune responses to rhGAA will provide the basis for the design of strategies to modulate both acute and long-term responses to the enzyme and to manage adverse events related to infusions.

PS2-244 / #328

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Increased prevalence of malignancy in adult mitochondrial disorders

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Objectives: there are indications that patients with a mitochondrial disorder (MID) develop more frequently malignomas or benign tumours than the general population. Aims of the study were to find out if the prevalence of tumours is actually increased in MID-patients and which of the malignomas or benign tumours are the most frequent.

Methods: Retrospectively evaluated were the charts of MID-patients for the presence of malign or benign tumours. MID was diagnosed according to the modified Walker-criteria.

Results: Among 475 MID-patients screened for tumours, at least a single malignoma was found in 65 patients (13.7%), and at least a single benign tumour in 35 patients (7.4%). Among those with malignancy, 22 were men and 43 female. Among those with a malignancy 1 had definite MID, 9 probable MID, and 55 possible MID. The most common of the malignancies was breast cancer, followed by dermatological, gynecological, and gastrointestinal malignancies. The most frequent of the benign tumours was lipoma, followed by pituitary adenoma, meningeomas, carcinoids, and suprarenal adenomas. Compared to the general population, the prevalence of malignancies and of benign tumours was markedly increased. The female preponderance was explained by the frequent maternal inheritance of MIDs.

Conclusions: Adult patients with a MID, particularly females, carry an increased risk to develop a malignancy or a benign tumour. Since malignancy is an important determinant for their outcome, these patients should be more accurately screened for neoplasms, not to overlook the point, at which an effective treatment can no longer be provided.

PS2-245 / #334

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

A dominant mutation in CHCHD10 causes neurodegenerative disorder with mitochondrial DNA instability

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Mutations in OPA1 and MFN2, two genes encoding membrane proteins involved in mitochondrial dynamics, are responsible for mitochondrial DNA (mtDNA) instability disorder with "Autosomal Dominant Optic Atrophy (ADOA) plus" phenotype. We report a large family with a late-onset complex phenotype including motor neuron disease, cerebellar ataxia, cognitive decline and myopathy. Muscle biopsy showed ragged-red and COX negative fibres with combined respiratory chain deficiency and abnormal assembly of complex V. The multiple mitochondrial DNA (mtDNA) deletions found in skeletal muscle revealed

a mtDNA instability disorder. By whole-exome sequencing (WES), we identified a missense mutation (c.176C>T; p.Ser59Leu) in the CHCHD10 gene that encodes a coiled-coil helix coiled-coil helix protein, whose function was unknown. We show that CHCHD10 is a mitochondrial protein located in the intermembrane space and enriched at cristae junctions. Patient fibroblasts carrying the CHCHD10 mutation present with a respiratory chain deficiency and a fragmentation of the mitochondrial network. Furthermore, we show that overexpression of CHCHD-10^{S59L} triggers mitochondrial fragmentation in HeLa cells, thus confirming the deleterious effect of this mutant on mitochondrial morphology and network. $DRP1^{K38A}$which is resistant to fission, did not modify the mitochondrial fragmentation observed in cells expressing $CHCHD10^{S59L}$, suggesting that the CHCHD10 mutant leads to impaired fusion activity. This work, suggesting that CHCHD10 plays a role in mitochondrial fusion and/or in maintenance of cristae morphology, highlights the critical role of mitochondrial dynamics in terms of human disease and mitochondrial genome stability.

PS2-246 / #352

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Exercise intolerance associated with ACAD9 mutations : A case report

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Isolated defect of respiratory chain complex I activity is a frequent biochemical abnormality in mitochondrial disorders. Acyl-coA Deshydrogenase 9 deficiency is an autosomal recessively inherited disorder of mitochondrial long chain fatty acid beta-oxidation caused by mutations in the ACAD9 gene. This flavoprotein is also involved in complex I assembly. First described patients with ACAD9 mutations presented with lactic acidosis and hypertrophic cardiomyopathy responsive to ribofavine treatment. Less than 10 cases were described with Reye syndrome or myopathic presentation. We report one sporadic case of mild mitochondrial myopathy with ACAD9 mutations. A 33 year-old woman developed during infancy exercise intolerance associated with hyperlactatemia. This mild myopathy was non progressive. Muscle biopsy showed lipidic accumulation and ragged-red fibers. An isolated and profound complex I deficiency was identified. Blue native-polyacrylamide gel electrophoresis revealed an assembly defect of complex I. Analysis of mitochondrial DNA showed multiple deletion of mtDNA, but exhaustive screening did not identify any mutation. These suggestive biochemical abnormalities led us to analyze ACAD9 gene. The homozygous missense mutation (p.Arg414Cys), identified in exon 12, was previously described in a patient with exercise intolerance and mild psychomotor retardation. In conclusion, the association of exercise intolerance with deficiency of complex I is strongly evocating of ACAD9 mutations.

PS2-247 / #391

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Severe early onset cardiomyopathy in females with Danon disease not caused by skewed X-chromosome inactivation

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Danon disease is caused by mutations in the lysosome-associated membrane protein-2 gene, LAMP2, which is located on the X chromosome. Although late onset cardiomyopathy is common in female carriers with LAMP2 mutations, there are unusual cases with an atypically early presentation and a very severe disease course. It has been proposed that this is a result of unfavorable skewing of X-chromosome inactivation, but knowledge of LAMP2 expression in severely affected young female carriers is very limited.

We have investigated two young girls aged ten and thirteen years who underwent cardiac transplantation because of hypertrophic cardiomyopathy due to de novo heterozygous LAMP2 mutations. We also investigated one adult female with a more typical clinical phenotype. We studied LAMP2 mRNA and protein in heart and skeletal muscle and also signs of skewed X-chromosome inactivation.

We found no evidence of skewed X-chromosome inactivation in the two young girls. In accordance with this finding skeletal muscle biopsy revealed no pathological changes. Expression of LAMP2 mRNA in cardiac muscle in the youngest patient revealed equal expression of both alleles also supporting the concept that there was no skewed X-chromosome inactivation. Immunohistochemistry in cardiac muscles of all three patients showed an unexpected and remarkable pattern with lack of LAMP2 protein large regions that also showed myocyte hypertrophy, lysosomal enlargement and disarray. In other large regions there was preserved LAMP2 expression and nearly normal histology.

Our results demonstrate that skewed X-chromosome inactivation may not be a common cause of severe cardiac phenotype seen in occasional female carriers of LAMP2 mutations. Instead an uneven distribution of LAMP2 protein may cause deleterious effects depending on which regions of the myocardium that are lacking LAMP2 protein in spite of an overall moderate reduction of LAMP2 protein.

★PF4

PS2-248 / #425

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Deoxypyrimidine Monophosphates treatment for Thymidine kinase 2 Deficiency

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Autosomal recessive TK2 mutations have been associated with severe depletion of mitochondrial DNA (mtDNA) and devastating neuromuscular diseases in infants and children, and with mtDNA multiple deletions and progressive external ophthalmoplegia in adults.

Similar to other mitochondrial disorders, only supportive treatments are available for TK2 deficiency. We generated a Tk2 H126N knock-in mouse model that manifests a phenotype strikingly similar to the human infantile encephalomyopathy. We demonstrated that lack of Tk2 activity cause nucleotide pools unbalance with severe reductions of deoxypyrimidine triphosphates (dTTP and dCTP) in brain and liver, leading to reduction of mtDNA copy number. To bypass Tk2 deficiency, we administered deoxypyrimidine monophosphates (dCMP+dTMP) to Tk2 knock-in mice by oral gavage from postnatal day 4, when mutant mice are biochemically affected but phenotypically normal.

Assessment of 13-day old Tk2-/- mice treated with dCMP+dTMP 200mg/kg/day each demonstrated that in mutant animals, the compounds: raise dCMP+dTMP concentrations; increase levels of mtDNA, augment quantity and activities of mitochondrial respiratory chain enzyme; and significantly prolong their lifespan

(34 days with treatment vs 13 days untreated). A second trial of dCMP+dTMP at 400 mg/kg/day showed even greater phenotypic and biochemical improvements. No adverse effects were observed with both doses of dCMP+dTMP. Oral dCMP+dTMP supplementation is the first effective and safe treatment for TK2 deficiency in mice. This treatment can potentially be applied to patients.

PS2-249 / #437

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Acid α-glucosidase rescue by splice-switching strategy using antisense oligonucleotides in patients with adult form of Pompe disease

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Glycogen storage disease type II, also termed Pompe disease, is an autosomal recessive lysosomal storage disorder (1/40,000). It is characterized by a deficiency of the acid α-glucosidase or acid maltase. This enzyme is needed to break down glycogen that is stored within the lysosome and its deficiency leads to accumulation of lysosomal glycogen in all cells of the body. Pompe disease comprises a continuous spectrum of phenotypes, based on age at onset and rate of progression, and sub-typed in non-classic infantile, childhood, juvenile and adult forms. The classic infantile form has virtually no residual acid α-glucosidase activity, while patients with adult forms do have some residual enzymatic activity.

The enzyme deficiency is caused by pathogenic mutations in the acid α-glucosidase gene (GAA) located on chromosome 17. The GAA gene spans over 20,000 base pairs and contains 20 exons giving rise to a full length mRNA of about 3.6 kb, translated into protein from the initiation codon located in exon 2. The nature and the combination of the mutations in the GAA gene determine the level of residual lysosomal acid α-glucosidase activity and subsequent clinical severity.

To date, more than three hundred private variants have been described in the GAA gene, randomly spread over the whole gene. Some mutations have a high frequency, such as c.-32-13 T>G mutation which is the most common in Caucasian patients with adult form. This intronic mutation within the pyrimidine tract leads to weaken splicing of exon 2, although low levels of normally spliced mRNA are still generated ensuring the production of low amount of residual GAA protein.

In order to rescue high levels of full length mRNA of GAA, we have developed a new splice switching approach by using Tricyclos DNA (Tc-DNA) antisense oligonucleotides (AON) promoting the inclusion of exon 2. We showed that GAA mRNA could be restored in fibroblasts from GSD II patients with the c.-32 IVS1-13 mutation after transfection with different tc-DNA AON targeting specific sequences of exon 2.

It is likely that this approach might represent a therapeutic avenue for the adult form of Pompe disease. Advantageously, Tc-DNA AON could be delivered systemically and target all skeletal muscles, liver and heart at least, to rescue the endogenous protein without risks of overexpression neither immune problems due to the fact that GAA is self of the considered patient population.

PS2-250 / #441

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

A multi-parametric protocol to study exercise intolerance in McArdle's disease

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Background: McArdle's disease is the most common metabolic myopathy of muscle carbohydrate metabolism, due to deficiency of myophosphorylase and alteration of glycogen breakdown in muscle. The clinical symptoms of McArdle's disease usually begin in young adulthood with exercise intolerance, exercise-induced muscle cramps, pain and recurrent episodes of myoglobinuria. Many patients experience the second wind phenomenon, characterized by an improved tolerance for aerobic exercise approximately after eight minutes of motor activity, secondary to the increased availability of blood glucose and free fatty acids associated to an enhanced glucose uptake by muscle cells.

Objective: The aim of the study has been to define a multi-parametric protocol in patients with McArdle's disease in order to detect and quantify the impairment of muscular metabolism and motor performance.

Methods: We enrolled 5 patients with McArdle's disease and 5 healthy subjects, who have been evaluated by: (1) monitoring of physical activity with an electronic armband; (2) testing of cardiopulmonary, metabolic and respiratory responses to exercise with a cardiopulmonary exercise test; (3) analyzing muscle fatigue during exercise test by surface electromyography (4) evaluating blood lactate and oxidative stress biomarkers at rest and during exercise.

Results: Armband monitoring of physical activity showed, compared to controls, low levels of physical activity in McArdle's subjects, this indicative of impairment of both anaerobic and oxidative metabolism in physical activity of these patients. We then tested the multi-parametric protocol to investigate the effect of a carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine on exercise intolerance and motor skills in these patients. We did not observe significant differences during the exercise test and physical activity monitoring between baseline and after three days of carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine. Discussion and conclusion: This protocol may be useful to obtain a multi-parametric evaluation of exercise intolerance in patients with McArdle's disease, also as measure of outcome in clinical or pharmacological trials.

PS2-251 / #457

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

A case of late-onset mitochondrial myopathy and ptosis due to a heterozygous DNA Polymerase gamma (POLG1) mutation

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Introduction: DNA polymerase gamma (POLG1) is crucially involved in mitochondrial DNA repair. More than 150 different mutations with various clinical presentations have already been described. The spectrum of symptoms comprises progressive external ophthalmoplegia (PEO), ptosis, paresis due to neuropathy or myopathy, cerebellar ataxia and seizures. A rare heterozygous variant (c. 803G>C; p.G268A) has been reported to be associated with a clinical phenotype comprising progressive external ophthalmoplegia. To our knowledge only three young patients (3-16 years) harbouring this variant without clinical PEO have been described so far.

Case presentation: We present a case of an 51 year old man with slowly progressing weakness, gait disturbance and muscular atrophy starting in his late thirties. Clinical evaluation showed a proximal tetraparesis with preserved ability to walk, mild bilateral ptosis as well as mild ataxia. Electromyography revealed findings that are typical for lower motor neuron lesions but electroneurography was normal. Testing with magnetic resonance imaging showed no myelopathy but fatty degeneration of muscle tissue in the lower extremities. Late onset spinal muscular atrophy has initially been suspected because of the electrophysiological findings, but SMN gene sequencing revealed no mutations.

Evaluation of muscle biopsy revealed neurogenic alterations. Moreover, COX negative/SDH positive and "ragged-red" -fibres suggestive of mitochondrial pathology were detected. Exome sequencing was performed and identified a heterozygous mutation in

the POLG1 gene (c. 803G>C; p.G268A), that has previously been described in patients predominantly presenting with PEO, finally.

Conclusion: We present a patient with late-onset myopathy and ptosis but without any signs of external ophthalmoplegia harbouring the heterozygous G268A mutation (c.803G>C) in the POLG1 gene. No strong genotype-phenotype correlation seems to exist for this mutation.

✶PF2

PS2-252 / #467

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Pathogenesis of the neuromuscular junction and motor neuron in Pompe disease

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Pompe disease is a neuromuscular disorder defined by lack of acid-alpha glucosidase (GAA) and characterized by the systemic depletion of GAA resulting in ubiquitous lysosomal glycogen accumulation. Respiratory and ambulatory dysfunction are prominent features in patients with Pompe yet the mechanism defining the development of muscle weakness is currently unclear. Transgenic animal models of Pompe disease mirroring the patient phenotype have been invaluable in mechanistic and therapeutic study. Here, we demonstrate significant pathogenesis in the neuromuscular junction (NMJ) of the diaphragm and tibialis anterior muscle as a prominent feature of disease pathology. Post-synaptic defects include an increase in motor endplate area and fragmentation accompanied by loss of endplate innervation in Gaa knockout mice. Pre-synaptic neuropathic changes were also evident in Gaaknockout mice, demonstrated by significant reduction in neurofilament-heavy chain within the sciatic nerve. More notably, an increased G-ratio was prevalent in the sciatic and phrenic nerve of affected animals. Our data suggest that loss of NMJ integrity and lower motor neuron pathology are novel contributors to the decline in respiratory and ambulatory function in Pompe disease. The observations of both pre- and post-synaptic pathology highlight the importance of systemic correction, particularly restoration of GAA to skeletal muscle and the central nervous system for treatment of Pompe disease.

PS2-253 / #469

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Clinical and genetic characteristics of chronic progressive external ophthalmoplegia (CPEO)

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Background: Chronic Progressive External Ophthalmoplegia (CPEO), either in isolation or together with other symptoms (CPEO+), is a prominent clinical manifestation in patients with mitochondrial disease. It may be caused by single or multiple deletions in mitochondrial DNA (mtDNA), as well as by mtDNA point mutations. Single deletions are usually sporadic, whilst multiple deletions arise due to mutations in nuclear encoded mitochondrial genes. Here, we examine the distribution of single and multiple mtDNA deletions and common mtDNA mutations in a cohort of 290 patients with CPEO, and compare the differentiating clinical features in the various groups.

Methods: Muscle, available from 201 of our patients, was screened for mtDNA deletions by Southern blotting or long-range PCR. Muscle and blood was screened for the most common mtDNA point mutations, m.3243A>G and m.8344A>G.

Results: Single deletions in mtDNA were identified in 112 (56%) of the patients. Of these patients, 36% had CPEO only, 16% had CPEO plus proximal muscle weakness, while the remaining patients were affected by additional clinical features. Multiple mtDNA deletions were found in 38 patients (19%): of

these 30 (79%) had a mutation in POLG, 3 (8%) had a mutation in C10orf2, and 5 patients were negative for mutations in these genes. 3 common mutations in POLG were identified. Polyneuropathy is common in patients with multiple mtDNA deletions, but rare in single deletion patients. The m.3243A>G mutation was identified in 7 (3.5%) of the patients, and 1 (0.5%) patient was positive for the m.8344A>G mutation.

Conclusion: Our genetic investigations in patients with CPEO and observations of the clinical features associated with the different causes of CPEO have allowed us to develop a diagnostic algorithm for the investigation of patients with CPEO.

PS2-254 / #479

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Late onset NLSDM with novel mutations in the PNLPA2 gene in an Italian family

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Mutations in the PNPLA2 gene cause the onset of Neutral Lipid Storage Disease with Myopathy (NLSDM), a rare autosomal recessive disorder characterized by an abnormal accumulation of triacylglycerol into cytoplasmic lipid droplets (LDs). PNPLA2 codes for adipose triglyceride lipase (ATGL), an enzyme that hydrolyses fatty acids from triacylglycerol. NLSDM patients are mainly affected by progressive myopathy, cardiomyopathy and hepatomegaly. However, their clinical severity appears to be highly variable. To our best knowledge, thirty one NLSDM patients have been clinically and genetically characterized. Twenty six PNPLA2 mutations have been described, which differently affect protein function or production. Here we report the clinical and genetic findings of a NLSDM Italian family with different affected members. In our patients we identified two novel PNPLA2 missense mutations (pL56R and pI193F). The oldest brotheris now 61yearsold.since age of 38 years, he had weakness and hypotrophy of right upper arm andkyphosis. He is now unable to raise arms in horizontal position. Plasma CK valuesvaried between 804 and 1118 IU/l (normal values: 0-180 IU/l). Spirometry showed a restrictive ventilator insufficiency (vital capacity: 57%). He had normal plasma and urine carnitine, but low total plasma carnitine (27.9 nanomoles/ml; normal 36.2-72.9 nanomoles/ml). The second brother, since 44 years of age, had exercise intolerance, cramps and pain in lower limbs. He currentlyhasa distal amyotrophy. PlasmaCK was 839 IU/l. Both patients underwent muscle biopsy that showed massive lipid storage. One sister, 57 years old, has high CK values, diabetes, hepato-steatosis andmild weakness in right upper arm. All three had Jordan's anomaly and lipid storage in cultured fibroblasts. Using a functional *in vitro* assay, we have observed that these mutations caused the production of ATGL proteins with diminish lipase activity, but able to bind to LDs. Our findings provide evidence that a small amount of correctly localized ATGL might explain a late-onset mild myopathy and probably preserves cardiac function in NLSDM. Molecular and functional analysis of PNPLA2 mutations is useful to explain the variation of clinical expression of this syndrome and it might improve prognosis. This is a very interesting family since it shows heterogeneity of clinical presentation from relatively asymptomatic phenotypeto fullexpression of a severe myopathy.

PS2-255 / #480

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Safety and efficacy of exercise training in 23 adults with Pompe disease receiving enzyme therapy

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Objective: Pompe disease is an inherited proximal myopathy. We investigated whether exercise training is a safe and useful adjuvant therapy for mildly affected adults receiving enzyme replacement therapy for this disease.

Methods: Training comprised 36 sessions of standardized aerobic, resistance and core stability exercises over 12 weeks. Before and after training, we evaluated safety, endurance, muscle strength, muscle function, core stability, and body composition.

Results: Of 25 patients enrolled, 23 successfully completed the training. Improvements in endurance were shown by increases in maximum workload capacity (100W ± 52 to 122W ± 53, $P<0.01$), maximal oxygen uptake capacity (69.4% of normal ± 17.4 to 75.9% ± 18.0, $P<0.01$), and maximum walking distance (6 minute walk test: 492 meters ± 89 to 508 ± 97, $P=0.01$). There were slight increases in total muscle strength (hand-held dynamometry), mainly due to an eight percentage point increase in hip flexors ($P<0.01$). Functional tests showed small reductions in the time needed to climb four steps (0.3 sec, $p=0.02$) and rise to standing position (1 sec, $p=0.05$), while time to run and the quick motor function test results remained unchanged. The number of patients who were able to perform the core stability exercises rose, as did the core stability balancing time ($P<0.05$, for all four exercises).

Conclusions: Our study shows that a combination of aerobic, strength and core stability exercises is feasible, safe and beneficial to mildly affected adults with Pompe disease. It should therefore be considered as an adjuvant treatment in Pompe patients receiving long-term ERT.

PS2-256 / #517

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

The Influence of a Polymorphism in the Gene Encoding Angiotensin Converting Enzyme (ACE) on Treatment Outcomes in Late-Onset Pompe Patients Receiving Alglucosidase Alfa

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Angiotensin Converting Enzyme (ACE) catalyzes the conversion of angiotensin I to the vasoactive peptide angiotensin II, and degrades bradykinin, a potent vasodilator. One outcome of these two actions is vasoconstriction and an increase in blood pressure. Half of the variation in human ACE activity can be accounted for by an insertion/deletion (I/D) allele in intron 16 of the ACE gene. An insertion of an Alu repeat in intron 16 results in lower ACE activity (I allele), and conversely, the deletion (D allele) results in higher ACE activity. Furthermore, the I allele has been reported to be associated with the predominance of slow-twitch muscle fibers (Type I) and the D allele with fast-twitch muscle fibers (Type II). Two publications recently suggested that the ACE I/D polymorphism modifies the clinical presentation of Pompe patients and influences treatment outcomes following alglucosidase alfa enzyme replacement therapy (ERT). Specifically, patients with the D/D genotype presented with an earlier onset of disease, higher CK levels at diagnosis, increased pain, and more severe disease progression (Fillippi et al, 2010), and had poorer treatment outcomes on ERT (Ravaglia et al, 2012). We investigated these findings in a large cohort of late-onset Pompe patients included in a randomized, placebo-controlled trial of alglucosidase alfa. Our results also suggest that patients carrying two DD alleles demonstrate an attenuated response to treatment relative to the I/D and I/I genotypes. This result was not associated with the antibody response to alglucosidase alfa. An investigation into the disease status of patients at entry into the clinical trial is ongoing.

✶PF4

PS2-257 / #528

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Co-administration of the pharmamcological chaperone AT2220 with recombinant human acid alpha-glucosidase as a potential next-generation enzyme replacement therapy for Pompe disease

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Pompe disease (PD) is an inherited lysosomal storage disorder that results from deficiency in acid α-glucosidase (GAA) activity, and is characterized by progressive accumulation of lysosomal glycogen primarily in muscle. Enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA) (Lumizyme®, Genzyme, a Sanofi company) is the only approved therapy for PD. While rhGAA slows disease progression and improves many of the pathophysiological manifestations of PD, the infused enzyme tends to be unstable at neutral pH/body temperature, shows low uptake into some disease-relevant tissues, and in some patients elicits immune responses that adversely affect tolerability and efficacy. We are investigating the use of the pharmacological chaperone AT2220 (deoxynojirimycin; duvoglustat HCl) in combination with rhGAA to increase the physical stability of the enzyme. Preclinical studies indicate that AT2220 protects rhGAA from denaturation and inactivation in human blood ex vivo, and leads to greater plasma exposure and tissue uptake of active rhGAA, as well as greater glycogen reduction, when orally co-administered to Gaa knockout mice. In an open-label, single dose Phase 2 study (AT2220-010), AT2220 (50 mg, 100 mg, 250 mg, and 600 mg) was orally co-administered to PD subjects 1 hour prior to ERT infusion. Drug safety monitoring following completion of each dose cohort concluded that single oral administrations of AT2220 were safe and well tolerated at all dose levels. Dose-dependent increases were observed in total plasma GAA activity of 1.2- to 2.8-fold relative to rhGAA infusion alone. Increases in total muscle GAA activity were also observed in 16 of 24 (67%) subjects with evaluable data. These Phase 2 data will be leveraged to advance the development of a proprietary rhGAA (designated AT-B200) that contains significantly higher mannose 6-phosphate content, which may lead to improved lysosomal targeting compared to the current standard ERT. Thus, combination therapy with AT2220 may provide further therapeutic benefit for PD patients.

PS2-258 / #531

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Intrafamiliar phenotype variability related to a new mutation in SUCLA2 gene

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Background: SUCLA2 gene is one of the known genes related to mtDNA depletion syndrome. Up to now, SUCLA2 mutations have been described in 30 patients with encephalomyopathy (Leigh syndrome), hearing loss and mild methylmalonic aciduria. Here we describe two Brazilian siblings with a new mutation in SUCLA2 gene, showing intrafamiliar phenotypic variability (mild and severe phenotypes).

Case report: The index case is a 7-year old boy, the youngest child of healthy and consanguineous parents (the mother is second-degree cousin of the father). He presented a severe movement disorder (dystonia and chorea), motor delay (unable to sit without support and to walk) and deafness. Brain MRI showed increased T2 and FLAIR signals in caudade and putamen nuclei bilaterally. His older brother, a 9-year-old boy, showed mild chorea, ataxia and deafness, being able to walk without any support. His brain MRI did not show any abnormalities in the basal nuclei. In both siblings, urine organic acids chromatography (GC-MS) showed mild increase of methylmalonic and methycitric acids. Exome sequencing was performed on both brothers and their parents, and identified a homozygous A998G

mutation in exon 8 of SUCLA2 gene in the two siblings, present in heterozigosity in the parents.

Conclusion: We described here the intrafamiliar variabililty of clinical presentation associated with a new SUCLA2 mutation. It suggests a broader spectrum of presentation associated to SUCLA2 defects, ranging from mild cases without any brain MRI abnormalities to severe cases, as described in the first publications.

PS2-259 / #532

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Recurrent rhabdomyolysis due to muscle?-enolase deficiency: Expanding the clinical spectrum

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Objective: To report clinical, morphological and genetic features in two cases with muscle β-enolase deficiency.

Background: Muscle β-enolase deficiency is the last described defect of distal glycolysis. In 2001, Comi et al. firstly reported a benign case of a patient characterised by exercise intolerance, myalgia after physical exertion with no pigmenturia. His β-enolase enzyme residual activity was about 5% and he harboured a double heterozygous mutations in ENO3 gene.

Cases report: We report herein two unrelated men, one Italian (pt1) and the other Turkish (pt2) who complained since their late childhood of several episodes characterised by intense myalgia, cramps, generalized muscle tenderness and marked presence of dark urines. Pt1 at age 42 developed an acute renal insufficiency with anuria, muscle aches and generalized muscle weakness after a strong physical exercise. Because of a massive rhabdomyolysis (serum CK was 214000 UI/l) and myoglobinuria he needed many dialysis cicles followed by a quite sudden restoration of renal function. After few weeks, he referred to our Center: neurological examination was normal, EMG studies revealed a myopathic pattern.

Results: Muscle biopsy in both patients revealed minimal changes with no lipid or glycogen accumulation. Biochemical studies evidenced normal activities of CPT II, AMP and glycolitic enzymes except for β-enolase that showed about 20% (pt1) and 10% (pt2) of residual activity.

Molecular genetic analysis of ENO3 gene revealed two novel missense mutations: in pt1, a c. A452G transition (p.N151S) and in pt. 2 an c.559G>A (p.Glu187Lys) both in exon 7. Genetic analysis in the family revealed that three asymptomatic brothers and the mother harboured the mutation in heterozygous state.

Conclusions: Although few patients with muscle enolase deficiency are so far described it seems that this enzymatic defect should be considered in the differential diagnosis of metabolic myopathies due to inherited defects of distal glycolysis presenting also a clinical heterogeneity.

✶PF4

PS2-260 / #533

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Early diagnosis and early treatment in LOPD: When asymptomatic patients should be treated

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Objective: to review the current treatment recommendations for Late Onset Pompe Disease (LOPD), focusing on early diagnosed LOPD patients .

Background: Pompe disease is a lysosomal disorder caused by GAA deficiency. LOPD is characterized by progressive muscle weakness and/or respiratory failure but, sometimes, only by an asymptomatic hyperCKemia. Being a muscle degenerative disorder, it has been suggested that an early diagnosis could be more useful for a timely ERT start and to maximize its efficacy. According to the current treatment guidelines, ERT is recommended for patients who have symptoms or signs of Pompe diseas and in presymptomatic patients who have detectable proximal muscle weakness or reduction in respiratory parameters.

In a recent high risk population study, involving several Neuromuscular Italian Centers, we were able to diagnose 17 new LOPD patients.

Among those patients, 35% showed an asymptomatic hyperCKemia, 59% hyperCKemia and limb girdle muscle weakness (LGMW) whereas 6% manifested only LGMW. In these patients, the median time from the onset of symptoms/signs to the diagnosis was 7.7 years. ERT has been initiated in 11 patients. 8 out of the 11 showed LGMW with hyperckemia and two of them also had severe respiratory involvement . The last 3 only had hyperCKemia without any symptoms. Despite the presymptomatic condition, muscle morphology showed severe muscle damage and the muscle MRI revealed an adipose substitution in proximal muscles at lower limbs. These results strongly suggest to start the treatment early.

Conclusions: Of the 17 newly diagnoses Pompe patients, remarkably 35% of patients with only asymptomatic hyperCKemia were early identified but a combination of clinical and morphological data prompted us to start ERT early. To initiate ERT we suggest to consider, apart from the clinical symptoms, different parameters such as muscle MRI or muscle morphology to optimize the treatment efficacy.

PS2-261 / #539

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Acid phosphatase-positive rimmed vacuoles as useful marker in the diagnosis of adult-onset Pompe disease lacking specific clinical and pathological features

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Pompe disease is an autosomal recessive glycogen storage disease caused by deficiency of a lysosomal acid maltase. In adult form, impairment of skeletal muscle leads to weakness and respiratory failure. Clinical signs may be suggestive and diagnosis can be easily confirmed by an assay of enzyme activity in the blood. But diagnosis can be challenging because of similarities to muscle dystrophy or atypical clinical presentation. Moreover muscle biopsy can lack disease specific pathology (PAS positive cytoplasmic vacuoles). We report here three cases with clinical atypical presentation for whom clinical diagnosis wasdelayed anddone on family history or systematic analysis of acid maltase activity. The first patient was

treated for pulmonary emphysema with respiratory failure with large bubbles and proximal motor deficit remained undiagnosed for many years.The second patient presented with only a unilateral ptosis and the initial diagnosis was oculo pharyngeal dystrophy. The third one presented with an axial muscle weakness. In these three cases, muscle biopsy showed only very sparse rimmed vacuoles. An acid phosphatase activity (studied in a second time) was present in these three cases.Such features may be a useful diagnostic marker for adult-onset Pompe disease lacking typical vacuoles and may allow an early enzyme replacement therapy.

PS2-262 / #540

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Functional study of a germinal heterozygous 2bp-deletion in the SDHA gene identified in a patient with severe myopathy and late-onset cerebellar syndrome

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Deficiency of complex II, which is exclusively nuclear encoded, is a rare cause of mitochondrial disease. Homozygous mutations in the SDHA gene have been found in young patients presenting a complex II deficiency neurological phenotype. Here we describe a patient presenting with early adult-onset myopathy and late-onset cerebellar syndrome. Weakness started when 20-y-old, the patient being wheelchair-dependent when 44 with severe respiratory failure requiring non -invasive positive pressure ventilation. His parents presented no clinical symptoms. But in the sibship of 2 brothers and 4 sisters, one brother (3-y older than the proband) complained of asthenia with a normal clinical examination. Muscle tissue was explored for histology, respiratory complex activity and molecular biology for the proband and his brother. Histological and histoenzymaticanalysis of the propositus's muscle revealed ragged red fibers. lipid storage, negative fibers on COX reaction and type 1 fiber atrophy and predominance on ATPase reaction. Peripheral hyperactivity in NADH and SDH evoked mitochondrial aggregates.Biopsy for the proband's brother showed the same types of morphological abnormalities but very less obvious.Biochemical analysis on muscular biopsies revealed a partial and combined deficit of complexes II, II+III, and III and low activity of complex IV for the proband. His brother did not present any deficit in complex II or III activities.We identified a germinal heterozygous 2-bp deletion in the SDHA gene in the proband and his brother, corresponding to a C-terminal truncated SDHA protein of 476 amino acids. Contrary to his brother, the patient harbored the rs6960 polymorphism that may influence the full-length SDHA stability and lead to a more aggressive phenotype for the propositus. Our results underline the importance of a molecular SDH screening for patients with late-onset myopathy, taking attention to associated polymorphisms.

PS2-263 / #543

Theme: *2.8 - Muscle diseases of genetic origin: Metabolic Myopathies / Mitochondrial Myopathies*

Molecular Basis of CoQ10 deficiency in the First Identified Patients

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Coenzyme Q10 (CoQ_{10}), located in the inner mitochondrial membrane, is responsible for carrying electrons from complex I and II to complex III in the mitochondrial respiratory chain. Selective deficiency of CoQ_{10} and partial response to oral CoQ_{10} replacement therapy was first documented in 1989 in two sisters suffering from abnormal fatigability, progressive muscle weakness, learning disability, generalized seizures, rhabdomyolysis and cerebellar ataxia with lipid and mitochondrial excess in muscle fibers (Ogasahara S, et al. PNAS; 1989, 86:2379-82). One of the sisters died at age 36 years of systemic lupus erythematosus. The second sister, currently 39 years of age, is seizure free and not weak but remains severely ataxic despite treatment with 900 mg per day of CoQ_{10} at another

medical center. The molecular basis of the CoQ_{10} deficiency in the two sisters remained unsolved. Since 1989, mutations in eight genes causing primary or secondary CoQ_{10} deficiency were reported with clinical phenotypes of (1) infantile-onset multisystem disorder with nephropathy and encephalopathy, (2) steroid resistant nephrotic syndrome with or without sensorineural hearing loss, (3) cerebellar ataxia, (4) Leigh syndrome, and (5) isolated myopathy with exercise intolerance. Whole exome sequencing of the surviving sister, confirmed by Sanger sequencing in both sisters, revealed a novel missense and a novel frameshift mutation in the ADCK3 (aarF-domain-containing kinase 3) gene. Previously reported patients harboring mutations in ADCK3 have presented with slowly progressive childhood-onset cerebellar ataxia with mild or no muscle symptoms. The findings highlight the phenotypic variability of the ADCK3 mutations as well as the power of whole exome sequencing, and bring to closure of a longstanding mystery.

PS2-264 / #93

Theme: *2.9 - Muscle diseases of genetic origin: Other myopathies including GNE - Hereditary Inclusion Body Myopathy*

X-linked myopathy with excessive autophagy (XMEA): NGS identifies a new in/del in the critic splicing region of the VMA21 gene

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X-linked myopathy with excessive autophagy (XMEA) is an inherited, slowly progressive myopathy, characterized by membrane-bound sarcoplasmic vacuoles in muscle fibers. Clinically, proximal muscle weakness is manifested in early childhood, but with no cardiac muscle involvement, nor cognitive impairment. Recent findings have shown that mutations in vacuolar membrane ATPase activity 21 (VMA21), one of the factors required for assembly of the vacuolar (V-) ATPase domains in the endoplasmic reticulum, are causative of XMEA. Six different single-nucleotide substitutions in this gene were identified in 14 families with XMEA. Four were intronic, and in two of them, the IVS1-27A base is involved. These mutations result in a 32 to 58% reduction in VMA21 mRNA, and protein, and a consequent elevated lysosomal pH with partial block in the common final degradation stage of autophagy.

Only a few XMEA families have been identified so far in Europe and North America, and one in Japan. Here we describe the first identified XMEA Brazilian family carrying a small in/del in the VMA21. The 5 year-old propositus presented a characteristic dystrophic phenotype. He walked at the age of 2 and showed difficulties for running , climbing stairs, and raising from the floor. No calf hypertrophy nor joint contractures were observed. CK level was 1330 U/l, and ECG showed altered conduction in the right branch. Muscle biopsy showed a dystrophic pattern and autophagic vacuoles. Emerin was normal . Family history revealed a clear X-linked recessive pattern of inheritance with 5 affected males linked through asymptomatic females. The affected maternal grandfather, aged 48, was wheelchair bound since the age of 30, presenting also cardiac alterations and joint contractures in the upper limbs. Exome sequencing identified a small insertion-deletion, including the IVS1-27A base previously described.

This new family/mutation reinforces the importance of this splice site branchpoint for the appropriate transcription/translation of VMA21, and normal lysosome function. Additionally, it expands the clinical variability, including cardiac involvement and joint contractures to the XMEA phenotype. FAPESP-CEPID, CNPq-INCT, FINEP, CAPES- COFECUB.

✶PF2

PS2-265 / #181

Theme: *2.9 - Muscle diseases of genetic origin: Other myopathies including GNE - Hereditary Inclusion Body Myopathy*

Two founder mutations within GNE gene and high prevalence of GNE myopathy identified in North of Britain

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Glucosamine(UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase (GNE) myopathy also known as hereditary inclusion body myopathy (HIBM) is an ultra-rare autosomal recessive muscular disorder. Typically HIBM is caused by GNE mutations resulting in reduced sialic acid synthesis. Clinical presentation of HIBM varies from asymptomatic carrier to severely disabling forms.

Here we present description of 26 HIBM patients recruited via the Newcastle MRC Neuromuscular Centre between 1987 and 2012. Genetic testing for GNE myopathy in the UK is available only through the Northern Genetic service in Newcastle therefore most of UK patients are captured in the presented analysis.

The highest prevalence of GNE myopathy was observed in Northern Ireland (0.44 per 100,000 population) which exceeds prevalence in Japan (0.3 per 100,000 population), one of the countries with the highest prevalence in the world. In other parts of the UK (Scotland and England) GNE myopathy prevalence was lower (0.19 and 0.01 per 100,000 population respectively). In the cohort, 2 patients were of Asian descent and others were of British descent.

Clinical data analysis confirmed classical pattern with quadriceps sparing in most of the patients and also revealed unusual asymmetrical pattern in some cases. Unilateral leg weakness was found in 9/26 (34.6%) patients. Asymmetric scapular winging was observed in 3/26 (11.5%) patients. All patients with unilateral foot drop progressed to developing bilateral foot drop. Muscle biopsies ($n=20$) showed myopathic features in most of the cases or normal appearance (2/20). Rimed vacuoles and inflammatory infiltrates were found in 5/20 (25%) and 3/20 (15%) cases respectively. MRI ($n=7$) showed varying degrees of symmetrical muscle atrophy with relative sparing of the quadriceps in all.

GNE gene analysis showed that 2 previously reported mutations (c.1985C>T, p.Ala662Val and c.1225G>T, p.Asp409Tyr) were prevalent in GNE patients in North Britain (including North England, Scotland and Northern Ireland). Majority the patients (90%) are carrying at least 1 of the 2 mutations.

We report, for the first time, two common mutations in the North Britain and highlight the broader spectrum of clinical phenotypes. Longitudinal studies on larger cohorts combining effort internationally are needed to further describe HIBM, optimise diagnostic and management

★PF4

PS2-266 / #234

Theme: *2.9 - Muscle diseases of genetic origin: Other myopathies including GNE - Hereditary Inclusion Body Myopathy*

A controlled Phase 2 study of extended release sialic acid (SA-ER) in GNE myopathy

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GNE myopathy is a rare disorder caused by a defect in sialic acid (SA) biosynthesis without an approved treatment. Sialic Acid-Extended Release (SA-ER) was investigated in a randomized, double-blind, placebo-controlled 48 week Ph 2 study as a potential therapy. The study evaluated dose, PK and clinical effects of treatment. A total of 47 subjects were enrolled and randomized to either receive placebo, 3 g or 6 g of SA-ER/day, stratified for baseline 6 min walk distance (6MWT). After wk 24, placebo patients were crossed to either 3 g or 6 g /day groups for an additional 24 wks. The analyses compared change from baseline at wk 24 for 6 g or 3 g vs. placebo and at wk 48 for the combined 6 g vs. combined 3 g groups. Study assessments included sialic acid PK, muscle strength by hand-held dynamometry (HHD) [predefined for upper extremity composite (UEC), lower extremity composite (LEC)], other clinical endpoints, patient reported outcomes (PRO) and safety.

A total of 47 patients were treated and 46 completed the study. PK results showed a dose dependent increase in serum SA levels with mean serum SA ~2x normal at 6 g dose. Free SA in muscle was very low at baseline and showed a variable increase at wk 48 with the 6 g dose. Muscle strength assessments showed a modest rise over time at 6 g vs. a decline in placebo and 3 g at wk 24, or the combined 3 g group at wk 48. At wk 24, the UEC in the 6 g group showed a statistically significant difference from placebo (+2.33kg, $p=0.04$) and trend better than 3 g (+1.74 kg, $p=0.12$); at wk 48, the combined 6 g group was improved over the combined 3 g groups (+3.44 kg, $p=0.0033$) and even more in the predefined >200m baseline 6MWT walking group (+4.50 kg, $p<0.001$). The LEC showed a similar pattern of response but with no significant decline in placebo or 3 g and no statistically significant difference. The GNEM-FAS, a novel PRO measure for GNE myopathy, did not show differences at wk 24 but at wk 48 showed a positive trend in total ($p=0.086$), mobility ($p=0.087$) and UE scores ($p=0.095$) in the combined 6 g group over the combined 3 g group by ANCOVA . Clinical endpoints related to walking (e.g. 6MWT) did not reveal significant changes in function. SA-ER appeared to be well tolerated with no serious adverse events observed in either dose group. These data suggest that the SA-ER 6 g dose is having a clinically meaningful effect stabilizing UEC muscle strength.

PS2-267 / #402

Theme: *2.9 - Muscle diseases of genetic origin: Other myopathies including GNE - Hereditary Inclusion Body Myopathy*

Transthyretin Amyloidosis Outcomes Survey (THAOS): Early symptom presentation in hereditary transthyretin amyloidosis

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Objective: To characterize early symptom presentation in transthyretin amyloidosis (ATTR) patients with Val30Met and nonVal30Met mutations.

Background: Hereditary ATTR is a progressive, life-threatening, systemic condition characterized by polyneuropathy and/or cardiomyopathy. It is caused by mutations in transthyretin (TTR), including the Val30Met and non-Val30Met mutations. These destabilize TTR protein causing it to misfold and accumulate as amyloid fibrils in nerve tissues, the heart, and other organs.

Methods: This analysis used cross-sectional data from the Transthyretin Amyloidosis Outcomes Survey (THAOS) patient registry. Patients with hereditary ATTR who never received disease-modifying treatment and had a disease duration of 0 to 2 or >2 to 4 years at their last THAOS visit were classified as: Val30Met early onset (< 50 years of age), Val30Met late onset (≥50 years of age), or non-Val30Met. Symptoms were recorded by the study site as present or absent at the last THAOS visit and the percentages of patients reporting symptoms classified as possibly or definitively related to ATTR by the treating investigator were calculated.

Results: See table.

Conclusions: Sensory and autonomic neuropathy were prominent early indicators of the disease regardless of the mutation and onset status.Motor neuropathy (walking disability, muscle weakness) and cardiac symptoms were more frequent in non-Val30Met patients in early years. Autonomic neuropathy, motor neuropathy, and cardiac symptoms were more frequent at >2 to 4 years than 0 to 2 years in non-Val30Met patients. There were no substantial differences in symptom presentation between early- and late-onset Val30Met patients, apart from more frequent carpal tunnel and cardiac symptoms in late-onset patients.

✶PF2

PS2-268 / #429

Theme: *2.9 - Muscle diseases of genetic origin: Other myopathies including GNE - Hereditary Inclusion Body Myopathy*

Analysis of baseline sialic acid and NCAM data in GNE myopathy patients & mouse model

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GNE Myopathy, also known as hereditary inclusion body myopathy (HIBM) is a late onset progressive myopathy caused by a defect in the biosynthetic pathway for sialic acid (SA). The disease is characterized by distal muscle weakness with the quadriceps muscle relatively spared until the late stages of disease. The objective of this study is to provide a comprehensive and systematic analysis of the biochemical defects underlying the disease in GNE patients at baseline from a phase 2 clinical trial and in a GNE mouse model. Using a novel LC/MS/MS method, free SA levels in muscle were shown to be significantly lower in patients vs. non HIBM individuals(1/3rd). Additionally, total SA in muscle and free SA levels in serum were moderately reduced by <20% when compared to the same controls. Moreover, free and total SA in muscle demonstrated a statistically significant positive correlation. Interestingly, quadriceps had lower SA(free and total) levels compared to gastrocnemius in patients suggesting a lower SA requirement for this muscle which could explain its sparing in HIBM patients. Analysis of muscle SA levels in the GNE mouse model revealed very similar results to the human study. All the above results suggest that free SA, particularly in quadriceps, is a sensitive potential biomarker in mouse as well as in human.

Muscle neural cell adhesion molecule (NCAM) is known to be a sensitive marker of the level of SA within the cell. In GNE mouseNCAM was hyposialylated on Western blot and present as a doublet distinct in size from control mice. In contrast, NCAM appeared as a single band in GNE patients with a molecular weight similar to normal NCAMbut with lower intensity compared to non HIBM individuals, suggesting a difference in extent of NCAM sialylation between human and mouse.

Overall, results from GNE patients and the GNE mouse model support that the levels of SA in muscle is a good indicator of the disease therefore the approach of reversing SA depletion would be a potentially useful treatment for patients with GNE myopathy.

PS2-269 / #434

Theme: *2.9 - Muscle diseases of genetic origin: Other myopathies including GNE - Hereditary Inclusion Body Myopathy*

Natural history study of GNE myopathy

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Background: GNE myopathy is a rare autosomal recessive muscle disease caused by mutations of GNE, encoding the key enzyme in sialic biosynthesis. GNE myopathy usually manifests in early adulthood with foot drop secondary to anterior tibialis muscle weakness and progresses slowly to involve more proximal muscles of the lower extremities, with relative sparing of the quadriceps. Upper extremity involvement is variable. Since its description, several case reports have been published but an overview of the natural history has been lacking.

Methods: We performed a prospective study of patients ($n = 34$, age range, 29 to 65 years) through NIH study 11-HG-0218 "A Natural History Study of Patients with GNE myopathy" (ClinicalTrials identifier: NCT01417533).

Results: Median age of onset was 27.5 years (range 12-40). In all but one case, presentation was distal lower extremity weakness. There was a mean diagnostic delay of 12 years (range: 0-43 years) and 79% of patients were previously misdiagnosed. Patients had a variety of GNE mutations, mostly missense. Assistive devices included braces (54%), cane (42%), walker (18%), occasional wheelchair (18%) and wheelchair required for mobility (15%). 25% of ambulatory patients were unable to complete a 6-minute walk test. Quantitative muscle strength showed a mean overall strength of 43%, ankle dorsiflexion of 0.3% (range 0-6%), hand grip of 38% (range 1.8-135%) and knee extension of 70% of predicted for age and gender. The Activity Balance Confidence scale predicted a risk for falls in 89% of patients. EKG abnormalities were encountered in 33% and cardiomyopathy was seen in 1 patient. Assessment of forced vital capacity, maximal inspiratory and expiratory pressures suggested mild involvement of respiratory muscles. Mean values were 262 U/L (range: 27-1152) for CPK and 0.41 mg/dl (range 0.15-0.92) for creatinine. Renal function, evaluated by cystatin C, was normal in all patients. Plasma glycan profiling showed abnormal ratios of Thomson-Friedenreich antigen (T) and its sialylated form ST. Plasma ManNAc and sialic acid levels were not significantly different from controls. Plasma sialic acid levels increased 3-fold after administration of a single dose of ManNAc and levels persisted for up to 48 hours.

Discussion: Potential therapies for GNE myopathy are being developed and a better understanding of the natural history of the disease will facilitate the timely diagnosis of patients and the design of clinical trials.

PS3-270 / #87

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Activation of the NFkappaB p65 subunit in myositis: Comparing autoimmune inflammatory myopathies with the secondary inflammation associated with muscular dystrophy

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Background: In muscle inflammation, the nuclear factor kappa B (NFkB) pathway switches on a pro-inflammatory gene repertoire of cytokines, chemokines and adhesion molecules. The cause of muscle inflammation can be either an autoimmune process, as

in most inflammatory myopathies, or can develop secondarily to tissue damage as in Duchenne muscular dystrophy (DMD).

Objective and Methods: We investigated the expression and distribution of the activated serine 536 phosphorylated form of the NFkB p65 subunit (pp65) in detail, using immunofluorescence and quantitative western blotting. We specifically compared dermatomyositis (DM), inclusion body myositis (IBM) and polymyositis (PM) samples with DMD biopsies, thus comparing primary and secondary muscle inflammation.

Results: Relative protein levels of pp65 were significantly higher in PM/IBM ($n=6$; 0.63 ± 0.11) compared to healthy controls ($n=4$; 0.31 ± 0.08; $p=0.0007$). In a single DMD sample, the pp65 protein level was also high (0.73). Fiber type-dependent sarcoplasmic pp65 staining was observed in healthy controls, showing negative and positive fibers. In patient samples however, a plethora of pp65 sarcoplasmic staining intensities was observed reflecting regeneration processes, with highest expression present in small regenerating NCAM+ fibers. Myonuclear pp65 staining was increased in the central myonuclei in patient biopsies. Satellite cells were generally and strongly pp65 positive. Blood vessel staining intensities in PM, IBM and DMD were higher than in controls. In DM endothelial pp65 staining was further increased, with highest expression levels in swollen capillaries. Staining patterns of inflammatory cells were heterogeneous, also within the same sections. Within necrotic muscle fibers, pp65 staining could be shown in both regenerative CD206+ and destructive CD68+ macrophages.

Discussion and Conclusion: The distribution of the NFkB pp65 component in inflammatory myopathies andDMD points to a general role in necrosis and regeneration, and to a more specific role in DM endotheliopathy.

PS3-271 / #143

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Myo-endothelial repair in juvenile dermatomyositis

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Background: Juvenile dermatomyositis (JDM) is a rare, potentially life-threatening inflammatory myopathy clinically characterized by proximal muscle weakness, skin rashes and varying additional organ involvement. An immune-mediated microangiopathy is considered to cause reduction of endomysial capillaries and perifascicular muscle atrophy. Whether injury induces regenerative processes in JDM muscle remains poorly investigated.

Objective: Our aim was to analyze expression of myogenic transcription factors involved in the regulation of reparative myogenesis and the frequency of endothelial progenitor cells in muscle biopsies from JDM patients.

Patients and Methods: Markers of satellite cells (Pax7), proliferating (MyoD) or differentiating (Myogenin) myoblasts, and regenerating fibers (developmental Myosin) were studied by immunohistochemistry in patients with JDM (n = 7) compared to controls (n = 4). In addition, capillary density and numbers of endothelial progenitor cells within the endomysium were determined by double-immunoflourescence for CD34 and laminin.

Results: Myogenic regulatory factors (Pax7, MyoD and Myogenin) and developmental Myosin were highly up-regulated in perifascicular regions of JDM (n = 5). Within intrafascicular regions of JDM (n = 7), the number of Pax7+ satellite cells was equivalent to controls. Expression of MyoD, Myogenin and developmental myosin was rarely observed intrafascicularly in JDM and absent in controls. Loss of capillaries was most pronounced in perifascicular regions of

JDM (n = 5). Quantification of CD34+ endothelial progenitor cells within the endomysium, which are implicated in the process of neovascularisation, revealed no difference between JDM and controls.

Conclusions: Our results indicate induction of regenerative myogenesis in perifascicular regions of JDM, despite pronounced loss of capillaries. No evidence was found for compensatory neovascularisation.

✶PF3

PS3-272 / #246

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

MDA-5 associated myositis : Towards a molecular and morphological definition of a distinct entity

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Introduction: The anti-MDA5 auto-antibody(Ab)is held to be specificly associated with dermatomyositis. MDA-5 patients suffer from mainly extramuscular involvment (severe interstitial lung disease, skin ulcers and arthritis)whereas clinical signs of myopathy are rather mild or absent. Althougth the general signs and symptoms of the disease are well described, nothing is known about the affection of the muscle in terms of morphology and immunology.

Objective: To describe the histological pattern of the skeletal muscle in depth and the intrinsic immune response.

Method: Muscle specimens are subjected to immunohistochemical analysis and molecular testing of a comprehensive panel of mediators invovled in the immune response by quantitative PCR. Morphometric analysis of vessel density is performed using imageJ software on digitally completely scanned slides. Results are compared to patients with classical dermatomyositis (cDM) based on ENMC criteria.

Results: Nine anti-MDA5Ab positive patients were included and 10 muscle biopsies were analyzed compared to 7 cDM patients biopsies.Only 6anti-MDA5Ab positive patients had a muscular deficit and the mean MRC score of the weakest musclar group was 4.5 ± 0.5. In anti-MDA5Ab positive patients the CK level was normal in 6 patients and the mean CK level was 498 ± 809 I.U/l. Hsitological analysis showed that anti-MDA5Ab positive patients do not present the classical feature of perifascicular fiber atrophy. Whereas allexcept one anti-MDA5Ab positive patient presented inflammatory infiltrates composed mainlyof CD68+ macrophages and CD4+ T cells. Inflammation was focal and located in perivascular regions. Inflammatory infiltrates were significnatlyless intense in anti-MDA5Ab positive patients compared to cDM patients as it is shown by the number of CD45+ cells (35.8 ± 28 vs. 5.9 ± 7.6 cells/mm2, $p < 0.05$). MHC-I over-expression was also less intense and more focal compared to cDM patients who had a diffuse over-expression with a perifascicular reinforcement.In anti-MDA5Ab positive patients the capillary:fiber ratio was $1.26 \pm 0.27$and tubuloreticular formationswere observed in only 50% of the patients.

Conclusion: These results show that myositisin patients withanti-MDA5Ab positivityhas a distinct pattern compared to cDM patients, which isin line with the differentclinical presentation. Nevertheless wedo not know yetif it is an attenuated form ofcDM or a distinctentity.

✶PF2

PS3-273 / #261

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Redifining the immune histolochemical pattern of Anti-SRP auto-antibody positive patients: A subgroup present significant inflammation

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Introduction: Among acquired idiopathic inflammatory myopathies necrotizing auto-immune myopathy (NAM) has been officially recognized as disctinct entity in 2004 based on morphological features. The anti-SRP auto-antibody (Ab) is specifically associated with NAM and can be considered the archetype of NAM. However to date, data concerning precise histological features are sparse.

Aim: To describe the histological pattern of the skeletal muscle of a large series of patients and to analyse the intrinsic immune response.

Methods: Fiber necrosis and myophagocytosis was defined based onconventional H&E stains. Fiber necrosis was manually counted and normal fibers were counted automatically using imageJ software on digitally completely scanned slides.Forimmohistochemical analysisa semi-quantitative score (0-5) wasused for MHC-Istaining (e.g. 0: no over expression, 2: :focal over-expression> 50% of fibers, 5: diffuse and intense over-expression(100%).Results are compared to those obtained in patients with dermatomyositis (DM).

Results: Twenty four anti-SRP Ab positive patients were included and compared to 5 DM patients. The percentage of fiber necrosis was $5.1 \pm 3.8\%$ in anti-SRP Ab positive patients compared to$2.3 \pm 1\%$ in DM patients ($p < 0.001$). Whereas there is no reliable threshold concerning the percentage of fiber necrosis to well segregate both group because of important overlap. Of note the muscle fibernecrosis was randomly distributed in anti-SRP Ab positive patients while it was mainly located in the perifascular areas in DM. The mean number of CD3 and CD8 positive T cells was significantly higher in DM patients ($p < 0.05$). Nevertheless one quarter of anti-SRP auto-antibody patients presents a T cell number in the range of those observed in DM patients.MHC-I expression was strong in all DM patients (5, 5)with a perifascicular enhancement, however expression was also detectable in all patientswith anti-SRP Ab's (except in one case) but significantly less intense (2, 0-5; $p < 0.05$).

Conclusion: anti-SRP Ab positive patients present mostly a'necrotizing myopathy'pattern without important inflammation and only slight MHC-I up-regulation. Neverthelss a quarter of patients present an inflammatory pattern. This finding suggests that the immune cellular response against fibers may play a more important role than previously estimated. In addition anti-SRP ab testing should be performed in 'polymyositis' patients that do not harbour a common Ab profile.

★PF2

PS3-274 / #264

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Myofiber HLA-DR expression is distinctive biomarker for antisynthetase myositis

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Anti-synthetase (AS) autoantibodies are characteristics of a subset of immune inflammatory myopathies (IIM). In present work, we evaluated the reliability of HLA -DR expression as a biomarker of antisynthetase myositis.

Methods: We investigated HLA-DR expression in muscle biopsies from 33 patients with AS syndrome(anti-Jo-1: $n=26$; anti-PL7: $n=2$; anti-PL12: $n=4$; anti-EJ:$n=1$),16 dermatomyositis (DM), and 10 histologically normal muscle. Evaluation included (i) the percentage of positive fibers on the whole fascicle, and (ii) the percentage of contiguous positive perifascicular fibers.

Results: HLA-DR myofiber expression was found in 84.8% (28/33) AS patients (anti-Jo1: 88,4%) and in 4/17(23,5%) patients with DM ($p< 0.0001$). No myofiber HLA-DR expression was found in normal muscles. The mean percentage of positive fibers was 36.3% in AS (40.5% in anti-Jo1) and 6.8% in DM (DM vs AS: $p=0.001$; DM vs Jo1: $p< 0.001$). All DM had less than 10% DR-positive myofiber. Myofiber HLA-DR expression was observed in perifascicular areas with ribbon-like pattern. The percentage of DR-positive perifascicular contiguous myofibers was 33.4% in AS and 2% in DM ($p< 0.001$).

Conclusions: Myofiber HLA-DR expression is specific biomarker of anti-synthetase myopathy suggesting a role for INF-γ in its pathophysiology.

PS3-275 / #269

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Brain perfusion defects correlate with cognitive deficits in patients with alum-induced macrophagic myofasciitis

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Patients with post-vaccinal aluminum hydroxide (alum)-induced macrophagic myofasciitis (MMF) complain of diffuse arthromyalgias, chronic fatigue and stereotyped cognitive deficits. The objective of the present study was to assess brain perfusion abnormalities as potential biomarker of neural dysfunction in MMF patients.

Methods: Brain perfusion SPECT was prospectively performed in 76 consecutive adult patients (49 ± 10 y) with muscle biopsy-proven MMF diagnosed in the Reference Centre for Neuromuscular Diseases of Créteil, France. Multiple sclerosis was excluded in all patients on the grounds of clinical manifestations and MRI. SPECT images were acquired 30 min after intravenous injection of 925 MBq ^{99m}Tc-ECD at rest. All patients had within 1.3 ± 5.5 mo a comprehensive battery of neuropsychological tests performed blind for SPECT results. Statistical parametric maps (SPM2) were obtained for each test using a correlation design between performance scores and perfusion, and ANCOVA group analysis was done after dividing the population in good vs. bad performers according to the cognitive scores.

Results: All patients had SPECT abnormalities. Their analysis revealed non-random distribution of hypoperfusion, diffusely affecting periventricular areas, posterior associative areas, cerebellum and the limbic system (including amygdalo-hippocampic complexes and anterior cingulate gyrus). SPECT abnormalities were significantly correlated with abnormal cognitive tests, including left ear dichotic listening, verbal memory, visual memory, and executive functions. Depression had virtually no impact on performance scores in the altered tests. ANCOVA

group analysis confirmed prominent involvement of relevant hypoperfusion areas in bad performers for each altered test.

Conclusion: Brain perfusion SPECT in MMF patients shows a characteristic pattern of cortical and subcortical abnormalities, matching well the previously described MMF-associated cognitive disorder. These results confirm that the mental dysfunction that seriously impacts life of patients with MMF has a conspicuous neurobiological substrate.

PS3-276 / #291

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Isolated polymyositis. Still a rare syndrome that needs long-term clinical follow-up in reaching a definite diagnosis

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Background: Isolated Polymyositis (PM) is considered a rare disease and even more as a syndrome with many etiological factors.

Aims: 1. To asses the etiological factors over time in two series of PM cases. 2. To compare these two cohorts of PM cases with respect to the reached final diagnosis.

Methods: Cohort 1. Forty-six muscle biopsies with the PM-pattern from January 1997 to may 2012 (174 months). Cohort 2: Nine muscle biopsies with the same histological pattern from June 2012 to December 2013 (18 months).

Results: A similar figure (78% and 66%, respectively) in the two cohorts was obtained with respect to the identification of an etiologic factor in PM cases.

In the first cohort ($n=46$), and after a long-term follow-up, only one patient remained with the diagnosis of isolated PM. In other 9 patients with such initial diagnosis, sporadic inclusion body myositis (s-IBM) ($n=4$), necrotizing autoimmune myopathy ($n=3$) and connective-tissue associated myositis ($n=2$) were the final diagnosis. With respect to the second cohort ($n=9$) while an associated condition was present in 6 cases (HIVand HCV infections, Sjögren syndrome, silicone breast prosthesis-related and sarcocystis parasite), three cases remained with the diagnosis of isolated PM. Taking together, the figures for isolated PM cases are 2% and 30% for the first and second cohort, respectively. On the basis of these results it seems that in many PM cases the final diagnosis will change over the time (configuring a typical s-IBM or developing signs or symptoms of a definite autoimmune disease, among others).

Conclusions: 1. In the majority of PM cases an etiological factor would be identified. 2. Long term follow-up of apparently isolated PM cases is needed in order to achieve a final definite diagnosis.

✶PF3

PS3-277 / #300

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Clinical presentation and response to treatment of patients with the necrotizing immune-mediated myopathy associated with statins

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Objective: To present our experience in the clinical presentation, diagnosis, treatment and outcome of a cohort of the necrotizing immune-mediated myopathy associated with statins (NIMAS), diagnosed and followed-up in the last 10 years in the Hospital Clinic of Barcelona.

Patients and Methods: An observational analysis of a retrospective cohort of 6 patients who received the diagnostic of NIMAS.The diagnosis was made on the basis of clinical phenotype together with muscle biopsy findings. Follow-up was 4-130 months. Muscular strength as well as serum CK levels were recorded in each visit.

Results: Five patients were women and mean age was 63,7 (range:59-72). The time elapsed from the initial symptoms to diagnosis was 7,8 months (1-24).

The most common symptom at diagnosis was limb girdle muscle weakness. Atorvastatin was the drug that received five patients and the mean time of treatment was 55,2 months (range 24-120)..Mean CK values were 6,300 UI/dL (range: 1,270-10,400). In all the cases the muscle pathological exam showed variability in fiber size, prominent necrotic findings together with regeneration and little or no inflammatory infiltrate. Class I antigens from the MHC were positive in all the abnormal cells as well as in the majority of the otherwise normal muscle cells. Statins were stopped in all patients and immunosuppressive treatment started. Prednisone plus azathioprine or cyclosporine was administered, but iv IG (two patients) and rituximab (one patient) should be administered because of a poor clinical response.

Discussion: Statin users occasionally experienced myotoxicity, usually self-limited after discontinuing the medication, although some patients as occurs in the present series, suffer severe worsening myopathy despite drug withdrawal. Most patients required an aggressive immunosuppressive regimen in order to improve their muscular strength. The main clinical data are reflected in table 1.

Conclusions: NIMAS represents a distinct and severe clinico-pathological condition requiring an aggressive immunosuppressive treatment despite the withdrawal of the statin.

✶PF4

PS3-278 / #311

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Effects of auto-antibodies anti- signal recognition particle (SRP) and anti-Hydroxyméthylglutaryl-CoA reductase (HMGCR) on muscle cells

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Necrotizing myopathies (NM) might be acquired auto-immune muscle diseases, in which muscle biopsy demonstrates marked muscle necrosis with regeneration, little or absence of inflammatory infiltrates and a particular patterns of complement C5b-9 deposition on muscle fibers. NM can be seropositive for some auto-antibodies (aAbs) such as anti-SRP as well as anti-HMGCR. The titer of those abs is correlated with the creatine kinase levels, but their role remains unclear.

In the current study, we investigated the effect of the aAbs anti-SRP and anti-HMGCR on *in vitro* primary human myoblasts/myotubes. Primary human myoblasts were isolated from human muscle biopsies of non myopathic patients. Myoblasts were sorted by CD56 immune-magnetic microbeads abs. To study the effect of the auto-abs on muscle cells, confluent myoblasts and 4 day myotubes were incubated with anti-SRP or anti-HMGCR positive human plasma for 72 hours or with seronegative human plasma.

The addition of the plasma containing the aAbs onto differentiated myotubes leads to atrophy, as measured by the reduction of the occupied surface (anti-SRP 51.6 ± 5.4%, anti-HMGCR 47 ± 2.6% vs control 78.3 ± 6.9%, $p < 0.001$). Furthermore, addition of the aAbs to a confluent myoblasts significantly reduce the capacity of myoblasts to differentiate (anti-SRP18.3 ± 5.1%, anti-HMGCR 15.3 ± 4.2% vs control 56.3 ± 7.1%, $p < 0.0001$).

These findings suggest that anti-SRP and anti-HMGCR aAbs have a pathogenic effect on muscle cells *in vitro* by both inhibiting cell fusion and triggering atrophy on fully differentiated myotubes.

PS3-279 / #329

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Myositis after anti-PD1 antibody therapy for malignancy – a case report

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Antibodies to programmed death 1 (PD1) protein can be used therapeutically to shrink tumours in melanoma, lung cancer and renal cancer since it increases the immune response to tumour cells. PD1 antibody is generally well-tolerated, but immune-related adverse events include pneumonitis, vitiligo, colitis, hepatitis,

hypophysitis and thyroiditis. No instance of myositis following PD1 antibody administration appears to have been described. Here we present a case of autoimmune myositis following anti-PD1 therapy.

A 51 year-old man was treated with PD1 antibody for widespread melanoma, and had no disease on PET scanning following administration of the antibody. He later presented with myalgia and peripheral edema and MRI revealed increased signal in muscles. A quadriceps muscle biopsy showed inflammatory infiltrates in perifascicular connective tissue and in the endomysium, composed mostly of macrophages, whose identity was confirmed on CD68 and CD163 immunohistochemistry. One large collection of epithelioid macrophages was admixed with lymphocytes and resembled a granuloma. Widespread upregulation of sarcolemmal MHC1 was present. No myofibre necrosis or regeneration, giant cells, vasculitis, rimmed vacoules, features of macrophagic myofasciitis, or perifascicular atrophy was present.

The widespread upregulation of MHC1 suggests an autoimmune process, though the inflammatory cell collections, containing mostly macrophages, are unusual for an autoimmune myositis such as polymyositis. Features seen in this case may therefore represent a new subgroup of PD1 therapy-induced tissue inflammation. Immunosuppressive therapy of the myositis would need to be carefully monitored to prevent recrudescence of the tumour.

PS3-280 / #340

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Rare symptoms in 4 Japanese patients with dermatomyositis

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Background: Dermatomyositis (DM) is a heterogeneous inflammatory muscular disease showing various findings in skin, muscle and other organs.

Purpose: We here show some rare symptoms and complications in order to pay attention to them in examining DM patients.

Patients and Methods: Four DM patients who were admitted to our hosipital since April 2012, were enrolled in this study. They were all female and late onset cases. The onset ages were Case A, 60; Case B, 74; Case C, 72; Case D, 71. We analyzed their clinical findings, laboratory data, therapeutic response and complications from their clinical records.

Results: Case A, B and D showed elevation of serum aldolase with normal creatine kinase. They all showed remarkable cutaneous manifestations. Case A and B showed prominent facial erythema like seborrheic dermatitis, and Case D showed systemic skin ulcers due to ectopic skin calcification. Skin ulcers in Case D were improved by corticosteroid therapy. Case B and C were accompanied with interstitial pneumotitis, and oral corticosteroid and tacrolimus therapy was started. Several days after the therapy, diffuse ulcerations in the mouth was seen in the both cases. The ulcerations were resistant for some therapies such as antifungal agent, but they quickly recovered after ceasing tacrolimus in Case C.

Discussion and Conclusion: (1)Elevation of aldolase with normal creatine kinase, (2)facial erythema like seborrheic dermatitis, (3)effectiveness of corticosteroid for ectopic skin calcification and skin ulcers, and (4)ulceration in mouth due to tacrolimus were very important aspects in this study. We should pay attention to these findings when we evaluate paients with DM.

PS3-281 / #410

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Polymyositis-associated to chronic Hepatitis C virus infection

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It is well known that chronic hepatitis C virus (HCV) infection has been associated with some extrahepatic manifestations such cryoglubinemic vasculitis or haemolytic autoimmune anemia, among others.

Case	Sex	Age	Weakness	CK (U/l)	Electromyography	Muscle biopsy	HCV diagnosis (year)	PM diagnosis (year)	HCV gentotype	Treatment for HCV prior to PM diagnosis	Tretment for PM	Response to PM treatment
1	M	48	Proximal upper and lower extremities	1,000	.	Polymyositis pattern	1989	2013	1b	Yes (interferon)	Prednisone + Azathioprine	Poor
2	F	68	Proximal upper and lower extremities	915	Myogenic	Polymyositis pattern	2000	2013	1b	No	.	Poor
3	F	64	Proximal upper extremities	600	.	Polymyositis pattern	1980	2001	1b	No	Prednisone	Poor
4	F	66	Proximal upper and lower extremities	5,558	Myogenic	Polymyositis pattern	1980	2005	1b	No	Prednisone + CyA	Poor
5	M	70	Proximal upper and lower extremities	731	Consistent with inflammatory myopathy	Polymyositis pattern	Before 1997	2006	1b	No	Prednisone + Azathioprine	Poor

Polymyositis (PM) has also been reported although with very low prevalence and similar to general population. Complement activation by HCV, specific virus induced autoantibodies, and unspecific autoantibodies against viral antigens are the proposed pathogenic mechanisms for HCV associated PM. Although some authors have demonstrated the presence of non-structural protein NS3 in muscle tissue, viral replication has never been documented. Moreover, it is not known if the virus itself may be responsible for muscle damage.

In this work we present our experience in PM associated to chronic HCV infection. From 1997 to 2013, 1,950 muscle biopsies were performed at our Unit. PM pattern consisting of endomysial inflammatory infiltrate for predominantly CD8+ T cells that invade healthy muscle fibres expressing the MHC-I antigen, necrosis and regenerating muscle cells, was observed in 55 patients. In five of them (9%) chronic infection by HCV genotype-1b was detected. They were referred to our unit because of proximal muscle weakness, raised creatin-kinase values or myogenic pattern in the electromyography. The most relevant clinical data are reflected in table 1. All patients received immunosuppressive therapy (cyclosporine, prednisone and azathioprine) with poor response in all cases. Two of them died because of septic shock.

To determine the true prevalence of PM associated to chronic HCV a well structured epidemiological studies are required. In addition, immunohistochemical and/or molecular approaches should be performed to clarify the pathogenic role of HCV in some PM cases.

PS3-282 / #440

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Clinical features and treatment outcomes in patients with necrotizing autoimmune myopathy

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Necrotizing Autoimmune Myopathy (NAM) is an immune-mediated myopathy characterized clinically by myalgia and weakness, and pathologically by the coexistence of necrotic and regenerating muscle fibers but absent or minimal inflammation. NAM can be associated with statin exposure, connective tissue disease, or cancer. Autoantibodies, such as anti-signal recognition particle (SRP) or anti-3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) can accompany NAM, but are not highly sensitive or specific. Treatment strategies for NAM have not been prospectively validated. We retrospectively reviewed clinical and laboratory features of adults diagnosed with NAM between 2004 and 2013 at Mayo Clinic, Rochester. Patients needed to have subacute weakness, hyperCKemia, and muscle biopsy with necrotic fibers as the predominant feature, with no or minimal inflammation. Data collection is ongoing. Thus far, 37 patients have been included, with a mean age of 54.8 years, and a mean of 6.2 months between symptom onset and clinical evaluation. Proximal lower limb weakness predominated in 73%; distal weakness (43%) and dysphagia (43%) were common. Almost half had respiratory involvement. Two patients had connective tissue disease (scleroderma, Sjogren

syndrome), and 2 had cancer (1 esophageal and 1 lung adenocarcinoma). Thirteen (35%) were on a statin at symptom onset, none improving with drug discontinuation alone. Mean presenting CK was 7,245 U/L. Minimal inflammation was present in muscle biopsies of 15 (42%), and restricted to perimysium in 87%. Monotherapy with prednisone or IVIG was insufficient to control disease in 35 (95%), and 41% required IVIG plus chronic immunosuppression. The most common agents used were corticosteroids, IVIG, mycophenolate mofetil, and methotrexate. Mean follow-up was 32 months, and 9% were able to discontinue all medications. Nine (65%) achieved moderate or marked improvement by 12 months. Clinical relapse occurred in 55% during medication taper. CK values correlated with clinical response and relapse. In summary, we report the clinical features and outcomes of a cohort of NAM patients. NAM is usually idiopathic, although statin use is the most common precipitant. Corticosteroids or IVIG alone did not control disease in most patients. Early aggressive treatment can result in good outcomes, with a high risk of relapse during medication taper.

PS3-283 / #529

Theme: *3.1 - Acquired myopathies: Inflammatory / dysimmune myopathies*

Case report of juvenile polymyositis

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Idiopathic inflammatory myopathy is a rare autoimmune disorder. Overall incidence incidence is 2.2- 7,7 per million with the incidence in children of 1-3.2 per million. Polymyositis is rare in children. This is a case report of an 8 year old with polymyositis.

This is a case presentation of an 8 yera old girl with-progressive difficulty walking over a period of one-year, following a motor vehicle accident 2 months prior. She required admission for pneumonia a few months preceding presentation.The ckinical features includedproximal muscle and respiratory muscle weakness with severe wasting, kyphoscoliosis and pectus excavatum deformity. Laboratory investigations revealed elevated muscle enzymes. Lung function test revealed a restrictive pattern.NCS supported a myopathic process.Post muscle biopsy she complicated with a re-sedation phenomenon and required short-term IPPV. Her muscle biopsy confirmed polymyositis with endomysial and epimysial CD8 and Cd3 inflamatory infiltrate.

Dermatomyosistis is the commonest inflammatory myopathy in childhood. This report describes a rare case of polymyositis at an extremely young age with severe weakness and subsequent post-sedation respiratory failure with a remarkable recovery to her pre-biopsy state following IVIG within 6 weeks.

PS3-284 / #80

Theme: *3.2 - Acquired myopathies: Inclusion body myositis*

Magnetic resonance imaging pattern recognition in sporadic Inclusion Body Myositis

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Background: In sporadic Inclusion Body Myositis (IBM), additional tools are needed to confirm or support the diagnosis especially in clinically atypical or not pathologically proven patients.

Objectives: Aims of our study were the refinement of muscle magnetic resonance imaging (MRI) pattern of involvement in IBM and the assessment of its accuracy, sensitivity and specificity in the differential diagnosis with other late-onset myopathies with clinical or pathological overlap with IBM.

Methods: To identify the MRI pattern we integrated existing knowledge with evaluation of IBM scans available in our Center. Subsequently, three different observers blindly assessed the MRI scans of definite IBM, possible IBM and patients affected by other myopathies and expressed a judgment of consistency or inconsistency of each scan with the described pattern.

Results: The pattern was defined based on the appearance of the distal anterior thigh, which showed characteristic signs of fatty infiltration and/or atrophy together with abnormalities on short tau inversion recovery sequences, accompanied by involvement of the gastrocnemius medialis and relative sparing of

pelvic muscles. Diagnostic accuracy of the recognition of this pattern to detect definite IBM was 95% considering a judgment of typical (with 100% specificity) and 97% considering a judgment of both typical and consistent (with 97% specificity) in our cohort.

Discussion:Our data suggest that recognition of a typical imaging pattern is specific for IBM, while recognition of a consistent pattern makes a diagnosis of IBM highly likely.

Conclusion: Visual assessment of the overall imaging pattern is a reliable and accurate tool in the diagnostic workup of suspect IBM patients even in early disease stages.

✶PF1

PS3-285 / #165

Theme: *3.2 - Acquired myopathies: Inclusion body myositis*

Phosphorylation of the autophagy receptor NBR1 by GSK3 modulates protein aggregation and is abnormal in muscles of sporadic inclusion body myositis patients

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The autophagy receptor NBR1 (Neighbor of BRCA1 gene 1) binds UB/ubiquitin and the autophagosome-conjugated MAP1LC3/LC3 (microtubule-associated protein 1 light chain 3) proteins, thereby ensuring ubiquitinated protein degradation. Numerous neurodegenerative and neuromuscular diseases are associated with inappropriate aggregation of ubiquitinated proteins and GSK3 (Glycogen Synthase Kinase 3) activity is involved in several of these proteinopathies. Here we show that NBR1 is a substrate of GSK3. NBR1 phosphorylation by GSK3 at Thr586 prevents the aggregation of ubiquitinated proteins and their selective autophagy degradation. Indeed, NBR1 phosphorylation decreases protein aggregation induced by puromycin or by the DES/desmin N342D mutant found in desminopathy patients and stabilizes ubiquitinated proteins. Importantly, decrease of protein aggregates is due to an inhibition of their formation and not to their autophagic degradation as confirmed by data on muscle-specific Atg7 knockout mice. The relevance of NBR1 phosphorylation in human pathology was investigated. Analysis of muscle biopsies of sporadic inclusion body myositis (sIBM) patients revealed a strong decrease of NBR1 phosphorylation in muscles of sIBM patients that directly correlated with the severity of protein aggregation. We propose that phosphorylation of NBR1 by GSK3 modulates the formation of protein aggregates and that this regulation mechanism is defective in a human muscle proteinopathy.

✶PF3

PS3-286 / #194

Theme: *3.2 - Acquired myopathies: Inclusion body myositis*

Clinical patterns in NT5C1A antibody positive sporadic Inclusion Body Myositis patients compared to seronegative patients

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Objective: To evaluate clinical patterns in seropositive sIBM patients (antibodies to NT5C1A antigen) vs. those sIBM patients without this antibody.

Background: Recent advances have potentially led to further understanding of the pathogenesis of sporadic IBM, including demonstration of clonal immunoglobulin transcripts inside rimmed vacuoles, plasma cell infiltrates and most recently a specific antibody, present in up to 70% of sIBM patients.

Design/Methods: Serological testing for NT5C1A antibody was done through Washington University (St. Louis Neuromuscular Laboratory) on all consecutive sIBM patients presenting to our clinic over the last six months. Clinical details of these patients were reviewed and functional data prospectively collected. Values were expressed as mean ± SD. Significance on unpaired t-tests was defined a priori at $p<0.05$.

Results: In the 15 patients tested thus far, there is a male predominance in the seronegative patients (M:F ratio 4:5 in seropositive vs. 5:1 in seronegative; $p<0.15$). Mean age at onset was 55.89 ± 5.64 years in seropositive vs. 63.33 ± 10.56 years in the seronegative group ($p=0.09$). Dysphagia was significantly more prevalent in seropositive patients (7/9 vs. 1/6; $p<0.01$). Odds of having dysphagia were 17 fold increased in seropositive patients (OR 17.5, 95% CI 1.222 to 250.5). Serum CK levels were 438.5 ± 326.4 IU/L in the seropositive vs. 523.2 ± 392.2 IU/L in the seronegative patients ($p=0.68$) and the IBMFRS score was 26 ± 3.36 in seropositive vs. 28.67 ± 6.50 in seronegative patients ($p=0.50$).

Conclusions: Seropositive sIBM patients are likely to present almost a decade earlier and are significantly more likely to have dysphagia than seronegative patients.

PS3-287 / #289

Theme: *3.2 - Acquired myopathies: Inclusion body myositis*

Frequency of sIBM in the Slovenian national biopsy collection

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It is known that the frequency of sIBM varies in different areas. No data for Slovenia are available. The objective of the study is to report on frequency of sIBM in Slovenia.

371 muscle biopsies of adult patients, performed at the national Slovenian centre for neuromuscular diseases, over a period of last ten years (2003-2013) were re-examined for the presence of histopathological criteria of sIBM and corresponding patient records analysed.

3 patients showed the sIBM phenotype: all three were men with the onset of the disease in the 7th decade or later (66 to 81years). The onset of the disease was 3 to 10 years before muscle biopsy was performed. Two of them had swallowing difficulties early in the disease course. Typical pattern of muscle involvement, that is combination of finger flexors and quadriceps muscle atrophy and/or muscle weakness, was detected in all. None of them was able to walk on the heels. Asymmetry of muscle weakness was present in one patient. At the onset serum CK activity was slightly to moderately elevated (1.5 to 8 times normal). None of them had an associated autoimmune disease. EMG findings displayed abnormal spontaneous activity and myopathic motor unit potentials in all. Two had an associated axonal neuropathy. Immunotherapy was unsuccessful in one and was not tried in other two. Endomysial inflammation and nonnecrotic muscle fibres invaded by CD8 positive lymphocytes were detected in all. Histopathological classification of the disease as sIBM was based on the findings of rimmed vacuoles and cytoplasmic tubolofilamentous inclusions. Congophilic inclusions, immunoreactivity with SMI-31 and TDP-43 were observed in two patients and were not performed in one. Immunoreactivity with p62 and LC3 was not tested.

From the biopsy data it seems that in Slovenia sIBM is rare (3 cases in 10 years). This is compatible with the view that the disease is infrequent in Mediterranean countries.

PS3-288 / #302

Theme: *3.2 - Acquired myopathies: Inclusion body myositis*

Mitochondrial DNA depletion in muscle from patients with sporadic inclusion body myositis

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Background: Sporadic inclusion body myositis (sIBM) represents one of the three major categories among inflammatory myopathies. Although its clinical and histopathological pattern is known, its pathogenesis remains unknown. Histologic inflammatory reaction, degenerative changes (vacuoles) and

mitochondrial abnormalities frequently coexist. Muscle biopsy is commonly used for diagnosis.

Objective: To analyze mitochondrial DNA content in muscle biopsies from patients with sIBM compared to age and sex-paired controls.

Methods: 24 sIBM patients and 18 controls were included in this study. Muscle biopsies were performed only for diagnosis purposes. Muscle biopsies from sex and age paired subjects not diagnosed as sIBM were used as controls. Exceeding biological material from the diagnostic procedure was used to isolate total DNA with the phenol-clorophorm technique. To perform the quantification of mitochondrial DNA (mtDNA content), a fragment of the mitochondrial and the nuclear genes 12SrRNA and RNAsa-P, respectively, were amplified in multiplex with Applied Biosystems RT-PCR technology. Results were expressed as the ratio between mitochondrial and nuclear DNA content.

Results: Muscle biopsies with sIBM presented a 37.4% decreased mtDNA content with respect to the controls (641.6 ± 141.2 vs 979.7 ± 111.1; $p=0.027$).

Conclusions: We found mtDNA depletion in muscles from sIBM patients compared to controls, suggesting that molecular mitochondrial changes might contribute in sIBM pathogenesis. Further studies must be done to asses downstream genetic mitochondrial consequences in sIBM etiopathology.

PS3-289 / #324

Theme: *3.2 - Acquired myopathies: Inclusion body myositis*

Pharmacological up-regulation of the heat shock response improves pathology in a transgenic mouse model of inclusion body myopathy

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Inclusion body myositis (IBM) is the commonest inflammatory myopathy affecting adults over the age of 50. This chronic condition leads to reduced muscle strength and impaired mobility in patients, however at present there is no effective treatment. Although the aetiology of this disease remains unclear, there is evidence for both inflammatory and myodegenerative processes in IBM muscle pathology. In particular, abnormal protein aggregation is a characteristic feature of affected muscle, with evidence of ubiquitinated inclusion body formation, TDP-43 mislocalisation, mitochondrial dysfunction and ER stress.

The heat shock response (HSR) is an endogenous cytoprotective mechanism involved both in the regulation of normal protein folding and prevention of protein aggregation. Using an *in vitro* model of IBM we have previously shown that pharmacological up-regulation of the HSR and the subsequent elevation in heat shock protein (HSP) expression improves pathology in muscle cells in culture. In this study we examine the potential of this approach *in vivo* in a mouse model. Due to the sporadic nature of IBM there is no animal model of this condition. However, mutations in the valosin containing protein (VCP) gene cause a hereditary form of the disease called Inclusion body myopathy associated with Pagets' disease and frontotemporal dementia (IBMPFD). In this study, we found that transgenic mice with an A232E mutation in the VCP gene had several characteristics of sIBM including decreased muscle strength, TDP-43 mislocalisation, ubiquitinated inclusion bodies, macrophage infiltration, centralised nuclei and necrotic and atrophied fibres. Treatment with Arimoclomol, a co-inducer of the heat shock response was able to significantly attenuate all of these pathogenic features. Arimoclomol may therefore be a potential therapeutic agent for the treatment of IBM.

PS3-290 / #481

Theme: *3.2 - Acquired myopathies: Inclusion body myositis*

Agreeing best practice guidelines for inclusion body myositis

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Inclusion body myositis (IBM) is the most common acquired adult onset muscle disease seen in specialised muscle clinics. It is an incurable disease that causes progressive muscle wasting and weakness resulting in significant disability. Our incomplete understanding

of the pathophysiology of IBM means that we do not have a specific marker for the disease and so the diagnosis of IBM is based on clinical features combined with muscle biopsy results. Lack of definitive treatment means that the mainstay of management is supportive. Even in the absence of a proven treatment a number of treatment options have been suggested but without consensus on whether these are appropriate. The lack of consensus on the diagnosis, treatment and management of IBM leads to inconsistencies in the care of those with IBM and variation in the resources allocated for that care. This project therefore proposes to obtain international consensus on the best standards of care for those with IBM. We will present the results of our systematic review of the current literature which offers a limited evidence base for much of the management of IBM. We will therefore supplement our evidence based guidelines with recommendations consolidated by a Delphi like approach to reaching consensus across the expertise of a large, international network of experts. These experts will be drawn from a variety of disciplines including clinicians, allied health professionals, expert patients, patient organisation representatives and commissioners of healthcare.

✶PF3

PS3-291 / #505

Theme: *3.2 - Acquired myopathies: Inclusion body myositis*

HMGB1 and RAGE expression in skeletal muscle inflammation: Implications for protein accumulation in inclusion body myositis (IBM)

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Background: GNE myopathy is a rare autosomal recessive muscle disease caused by mutations of GNE, encoding the key enzyme in sialic biosynthesis. GNE myopathy usually manifests in early adulthood with foot drop secondary to anterior tibialis muscle weakness and progresses slowly to involve more proximal muscles of the lower extremities, with relative sparing of the quadriceps. Upper extremity involvement is variable. Since its description, several case reports have been published but an overview of the natural history has been lacking.

Methods: We performed a prospective study of patients ($n=34$, age range, 29 to 65 years) through NIH study 11-HG-0218 "A Natural History Study of Patients with GNE myopathy" (ClinicalTrials identifier: NCT01417533).

Results: Median age of onset was 27.5 years (range 12-40). In all but one case, presentation was distal lower extremity weakness. There was a mean diagnostic delay of 12 years (range: 0-43 years) and 79% of patients were previously misdiagnosed. Patients had a variety of GNE mutations, mostly missense. Assistive devices included braces (54%), cane (42%), walker (18%), occasional wheelchair (18%) and wheelchair required for mobility (15%). 25% of ambulatory patients were unable to complete a 6-minute walk test. Quantitative muscle strength showed a mean overall strength of 43%, ankle dorsiflexion of 0.3% (range 0-6%), hand grip of 38% (range 1.8-135%) and knee extension of 70% of predicted for age and gender. The Activity Balance Confidence scale predicted a risk for falls in 89% of patients. EKG abnormalities were encountered in 33% and cardiomyopathy was seen in 1 patient. Assessment of forced vital capacity, maximal inspiratory and expiratory pressures suggested mild involvement of respiratory muscles. Mean values were 262 U/L (range: 27-1152) for CPK and 0.41 mg/dl (range 0.15-0.92) for creatinine. Renal function, evaluated by cystatin C, was normal in all patients. Plasma glycan profiling showed abnormal ratios of Thomson-Friedenreich antigen (T) and its sialylated form ST. Plasma ManNAc and sialic acid levels were not significantly different from controls. Plasma sialic acid levels increased 3-fold after administration of a single dose of ManNAc and levels persisted for up to 48 hours.

Discussion: Potential therapies for GNE myopathy are being developed and a better understanding of the natural history of the disease will facilitate the timely diagnosis of patients and the design of clinical trials.

★PF4

PS3-292 / #523

Theme: *3.2 - Acquired myopathies: Inclusion body myositis*

Molecular treatment effects of alemtuzumab in skeletal muscle from patients with IBM

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Background: Mechanisms of inflammation and accumulation of unwanted proteins are believed to be crucial during muscle fiber damage in inclusion body myositis (IBM). Recent evidence from a treatment study with intravenous immunoglobulin G demonstrated that particularly nitric oxide as a mediator between inflammation and degeneration in IBM was not reduced. Here we studied the molecular changes in skeletal muscle biopsies from patients with IBM before and after treatment with alemtuzumab.

Methods: Relevant inflammatory and degeneration-associated markers were assessed by quantitative PCR and immunohistochemistry in repeated muscle biopsy specimens performed before and six months after therapy from the same extremity. Skeletal muscle biopsies were used from thirteen patients with IBM treated in a previously published, uncontrolled, proof-of-concept trial with alemtuzumab.

Results: There were no significant changes of the mRNA expression levels of the pro-inflammatory chemokines CXCL-9, CCL-4, and the cytokines IFN-γ, TGF-β, TNF-α, and IL-1β six months after therapy. Similarly, the degeneration-associated molecules ubiquitin, APP, iNOS, desmin, and αB-crystallin remained without major variation. Although no overall beneficial treatment effect was noted, some patients displayed a transient stabilization. In such responders, a trend towards reduced expression of inflammatory markers was noted. By contrast, the expression remained unchanged in non-responders. The expression levels of IL-1β displayed a significant inverse correlation with the clinical response. By immunohistochemistry, some inflammatory markers like CD8, CXCL-9, and MHC-I were downmodulated. However, no consistent changes were noted for ubiquitin, nitrotyrosine, and β-amyloid. The staining intensity of MHC-I inversely correlated with the clinical response.

Conclusion: Collectively, in a subset of IBM patients alemtuzumab downmodulates the overexpression of some inflammatory molecules in skeletal muscle. Yet, several crucial markers of cell stress and degeneration remain without a relevant change. Our data help to explain the molecular treatment effects of a lymphocyte-targeted immunotherapy in IBM.

PS3-293 / #49

Theme: *3.3 - Acquired myopathies: Toxic / Endocrine / other acquired myopathies*

Multiple acyl coA dehydrogenase deficiency and severe rhabdomyolysis caused by ingestion of hypoglycin A in seeds of Acer negundo and Acer pseudoplatanus

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A highly fatal muscle disease has been described for many decades in thousands of horses grazing fall pastures in the USA, Canada and Europe. It is characterized by an acquired deficiency of multiple acyl-CoA dehydrogenases (MADD) and its cause has remained elusive. We hypothesized that equine acquired MADD was induced by ingestion of seeds containing hypoglycin A that were abundant in autumn pastures grazed by affected horses. We sought to identify a common seed bearing plant amongst autumn pastures grazed by affected horses in North America

and Switzerland, to determine if the toxic amino acid hypoglycin A was present in the seeds and to determine if the toxic metabolite of hypoglycin A, methylenecyclopropylacetic acid (MCPA) was present in serum or urine of affected horses.

Eleven farms in North America and six farms in Switzerland were visited in autumn to identify a plant common to all affected pastures. Acer Negundo (box elder) or Acer Pseudoplatanus (European sycamore maple) trees were present in all affected North American and Swiss pastures, respectively. Amino acid analysis of seeds analyzed by GC-MS identified hypoglycin A as the most abundant amino acid with maximal concentrations of 160 microg/seedfor Acer Negundo and253 microg/seed for Acer pseudoplatanus. Serum acylcarnitine or urine organic acid profiles were typical of MADD and carnitine conjugates of MCPA were identified in serum and urine of affected but not control horses.

For the first time, the cause of a decades old form of seasonal pasture-associated rhabdomyolysis has been identified; ingestion of Acer sp. seeds containing hypoglycin A. While acquired MADD occurs in humans and horses, humans consuming hypoglycin A in unripe Jamaican Ackee fruit present with seizures and hypoglycemia whereas horses ingesting hypoglycin A in Acer sp. seeds present with severe rhabdomyolysis, respiratory failure and hyperglycemia. The disease in horses can now be prevented by minimizing exposure to pastures containing seed bearing Acer negundo or pseudoplatanus trees during the autumn.

✶PF3

PS3-294 / #54

Theme: *3.3 - Acquired myopathies: Toxic / Endocrine / other acquired myopathies*

Sporadic late onset nemaline myopathy with MGUS: Long term follow-up after SCT

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Sporadic late-onset nemaline myopathy (SLONM) is a rare, late-onset, subacute myopathy that progresses subacutely. Limb-girdle and axial weakness and atrophy predominate the clinical picture. In addition, distal weakness, head drop, respiratory insufficiency, and dysphagia can occur. Recognition of nemaline rods on trichrome staining in the biopsy is crucial. This can be confirmed by immunohistochemical staining of the muscle biopsy with alfa-actinin antibodies. However, repeated biopsies might be required. SLONM is in a significant proportion of cases associated with a monoclonal gammopathy of unknown significance (MGUS), a combination which portends an unfavorable outcome: the majority of these patients (71%) die within 1 to 5 years of respiratory failure.

Treatment with high dose melphalan (HDM) followed by autologous stem cell transplantation (SCT) has proven to be effective in individual cases. We here present the long-term follow-up of eight patients with SLONM and MGUS. They were treated aggressively with HDM and SCT. Seven patients showed a partial or complete response, both hematologically and clinically. One patient showed no response and detoriated. Two patients had a relapse and underwent a second cycle of HDM with SCT. The association between the

hematological response, the disappearance of rods in the muscle biopsy, and the clinical improvement strongly suggests a direct or indirect effect of the M protein on the myopathy.

Factors which may portend an unfavorable outcome are a long disease course before the hematological treatment and a poor hematological response to treatment. Age at onset of muscle weakness, level and kind of M-protein (kappa vs lambda), and severity of muscle weakness before the graft did not seem to be associated with outcome.

In conclusion, this analysis demonstrates the positive effect of HDM and autologous SCT in SLOMN patients. We advise M-protein screening in this subacute, late-onset myopathy with predominantly limb-girdle and axial weakness and atrophy, and (repeated) muscle biopsies to detect the characteristic rods.

PS3-295 / #265

Theme: *3.3 - Acquired myopathies: Toxic / Endocrine / other acquired myopathies*

Two cases with telbivudine-induced myopathy

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Telbivudine (β-L-2'-deoxythymidine) is a new synthetic L-nucleoside analogue with potent antiviral activity against hepatitis B virus. Anecdotal reports on adverse reactions associated with telbivudine challenges its safety profile, although it is not supposed to influence mitochondrial function according to *in vitro* data. We report two cases of telbivudine-induced myopathy in patients with chronic hepatitis B.

Patient 1 (48-year-old woman) was presented with progressive weakness of both lower legs over the past 4 months. Diagnosed to have chronic hepatitis B, she has been taking and taking telbivudine 600 mg once daily for 3 years. Her serum level of creatine kinase (CK) was as high as 8882 U/L, electromyography findings were compatible with acute myopathy, and muscle biopsy revealed ragged red fibers. Telbivudine was discontinued and switched to tenofovir, which successfully reduced her CK level down to 200 U/L after 1 month.

Patient 2 (51-year-old woman) was taking telbivudine 600 mg once daily for 2 years. Her weakness of both legs progressed for 18 months, and she could walk with the aid of a cane at the time of consultation. Her serum CK level was up to 1547 U/L. Electromyography showed acute myopathy and muscle biopsy revealed mitochondrial myopathy. Discontinuing telbivudine leads to mild improvement of her weakness.

We had previously reported 7 cases of clevudine-induced myopathy with evidence of mitochondrial toxicity including numerous ragged red fibers, cytochrome oxidase-negative fibers in muscle pathology and depletion of mitochondrial DNA (mtDNA) on quantitative PCR analysis. DNA polymerase is highly sensitive to inhibition by nucleoside analogues, which may leads to mtDNA depletion in muscle. Evidence of mitochondrial dysfunctions has also been reported with nucleoside analogues such as zidovudine, lamivudine and fialuridine.

Cases with telbivudine-induced myopathy prove telbivudine is not an exception from antiviral-induced mitochondrial toxicity. This nucleoside analogue-related cellular toxicity has been attributed to decreased mtDNA content and altered mitochondrial function by inhibiting mitochondrial DNA polymerase. New nucleoside analogue like entecavir is considered alternative choice, though no nucleoside analogue can be free from mitochondrial toxicity. Careful clinical observation to the muscle-related symptoms and regular measurement of serum CK is warranted in all chronic hepatitis B patients taking nucleoside analogues.

PS3-296 / #283

Theme: *3.3 - Acquired myopathies: Toxic / Endocrine / other acquired myopathies*

Two Australian cases of Anncaliia algerae microsporidial myositis - the first non-fatal outcome

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Two cases of Anncaliia (formerly Nosema then Brachiola) algerae microsporidial parasitic myositis in patients with rheumatoid arthritis (RA) treated with immunosuppression are presented with one surviving; the factors that may have contributed to survival are discussed.

Case 1. A 66 year old man with RA and renal impairment treated with methotrexate; the prednisone dose had recently increased as high as 50mg daily for increasing muscle pain over a month. He was admitted febrile with a peak CK 6630 U/L (<200). He was treated empirically for sepsis, viral myositis, then fungal myositis; corticosteroids were continued at a reducing dose. He died 4 weeks after admission. Subsequently, Anncaliia algerae organisms were demonstrated on electron microscopy of biopsied muscle and confirmed on PCR.

Case 2. A 67 year old man with RA treated with methotrexate, etanercept and leflunomide; these were sequentially ceased for diarrhoea two weeks prior to admission; the prednisone dose had recently increased to 35mg daily for increasing muscle pain over a month. He was admitted afebrile with a peak CK of 1141. A muscle biopsy was immediately performed for infective myositis as the critical diagnosis of exclusion. This demonstrated microsporidial structures later confirmed as Anncaliia algerae. Prednisone was weaned to 5mg daily whereupon he became febrile, leflunomide was "washed out". Albendazole, pyrimethamine and sulfadiazine were administered. He reached a clinical nadir after a further week and recovered over two months.

To date, four of the five reported cases of Anncaliia algerae myositis have been from the east coast of Australia. Our second case is the first report of a patient to survive Anncaliia algerae myositis and one of the few to survive a parasitic myositis with systemic infection. The timely diagnosis, reversal of immunosuppression, management of complications and the administration of albendazolelikely contributed to the survival of the patient in case 2.

PS3-297 / #460

Theme: *3.3 - Acquired myopathies: Toxic / Endocrine / other acquired myopathies*

Admission to an intensive care unit as first event of neuromuscular disease in adult patients.

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Background: Neuromuscular diseases in adult patients are rarely diagnosed in an intensive care unit (ICU) with a severe debut as a first clinical manifestation. In consequence there are no much reported cases in medical literature on this issue. Nevertheless, in our experience, there was a significant number of patients in whom their neuromuscular clinical picture was diagnosed in ICU.

Objectives: Our main objective was to assess the number of patients with an undiagnosed neuromuscular disorder who where admitted to an ICU, from January 2005 to December 2013, as well as the main clinical features and the final diagnosis.

Methods and results: We conducted a retrospective study, collecting data from the clinical records from both the ICU and the Muscle Research Unit. In the period of time from January 2005 to December 2013, there were 2,917 patients admitted to the ICU, nine of them receiving the diagnosis of previously undiagnosed neuromuscular disease.

The main cause of ICU admission was hypercapnic respiratory failure (7 patients), while the other two patients entered the UCI because of normocapnic respiratory failure and convulsive status respectively. Seven required mechanical ventilation for a median time of 23 days, Five electromyograms and seven muscle biopsies were performed for diagnostic puposes. Furthermore, only after through directed anamnesis and careful evaluation, five patients had previous slight symptoms suggestive of neuromuscular disorder. The final diagnoses of the nine patients are compiled in the attached table.

When discharged from the hospital, four patients still matched criteria for respiratory insufficiency. One of the patients needed ventilatory support and four remained with a permanent tracheostomy Three

patients died during their ICU admission, being the cause of death indirectly related to the newly diagnosed neuromuscular disease.

Conclusions: Although this is not a frequent issue for day-to-day medical practice, neuromuscular disorders could be diagnosed in the wake of a ICU admission, mainly due to pulmonary complaints. Such diagnoses hold great importance since they can determine the final outcome.

PS3-298 / #548

Theme: *3.3 - Acquired myopathies: Toxic / Endocrine / other acquired myopathies*

A case of rhabdomyolysis due to cyanide poisoning following the consumption of European black elderberries

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Background: European black elderberry (Sambucus nigra) is a traditionally used medicinal and culinary plant in Central- and Southern-Europe. When unripe the berries are poisonous containing the cyanogenic glycoside sambunigrin, which can be activated by cytoplasmic enzymes releasing toxic hydrogen cyanide. It has been reported that cyanides, as mitochondrial toxins, may play an important role in the pathogenesis of some rare neurotoxic diseases such as neurocassavaism, characterised by clinical picture of self-limited and irreversible spastic paraparesis due to the motor neuron degeneration.

Case Report: In this article we present a case of a 63-year-old male with a history of muscle cramps and a slowly progressive tetraparesis. Three years before he had been hospitalised with acute symptoms of intoxication after the consumption of unripe European black elderberries with drinking water. He was presenting with diarrhea, nausea and dizziness initially. Laboratory testing detected increased creatine kinase levels 5.914 U/l four days after the intoxication. Unfortunately, the blood cyanide concentration was not obtained. He eventually developed symptoms of forgetfulness and progressive weakness, and suffered from painful muscle cramps of the upper and lower limbs. We performed electrophysiological testing revealing signs suggestive of a slowly progressive axonal damage especially of the lower extremities. Magnetic resonance imaging of the lower limbs showed no alteration of the muscles. Evaluation of muscle and nerve biopsies revealed neurogenic alterations and a primary axonal degeneration, respectively.

Conclusion: To our knowledge this is the first case of rhabdomyolysis after the intoxication with unripe European black elderberries. Interestingly, our patient developed a slowly progressive tetraparesis due to axonal polyneuropathy thereafter. Since similar clinical picutures of progressive paraparesis due to motor neuron degeneration have been reported after the ingestion of improperly processed bitter cassava roots (Manihot esculenta) containg cyanogenic glycosides, an association between the polyneuropathy of our patient and the intoxication of European black elderberries seems possible. However, no other potential causes of the polyneruopathy could be determined through an extensive diagnostic testing.

PS3-299 / #63

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Both binding and blocking antibodies correlate with disease severity in myasthenia gravis

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Introduction: Myasthenia gravis (MG) is an autoimmune disease associated with antibodies directed to the postsynaptic muscle components of the neuromuscular junction. The heterogeneous nature of the acetylcholine receptor (AChR) antibody response had led to the categorization of AChR antibodies into 3 types: binding, blocking, and modulating antibodies. The purpose of this study was to compare assays of AChR binding and blocking antibodies for their ability to the diagnosis of MG and to estimate the clinical severity of MG patients.

Methods: We analyzed the antibody type and medical records of 44 patients whose MG had been confirmed by serological testing. The patients enrolled in the study had received both the binding and blocking AChR antibodies tests and disease duration exceeding 2 years since diagnosis. Patients who had only a binding or blocking antibody test, and who were

diagnosed with seronegative MG were excluded. The patients were divided into five main classes by the Myasthenia Gravis Foundation of America (MGFA) clinical classification. Again, the enrolled patients were divided into ocular and generalized group according to MGFA classification. We compared the type and titer of antibodies and the thymus status between the ocular and generalized group.

Results: Thirty-five patients met the inclusion criteria. Of these, 16 patients (47%) had both blocking and binding AChR antibodies, 11 patients (31%) had only binding antibodies, and 8 patients (22%) had only blocking antibodies. According to MGFA classification, we classified 35 patients into five classes; 10 patients in class I, 12 patients in class II, 7 patients in class III, 2 patients in class IV, 4 patients in class V. By defined clinical classification, the ocular and generalized groups included 10 and 25 patients, respectively. Sixteen patients in the generalized group possessed both AChR antibodies, with the remaining patients displaying only the binding antibody. All the patients with only blocking antibody were classified into ocular group.

Conclusions: Our study demonstrates that MG patients with both binding and blocking antibodies show more severe generalized MG or myasthenic crisis. We suggest that both antibodies tests are useful in determining whether the disease will generalize.

PS3-300 / #72

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Autoimmunity - Myasthenia Gravis, Graves' disease and Vitiligo: a case report

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Introduction: Myasthenia gravis (MG) is an autoimmune neuromuscular junction disease that rarely can be associated with other autoimmune conditions. Epidemiologic studies report 5-10% occurrence of autoimmune thyroid disease (autoimmune thyroiditis and Graves' disease) in patients with MG; whereas MG occurs in only 0.2% of patients with autoimmune thyroid disease. The clinical presentation of MG associated with autoimmune thyroid disease is frequently restricted to the eye muscles. There are only few cases, like the one reported here, with the very rare association of ocular MG, Graves' disease and Vitiligo. The reason of the autoimmune cluster is unknown, but hypothesized as immunological cross-reactivity of the epitopes.

Case report: Twenty year old right-handed man presented with diplopia, variable ptosis, severely restricted eye movements with disconjugated gaze, having onset two months after Graves' disease and Vitiligo were diagnosed. He gradually developed generalized axial muscle weakness and shortness of breath. High titers of anti-acetylcholine receptor antibodies were detected. MUSK antibodies were absent. Repetitive nerve stimulation (RNS) of the facial and accessory nerves at 3 Hz revealed significant decrement and needle EMG revealed short-duration polyphasic potentials in the face, deltoid and biceps, without spontaneous muscle activity. His chest CT identified a thymic mass and he underwent thymectomy. Pathological exam revealed thymic hyperplasia and no malignant cells. The replacement hormonal treatment for Graves' disease did not improve diplopia and nor the disconjugated eye movements, but he showed partial response to plasma exchange, anticholinesterase therapy and steroids.

Conclusions: The association of multiple autoimmune conditions is very rare, but represents a diagnostic and therapeutic challenge, in particular MG preceded by Graves' disease and Vitiligo. Further investigation is needed for better understanding of the phenomena.

PS3-301 / #81

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Myasthenia gravis: Epidemiological study in the North of Portugal

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Background: Myasthenia Gravis (MG) prevalence has been studied in several populations all over the globe, and it varies greatly - ranging from 15 to 179/1000000 (Carr, 2010). There have been reports showing changes in the age distribution of the patients, with an increase in those over 50 years old, particularly males. There have been also changes in the geographical distribution of the different clinical phenotypes and serology status. Objective: To know the prevalence of MG in the North of Portugal and characterize clinical and serological features of MG patients from this geographical area. Patients and *Methods*: We identified patients with the diagnosis of MG (seropositive and seronegative) followed in the hospitals of the North of Portugal with a neurologist. We collected the clinical data and blood to characterize their clinical phenotypes/serologies.

Results: So far were identified 303 patients in the population of the North of Portugal (3,689,609 inhabitants), corresponding to a prevalence of 82.1/1,000,000. Age of onset ranged between 7-85y; female/male ratio was 1.9/1. We found a prevalence of 4.3/1,000,000 for anti-MuSK MG, corresponding to 5.2% of this MG population. In this group the female/male ratio was much higher 7/1 and the age of onset was similar 17-86y. The same ratio was 3.45/1 in the EOMG group and 0.88/1 in the LOMG group, suggesting a male predominance in the latter. LOMG corresponded to 31.3% (95/303pts), 34 the LOMG patients were ocular and 61 generalized, only one presented with myasthenic crisis. In this group the frequency of Musk MG patients was similiar, 5.2%. (4/95pts). In the last decade we observed an increase of the number of LOMG patients, 19 new cases between 2001-2006 and 62 new cases between 2007-2012.

Comments: This is on going work, and so far it seems that Portugal has a higher prevalence of MuSK than the northern countries of Europe. Also we show evidence of an increase of the prevalence and incidence of LOMG.

PS3-302 / #123

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Myasthenia gravis and inflammatory bowel disease in a cohort of Brazilian patients

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Introduction: Auto-immune disorders may affect up to 5% of the population, but co-existence of 2 autoimmune disorders is far less common: 0.2% (Sardu et al., 2012).

Objective: To present the clinical and electrodiagnostic findings of 2 patients with inflammatory bowel disease (IBD) and myasthenia gravis (MG) in a cohort of Brazilian patients with IBD.

Patients and Methods: We evaluated the presence of neurological disorders in all patients with IBD seen at a tertiary IBD Clinic from the Universidade Federal do Cearà, Brazil. This evaluation consisted in a cohort of 218 IBD patients, seen over a 9-year period. We have also conducted a comprehensive literature review about the overlap of the MG diagnosis in IBD patients.

Results: We have found 2 patients with IBD and MG over 9 years. Patient 1: Three years after being diagnosed with ulcerative colitis (UC) at age 37, a man underwent total colectomy and partial gastrectomy (previously undiagnosed gastro-colonic fistula). His diagnosis was then changed toCrohn's disease

(CD). After tapering off prednisone on his own, he experienced quadriparesis, bilateral ptosis, dysphagia and dysarthria. Patient 2: A 41 year-old woman (diagnosed with UC and primary sclerosing cholangitis at age 35) developed speech impairment and ptosis. On both patients, MG was diagnosed and confirmed by abnormal repetitive nerve stimulation (decrement >10% with 3Hz stimulation) and elevated anti-acetylcholine receptor antibody titers. Pyridostigmine and prednisone successfully controlled MG on both. The literature review disclosed 15 papers and 21 patients with IBD and MG (7 CD and 14 UC). Ocular symptoms were the most common complaints and most had positive AchRAb. Thymectomy usually produced good results (improving both IBD and MG symptoms). Patients were usually diagnosed with MG several years after IBD onset and the disease course was usually similar to MG patients without IBD.

Conclusions: MG prevalence in this Brazilian cohort of IBD patients over 9 years was 0.9%. Otherwise, there are few cases of combined MG and IBD diagnosis reported in the medical literature ($N=21$). MG clinical course was not significantly modified by IBD. MG should be considered in all IBD patients with new onset ocular, bulbar or limb weaness, particularly after changes in immunosupression.

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✶PF1

PS3-303 / #131

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

In vitro characterization of satellite cells from myasthenic patients

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Myasthenia gravis (MG) is a relatively uncommon neuromuscular disease caused by circulating autoantibodies against proteins of the neuromuscular junction that lead to impaired neuromuscular transmission (NMT). MG is characterized by fatigability and fluctuating muscle weakness as well as muscle atrophy. The regeneration of atrophied muscle is carried out by local stem cells called satellite cells (SC), however, molecular and cellular mechanisms of myogenesis in MG disease are still unknown.

Muscle biopsies from 6 MG patients and 6 healthy age-matched controls were collected. SCs were isolated from these muscle biopsies using explant method and positive selection of $CD56^+$ cells using magnetic microbeads. Proliferation and differentiation of SC *in vitro* was measured respectively by cell counting (flow cytometer) and MF-20 immunolabelling in a kinetics study (from day 0 to day 4). We observed that SCs from MG biopsies proliferate as well as differentiate more actively than SCs from healthy ones. This could be due to the known role of growth factors (IL-6, IGF-1, SDF-1) and myogenic factors (Myf5, MyoD, MRF4, MyoG) in myogenesis. Using real time qPCR, we observed that during SCs proliferation, Myf5, MyoD and Myogenin were more expressed in MG SCs compared to controls. Using immunoloabeling and western blot analyses, we observed that MF-20 and TnT, which are the specific markers of the differentiation, were more expressed in MG SCs compared to controls. In parallel, the number of SCs in muscle biopsies (using anti-Pax7 antibody) was significantly increased in the MG muscle compared to controls.

These findings demonstrate the activation of SCs in MG muscle as well as functional differences between SC properties from healthy and MG muscles. The autoimmune attack in MG might lead to important changes in the number and function of SC that could represent a mechanism of compensation to regenerate muscle fibres that have been damaged by the autoantibodies.

PS3-304 / #186

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Increased skeletal muscle expression of the endoplasmic reticulum chaperone GRP78 in patients with myasthenia gravis

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Background: An endoplasmic reticulum (ER) stress in muscle has been observed in some muscular diseases and ER stress might influence muscle weakness and degeneration. There have been reports of Glucose-regulated protein 94 and MHC class I overexpression in skeletal muscles with MG. These expressions in muscle might have been attributed to the ER stress response.

Objective: Glucose-regulated protein 78 (GRP78) belongs to the heat shock protein 70 kDa family and GRP78 induction has been widely used as a marker of ER stress. The aim of the current study was to evaluate the upregulation of the ER stress chaperone GRP78 in the skeletal muscles of patients with MG.

Methods: Patients with MG included six with thymoma and seven without thymoma. During thymectomy, tissue samples were resected from the musculus pectoralis major. In addition, five patients with inflammatory myopathy and five patients with non-myopathy provided biopsy specimens as control samples.

Immunohistochemistry using anti-GRP78 antibody was completed according to standard methodology. qRT-PCR was performed and calculated mRNA level of GRP78 using the 2-ΔΔCt method.

Results: The skeletal muscles obtained from patients with MG exhibited upregulation of GRP78 mRNA. We also observed a significant positive correlation between GRP78 mRNA expression and GRP78 protein levels and between GRP78 mRNA expression and age of MG onset.

Discussion: In MG, neuromuscular junction damage may induce ER stress in the skeletal muscles and may be implicated in GRP78 expression. Lymphocytic infiltration in MG muscles has been reported and infiltrated lymphocytes may lead to secretion of cytokines that could then stimulate the ER and induce GRP78 expression. GRP78 gene expression was correlated with age of MG onset. Autoantibodies directed against non-AChR skeletal muscle proteins are more frequent in older patients with MG. It is possible that a heterogeneous immune attack against muscle antigens could induce ER stress. In addition, ER stress in muscles is important for muscle repair after injury. Thus, upregulation of GRP78 in muscle cells may play an important role in muscle repair and improvement of MG.

Conclusion: The muscles with MG might not only be damaged in the neuromuscular junction, but also in intracellular areas. Our findings may help to understand muscle responses to ER stress conditions in MG.

PS3-305 / #243

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

The West of Scotland Myasthenia Service

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The myasthenia gravis (MG) clinic was set up in August 2007, initially run by a physician (MEF). The Myasthenia Gravis Association funded the post of a myasthenia nurse specialist in 2009. Since then the myasthenia nurse (CC) has helped in running the West of Scotland myasthenia service, receiving telephone calls for advice from all over Scotland. Since 2009, we have established 3/month myasthenia clinics and weekly nurse-led myasthenia clinics.

We now have 431 MG patients on our database, 9 patients with Lambert-Eaton myasthenic syndrome and 5 patients with molecularly proven congenital myasthenic syndrome (1 slow channel syndrome, 1 RAPSN, 1 DOK7 and 2 with epsilon mutations of the acetylcholine receptor (AChR) subunit). Out of the cohort, we have 109 ocular MG patients (OMG) and 296 generalised MG patients (GMG). We have very limited information on another 26 MG patients and have excluded them for the purpose of this abstract.

In the GMG cohort (296) , 56% are female (166). 112 patients (39%) presented under the age of 50y. 229 patients (77%) are AChR antibody positive, 58 are AChR antibody negative, 3 are positive for MuSK antibodies , 3 are positive for low-affinity MuSK antibodies and in 3 their antibody status is unknown. 75 patients have had thymectomy, 19 of these had thymoma. In the OMG cohort (109), 44 patients (40%) are female. Only 27 patients (25%) presented under the age of 50. 66 (61%) had AChR antibodies, 41 were negative and 2 were of unknown antibody status.

Only 48 OMG and 54 GMG patients were treated with pyridostigmine alone. 283 patients were on steroid treatment at some stage (57 OMG and 226 GMG). 40 OMG and 150 GMG patients are still receiving steroids. 40 OMG patients and 192 GMG patients received azathioprine, mycophenolate mofetil, ciclosporin or methotrexate with 134 patients still on steroids and immunosuppression (23 OMG, 111 GMG). 17 patients receive regular intravenous immunoglobulin (monthly to 6 monthly) and 2 receive outpatient plasmapheresis to further improve their MG status.

Our MG practice has steadily changed and we are encountering older patients presenting with MG. These patients often have several underlying comorbidities and this influences the physician's attitude towards how aggressive treatment should be to control their MG symptoms.

PS3-306 / #262

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Factors associated with generalization of ocular onset myasthenia gravis

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Background: Several small retrospective studies have suggested that patients with purely ocular manifestations of myasthenia gravis (OMG) are significantly less likely to convert to a generalized disease when treated early in with oral steroids.

Objective: This study analyze the effect of oral steroids in preventing secondary generalization of ocular myasthenia gravis (OMG-G) and review the prognostic factors associated withthis development.

Methods: Retrospective observational study. Inclusion criteria: typical clinical findings of MG and absence of clinical findings of generalized MG for at least one month, one confirmatory diagnostic tests including antiacetylcholine receptor antibody titers or suggestive findings in one electrophysiological study and no timoma in chest image studies. The patiens were retrospectively selected and then grouped depending of the evolution. Sixty four remained OMG (OMG-R) and 47were OMG-G. The mean follow-up time was 7.6 years. We performed univariate and multivariate analysis.

Results and discussion: There were 47 (42.3%) patients who developed generalized myasthenia gravis. Antibody-positive cases were more frequent in OMG-G group than in OMG-R (78.2% and 56.2% respectively; p <0.041), as well as women (53.1% and 29.7% respectively; p <0.012). There were no differences in age or neurophysiologic studies performed for diagnosis. There were 17/64 (26,6%) in the OMG-R and 9/38 (19%) patients in the OMG-G patients who received steroids prior to generalization; p >0.05. Survival analysis showed a shorter time to generalization in the group that was not treated with steroids than in those who were treated (median 6.9 and 14.7 years respectively, P <.04). In multivariate analysis (Cox Regression) only the absence of early treatment with prednisone was associated with shorter time to generalization (HR 2,18; IC 95%= 3,18-1,49; p=0.039).

Conclusions: Early use of oral steroids in the OMG could delay progression to OMG-G.

★PF3

PS3-307 / #272

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Late-onset and very late-onset non-thymomatous anti-acetylcholine receptor antibody positive generalized myasthenia gravis: Clinical features

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Background: Recently there has been an increase in the incidence of myasthenia gravis (MG) in patients over 65 years. Several studies have described the clinical features of late-onset myasthenia gravis (LOMG).

Objective: The objective of this study is to compare the clinical features of LOMG and the very late-onset myasthenia gravis (VLOMG) subgroups with classical early-onset myasthenia gravis (EOMG).

Methods: We reviewed our patients diagnosed with non-thymomatous anti-acetylcholine receptor antibody (AChRAb) positive generalized from 2002 to 2009 and with one to five years of follow-up. Patients were classified according to age of onset as EOMG (onset before age of 50), LOMG (onset after age of 50) and VLOMG (onset after age of 70).

Results: In the LOMG and the VLOMG subgroup more patients debuted with bulbar weakness ($p=0.00154$ and $p=0.046$ respectively) and more corticotherapy was prescribed compared with EOMG patients ($p=0.0055$ and $p=0.00054$ respectively). There were no differences with regard to other immunosuppressive therapies. The age was the only variable that influences clinical evolution during the first year of follow-up ($p=0,0063$). In the first year of follow-up more patients in the LOMG and VLOMG subgroups presented good clinical evolution (CSR, PR and MM) on MGFA PIS ($p=0.0028$ and $p=0.0312$ respectively).

Discussion: In our series, unlike those reported in the literature, we found that in LOMG and VLOMG patients more corticotherapy were prescribed than EOMG patients. Also, we found the the only variable that influenced the outcome in the first year of follow-up was the age, with better outcomes being recorded for older patients. Surprisingly, immunosuppressive treatment, sex or the onset MGFA status did not directly influence the clinical course of these patients.

Conclusions: Non-thymomatous AChRAb positive generalized LOMG patients were predominantly male and presented more bulbar muscle weakness. Age is the only variable that influences the clinical evolution of the first year of follow-up. Though they presented a trend towards worse clinical onset, LOMG and VLOMG patients achieved good clinical outcome before EOMG patients. LOMG group and VLOMG subgroup presented similar clinical features, distinct from those of the classical EOMG group.

PS3-308 / #293

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Muscle cells undergo metabolic changes in Acetylcholine Receptor positive (AChR+) Myasthenia Gravis

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Clinical features of Myasthenia Gravis (MG) are attributed to defects of the neuromuscular junction resulting from autoantibodies action and little is known of modifications in muscle physiology. Transcriptomic analyses of muscle biopsies from patients and from experimental autoimmune myasthenic rats have demonstrated the involvement of several major pathways, among which the IL-6 and IGF-1 pathways. Both pathways are central to muscle physiology and involve the Akt/PKB signalling cascade.

Treatment of myotubes *in vitro* with antibodies against AChR upregulates IL-6 at both the transcript and protein level. IL-6 protein up-regulation was also confirmed in muscles from myasthenic patients. To test whether anti-AChR antibodies could alter Akt phosphorylation, human myotubes were treated for 24h with two monoclonal anti-AChR antibodies (198 and 155) or control isotype. Phosphorylation of Akt in

response to insulin was analysed by Western Blot. As expected, insulin stimulated the phosphorylation of Akt. We found that both AChR antibodies reduced the phosphorylation of Akt in response to insulin, suggesting that anti-AChR antibodies could interfere with the IGF1/Akt pathway. IGF-1/Akt regulates several cellular processes such as cell growth, survival and glucose metabolism. Therefore, we investigated the glucose homeostasis in the experimental autoimmune animal model of myasthenia gravis (EAMG). Preliminary results show that EAMG mice have higher glycaemia in response to an intraperitoneal glucose tolerance test. We hypothesize that this is due to glucose uptake inhibition in the muscles under the action of AChR antibodies.

Altogether, our data demonstrate a new mechanism of action of anti-AChR antibodies, that alter the metabolism of the muscle cells. These effects may participate to pathological mechanisms observed in the muscle of MG patients and could contribute to comorbidities like diabetes. Chemical action to re-activate the pathway may therefore counteract the specific effect of antibodies on muscle cells and supplement existing systemic treatments.

PS3-309 / #350

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

The incidence of Myasthenia Gravis in South Africa

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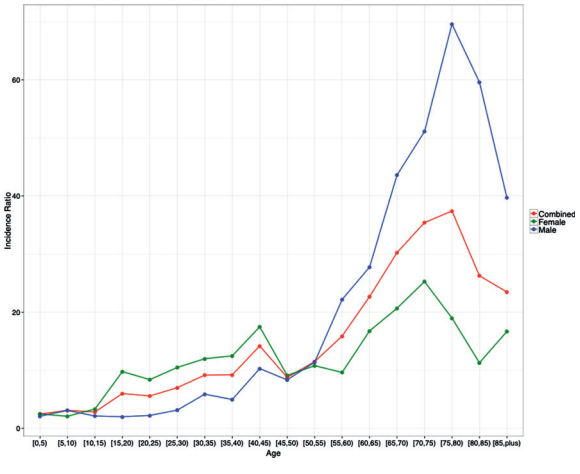
Objectives: To assess the age- and sex- specific incidence rates (IR) of acetylcholine receptor (AChR) antibody-positive myasthenia gravis (MG) in South Africa (SA), and to examine variation in incidence in different regions of the country.

Methods: Age and sex-specific incidence rates (per million inhabitants per year) were calculated from laboratory data between January 1, 2011 and January 1, 2013. Direct age-adjusted rates were calculated from the WHO world population.

Results: We identified 890 patients with AChR-positive MG during the study period, with an average pooled crude annual IR of 8.5 (95% CI 8.0, 9.1). The age standardized IRs for early onset (age <50) and late onset MG were 4.1 (95% CI 3.5, 4.7) and 24 (95% CI 21, 28), respectively. For juvenile MG (age < 20), the age standardized IR was 4.3 (95% CI 3.6, 5.1). The crude incidence between provinces ranged from 1.3 to 18.8 per million.

Discussion: This is the first report of the geographical variation in the incidence of seropositive MG in an African country, highlighting inequities in specialist care delivery. The annual IR for AChR-antibody positive MG has shown an apparent increase from 2.6 to 8.6 since the previous analysis (2003-2004), and is now comparable to the worldwide estimated pooled IR (eIR) of 7.3 (95% CI 5.5, 7.8), suggesting that health care delivery in this country is improving. Overall, the data shows a similar trend and gender distribution to cohorts from Canada, Scandinavia and Europe, with the highest age-adjusted IR over the age of 65 years, with a male preponderance in late onset MG. There are few comparable studies reporting the frequency of AChR-positive MG among juveniles.

Conclusions: The overall incidence of AChR-positive MG in SA is similar to comparable studies from Europe and Japan, and shows the highest incidence amongst the elderly.The geographical variation in incidence underscores the importance of outreach programs for regions with limited resources.



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PS3-310 / #374

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Epitope spreading is rare in MuSK myasthenia gravis

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In myasthenia gravis (MG) autoantibodies against essential neuromuscular junction proteins inhibit neuromuscular transmission and maintenance and thereby cause muscle weakness and fatigue. A few percent of MG patients have autoantibodies against muscle-specific kinase (MuSK), which have several unique features. The pathogenic MuSK autoantibodies are of the IgG4 subclass, and cause myasthenia by directly interfering with protein function rather than by activation of the complement system. Recently, we showed that the pathogenic MuSK autoantibodies bind to the N-terminal Ig-like 1 domain of MuSK and inhibit the interaction between MuSK and Lrp4. MuSK Lrp4 interaction is mediated by the Ig-like 1 domain of MuSK and is essential for AChR clustering and neuromuscular junction maintenance. The loss of this trophic signal is the main cause of myasthenia. The location of autoantibody binding (epitope) is therefore crucial for its pathogenic effects.

An initial immune response is often aimed at a restricted number of epitopes (main immunogenic region (MIR)). Subsequently, the antibody specificity spreads to other regions of the protein and/or other closely associated proteins, known as "epitope spreading". The cause and effect of epitope spreading in the pathogenesis of autoimmune diseases is unclear.

Since the binding of MuSK autoantibodies is of particular importance for it pathogenic effects, we investigated epitopes spreading in MuSK myasthenia gravis patient samples. We included 231 unique longitudinal serum samples of 20 Dutch patients and 29 sera samples of 9 Spanish patients. All patients were tested positive for MuSK autoantibodies in a commercial MuSK RIA. The mean follow up of the Dutch patients was 6,1 years (1.01-19.17) and for the Spanish patients 5,8 years (1.74-11.17). Six healthy controls, eight Lambert Eaton myasthenia gravis patients and nine seronegative myasthenia gravis patients served as negative controls. Epitopes were mapped using recombinant overlapping MuSK proteins expressing either the full length protein or parts of this protein in ELISA.

All patients harboured autoantibodies against the N-terminal Ig-like 1 domain throughout the course of the disease. Some patients harboured additional reactivity against the Ig-like 2 domain and the Fz-domain. Epitope spreading was rare and observed sporadically in patients. Detailed results will be discussed at the conference.

★PF3

PS3-311 / #381

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Seronegative myasthenia gravis- clinical and serological features

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Acquired Myasthenia Gravis is an autoimmune channelopathy of the post-synaptic neuromuscular junction. The diagnosis is supported by clinical presentation, neurophysiology, and detection of Acetylcholine Receptor (AChR), Muscle Specific Kinase (MuSK), or Low Density Lipoprotein Related Protein (LRP4) antibodies. Despite clear autoimmune aetiology, a proportion of patients remain 'seronegative' (SNMG) and diagnostic confirmation can be challenging.

Our aim was to characterise the clinical and functional features of SNMG.

An international cohort of 293 suspected SNMG patients were tested by radioimmunoassay (RIA) and cell-based assays (CBA) for AChR, MuSK or LRP4 antibodies. SNMG sera were screened for binding to

primary muscle cell lines (TE671 and CN21) and inhibition of agrin-induced AChR clustering in myotube cultures (C2C12).

Of the 293 patients 7 patients were RIA/CBA(+) and 81 patients were RIA(-)/CBA(+). 212 patients were negative by both methods [M:F 1:1.4, median onset age 35.5 (1-63)]. Most of these patients had mild disease (MGFA ≤IIb) at onset (17/17) and follow-up (70% $n=61/87$). Neurophysiology was positive in 65% ($n=28/43$). Preliminary results suggest that these sera do not affect agrin-induced AChR clustering in C2C12s, although some seronegative sera bound to TE671 and CN21 muscle cell lines.

Myasthenia gravis patients without AChR, MuSK or LPR4 antibodies have relatively mild disease according to MGFA grading. Neurophysiology was supportive in only 65% of these patients. Our evidence suggests that some of these sera do bind to novel antigen(s) on muscle cells. The next stage will be to try to define the antigen(s) using immunoprecipitation and mass spectroscopy.

PS3-312 / #392

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Initial single-fiber electromyography has prognostic value in myasthenia gravis

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Initial single-fiber electromyography has prognostic value in myasthenia gravis.

For myasthenia gravis (MG), single-fiber electromyography (SFEMG) is the most sensitive diagnostic tool. The typical SFEMG findings in MG are increased jitter and/or impulse blocking in some of the examined motor end-plates. In most patients with MG, the trend of the disease severity correlates with SFEMG jitter and blocks changes, but the prognostic value of the initial SFEMG data for the long term clinical course is not known, as yet.

The aim of our study was to find out whether the initial SFEMG changes in MG are predictive of the severity of the long term clinical course of the disease.

We reviewed medical files of MG patients, diagnosed between 01.01.2003 and 31.12.2012. We correlated the patients' percentages of increased jitter and conduction blocks with their later disease clinical course. The SFEMG was performed on the orbicularis oculi muscle at the time of the diagnosis. The severity of the disease clinical course was defined by the worst MGFA clinical classification score that was reached by the patient during the observation period.

229 MG patients were included in the study (122 women, 107 men). The mean age was 56.9 years (52.4 years for women and 62.1 for men). The longest and shortest observation periods were 114.5 and 6 months, respectively. According to the MGFA clinical classification score, 74 patients were in stage I, 71 in stage II (a and b), 54 in stage III (a and b), 19 in stage IV (a and b), and 11 in stage V. Worse MGFA clinical classification score in the observation period correlated with the median value of the increased jitter and with conduction blocks on the initial SFEMG: the median percentages of increased jitter and blocks were 97 and 60 for stage V, respectively; 90 and 50 for stage IV; 94 and 41 for stage III; 33 and 9 for stage II; and 30 and 7 for stage I, respectively.

The extent of the initial SFEMG abnormalities correlates with the severity of the later clinical course of MG. The prognostic value of the initial SFEMG results for the long term MG course thus seems confirmed.

✶PF3

PS3-313 / #423

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Prognostic factors in autoimmune myasthenia gravis

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Background: Predicting which myasthenia gravis (MG) patients are at risk for a more severe disease course would help in planning therapeutic interventions.

Objectives: To identify prognostic factors for the disease course in myasthenia gravis.

Methods: We included patients diagnosed with MG and under treatment at our tertiary medical centre between 1993 and 2013. Autoimmune MG was defined as clinically confirmed fluctuating muscle weakness with autoantibodies to the acetylcholine receptor (AChR-MG), or muscle-specific kinase (MuSK-MG), or no detectable antibody together with abnormal decrement (at least 10%), increased jitter in single-fiber EMG or a positive neostigmine test (seronegative myasthenia gravis, SNMG).

An exacerbation was defined as a clinical deterioration that required an increase of the daily prednisone dose of at least 20 mg or the undergoing of an emergency treatment, defined as administration of intravenous immunoglobulins, plasmapheresis or intubation.

Results: We included 96 patients with MG. Late age at onset (50 years or older) was significantly associated with a higher risk of experiencing one or more exacerbation(s) within 3 years of follow-up (odds ratio [OR] = 9.33, 95% confidence interval [CI] = 2.43-35.87; $p=0.001$). Late age at onset also predicted a higher occurrence of one or more emergency treatment(s) within 3 years (O$R=5.25$, 95% CI = 1.21-22.80; $p=0.027$). The presence of one or more other autoimmune disease(s) in the patient correlated with a higher risk of experiencing one or more exacerbation(s) within 3 years (O$R=4.03$, 95% CI = 1.09-14.85; $p=0.036$). The same trend was observed for the occurrence of one or more emergency treatment(s) within 3 years, although non-significant (O$R=3.64$, 95% CI = 0.94 - 14.11; $p=0.062$). Patients with one or more other autoimmune disease(s) and with late onset of disease had a higher risk for both exacerbations (O$R=47.00$, 95% CI = 6.49-340.65; $P<0.001$) and emergency treatments (O$R=26.11$, 95% CI = 4.12-165.55; $p=0.001$) as compared to patients with no other autoimmune diseases and early onset of disease.

Conclusion: Late age at onset and the presence of one or more other autoimmune disease(s) were associated with a higher risk of experiencing an exacerbation of the MG and undergoing an emergency treatment within 3 years of follow-up.

★PF3

PS3-314 / #443

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

HLA-DRB1*01 in late onset Myasthenia gravis

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Introduction: The most important genetic loci implicated in the etiology of Myasthenia gravis (MG) are located in the Human MHC chr 6p21.3 region. Several studies aimed at defining the disease causative loci in MG, but the strong linkage disequilibrium of the HLA complex has made this search difficult. The first genetic studies suggested that in European descendent populations the HLA-DRB1*03 allele strongly influences susceptibility to MG. Compston in 1980 first addressed the role of different HLA alleles in early onset MG (EOMG) and late onset MG (LOMG), subgroups. In 2012, Harbo et al pointed out that HLA-DRB1*15:01 is the strongest risk allele for LOMG in the Norwegian population. Objective: To investigate the role of HLA-DRB1 alleles in EOMG and LOMG subgroups in a Portuguese population. Material and methods: A total of 89 MG patients (66 female and 23 male) and 282 healthy individuals (controls) were studied. Patients were classified according to the age of onset (EOMG ≤40yo, $n=55$ and LOMG ≥ 50yo, $n=10$). Patients with thymoma were excluded. The genotyping method used was PCR-SSP. Statistical analyses were performed using the Chi-square or Fisher's exact test as appropriate. Results: The frequency of HLA-DRB1*01 allele was significantly higher in LOMG patients when compared with EOMG (70.0% vs 20.0% O$R=9.333$, CI=2.072-42.051, $p=0.001$) and with controls (70.0% vs 23.4% O$R=7.636$, CI=1.921-30.364, $p=0.001$).

As expected HLA-DRB1*03 was increased in both subgroups. Conclusion: Our results demonstrate an association between HLA-DRB1*01 allele and LOMG, suggesting that this allele could be a susceptibility factor for LOMG in the Portuguese population. To our knowledge this is the first study to report this association.

PS3-315 / #445

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

HLA-DRB1 is positively and negatively associated with Myasthenia Gravis

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Introduction: Myasthenia gravis (MG) is a rare antibody-mediated autoimmune disease characterized by progressive weakness and fatigue of the voluntary musculature. Like most autoimmune disorders, MG is a multifactorial, non-inherited disease with an established genetic constituent. Over the years association of MG with Human Leucocyte Antigens (HLA) has been described in different populations. The first genetic studies suggested that in European descendent populations the HLA-DRB1*03 allele strongly influences susceptibility to MG.

Objective: To investigate if different HLA-DRB1 alleles have different roles in MG.

Material and methods: Eighty nine MG patients (66 female and 23 male) and 282 healthy individuals (controls) were studied. HLA-DRB1 genotyping was performed using polymerase chain reaction with sequence-specific primers (PCR-SSP). Statistical analyses were performed using the Chi-square or Fisher's exact test, as appropriate. Results: The frequency of HLA-DRB1*03 allele was significantly higher in patients in comparison with controls (34% vs 16%, O*R*=2.821, CI=1.682-4.970, *p*=0.0001). The HLA-DRB1*13 allele frequency was significantly lower in patients when compared with controls (18% vs 30%, O*R*=0,517, CI=0,284-0,940, *p*=0.029).

Conclusion: These results demonstrate a strong association of HLA-DRB1*03 with MG, confirming that this allele is an important susceptibility factor for this disease. Our data also supports the role of HLA-DRB1*13 as a protective allele for MG. This association was also recently reported in a Norwegian population and is frequently observed in other autoimmune diseases such as Multiple Sclerosis and Systemic Lupus Erythematous. This fact deserves our attention.

PS3-316 / #453

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

The Follow-up of the patients with Myasthenia Gravis in a period of 5 years

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Background: MG is an autoimmune neuromuscular disease caused by circulating antibodies that block acetylcholine receptors at the postsynaptic neuromuscular junction.

Objective: To evaluate the progression of the disease in different patients, with different classes of MGFA at the beginning of the evaluation.

Methods: In our prospective study a group of 45 patients with MG was evaluated in a first moment, and after 5 years. The evaluation was made using the MGFA clinical classification.

Results: We have evaluated 45 patients with the MGFA clinical classification. The clinical evaluation in the first presentation to our Neurology Service was: 23 patients were in class II-A, 5 patients were in class I and 17 patients were in class II-B. Someone of these patients have used PEX procedure, Ig-iv. Some of the patients have done thymectomia. The majority of the patients were positive for the AcACH and a small number of cases were AntiMusk positive.

We have evaluated the patients after 5 years and most patients of class I and II-A at the beginning, were better after this period of time(with therapy: farmacological therapy e/or PEX e/or Ig-iv e/or thymectomia), but we found that patients in class II-B at the first examination, had not the same clinical improvement as the group of patients in class I and II-A and some of them were in class III-B after 5 years.

Conclusions: We have found a better clinical improvement in the patients in class I, II-A then in patients that were in class II-B at the first clinical examination.

PS3-317 / #456

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Clinical characteristics of a sample of myasthenia gravis patients with dropped head syndrome

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Dropped head syndrome (DHS) refers to a clinical sign secondary to prominent weakness of neck extensor muscles and has been described in different neuromuscular conditions including MG. So far, the majority of patients with myasthenia and DHS were described as single case reports.

We reviewed the clinical characteristics of a sample of eleven patients with MG admitted at two different centres in Buenos Aires (Hospital Churruca-Visca and FAIAM) from 2008 to 2013 who presented with DHS at any stage of the disease. All the patients had a diagnosis of MG confirmed either by electromyography or antibody testing.

The median age was 64 years (54.5% men). Bulbar symptoms were detected in 72.7% of the sample whereas ocular symptoms appeared in 45.5%. We observed that 63.6% of the patients tested positive for acetylcholine receptor antibodies (AChRA) and 18.2% for antibodies against Muscle-Specific Kinase Receptor (anti-MuSK). Three patients had thymic pathology (2 thymic hyperplasia and 1 thymoma). Regarding treatment 81.8% of the patients required treatment with steroids; within this group 55.5% needed additional treatment with another immunesupressor. In this sample the majority of patients with DHS had bulbar features, were positive for either antibody and required immunesuppresive treatment to manage clinical symptoms. These results might indicate that the clinical characteristics and course of MG patients with DHS could be different from those without this clinical sign.

PS3-318 / #521

Theme: *4.1 - Diseases of neuromuscular junction: Mysthenia gravis*

Myasthenia gravis in senegalese children: 10 years follow up at Fann teaching hospital, Dakar

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Background: Many studies enlights differences between the characteristics of myasthenia in children and adults depending on age of onset of first symptoms. Similarly evolution would depend on conditions of care.

Objectives: Determine epidemiological, clinical, biological, electromyographic, evolutive aspects of myasthenia gravis in children in Senegal

Methods: Retrospective study of a cohort of children from july 2003 to july2013

Results: 20 children aged from 1 to 17 years with an average of 6.75 years were followed. Most of them had between 6 and 10, 70% were girls. Clinical signs were dominated by ptosis (65%) and fatigability. The blood level of acetylcholine receptor antibodies was

normal in 8 patients, high in 11 patients. Electroneuromyography found decrements. Radiography performed in all our patients, allowed to find an enlargement of the anterior mediastinum in 2 patients who have had a chest CT scan with strongly evocative images of thymoma. Classification according to Osserman had found 15 patients with stage I, stage IIa 01 patient and 04 other stage IIb. All patients received anticholinesterasic , 2 had a one-time corticotherapy. None received immunotherapy or had had plasmapheresis. Evolution has been marked by an improvement in 14 patients, a regression of clinical signs in 4 patients and 2 died in a myasthenic crisis by respiratory failure.

Conclusion: Lack of availability of certain molecules and inappropriate emergency care infrastructure underscore the disparities that exist in evolutivity in our patients.

PS3-319 / #559

Theme: *4.1 – Mysthenia gravis*

Myasthenia gravis with double antibodies positivity (AchRAb and anti-MuSK) associated with severe thymic maligrancy: Description of 6 patients

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Introduction: Antibodies to muscle-specific kinase (anti-Musk Ab) are usually identified in the serum of 10% myastenic patients without anti-AChR Ab. Presence of both AChR-Ab and MuSK-Ab in the same patient affected by Myasthenia Gravis (MG) is a very rare phenomenon.

The aim of this study was to analyze the clinical features of 16 positive MG patients for both AChR and Musk antibodies associated with thymoma and to correlate this singular finding with the patients' thymic pathology.

Materials and Methods: Anti-AChR and anti-Musk antibodies were both detected in the serum of 16 MG patients (8 women, 8 men; mean age at the onset disease: 42.18 ± 13).

Follow-up has been conducted from 1998 to 2013 (average length of follow-up: 7.8 ± 6 years). According to MGFA Classification, 5 patients have been considered as type IIB, 7 patients as type IIIB, 2 patients as type IVB and 2 patients as type V.

All patients underwent thymectomy (mean age at thymectomy: 43.61 ± 13). The presence of thymoma was confirmed by histological findings. ot useful to improve the clinical status of the disease

All patients had thymoma and a significant presence of malignity has been highlighted: 10/16 thymomas were highly invasive and 2 patients had a relapsed thymoma.

Coexistence of anti-Musk and anti-AChR antibodies in the same MG patient is very rare. ACHEI treatment often causes cholinergic side effects in MG patients with double positivity. Additionally, double positive patients have often a severe thymic disease characterized by a thymoma with high grade of malignity. We can conclude that the coexistence of the two types of antibodies is correlated with a poor tolerance to ACHEI treatment, as it happens in anti-MuSK patients, and with a severe thymic malignancy.

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✶PF3

PS3-320 / #109

Theme: *4.2 - Diseases of neuromuscular junction: Myasthenic syndromes*

Missense mutations of agrin are responsible for a presynaptic form of congenital myasthenic syndrome with distal myopathy

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Congenital myasthenic syndromes (CMS) result from impaired neuromuscular transmission at the neuromuscular junction (NMJ). Their clinical hallmark is fatigable muscle weakness with decremental muscle response to repetitive nerve stimulation at EMG. Distal myopathies are another group with muscle weakness restricted to distal muscles and related to primary muscle damages. In both groups remain a portion of patients without any mutation in the known genes. We report a new and homogenous clinical entity combining CMS with distal myopathy in 5 patients from 3 families. All patients shared distal limb muscle wasting and weakness that did not respond to cholinesterase inhibitors. MRI and neurophysiological changes were in accordance with mild myopathy restricted to distal limb muscles, as were decremental responses recorded in response to repetitive nerve stimulation. Post-exercise facilitation was observed in the distal muscles in all cases, which is a feature characteristic to presynaptic CMS. Muscle biopsies analyses from 2 unrelated patients did not detect myopathic changes. Immunofluorescent and ultrastructural analyses of muscle end-plate regions showed synaptic remodelling with denervation-reinnervation. Whole-exome sequencing performed in 2 kinships identified 4 recessive mutations in the gene encoding agrin, the basement membrane proteoglycan with critical function for the NMJ, which was already associated with unspecific forms of CMS. This new association was further confirmed by the identification of a fifth AGRN mutation in an unrelated consanguineous patient with CMS and distal myopathy. Our findings expand the spectrum of CMS due to agrin mutations and show a striking correlation between the mutated gene and the associated phenotype. They provide a good rationale to search for agrin mutation in patients with distal myopathy who might indeed suffer from CMS.

PS3-321 / #362

Theme: *4.2 - Diseases of neuromuscular junction: Myasthenic syndromes*

T cell activation and differentiation in the Lambert-Eaton myasthenic syndrome

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Background: Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disorder associated with pathogenic antibodies against voltage-gated calcium channels. Although the humoral immune response has been well-characterized, few studies have described the cellular immune response in LEMS patients.

Methods: We prospectively studied the peripheral blood lymphocyte subpopulations of LEMS patients visiting our tertiary centre. Control data were obtained from age-matched transplantation donors. All lymphocyte subpopulations were analysed by flow cytometry (BD FACS Calibur II, Franklin Lakes, NJ, USA).

Results: A total of 13 LEMS patients (including 4 with associated small cell lung cancer (SCLC)) were studied and compared to 18 healthy controls. An increase in HLA-DR+ CD8 T cells was observed in LEMS patients, especially in those with SCLC (Figure 1). T cell differentiation stages in LEMS showed a variable distribution in CD8 T cells, with a trend to an increased proportion of end-stage effector cells (EMRA) and a decrease of naïve cells, especially in SCLC (Figure 1). CD4 T-cell activation and differentiation stages were normal. A decrease in NK cells in three patients and lymphopenia in another was most likely related to treatment.

Discussion: We have shown an increase in activated CD8 T cells in LEMS patients. Although the current study is explorative in nature, CD8 T cell differentiation stages in individual LEMS patients appear to differ markedly from our healthy controls and previously established normal values. These observations in the CD8 T cell compartment suggest a role for the cellular immune response in paraneoplastic as well as non-tumour LEMS.

PS3-322 / #155

Theme: *4.3 - Diseases of neuromuscular junction: Congenital myasthenia*

ColQ controls Acetylcholine receptor mRNA levels by a post-transcriptional mechanism : Implication for the Congenital Myasthenic Syndrome with Acetylcholinesterase deficiency

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Collagen Q (ColQ) is a specific collagen that anchors acetylcholinesterase (AChE) in the extracellular matrix at the neuromuscular junction (NMJ). The an-

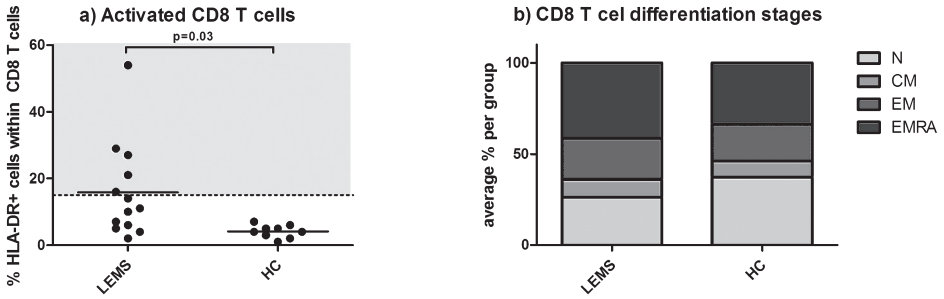

Figure 1 CD8 T cell activation and differentiation in Lambert-Eaton myasthenic syndrome (LEMS)
a) Activated CD8 T cells as measured by HLA-DR+ surface expression in patients with LEMS and healthy controls (HC).
b) CD8 T cell differentiation stages: naïve (N), central memory (CM), effector memory (EM) and end-stage effector T cells (EMRA).

choring of AChE requires the interaction of ColQ with Muscle-Specific Kinase (MuSK) (Cartaud et al, 2004), a tyrosine kinase receptor expressed on the muscle membrane that is necessary for the formation and the maintenance of the NMJ. MuSKforms with its co-receptor LRP4 a complex which binds several ligands including agrin, Wnts and ColQ. This complex represents the core system from which the postsynaptic domain is built. The discovery that ColQ binds to MuSK prompted us to explore a possible muscle signalling function of ColQ. We have previously shown that the absence of ColQ induces an increase in mRNA levels of all Acetylcholine Receptor (AChR) subunits, a mechanism that is believed to be an adaptation to Acetylcholine increase in the synaptic cleft (Sigoillot et al., 2010). Our objective is to now dissect the molecular cascade which links ColQ to AChR mRNA regulation. Our data demonstrate that[1] ColQ regulates the AChR subunit mRNA levels by a post-transcriptional mechanism of mRNA stabilization,[2] AChR transcripts contain in their 3'UTR a consensus binding sites for HuR (ARE binding element),[3] HuR, a RNA binding protein, binds the 3' end of AChR subunit mRNA and stabilizes these transcripts,[4] ColQ and the p38 pathway regulate HuR levels,[5] This molecular cascade is at least partially mediated by MuSK. These results were obtained from a ColQ muscle deficient cell line and from ColQ deficient mice, an animal model for the Congenital Myasthenic Syndrome (CMS) with AChE deiciency. These results open the door to new therapeutical targets for this CMS and potentially other diseases of the neuromuscular junction.

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★PF1

PS3-323 / #519

Theme: *4.3 - Diseases of neuromuscular junction: Congenital myasthenia*

Molecular mechanisms of RAPSN mutations in congenital myasthenic syndromes

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Rapsyn mutations form a major subtype of the congenital myasthenic syndromes (CMS). At the postsynaptic membrane of the neuromuscular junction, RAPSN interacts directly with acetylcholine receptors (AChR) and is essential for the formation of AChR clusters. Mutations in the RAPSN gene have an overall effect of causing a deficiency of AChR at patient endplates, possibly through a variety of molecular mechanisms that disrupt RAPSN function. RAPSN-CMS is autosomal recessive, with a common mutation, p.N88K, either homozygous or heterozygous in over 90% of patients. We have investigated the pathogenic properties, of rarer mutations identified from patients who do not harbor promoter mutations or the common mutation pN88K: p.V45M, p.R91L and p.A153T.

Mutations identified from patients were introduced into wild-type RAPSN cDNA, and then subcloned into expression vectors. These RAPSN variants were transiently expressed in TE671 cells to determine the expression and stability of the protein. To further explore pathogenic mechanisms, the RAPSN variants were introduced into rapsn$^{-/-}$ myoblasts using the pBabe-PURO retroviral system for AChR clustering assay where the effect of the mutations on the formation of AChR clusters and the stability of AChR clusters formed were assessed following differentiation to form rapsn$^{-/-}$ myotubes. . The mutation p.V45M was found to have a low steady state level of expression ($p<0.001$ vs wild type). The stability of the other RAPSN protein variants were also found to be affected by the mutations, though the effect is less marked than for p.V45M. The mutations impair AChR clustering ($p<0.001$) and carbachol induced AChR dis-

persion studies reveal that the AChR clusters formed by mutant RAPSN disperse more rapidly than wild type ($p < 0.01$).

The mutations investigated were all found to impair AChR cluster formation. Present data suggest that RAPSN protein stability is likely to contribute to an increased instability of AChR clusters formed with mutant RAPSN. Thus, this group of RAPSN mutations, which have all been found to cause a myasthenic syndrome in the absence of the N88K mutation, may all have a similar underlying molecular mechanism of causing reduced endplate AChR expression through reduced RAPSN protein and clustered AChR stability.

PS3-324 / #544

Theme: *4.3 - Diseases of neuromuscular junction: Congenital myasthenia*

The spectrum of DOK7 congenital myasthenia in Northern Ireland

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DOK7 congenital myasthenic syndromes (CMS) are reported to be the third most common cause of CMS in the UK. They are inherited in an autosomal recessive manner and are most often caused by missense or frameshift mutations in exon 7 causing premature truncation of the protein. They are and have a broad phenotype. Most importantly symptoms improve with salbutamol or ephedrine but can be exacerbated by acetylcholinesterases.

The cases described were identified as part of a population-based epidemiological study of myasthenic syndromes in Northern Ireland between 2000 and 2009. All individuals and their parents and/or relatives (when available) were examined clinically and electrophysiologically. Sequencing of DOK7 was performed by direct sequencing of PCR amplicons containing the seven exons and the ?anking non-coding regions of the DOK7 gene and con?rmed by restriction digest analysis (enzymes from New England Biolabs)

DOK7 mutations were the second most common cause of CMS in the region (population 1.7 million) after AChR subunit mutations. Seven genetically confirmed cases were identified giving a crude prevalence of 4.2 cases/million. There were 4 unrelated individuals and 3 siblings; 5 females, 2 males; mean (S.D.) age at onset 22.4 (27.1)years; range 0-62 years. Early-onset cases had generalised weakness and low muscle bulk without ophthalmoplegia and a characteristic gait. Late-onset cases had exclusively limb-girdle weakness. Fatiguability was reported by most (4/7) as variable week-to-week and was not easily demonstrable clinically. Muscle histology showed minimal changes. All patients had at least 1 mutation in exon 7 and 4 carried the common c.1124_1127dupTGCC mutation. All treated patients showed significant functional improvement on salbutamol (4/7) or ephedrine (2/7). One patient died from neuromuscular respiratory failure and was diagnosed post-mortem.

DOK7 mutations are a common and treatable cause of CMS. The broad phenotype and lack of characteristic myasthenic features can make diagnosis challenging.

PS3-325 / #555

Theme: *4.3 - Diseases of neuromuscular junction: Congenital myasthenia*

Congenital myasthenic syndrome: Identification of a MuSK mutation that results in exon 9 skipping and could impair the agrin system

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We report the case of a severe CMS due to a mutation in MUSK. Gene analysis identified a homozygous missense mutation, c.1182C>T in exon 9. Total ARN extracted from the muscle biopsy of the patient revealed a deletion of the whole exon 9. The result of this mutation is a protein (MuSK del_ex9) which lacks 88 aminoacids, truncating a large part of the extracellular CRD domain of MuSK.

Two muscle biopsies were made at 15 y and 17 y and analyzed. In the 1st biopsy, there was a simplification of the neuromuscular junctions characterized by fragmentation and dispersion of the synaptic gutters and decreased nerve terminal branches. In the 2nd biopsy, neuromuscular junctions were small with a few synaptic cups connected by an "en passant" innervation through without ramifications. In addition several neuromuscular junctions did not express agrin associated with a decrease in the number of acetylcholine receptors (AChRs).

To analyze the consequences of the mutation, vectors expressing wild-type or del_ex9 MuSK were used. Agrin-dependent phosphorylation of del_ex9 MuSK was decreased by 75% while Dok7-dependent phosphorylation of del_ex9 MuSK was decreased by 50% both in comparison with wild-type MuSK. In electroporated mouse muscle, overexpression of the del_ex9 mutation induced within 3 weeks major changes. Indeed while one third of the neuromuscular junctions expressed agrin, MuSK and AChR altogether, the two other thirds had a diminished expression of agrin or MuSK but still expression of AChRs.

The question arises: what allows the expression of AChR in the absence of agrin and MuSK? We quantified the mRNAs of the human biopsy and showed that neuregulin-2 mRNA was 5 times more expressed than in control human muscle. It has been shown that neuregulin-2 could be synthesized by terminal Schwann cells and activates transcription of AChR (Rimer et al, Mol Cell Neurosci 2004; 26:271-281). We hypothesize that neuregulin-2 could be responsible for the expression of AChR in the absence of the agrin-MuSK system.

PS3-326 / #556

Theme: *4.3 - Diseases of neuromuscular junction: Congenital myasthenia*

Congenital myasthenia due to AGRN c.5125G>C: Does the mutated agrin perturb motoneuron differentiation?

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We reported a case of a CMS patient carrying a homozygous mutation c.5125G>C in AGRN. Agrin recombinant proteins, either normal or reproducing the mutation, were produced and have allowed us to show that the mutation does not affect the formation of the neuromuscular junction (NMJ) but is deleterious for its maintenance. The main changes observed were fragmentation of the synaptic gutters and disassembly of neurofilaments in nerve terminals both in patient muscle biopsy and in rat injected with mutated recombinant agrin (Huzé et al, Am J Hum Genet 2009 ; 85:155-167).

The purpose of this study was to analyze whether the observed changes in nerve terminal cytoskeleton reflected the perturbation of the motoneuron differentiation. To analyze this hypothesis, we injected the 2 recombinant agrins (wild-type and mutated) in the soleus muscle of rodents and collected muscle specimens up to 15 days after the injections. The wild-type agrin only slightly modified the native NMJs. The mutated agrin however significantly increased the area of the synaptic gutters and the number of synaptic nerve endings.

The injection of both types of agrin induced the formation of many extrasynaptic AChR clusters. However mutated agrin alone induced an axonal

exuberance that led to the innervation of AChR clusters sometimes located on the same muscle fiber (multiinnervation).

Finally we analyzed the pattern of innervation of a motor nerve innervating the Levator auris longus muscle and found that application of mutated agrin increased both the number of primary branches of this nerve as it enters the muscle and the number of nerve endings (either increased number of axonal branches innervating a given NMJ or induction of the formation of new NMJs).

To check if mutated agrin mutant had a similar effect *in vitro*, we added the two types of agrin to cultured rat embryo motoneurons and found that mutated agrin increased 2.5 the length of the axons.

One can hypothesize that abnormality of axon growth *in vivo* and *in vitro* after application of mutated agrin depends on a trophic factor still to be determined.

PS3-327 / #50

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Takotsubo cardiomyopathy associated with Guillain–Barré syndrome

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The Guillain-Barré syndrome (GBS) is an autoimmune disease involving the peripheral nervous system. Autonomic dysfunction is a common complication in GBS. Among them, a cardiac involvement is relatively common and fatal.

Takotsubo cardiomyopathy is a transient heart failure of unknown origin characterized by a hypokinesis of the left ventricle. This cardiomyopathy is secondary to physical or emotional stress affecting mainly postmenopausal women. Electrocardiographic and echocardiographic abnormalities are often regressive in days or weeks.

In GBS, in addition to autonomic dysfunction involving the heart, Takotsubo cardiomyopathy often occurs.

A 69-year-old woman presented with a 1-day progressive quadriparesis and a hyporeflexia. Cervical spine MR imaging was not significant. Nerve conduction study was suggestive of sensorimotor polyneuropathy, axonal type. These findings were consistent with diagnosis of GBS and a treatment by intravenous immunoglobulin over a 5-day period was started.

At hospital day 2, she was in respiratory distress. And mechanical ventilation was started. On the same day, electrocardiography was suggestive of acute coronary syndrome and cardiac marker was elevated. She did not have any symptoms. Trans-thoracic echocardiography revealed left ventricular inferior wall and apical akinesia, and decreased ejection fraction. But, coronary arteriography did not reveal coronary artery stenosis. A diagnosis of Takotsubo cardiomyopathy was then made. Low blood pressure was treated with intravenous dobutamine. After the blood pressure was stable, angiotensin-converting enzyme inhibitor was administered. Left ventricular dysfunction and electrocardiography normalized within one month.

In GBS, in addition to autonomic dysfunction involving the heart, Takotsubo cardiomyopathy can occur. GBS occurrence can be the stressful trigger of Takotsubo cardiomyopathy. This cardiomyopathy needs adequate management. Therefore, frequent monitoring is needed and trans-thoracic echocardiography should be performed when electrocardiographic abnormalities are present in this disease to rule out a Takotsubo cardiomyopathy.

PS3-328 / #95

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Steroid responsive chronic hypertrophic brachial plexus neuropathy – A case of focal CIDP

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Hypertrophic neuropathy of brachial plexus is a rare condition that shows brachial plexus dysfunction with evidence of brachial plexus enlargement, often detected by MRI. This can be a form of localized hypertrophic neuropathy which is now considered a benign peripheral neoplasm, but there are also some reports of other conditions such as multifocal motor neuropathy, or focal CIDP.

Case report: A 58 year old patient visited our out patient clinic due to 10 year history of slowly progressive left arm weakness, tingling and sensory disturbance. He had taken oral antihypertensive medication but had no other past medical history including diabetes, and denied family history of neurological disease. On neurological examination, there were motor deficit involving distal left arm: elbow flexion MRC grade IV, extension IV+, pronation/supination GIV, wrist flexion/extension GIV-, hand grip GIV-. There were sensory deficit of light touch, position, vibration sense with sparing of pin prick and temperature sensation. Deep tendon reflexes couldn't be elicited. He had mild muscle atrophy of left forearm and hand. Spine MRI which was taken 1 year ago was not remarkable. Electromyographic study result was compatible with brachial plexopathy involving middle and lower trunk. So, we took MRI and ultrasound study for brachial plexus, and revealed brachial plexus enlargement and proximal median nerve enlargement. With the impression of focal CIDP, we wanted to perform CSF study, but the patient refused. One month after oral steroid therapy, we could find improvement of motor power: elbow flexion/extension V, pronation IV+, supination V, wrist flexion IV+, extension IV+, hand grip IV+, but sensory deficit and areflexia were not improved.

There is one report of chronic hypertrophic brachial plexus neuropathy responsive to intravenous immunoglobulin. This case and our case have something in common: long history of localized hypertrophic brachial plexus neuropathy and rapid response to standard CIDP treatment. Furthermore, our case showed other characteristics of CIDP: generalized areflexia and large fiber sensory deficit. So, we concluded that this case is, most likely, a focal form of CIDP.

✶PF4

PS3-329 / #110

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Anti-myelin-associated glycoprotein neuropathy - a carbohydrate polymer effectively blocks pathogenic anti-myelin-associated glycoprotein antibodies

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Anti-myelin-associated glycoprotein neuropathy is an antibody-mediated demyelinating peripheral neuropathy. The disease is caused by immunoglobulin M (IgM) autoantibodies recognizing the Human Natural Killer-1 (HNK?1) carbohydrate epitope. A specific hallmark of this epitope is an unusual sulfated glucuronic acid moiety. This glyco-epitope is highly expressed in adhesion molecules such as the myelin-associated glycoprotein (MAG), present in myelinated nerve fibers. Since MAG is involved in adhesion and signaling processes at the axon-glia interface, antagonists that block the antibody binding to MAG are of therapeutic and diagnostic interest.

Our approach was to design carbohydrate ligands that block the IgM antibody binding sites by mimicking the natural epitope. A minimal HNK-1 epitope, consisting of a sulfated saccharide, was synthesized. In a competitive binding assay, it successfully inhibited the binding of pathogenic IgM antibodies from patient sera to MAG, although only at micromolar concentrations. A comparison with the unsulfated saccharide indicated the prerequisite of the sulfate group for antibody binding. To mimic the multivalent nature of the MAG / IgM antibody interaction, a polylysine polymer presenting the minimal HNK-1 epitope in a multivalent manner was prepared. Binding affinity was thereby increased by a factor of at least 34000, with inhibitory activity now being in the low nanomolar range. Potential applications of this glycopolymer are in the diagnosis as well as the treatment of anti-MAG neuropathy.

PS3-330 / #120

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Long –term prognosis and health – related quality of life (HRQol) in multifocal motor neuropathy (MMN)

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Introduction: MMN evolves with asymmetric weakness,conduction blocks (CB), antibodies to glycolipid GM1. Purpose of our study was to assess if de mographic,clinical,neurophysiological variables could be useful to identify disease progression in MMN.

Methods: Forty one Caucasian patients (34 males and 7 females,median age of onset 47 yrs) were followed for median duration of 92 months (range 12-264).Eight patients (19,5%) had GM1 IgM antibodies at diagnosis,36,5% became positive during study frame.Upper extremity (UE) tremor was observed 60% of patients.Strength was assessed separately in UE,LE with Medical Research Council Scale (MRC),disability with Overall Disability Sum Score(ODSS)and Ranking scale.Effects of IVIg treatment on progression was included in analyses conducted at 1,3,5,10,15yrs since onset by separate Mann-Whitney U test and Wilcoxon matched pair test.Human leukocyte antigen (HLA) antigen distribution was compared between patients and 3,528 controls.Health-related quality of life (HRQol) was assessed using Short-Form Health Survey (SF-36)

Results: .At 1 and 3 years,total MRC score and the subscore related to lower extremities significantly decreased (T=113; $p=0.009$ and T=70,5;$p=0.002$,respectively)without benefit from IVIg.At 10 years,overall MRC subscores significantly decreased($p=0.003$ and 0,001).There was no significant differences between demographic features,number of definite CBs,disability outcome measures.Analysis of distribution of 9 selected HLA allelles with frequency > o$r=15\%$ either in patients and controls showed that DQB1*06 prevailed in anti GM1 -positive MMN ($p=0,02$)

Conclusions: Our results provide evidence that MRC grading is reliable prognostic marker The finding of HLA DQB1*06 prevalence in patients with detectable anti GM1 confirms that HLA locus contributes to immune response

★PF4

PS3-331 / #184

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Immunoglobulin treatment in patients with Multifocal Motor Neuropathy: Insights from the SIGNS Registry

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Human immunoglobulins (IG) are established for substitution therapy in patients with primary (PID) or secondary immunodeficiencies (SID), and for immunomodulation in patients with neurologic autoimmune diseases (NAID, e.g. Chronic Inflammatory Demyelinating Polyneuropathy or Multifocal Motoric Neuropathy (MMN). In Germany, of the available 16 IG, 4 intravenous (IV) preparations have been approved for NAID (3 in CIDP, 1 in MMN [Kiovig], all 4 for Guillain-Barré Syndrome). Data on the utilisation of IG in patients with MMN are limited and thus of particular interest.

The SIGNS registry assesses all licensed IG in a long-term non-interventional study (ClinTrials.gov Identifier NCT01287689) in 66 centres throughout Germany. Currently 616 patients with PID, SID or NAID on maintenance or newly initiated IG therapy are documented. Outcomes comprise drug utilization (choice of drugs, dosage), effectiveness (function in NAID), tolerability, quality of life and therapy costs up to 4.5 years of follow-up. In an interim analysis dated 19. January 2014, 60 patients with MMN were included (age 55.3 ± 10.5 years, 68% men, duration between first symptoms until study entry 11.6 ± 11.2 years; duration between diagnosis until study entry 6.4 ± 6.3 years. For 52 patients detailed information were provided on a specific MMN data form. Conduction block was reported in 39 patients (75%), of whom 27 according to EFNS/PNS criteria. Clinical status was reported as stable without deficits in 6 patients, as stable with deficits in 38 patients, and as progressive disease in 5 patients (3 patients no information). No reduction in strength overall within 4 weeks prior therapy was reported in 30/52 patients (57.7%), reduced arm muscle strength in 21 (40.4%) and reduced leg muscle strength in 9 (17.3%) patients.

At entry, 5 different IV and 1 SC IG preparations were used in the 60 MMN patients. Patients received IV IG at a mean dosage of 0.75 g/kg body weight (range 0.2 - 2.0 g/kg BW). The mean infusion interval was 4.8 weeks (range 0.4-17.3). Patients received IV IG as maintenance treatment at a mean cumulative dosage of 0.9 g/kg BW/ 4 weeks (range 0.1 - 8.0 g/kg BW/ 4 weeks). The majority of MMN patients (83.0%) were treated with the recommended dose of ≥0.2g/kg BW /4 weeks.

As expected patients with MMN had substantially reduced motor function. IG are used in a wide range in MMN, however compared to other NAID indications, on average at higher IG doses.

PS3-332 / #189

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Prognostic factors and health-related quality of life (HRQol) in polyneuropathy with IgM antibodies to myelin associated glycoprotein (MAG)

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Introduction: Polyneuropathies with IgM antibodies to MAG are immunologically mediated disorders. Purpose of this cohort study was to assess effects on disease progression. of demographic(age of onset,gender),neurophysiological variables,as type of neuropathy either axonal or demyelinating at the diagnosis .

Methods: Forty Caucasian patients (25 males,15 females, median age 70.5 yrs) were followed for a median duration of 91 months (range 12-225).Median anti-MAG titer determined by ELISA was 17,452 U. Electrophysiological type of neuropathy (demyelinating,axonal or mixed),muscle strength (assessed with Medical Research Council Scale,MRC),disability (assessed with Overall Disability Sum Score,ODSS,Ranking scale),type of treatment,serum IgM level were included in the analyses.Worsening was considered significant if MRC difference between first and last esamination was at least 12 points .Survival analysis with Cox regression model was performed. Human leukocyte antigen (HLA) antigen distribution was compared between patients and to 3,528 controls. Health-related quality of life (HRQol) was assessed using Short-Form Health Survey (SF-36)

Results: Survival analysis showed that patients with higher IgM level ($p = 0.11$), electrophysiological

evidence of demyelinating damage ($p=0.05$),absence of either immnunomodulating, immunosuppressive during disease course (p.0021) had significantly higher risk of clinical worsening.Analysis of distribution of 9 selected HLA allelles with frequency > o$r=15\%$ either in patients or controls showed that B44 and DRB1*07 prevailed significantly in patients ($p=0.004$ and=0.03 respectively) Variations of clinical measures did not affect HRQol.

Conclusion: IgM level,electrophysiological type of neuropathy at onset/diagnosis could be considered prognostic markers in polyneuropathies with IgM antibodies to MAG. The finding of HLA B44 and DRB1*07 prevalence in patients could point out possible association of anti-MAG antibody production with this molecule

PS3-333 / #190

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Retrospective review of the safety of Hizentra in three neuromuscular patients with renal failure

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Background: All forms of immune globulin contain a black box warning for the potential for renal failure. This risk is increased in patients with pre-existing renal disease and often makes it impossible to treat these patients with intravenous immune globulin. To date there is no data on the safety of subcutaneous immune globulin in patients with renal disease.

Methods: Three patients with neuromuscular diseases who had renal failure were treated with Hizentra subcutaneously. Patients were monitored with labs to evaluate electrolytes and glomerular filtration rate.

Case 1. A 56 year old male was status post renal transplant and developed CIDP. The patient was on tacrolimus, cyclosporine, and prednisone. He had significant chronic renal disease with a GFR of 37. He responded initially to plasmaphresis. He was started on Hizentra 5 grams twice a week and over 1 year he had a return to normal strength in almost all muscles. His renal function iremained stable with a his last GFR of 47.

Case 2. A 65 year old woman was diagnosed with myasthenia gravis and a stage IV thymoma. She underwent thymectomy and was started on prednisone, mestinon, and chemotherapy. 2 years later a recurrence of her thymoma led to increased weakness. She had mycophenylate added and she was started on 2 grams/kg/month of IVIG and had a marked improvement in her myasthenia. However her GFR fell from 67 to 29 and the IVIG was stopped. She then developed increased muscle weakness and she was started on Hizentra 10 grams twice a week. She has continued on this dose for six months and her GFR has stabilized at 30.

Case 3. A 37 year old male with diabetes was diagnosed with CIDP and was started on IVIG 2 grams/kg/month. He had an excellent recovery in his strength but developed progressive renal failure with a GFR of 47. He was switched to Hizentra 10 grams twice a week with excellent management of his symptoms. His GFR has remained stable.

Conclusion: We have treated three patients with Hizentra who have been unable to receive IVIG because of renal failure. These patients tolerated the Hizentra well and despite significant pre-existing renal dysfunction were able to receive the subcutaneous immune globulin with no decline in their renal function. Although larger studies are needed this suggests that some patients may be able to tolerate Hizentra who have pre-existing renal disease. If these patients are treated with Hizentra they require very careful monitoring of their renal function.

PS3-334 / #219

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Clinical and electrophysiological factors associated with response to steroids treatment in chronic inflammatory demyelinating polyneuropathy (CIDP)

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Background: Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) is an immune neuropathy usually improved by intravenous immunoglobulins (IVIG) and steroids. The choice of the first line therapy is based on few controlled trials and on the risk of clinical worsening under steroids therapy.

Objectives To determinate predictive factors of a clinical response to steroids treatment in CIDP.

Methods: We retrospectively reviewed the demographic, clinical, biological, histological and electrophysiological data of all the patients treated with steroids in the department. Diagnostic criteria of CIDP were fulfilled according to EFNS/PNS (2011).

Clinical scale of motor deficiency (MRC scale, Medical Research Council), sensory deficiency (ISS, INCAT sensory sumscore), incapacity (ONLS, Overall Neuropathy Limitation Scale) and disability (modified Rankin scale) were evaluated at the diagnosis, when corticosteroids were introduced and at the last visit.

Clinical improvement or worsening after steroids was respectively defined by a decrease or an increase of at least one step on the ONLS scale.

Results: 33 patients received steroids therapy between January 1998 and January 2013. Median follow-up was 3.5 years (IQR 2-6). 16 patients (49%) were improved by steroids. 7 patients were cured (21%) and 6 were dependent to steroids (18%). 17 patients (52%) did not respond to steroids meanwhile 12 patients (36%) were clinically deteriorated after this treatment. There was 67% of good response to the steroids in the group treated in first intention versus 28% in the group treated in second intention with steroids ($p = 0.022$).

On univariate analysis, significant improvement with steroids was associated with low Rankin score at the beginning of the treatment, absence of cranial nerves involvement, disease duration less than 2 years, steroids given in first line therapy and nerve conduction study on median nerve (terminal index latency, proximal and distal latencies). On multivariate regression analysis, improvement under steroids therapy was correlated with an initial Rankin score below 1 (positive predictive value PPV 75%), absence of cranial nerves involvement (PPV 60%), and terminal index latency below 0.20 on median nerves (PPV 75%).

Conclusion: Steroids are efficient in 50% of the CIDP patients with 20% being cured. Absence of cranial nerve involvement, low initial Rankin score, and distal demyelination on median nerves are associated with good outcome under steroids therapy.

PS3-335 / #236

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Sensory mononeuritis: Differences between pure neural leprosy and non systemic vasculitic neuropathy

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Introduction: Our aim was to determine the distinguishing features of two cases of sensory mononeuritis who presented with similar clinical, electrophysiological and past medical history features.

Methods: We systematically reviewed the clinical features, laboratory studies, neurophysiologic findings, and histopathological changes of two patients with sensory mononeuritis. In one, the final diagnosis was pure neural leprosy (PNL) and the other non-systemic vasculitic neuropathy (NSVN).

Results: Our patients were females who had resided in areas endemic for leprosy (Brazil). They both developed a progressive, purely sensory, painful mononeuritis distally in the lower limbs followed, in patient 1, by asymmetric ankle edema and nodular induration without skin changes. In both cases, sensory nerve potentials were asymmetrically reduced in amplitude, and sural nerve biopsy revealed nonspecific inflammatory infiltration of the vasa nervosum in the epi- and perineurium. An axonal neuropathy, granulomas with epithelioid cells and caseous necrosis were observed in patient 1 confirming paucibacillary PNL; a skin punch biopsy revealed similar changes. Multifocal axono-demyelinating changes in patient 2 were compatible with NSVN. Both patients improved following targeted treatment (rifampicin and dapsone in case 1 and rituximab in case 2).

Conclusions: Both patients were surprisingly homogeneous in their clinical and electrophysiological manifestations. Late appearance of edema and nodular induration in the vicinity of affected nerves, as

well as, distinct pathological features with granulomas and caseous necrosis in skin or nerve biopsies appeared to be the cardinal features distinguishing PNL from NSVN.

✶PF2

PS3-336 / #238

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Gene expression changes in chronic inflammatory demyelinating polyneuropathy skin biopsies

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Objective: To determine molecular changes occurring in the skin biopsies of Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) patients and to identify biomarkers for the disease.

Methods: We performed transcriptional profiling microarray analysis on lower leg skin punch biopsies from 20 CIDP patients and 17 healthy controls to identify disease-associated expression changes. The differential expression of genes with a possible role in the pathogenesis of CIDP from ontological studies was validated by quantitative PCR (qPCR) analysis.

Results: Most of the 190 differentially regulated genes were involved in immune and inflammatory responses, nervous system development, cell adhesion, wound response, angiogenesis and apoptotic processes. The differential expression of 26 genes with a putative role in CIDP pathogenesis was confirmed by qPCR. Four downregulated genes encoded members of the MHC class II family, while 22 upregulated genes were involved in cell proliferation and tissue repair such as PDGF1, VEGFR or KDR, A2M, CAV2 and NOSTRIN. The combined upregulation of KDR/DDR2 was found in 95% of patients.

Conclusions: These findings indicate that gene expression is modified in skin biopsies of CIDP patients, with prominent changes in inflammatory pathway markers. Several repair and protective factors are also activated. The downregulation of HLA II genes may be indirect evidence of activation of dormant multiple sclerosis retrovirus (MSRV) viral particles. Importantly, this study provides a new set of prospective CIDP biomarkers.

PS3-337 / #254

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Somatosensory evoked potentials in 'Axonal' forms of chronic inflammatory demyelinating polyradiculopathy

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Objective: To assess the diagnostic value of somatosensory evoked potentials (SEPs) in cases of chronic inflammatory demyelinatingpolyneuropathy (CIDP) whose diagnostic remained incertain due to prominent secondary axonal degeneration.

Methods: We conducted a retrospective study of patients who presented with clinical criteria of CIDP but did not fulfill the electrophysiological criteria of definite or probable CIDP according to the 2010 Peripheral Nerve Society guidelines. 26patients were evaluated with SEPs, spinal roots magnetic resonance imaging, cerebrospinal fluid analysis and/or nerve biopsy. All patients received immunomodulatory treatment by IV immunoglobulins, eventually completed by oral prednisolone or plasma exchanges. Diagnosis of CIDP was considered as definite in patients who responded to immunotherapy and/or had evidence of demyelination on nerve biopsy.

Results: (table 1): 17 out of 21 patients considered as definite CIDP had evidence of proximal nerve segment involvement when evaluated with SEPs,of whom 10 had no diagnostic clue in favor of CIDP other than clinical and SEP data. SEPs abnormalities

included reduced brachial plexus responses, increased radicular conduction time or reduction of segmental spinal potentials. Exploration of asymptomatic nerves often yielded infra-clinical SEP abnormalities. The diagnosticsensibility of SEPs was 81% in patients with clinically defined CIDP who showed either nerve biopsy abnormalities or improvement under immunotherapy.

Conclusion: SEPs recording proves helpful to achieve the diagnosis of CIDP when ENMG fails to detect peripheral demyelination and allows increasing the number of patients who may benefit from effective immunotherapy.

PS3-338 / #284

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Treatments with immunoglobulin and thrombotic adverse events in dysimmune neuropathies

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Intravenous immunoglobulins (IVIg) are widely used especially for dysimmune neuropathies (Multifocal Motor Neuropathy, Chronic Inflammatory Demyelinating Polyneuropathy). They are generally considered safe, but complications such as thrombotic manifestations may occur.

Methods: Data from literature between 1986 and 2013 have been analyzed.

Results: Thrombotic complications after treatments with immunoglobulin occur in 0.6 to 13% of cases. The risk seems higher in dysimmune patients (11%) treated by IVIg (Rajabally and al. 2011). Thromboembolic complications affect arterial or venous territories, rarely both, mainly within 24h after perfusion. The risk factors are known: advanced age, cardiovascular risk factors and previous coronary disease, high IVIg dose (≥35 g/day), immobility... Patients with early thrombotic adverse events were more likely to have ≥4 risk factors. Several mechanisms are suggested to explain this increased risk of thrombotic complications. Indeed, Ig treatments increase the plasma viscosity, increase and activate platelets, can trigger the coagulation cascade through the presence of activated XI factor in some Ig preparations, and release vasoactive molecules responsible for vasospasm.

Conclusion: Risk factors should be identified before Ig administration. Preventive care accompanied by monitoring is recommended in at risk patients (hydration, slow perfusion rate, avoid high daily Ig dose, choice of an adequate Ig...). The use of antiplatelet or anticoagulation drugs may be considered in at risk patients, especially in dysimmune neuropathies.

PS3-339 / #286

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Interest of Home-treatment in auto-immune diseases patients treated by IVIg: Results of two French clinical trials

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Intravenous immunoglobulins (IVIg) are widely used especially in auto-immune diseases (dysimmune neuropathies, myositis...), at the hospital. IVIg infusions are time-consuming, and induce high costs for French society. The frequent visits to the hospital may be burdensome for some patients. LFB BIOMEDICAMENTS performed a French study to compare the safety of IgIV 5% (TEGELINE®) administered in hospital versus home in auto-immune diseases patients then a second study to evaluate the costs of TEGELINE administered in hospital versus home, in dysimmune neuropathies patients.

Methods: The first study (TOTEM), a French retrospective, multicentre study was performed to compare the safety of TEGELINE® at hospital versus home in

patients with auto-immune diseases. Patients were included in this study if treated by TEGELINE during 3 courses at hospital then at least one course at home with posology between 1-2 g/kg. The second study (CAT), a French prospective, bicentre study was realized to evaluate costs of TEGELINE® administration at home versus hospital in dysimmune neuropathies (Multifocal Motor Neuropathy-MMN, Chronic Inflammatory Demyelinating Neuropathy-CIDP, Lewis and Sumner Syndrome-LSS).

Results: Selected 46 patients were included in TOTEM study and received 461 cures of TEGELINE® (138 at hospital and 323 at home) with a posology (g/kg) at hospital 1.6 ± 0.4 versus 1.57 ± 0.4 at home.45 side effects (14 %) arised in 17 patients (37 %) receiving their cures at home compared to 24 side effects (17 %) arisen in15 patients (32 %) treated at the hospital. Thus, the average number of side effects per cure and per month is not significantly different between hospital and home,allowing to conclude that there is no significant link between the place of administration of TEGELINE® and the arisen of side effects. 85 % of the patients ($n = 39$) had pursued their cures of TEGELINE® at home.

For CAT study, 24 patients were included (9 patients with MMN, 8 CIDP and 7 LSS). Posology of TEGELINE® (g/kg) was 1.5 ± 0.43 at hospital and 1.5 ± 0.39 at home. Cost of home administration is lower than hospital administration.

Conclusion: The interest of home-treatment in auto-immune diseases patients treated by IVIg was demonstrated from 2 points of view:safetyandcosts.

PS3-340 / #336

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Haemolysis: Why this side effect increases and how to prevent it in dysimmune neuropathies?

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Introduction: Haemolysis are known as a side effect of the IVIg; 1st cases having been published in 1987. Their frequency was low but increased since the marketing of the new generation IVIg, in spite of the fact that pharmaceutical companies respect in Europe the recommendations of the European Pharmacopoeia: maximum titers of anti-A and anti-B antibodies should be 64. Since 2012, several Health Authorities (Canada, Swiss, EMEA, FDA) alerted on haemolysis condition after IVIg infusion.

Methods: Data from literature between 1987 and 2013 have been analyzed.

Results: IVIg may cause extravascular or intravascular haemolysis due to the presence of blood group antibodies in the IVIg final preparation (anti-A, anti-B) especially for high dose infusions (posology greater than 1-2 g/kg/cure) and in patients with some risk factors. A, B or AB blood group and inflammatory diseases (such dysimmune neuropathies) or HLA immunisation have been recognized as the major risk factors for haemolysis. Haemolysis can even occur between 12 hours and up to 10 days after IVIg infusion. They are biologically translated by a decrease of the haemoglobin of at least 1 g/dl and positive result in the direct Coombs test. Clinical signs (asthenia, icterus, failure of organs...) can be observed but they are not specific to haemolysis. In the published cases, the haemoglobin can fall from 1.3 to 7.8 g/dl with the need of blood transfusion in some patients. The described mechanism of IVIG-induced haemolysis is a transfer of the anti-A and anti-B antibodies from the IVIg while the second hit is the inflammatory condition in the recipient which can increase the risk of haemolysis. Preventive care consists in the identification of the at risk patients, the biological surveillance and the use of IVIg with low levels of anti-A and anti-B antibodies.

Conclusion: The number of haemolysis increased upon the arrival of new generation IVIg with high levels of anti-A and anti-B antibodies in their preparations. These haemolysis arise during infusions of a high posology of IVIg and at patients with underling inflammatory disease. They can be prevented.

PS3-341 / #337

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

French Clinical Trial, comparative, double-blind, randomized, multicentre efficacy and safety study of ClairYg® versus TEGELINE® in maintenance treatment of Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP)

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The clinical study is performed in 20 French sites*.

Introduction: The European Federation of Neurological Societies/Peripheral Nerve Society guidelines recommend the use of intravenous immunoglobulin (IVIg) or corticosteroids as first line treatment in CIDP. For IVIg, a meta-analysis in a Cochrane review of 5 double blind placebo-controlled randomized trials showed that a significantly higher proportion of patients improved in disability within one month after IVIg treatment compared with placebo. Having a new 5% liquid IVIg (ClairYg®), LFB BIOMEDICAMENTS wished to compare this new IVIg with TEGELINE®, another IVIg which efficacy in CIDP was already demonstrated.

Objectives: LFB BIOTECHNOLOGIES is currently conducting the first clinical trial comparing two IVIg (ClairYg® and TEGELINE®) in CIDP. This is the first time a clinical trial evaluates the IVIg efficacy in patients on maintenance treatment for at least 6 months, and for whom the minimal efficient treatment schedule has been ascertained.

Methods/Study design: This study is a phase III, comparative (ClairYg® versus TEGELINE® for 6 months), randomized, parallel-groups, double-blind, multicentre study. Patients meeting all eligibility criteria are randomised in two parallel groups. The primary criteria of efficacy is assessed by the adjusted INCAT disability score. The secondary criteria are: efficacy assessment (measured by INCAT Sensory Sumscore, Medical Research Council-sumscore, Grip strength) and safety assessment of ClairYg®.

Main inclusion criteria: - Patient whose CIDP has already induce a disability scored at least "2" on the adjusted INCAT disability score.

- Patient receiving a dose per course within 0.4 to 2 g/kg, at a course frequency within every 2 to 9 weeks.

Results: This study is on-going and 44 are expected.

*French sites : J.Pouget and J.Franques (Marseille), D.Adams and C.Labeyrie (Kremlin-Bicêtre), J-C. Antoine and J-P.Camdessanché (Saint-Etienne), A.Créange (Créteil), J. de Sèze and J-B Chanson (Strasbourg), M. Debouverie (Nancy), E.Delmont, C.Desnuelle and S.Bresch (Nice), A.Lacour (Lille), E.Lagrange (Grenoble), J-C.Vial and H.Gervais-Bernard (Lyon), G.Solé (Bordeaux), J.Cassereau and V. Pautot (Angers), Y.Péréon and S.Wiertlewski (Nantes), J-M.Vallat and L.Magy (Limoges), P.Cintas (Toulouse), P.Corcia (Tours), T.Moreau (Dijon), A-L. Bedat-Millet and V.Guyant-Maréchal (Rouen), S. Mathis and J.Ciron (Poitiers), P.Clavelou (Clermont-Ferrand)

PS3-342 / #364

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

The significance of focal mitochondrial congestion in susceptibility of small diameter axons to degeneration revealed by *in vivo* confocal imaging of mitochondrial dynamics in a model of inflammatory neuropathy

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The role of impaired mitochondrial traffic is increasingly recognised in the pathogenesis of peripheral neuropathies, but their dynamics *in vivo* is very

poorly understood. To study the effect of inflammation on mitochondrial function and transport, mitochondria were observed by time lapse confocal imaging in the exposed saphenous nerves of anaesthetised mice with experimental autoimmune neuritis (EAN). We found that the number of mobile mitochondria was increased in both EAN and adjuvant-only control animals in comparison with naive animals. However, the number of mobile mitochondria was significantly lower in animals with a neurological deficit due to EAN compared with adjuvant controls ($p<0.001$) or asymptomatic animals with EAN ($p<0.001$). Also, the stationary mitochondria were significantly shorter in symptomatic EAN animals than in controls ($p<0.001$), suggesting increased mitochondrial fission. Interestingly, at the onset of EAN, but not in matched asymptomatic or adjuvant-only control animals, we observed a number of small diameter fibres (3.34+/-0.61μm) which contained focal accumulations of stationary mitochondria. The accumulations started abruptly at the proximal end, but 'tailed off' gradually distally, over several tens of microns. Mitochondrial movement was absent at this tail end, and further distally. The accumulated mitochondria were polarised, thus seemingly healthy. Time-matched, asymptomatic animals with EAN showed few, if any such accumulations, but we were able faithfully to reproduce these accumulations by laser damaging (focal photo-bleaching) the axonal mitochondria. The damaged mitochondria became depolarised, fragmented and immobile, presumably depleting the energy supply of the affected portion of the axons. Interestingly, the more proximal mitochondria in these axons started to move towards the damaged region in significantly increased numbers than before photo-bleaching ($p=0.007$). Upon arriving they slowed or stopped moving, seemingly obstructed by the damaged mitochondria. The increase in proximal mitochondrial movement occurred in all axons, but it was only in small axons (2.7+/-0.45 μm) that the accumulations occurred. Thus in the larger axons alone the mitochondria passed unobstructed into the damaged field, repopulating it with healthy mitochondria. We suggest that failure of mitochondria to repopulate small axons may help to explain the selective loss of smaller axons in some peripheral neuropathies.

PS3-343 / #400

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

The challenging diagnosis of Guillain-Barre syndrome in the early childhood

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The classical picture of Guillain-Barré syndrome (GBS) is the ascending flaccid paralysis with areflexia however the diagnosis can be challenging and initially overlooked in the early childhood. The epidemiologic and clinical profile of GBS is described in three pediatric patients aged 1, 3 and 6 years old with special emphasis on the unusual and mimicking presenting symptoms.

The electrophysiology studies included motor and sensory nerve conductions at least of two different nerves at upper and lower extremities. Facial and phrenic nerve conduction studies were also performed.

The epidemiological and clinical features are summarized in the table. The predominant symptoms of GBS at presentation were leg/back pain. The painful symptoms in case 1, initially localized in one leg, were attributed to musculoskeletal sources. A pseudo-encephalopathic form was observed in the remaining cases. An initial diagnosis of acute meningitis was made in case 2. This atypical clinical picture led to the need of brain scan and cerebrospinal fluid analysis. Acute osteomyelitis, discitis or synovitis were also suspected until an unexpected albuminocytologic dissociation led to the electrophysiological studies and appropriate diagnosis. Irritability, gait ataxia, refusal or inability to walk or frequent falls were another prominent clinical features. Findings such as weakness or diminished/abolished reflexes were initially overlooked due to a reluctance to move owing to intense pain. Dysautonomic symptoms were disclosed in case 2 (constipation and intense abdominal pain). Bulbar dysfunction (IX-X cranial nerve involvement) were observed during the peak phase of the illness. The appropriate diagnosis by the neuropediatrician was delayed after excluding gastrointestinal, orthopedic or reumathologic causes; or even processes affecting the central nervous system. The

electrophysiological studies were performed in the early stages of disease and disclosed a demyelinating pattern with bilateral facial and phrenic nerve dysfunction. Following to the immunosuppressive treatment all patients experimented a satisfactory response with a rapid an excellent recovery.

GBS in early childhood can be a challenging diagnosis and thepatient can be referred to several specialists before reaching the appropriate diagnosis. Pain was the most disturbing symptom. Electrophysiological testing should be performed early due to the great diagnostic value.

Epidemiological and clinical features

	Case 1 (6 yo/♀)	Case 2 (3 yo/♂)	Case 3 (1 yo/♀)
Days from GBS onset to admission	7 days	2 days	6 days
Preceding events (time to GBS onset)	Influenza-like syndrome (10 days)	Influenza-like syndrome (2 days)	Influenza-like syndrome (15 days)
	DPTP vaccine (2 weeks)		
Application season	Winter (February)	Winter (February)	Summer (July)
Presenting symptoms	Abdominal pain. Constipation. Severe right leg pain, then pain confined to lower limbs. Persistent guarding, refusal to ambulate Screaming with passive movement	"Pseudo-encephalopathic" form: fever, severe headache, paraspinal/leg pain, meningismus. Drowsiness. Irritability.	"Pseudo-encephalopathic" form: Poor feeding, intense irritability, crying, tendency to opisthotonus, nuchal rigidity and restricted movements. Screaming with passive manipulation.
Specialty referral process	General practitioner→Rheumatolologist →General pediatrician↔ Neurophysiologist→ Neuropediatrician	Ambulatory pediatrician→Emergency department→General pediatrician↔ Traumatologist↔Radiology/Nuclear Medicine→ General pediatrician↔Neurophysiologist→ Neuropediatrician	Emergency department (5 return visits)→ Neuropediatrician
Initial clinical suspicions	Rheumatologic causes (synovitis)	Acute meningitis/meningoencephalitis Rheumatologic/orthopedic causes (synovitis, discitis, osteomyelitis, etc.)	NSAP, "fecal retention", "generalized abdominal pain", "constant crying and irritability",
First-line treatment	NSAIDs	NSAIDs	
Nadir phase clinical features	Lower limb weakness and sensitive gait ataxia with frequent falls. Gower and Romberg sign. Areflexia.	Flaccid weakness. Unable to rise from the sitting position. Romberg sign. Wide-based gait, with frequent falls. Areflexia.	Areflexia. bulbar dysfunction (CN IX and X)
Laboratory features	CSF albuminocytologic dissociation (PC: 90 mg/dl)	CSF albuminocytologic dissociation (PC: 158 mg/dl)	CSF albuminocytologic dissociation (PC: 66 mg/dl)
	Anti-CMV Ig M and Ig G (avidity score < 35%)		
Subclinical facial neuropathy (Electrophysiological tests)	+	+	+
Subclinical phrenic neuropathy	+	+	+
Specific treatment	IV Ig (0.4 g/kg x 5 days)	IV Ig (0.4 g/kg x 5 days)	IV Ig (0.4 g/kg x 5 days)
Specific treatment onset (days from admission)	3	8	0
Hospital stay (days)	12	19	18
Total functional recovery (days from clinical onset)	85	142	101

Abreviattures.
GBS: Guillain-Barré syndrome; DPTP vaccine: diphtheria, pertussis, tetanus and polio vaccine; NSAP: non-specific abdominal pain; NSAIDs: nonsteroidal anti-inflammatory drugs; CMV: cytomegalovirus; CSF: cerebrospinal fluid. ↔: medical interconsultation

✶PF3

PS3-344 / #426

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Antibodies against paranodal proteins detect CIDP patients with specific clinical phenotypes and poor response to conventional therapies

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Background: Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a heterogeneous disease with autoimmune origin but the antigenic targets remain largely unknown. Our group and others described antibodies against node of Ranvier structures but the clinical features associated to them remain uncertain. We describe a group of 9 patients with antibodies against contactin-1 (CNTN1) or neurofascin 155 (NF155) that associate with specific clinical features.

Methods: Systematic screening of anti-CNTN1 and -NF155 antibodies was performed in patients fulfilling EFNS/PNS CIDP diagnostic criteria from two sources: 53 patients from our clinics and 8 IVIg-resistant patients from the CIBERNED-CIDP registry. Screening for anti-CNTN1 and anti-NF155 antibodies was performed with immunocytochemistry. Anti-CNTN1 and NF155 IgG isotype was analyzed by ELISA. Immunohistochemistry (IHC) over rat teased nerve fibers was used to detect reactivity against nerve structures. Rat brain IHC was performed to detect serum reactivity against cerebellar structures. Clinical features were reviewed.

Results: Five patients with anti-CNTN1 and 4 with anti-NF155 antibodies were detected. All 9 sera reacted against paranodal structures. Anti-CNTN1 and anti-NF155 antibodies were of the IgG4 isotype in all patients. Anti-CNTN1+ patients presented with an aggressive, predominantly motor neuropathy. Acute denervation was present in the first EMG study. None of them responded to IVIg and only one to steroids. All 4 anti-NF155 positive patients presented with a subacute, severe neuropathy with predominantly distal motor involvement. EMG showed demyelinating features with no acute denervation. None of them responded to IVIg or steroids. Three of the 4 anti-NF155+ patients showed a disabling, slow action tremor. Sera from all anti-NF155+ patients reacted against cerebellar and brain neuropil structures, while anti-NF155 negative patients did not.

Discussion: Classification of CIDP following clinical and electrophysiological criteria includes patients with diverse phenotypes. Biomarkers, such as antibodies against CNTN1 or NF155, can help identifying homogeneous CIDP subgroups and, thus, have diagnostic and prognostic implications. The identification of these antibodies of the IgG4 isotype can also have therapeutic relevance.

Conclusion: Antibodies against CNTN1 and NF155 identify homogeneous subsets of CIDP patients and can have diagnostic, prognostic and therapeutic implications

PS3-345 / #439

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Hepatitis E and acute meningoradiculitis: viral loads in a repeated PCR study in CSF and blood

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Introduction: Hepatitis E (HEV) is a newly recognized infection with potential extra-hepatic manifestations, of which neuropathies may be encountered.

Options of treatment may depend on time and load of infected virus.

Case report: Over one week, a 51-year-old male reported initial back pain with episodes of fever, followed by radiating pain in the left and right arms. He was seen when he was unable to lift his arms. On examination, a MRC3 to 4 weakness was demonstrated in the C5-7 innervated muscles on the right side, and in C6-Th1 on the left. No other neurological deficits were observed. Nerve conduction studies were normal except for reduced amplitudes of motor responses. The sensory potentials were normal, as well as brain and spinal MRI. Liver enzyme levels were elevated 10 times the normal values. CSF showed 73 monocytes, and PCR analysis for common virus and bacteria were normal. A series of IVIG (2g/kg over 5 days) did not improve muscle weakness.

Results: Anti-HEV IgM levels were elevated in the serum, and diagnosis was confirmed by demonstrating HEV RNA in serum and CSF. Repeated analysis revealed HEV RNA disappearance in the CSF within days, and a rapid decrease of HEV RNA in the blood. The maximal viral load in the blood was calculated to be 5.8×10^5 GCE/ml. No antiviral treatment was administrated.

Discussion: This study demonstrates how fast is the infection, which may directly invade CSF and induce severe radiculitis. There is no evidence of beneficial usefulness of IVIG nor of anti-viral therapy.

PS3-346 / #452

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Clinical profile, treatment and outcomes of 121 Guillain-Barré syndrome episodes in 119 patients prospectively registered in the GBS National Czech Registry

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Introduction: The incidence of Guillain-Barré syndrome (GBS) in a 10 million population of the Czech republic oscillates about 175 cases per year according to the officially reported data. Prospectively acquired data are, however necessary to verify whether international standards for care are met on a national basis.

Methods: Ten neuromuscular centers (NMC) participate in the online web-based national Czech GBS registry based on a standard protocol created by a group of neuromuscular experts to follow clinical profile, treatment options and 6-months outcome of GBS patients.

Results: Data from 121 GBS episodes from 119 patients (65 men, 54 women, mean age 51,4 years) registered during 2012-2013has been analysed. Seventy-eight percent of patients have been primarily referred NMCs, while 22% weretransferred secondarily.Sixty percent of them were treated with plasma exchange, 30% with intravenous humane immunoglobulinand 10% received no immunomodulatory treatment (because of low GBS scale). After 6 months, 66% patients have GBS scale of 0-1. Two relapses during 6 months (1.7%) were found; the 6-month mortality reached 6%. There was no difference in outcomes between treatment modalities and sexes.

Conclusions: Prospective acquisition of data using standard protocol and web-based registry is a useful source of information that could be used for assessment of quality of care in particular centers and on a national scale. Centralization of care and following the care standards resulted in good outcome of GBS patients comparable with other relevant sources.

PS3-347 / #463

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Unusual presentation of Axonal Neuropathy associated with MGUS IgM kappa paraproteins - Case Report

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Background: Monoclonal gammopathy of undetermined significance (MGUS) is a benign hematological disease within which a polyneuropathy, mainly of demyelinating type, may develop. Based on the type of paraprotein MGUS polyneuropathies are divided in two groups: 1. IgM which are mostly demyelinating, 2. IgG , IgA ,which can be demyelinating or axonal. Axonal polyneuropathy associated with MGUS IgA IgG is more common when compared to axonal MGUS IgM polyneuropathy. In patients with IgM paraproteins with axonal polyneuropathy there is a connection of antibodies to glucoconjugates such as GM1, sulphatide or chondroitin-sulphatide. In patient with ALS and MGUS may be found significant increase the serum monoclonal paraproteins. These results corroborate the concept of a probable association between ALS and benign monoclonal gammopathy. This is very important from a therapeutic point of view.

Case Report: We report a 60-year-old man with axonal neuropathy associated with MGUS IgM kappa paraprotein. The disease began in December, 2012. with mild weakness of proximal and distal muscles of the right and then the left leg with fasciculations in the thighs and lower parts of the abdomen. Since May, 2013 the progressive evolution has led to the inability of independent walking, without muscle weakness of the upper extremities. Neurologic examination revealed hypotrophy and weakness of the lower limbs muscles. Motor examination found grade 0/5 weakness with dorsiflexion of the left foot and grade 2/5 weakness of the right foot, grade of plantar flexion both side was 3/5, the strength of the proximal leg muscles was 4/5. Deep tendon reflexes were 2+patellas and 1+ Achilles bilaterally. Sensibility was normal. Walking with crutches. Laboratory Findings: Protein electrophoresis shows gammopathy IgM kappa. ENMG results show sensorymotor polyneuropathy , predominantly motor axonal in the lower limbs, with spontaneous activity. A lymphoproliferative disorder was excluded with skeletal bone survey, bone marrow biopsy, chest and abdominal CT. The patient was treated with intravenous immunoglobulin with improving muscle strength to independent walking.

Conclusion: Our case report demonstrated axonal polyneuropathy associated with MGUS type IgM kappa , which is rarely described in the available literature.

PS3-348 / #504

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

A case of Multifocal acquired demyelinating motor sensory neuropathy complicated by phrenic nerve palsy

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We report on a patient with Multifocal acquired demyelinating motor sensory neuropathy (MADSAM), variant of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), complicated by phrenic nerve palsy. A 76-year old man was admitted to our hospital due to dyspnea, numbness in his limbs and hoarseness. On admission, intrinsic hand muscle atrophy, bilateral vocal cord palsy, weakness and sensory disturbance were seen in the distal part of his extremities. Deep tendon reflexes were absent in all the limbs. Routine laboratory examinations including autoimmune disease, thyroid function tests and collagen vascular disease were normal. Titers of GM1 ganglioside antibodies were normal and M protein was negative in immunofixation electrophoresis. Analysis of cerebrospinal fluid showed normal protein level and negative results for HIV, CMV, herpes simplex antibodies and VDRL. Electrophysiologic investigation disclosed multifocal demyelinating sensory and motor neuropathy. Severe restrictive extraparenchymal lung disease was suspected by pulmonary function test with lung volume measurement. % of Total Lung Capacity, Residual Volume was 78% and 111%. Chest

X-ray showed elevated diaphragm. Bilateral phrenic nerve palsy was confirmed by chest fluoroscopy. Although phrenic nerve palsy has been reported rarely in patients with CIDP, this is a first report of it in patients with MADSAM

PS3-349 / #511

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Primary neurolymphomatosis from low-grade B-cell lymphoma presenting as slowly progressive length dependent polyneuropathy

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Objective: Report an atypical presentation of primary neurolymphomatosis due to low-grade lymphoma.

Background: Neurolymphomatosis (NL) is a rare disorder, characterized by invasion of cranial or peripheral nerves by aggressive subtypes of non-Hodgkin's lymphoma. Most common clinical presentation is that of a rapidly progressive painful neuropathy, followed by cranial neuropathies, and less frequently painless polyneuropathy. Primary NL due to low-grade lymphoma is extremely rare, and the diagnosis requires histopathologic examination of the involved nerves.

Methods: Case Report.

Results: A 58-year-old, previously independent man presented with one-year history of aching legs and progressive weakness leading to wheelchair dependence. He reported no systemic symptoms. Short courses of prednisone in the past had resulted in transient improvement of muscle strength, though he continued to weaken. Neurologic examination revealed mild proximal and moderate distal asymmetric weakness in upper and lower limbs, preserved reflexes, and vibration loss below the knees. EMG showed a length dependent axonal sensorimotor neuropathy. Extensive blood workup was positive for mild anemia, and a small IgM kappa paraprotein detected only by immunofixation. Fat aspirate was negative for amyloid; skeletal survey was normal. MRI of the spine and cauda equina demonstrated no signal abnormality or abnormal enhancement. CSF analysis showed mild protein elevation at 68 mg/dl but no other abnormality. Paraneoplastic evaluation on serum and CSF was negative. Bone marrow study showed minimal focal involvement of <5% of the total marrow cellularity by a B-cell lymphoproliferative disorder. On staging CT, primary stage 1 squamous cell lung cancer was detected; no lymphadenopathy was present. Sural nerve biopsy revealed mild reduction in myelinated nerve fiber density, multiple epineurial perivascular CD20 positive collections and no CD4 positive cells, consistent with a diagnosis of B-cell neurolymphomatosis. The patient underwent surgical resection of his lung cancer followed by chemoradiation; treatment with rituximab for neurolymphomatosis stabilized his neurologic status in two months.

Conclusions: 1.Primary neurolymphomatosis is rare and can occur with low grade B-cell lymphoproliferative disorder. 2) Clinical presentation is variable including a length dependent axonal peripheral neuropathy. 3) Nerve biopsy remains the gold standard for diagnosis of primary neurolymphomatosis.

PS3-350 / #535

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

Nerve thickening in ultrasound in a patient with Lewis Sumner Syndrome (MADSAM)

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Background: MADSAM is a rare multifocal chronic inflammatory demyelinating polyneuropathy. The presentation can be misleading and sensory symptoms can be confused with mononeuropathies.

Objective: Aim of this case report is the rare appearance of a patient with MADSAM and its clinical presentation, ancillary findings including nerve ultrasound and the observation over one year.

Methods: Single case report.

Results: We report a 60-year old patient presenting with sensory loss in both hands resulting in clumsiness

and mild dysaesthesia in the toes. Initially a median and ulnar entrapment syndrome was suspected and also a surgical intervention took place. The symptoms increased and burning pain occured, in particular in the palms. Nerve conduction studies showed extremely prolonged distal latencies, demyelinating neuropathy, and absent antidromic sensory potentials. Laboratory studies including antimyelin antibodies were normal, no paraprotein was detected. A search for neoplasia was negative. Intravenous immunoglobulin (IVIG) treatment (0,4 g/kg for 5 days) was initiated at standard dose and is repeated in 6-10 weeks cycles. The effect of IVIG lasted 6-8 weeks after each cycle.

Discussion: This patient presented with a demyelinating distal neuropathy, classified as Lewis Sumner syndrome (MADSAM). No apparent cause for this condition could be found and no immunologic associations could be detected. In addition to the stricking electrophysiologic findings, thickening of nerves in ultrasound were detected, and a favourable, although incomplete and transient therapeutic improvement could be found.

MADSAM is a rare distal demyelinating neuropathy. It occurs in middle age; asymmetric distal (more often than proximal) weakness occurs. Upper extremities are more often affected. Patients report distal sensory loss. Tendon reflexes show focal loss. In electrophysiology multifocal conduction blocks and variably slowing in nerve conduction velocities are seen. Distal latencies are variably prolonged. In MRI swollen nerves in brachial plexus with high T2 signal have been described. The addition of nerve ultrasound is helpful.

Conclusion: MADSAM is rare but has to be considered in differential diagnosis of multifocal demyelinisating neuropathy with sensory and motor symptoms.

PS3-351 / #553

Theme: *5.1 - Peripheral neuropathy: inflammatory / dysimmune / associated with haemopathy*

A Guillain-Barre syndrome epidemic following a Zika virus epidemic in French polynesia.

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Since October 2013, French Polynesia (FP) has been affected by a Zika Virus (ZIKV) epidemic, an emerging arbovirus of the flaviviridae family, like dengue, transmitted by Aedes-type mosquitoes discovered in Uganda in 1947.

Over a 3 month period, one fifth of the Polynesian population has been affected. Described as a benign infection (sub-febrile flu-like syndrome, cutaneous rash, conjunctivitis), there is no report of hospitalization or complication due to ZIKV. In spite of this, immediately after the epidemic (from November 2013), we have diagnosed 38 Guillain-Barre syndromes (GBS) by 01/21/2014.

It is remarkable on several points:

There have been within 3 months more than 10 times the number of cases we usually expect in a year. Sex-ratio M/F is 2.5 (whereas 1.78 is usually found); the average age is 46.5 (27-74 years old). Patients all have Polynesian origins except one from cayenne, where dengue 1 and 3 exist.

11 patients (ie. 29%; 9M and 2F) were admitted in recovery unit; among them, 8 underwent tracheotomy. 4 patients required intensive care supervision.

Cranial nerves are nearly always affected, with a high frequency of bilateral facial palsies.

EMG were all pathological, rather showing acute motor axonal neuropathies (AMAN), and the presence of severity criteria such as denervation and conduction blocks at diagnosis.

Albuminocytologic dissociation is systematically found with proteins reaching more than 6g/l.

The anti-ganglioside antibodies routinely researched (GM1, GD1a, GT1a, GQ1b) were negative in the first results, but anti-GA1 are positive for some patients (result still in progress by 01/21/14, 6 over 8 tested are positive), and this type of anti-ganglioside is rather associated with AMANs.

This exceptional GBS epidemic, which is still going on, occuring immediately after a ZIKV epidemic, raises several questions:

Why these Polynesian patients, mostly male, all born before 1987?. Could it be linked to the Polynesian HLA system, which would have been stimulated by dengue 1 and 3 1989-1990 epidemics, reactivated during a dengue 3 epidemic in february 2013, and leading to an "immulogical storm" during ZIKV epidemic?

Why is there a predominance of AMAN associated with anti-GA1 anti-bodies?

Is there molecular mimicry between surface glycoproteins of ZIKV and GA1?

We will try to answer these questions.

PS3-352 / #56

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Peripheral nerve ultrasound in Charcot-Marie-Tooth disease type 1A

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Background: Charcot-Marie-Tooth disease type 1A (CMT1A), due to duplications in the PMP22 gene, is the most common cause of neuropathy in childhood. Peripheral nerve ultrasound provides a rapid, painless and non-invasive method of imaging the peripheral nervous system.

Methods: This cross-sectional, matched, case-control study evaluated differences in nerve cross-sectional area (CSA) measured by peripheral nerve ultrasound in children with CMT1A compared to healthy controls. Nerve CSA of the median, ulnar, tibial and sural nerves was measured in the dominant limb. Correlations between nerve CSA and neurologic disability (measured using the CMT Pediatric Scale) were explored.

Results: 29 children with CMT1A and 29 age- and gender-matched controls were enrolled. Nerve CSA was significantly increased in CMT1A - nerves were 2-3 fold larger in children with CMT1A compared to controls ($p<0.001$) at all sites, including in children as young as 19 months of age. Nerve CSA showed a strong positive linear correlation with age, height and weight in both the CMT1A and control groups. The increase in nerve CSA with age was disproportionately greater in those with CMT1A ($p<0.001$), suggesting that ongoing pathological nerve hypertrophy occurs throughout childhood. Nerve CSA correlated with neurologic disability.

Conclusions: Children with CMT1A have significantly greater nerve CSA compared to controls, and the increase in nerve CSA with age is disproportionately greater in CMT1A, suggesting ongoing pathological nerve hypertrophy throughout childhood. Nerve CSA correlates with neurologic disability. These findings demonstrate the utility of peripheral nerve ultrasound as an adjunctive diagnostic tool in CMT1A, as well as a biomarker for disease progression in natural history studies and clinical trials.

PS3-353 / #102

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Non-length dependent neuropathy in transthyretin amyloidosis due to A60T mutation

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Transthyretin related amyloid neuropathy (ATTR) is an autosomal dominant disorder caused by amyloid deposition into the peripheral nerves. The amyloid fibrils derive from circulating variant transthyretin, which results from mutations in its coding gene.

Familial amyloid polyneuropathy (FAP) due to variant transthyretin usually develops following a length-dependent pattern, being the distal parts of the lower limbs involved earlier than the upper limbs.

Here we describe the clinical features of an individual carrying the A60T mutation, which presented with cardiomyopathy and a non-length dependent peripheral neuropathy involving the upper limbs.

A 57 year-old man with a history of poliomielitis and lower limb's sequelae, developedamyotrophy and sensory loss in both hands at the age of 46 years. At

around the same time, a restrictive cardiomyopathy was recognized. Later, diarrhea, orthostatic hypotension and dysphonia, became prominent.

At the time of our examination the patient weighted 36 kg and was wheelchair-bound.

His legs were paralytic and flaccid since polio.

Sensation in his lower limbs was intact, with no trophic skin lesions. In the upper limbs instead, there was total loss of sensation to touch, pinprick and temperature with a symmetrical distribution.

Nerve conduction studies revealed absent sensory nerve action potentials (SNAPs) from the ulnar, median and radial nerves, with relative preservation of the sural SNAP. The amplitude of the compound muscle action potentials (cmap) in the ulnar and median nerves was reduced, and cmap were not recordable in the lower limbs.

The quantitative sensory thresholds for cold and vibration were severely abnormal in the hand dorsum, but were within normal range in the feet.

The heart rate did not show the normal variation with deep breathing, indicating cardiac autonomic involvement.

Previously, a rectal biopsy had revealed amyloid deposition and the TTR gene sequencing disclosed the A60T mutation.

An older brother, who died at the age of 63 years due to amyloid cardiomyopathy carried the same mutation. The mother and a sister also had cardiomyopathy and peripheral neuropathy.

In conclusion, this clinical case highlights the fact that ATTR might present with a non-length dependent neuropathy. Asymmetric, upper-limb presentation has also been recognized by other clinicians, emphasizing the need to include FAP in the differential diagnosis of non-length dependent peripheral neuropathy.

PS3-354 / #150

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Exome sequencing reveals a TFG mutation causing dominant axonal CMT

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Background and Objective: Charcot-Marie-Tooth disease (CMT) is a group of inherited neuropathies and the genetic etiologies of at least 30% of CMTs have yet to be elucidated. Herein, we describe a novel mutation in the TRK-fused gene (TFG) as a new cause of dominant axonal CMT (AD-CMT2) identified by exome sequencing and further characterized by *in vitro* functional studies.

Methods: Exome sequencing was utilized to investigate a large Taiwanese family with a AD-CMT2 in which mutations in common CMT2 implicated-genes were excluded by Sanger sequencing. Mutant gene products were surveyed *in vitro* to investigate their functional impact.

Results: Exome sequencing revealed a novel heterozygous mutation, c.806G>T (p.Gly269Val), in TFG that co-segregates with the CMT2 phenotype in all 27 family members. This mutation alters a conserved residue and is absent in 1,140 ethnically matched control chromosomes. Cell transfection studies showed that the TFG p.Gly269Val mutation increased the propensity of TFG proteins to form aggregates, resulting in sequestration of both mutant and wild-type TFG, and might thus deplete functional TFG molecules. The secreted Gaussia luciferase reporter assay demonstrated that inhibition of endogenous TFG compromised the protein secretion pathways, which could only be rescued by expressing wild-type TFG but not the p.Gly269Val altered proteins.

Conclusion: This study identifies a novel cause of dominant CMT2 and highlights the importance of TFG in the protein secretory pathways which are essential for proper functioning of human peripheral nervous system.

PS3-355 / #176

Theme: *5.2 - Peripheral neuropathy of genetic origin*

The broad spectrum of TRPV4 axonal neuropathies

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The transient receptor potential vanilloid 4 (TRPV4) gene is located on chromosome 12q23-24 and encodes for a non-selective, calcium-permeable channel. Mutations in the TRPV4 gene have been identified in patients with skeletal diseases and in patients with peripheral nervous system (PNS) compromise. These conditions are autosomal dominant and in the case of PNS syndromes the penetrance is incomplete. Patients with PNS manifestations present predominantly with an axonal neuropathy and 3 conditions have been associated with mutations in the TRPV4 gene: congenital distal spinal muscular atrophy (CDSMA) or distal hereditary motor neuropathy (dHMN), scapuloperoneal spinal muscular atrophy (SPSMA) and hereditary motor and sensory neuropathy type IIC (CMT I2C). Additional signs that may occur are vocal cord paralysis, sensorineural hearing loss and bladder urgency and incontinence. There are also 3 reports of an overlap phenotype with neurological and skeletal manifestations.

We report the clinical, electrophysiological and muscle biopsy findings in two unrelated patients with two novel heterozygous missense mutations in the TRPV4 gene (patient 1: p. [Arg269Cys]; patient 2: p. [Asp62Asn]). Patient 1 is a boy first referred to us at the age of 30 months due to motor development delay. The phenotype in this case is compatible with an overlap syndrome. The second patient is a female patient that presented first by the age of 40 years old with signs and symptoms suggestive of dHMN and with the particularity of harbouring intracitoplasmic basophilic inclusions in the muscle biopsy.

In conclusion mutations in TRPV4 produce a broad spectrum of phenotypic manifestations with marked variability in disease severity.

PS3-356 / #195

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Tongue atrophy and fasciculations in Familial Amyloid Polyneuropathy: an atypical presentation

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Objective: To describe atypical features of tongue atrophy, fasciculations and bulbar dysfunction in two unrelated patients with familial amyloid polyneuropathy (FAP).

Background: Transthyretin (TTR)-associated FAP typically causes a nerve length-dependent sensorymotor polyneuropathy with variable autonomic dysfunction and extra-neurologic manifestations resulting from focal deposits of amyloid. Tongue enlargement (macroglossia), from amyloid infiltration of the tongue muscles, is a well-known feature of the disease, but tongue atrophy and fasciculations are not well recognized.

Case Reports: Patient 1 is a 75-year-old female and patient 2 is a 60-year-old male. Both had a 2-3 year history of progressive painless weakness and sensory loss. Both are severely disabled; patient 1 walks with the assistance of a walker, while patient 2 requires a wheelchair for mobility. Examination of both patients demonstrated profound sensory loss to all modalities, areflexia and sensory ataxia. Motor strength testing shows distal greater than proximal motor weakness (asymmetric in patient 1 and symmetric in patient 2). Both patients had marked tongue atrophy, fasciculations and weakness. Patient 2 has an additional family history of a 61-year-old sibling who recently passed away with a reported diagnosis of amyotrophic lateral sclerosis. Electrodiagnostic studies showed severe axonal sensory and motor polyneuropathy with active denervation in multiple muscles. Sural nerve biopsy in patient 1 showed marked axonal loss but no amyloid (by Congo Red staining). Fat pad biopsy on patient 2 showed no amyloid deposition. TTR DNA testing revealed a mutation c.379A>G (p.I107V) in patient 1 and c.148G>A mutation (p.V30M) in patient 2.

Discussion: Focal manifestations with tongue enlargement have been reported in systemic primary amyloidosis secondary to amyloid accumulation; bulbar involvement with tongue atrophy and fasciculations has rarely been reported in patients with amyloidosis resulting in potential misdiagnosis of amyotrophic lateral sclerosis. We report 2 additional patients with tongue atrophy and fasciculations with genetically confirmed TTR FAP. In patients with severe rapidly progressive polyneuropathy and bulbar weakness, the diagnosis of FAP should be considered in the differential.

PS3-357 / #226

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Extensive genetic analysis of a taiwanese cohort with cmt diseases

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To assess the frequency and spectrum of mutations of major Charcot-Marie-Tooth disease (CMT) genes in a Taiwanese CMT cohort.

Mutational analyses of the PMP22, MPZ, GJB1, LITAF, EGR2, NEFL, FBLN5, MFN2, RAB7, TRPV4, GARS, HSPB1, HSPB8, GDAP1, KIFIB, DNM2, YARS, KARS and GNB4 genes were carried out by direct sequencing in 300 unrelated patients with CMT, who had been recruited at the Neurology Service of Taipei Veterans General Hospital, Taiwan.

Mutations have been identified in 202 of the 300 patients (67.3%), including 173 with demyelinating CMT (79.7%; 173/217) and 29 with axonal CMT (34.9%; 29/83). Among the 173 patients with demyelinating CMT, 132 (60.8%) were found to have PMP22 duplication, 18 (8.3%) had GJB1 mutations, 11 (5.1%) had MPZ mutations, 4 (1.8%) had PMP22 point mutations, 2 (0.9%) had EGR2 mutations, 2 (0.9%) had FBLN5 mutation, 2 (0.9%) had GNB4 mutations, 1 (0.5%) had LITAF mutation and 1 (0.5%) had NEFL mutation. Among the 83 patients with axonal CMT, 9 (10.8%) were found to have GJB1 mutations, 8 (9.6%) had MFN2 mutations, 7 (8.4%) had NEFL mutations, 1 (1.2%) had MPZ mutation, 1 (1.2%) had HSPB1 mutation, 1 (1.2%) had GDAP1 mutation, 1 (1.2%) had AARS mutation and 1 (1.2%) had TFG mutation.

This study clearly demonstrates the spectrum of CMT mutations in a Taiwanese cohort. Five commonly available genes account for 92.6% of all CMT. Genetic testing for PMP22 duplication, GJB1, MPZ, NEFL and MFN2 mutations should, therefore, be the first consideration in the molecular diagnosis of CMT in ethnic Chinese.

✶PF2

PS3-358 / #229

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Improper mitochondrial calcium homeostasis is responsible for the Friedreich ataxia neural pathophysiology

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Friedreich ataxia (FRDA) is a neurodegenerative disorder caused by an unstable GAA repeat expansion mutation within intron 1 of the FXN gene. A major feature of the pathology of FRDA is the loss of large primary sensory neurons, namely proprioceptive neurons, which induces degeneration of the posterior columns of the spinal cord, and is associated with degeneration of central and peripheral large myelinated axons.

It is known that lack of frataxin causes mitochondrial dysfunction and its consequences on the nervous system are responsible for the neural pathophysiology of the disease. Proper mitochondrial function is essential for the neuronal survival by different physiological functions. We have investigated several of these mitochondrial functions such as maintenance of mitochondrial transmembrane gradient and regulation of cellular Ca^{2+} metabolism, in sensory neurons of DRG from the YG8R mouse (B6.Cg-Fxntm1Mkn Tg(FXN)YG8Pook/J), a GAA-repeat-based mouse model of FRDA.

We measured cytosolic Ca^{2+} levels with FURA-2 AM probe in primary culture of DRG sensory neurons and we investigated store-operated Ca^{2+} entry mechanism (SOCE) after ER-calcium release by inhibition of the SERCA pump with tBuHBQ. We observed an increment of basal Ca^{2+} cytosolic concentrations, abnormal buffering of intracellular Ca^{2+} levels and the induction of SOCE by ER-calcium released was reduced in the YG8R mice. Changes in calcium homeostasis induced by frataxin deficiency are associated with reduction of mitochondrial transmembrane gradient and increase in ROS production.

Our results suggest that frataxin deficiency induces global failure of mitochondria that involve proper function of oxidative stress and mitochondrial management of Ca^{2+} signaling in sensory neurons of DRG responsible for the neural pathophysiology of FRDA.

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PS3-359 / #267

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Combined skin biopsy and neurophysiological study in TTR-amyloidosis allows early detection of small fiber neuropathy

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Small-fiber neuropathy (SFN) is the most frequent and early manifestation of transthyretin familial amyloid polyneuropathy (TTR-FAP). In this study, we evaluated the value of intraepidermal nerve fibers density (IENFD) quantification by skin biopsy and neurophysiological investigation of small nerve fibers to detect SFN in TTR-FAP.

Methods: We evaluated 7 patients with polyneuropathy (5M/2F; age 40-75 ys) and 5 asymptomatic carriers of TTR variants (5M; age 30-63 ys). Skin biopsies were performed at thigh (proximal) and leg (distal); IENFD was measured after immunofluorescence staining of PGP9.5 in nerve terminals. Congo red staining was performed to detect amyloid deposits. Neurophysiological investigation including laser evoked potentials (LEP), quantitative sensory testing (QST), sympathetic skin response (SSR) and heart-rate variability (HRV).

Results: In the 7 patients with overt neuropathy, skin biopsy evidenced SFN, with proximal IENFD (mean ± SD) at 4.3 ± 3.9 f/mm, and distal IENFD at 2.3 ± 1.6 f/mm. Neurophysiological investigation showed abnormal LEP (n = 5), QST(n = 5), SSR (n = 6), and HRV (n = 7). In the 5 asymptomatic carriers, proximal IEFND was decreased in 5/5 at 7.1 ± 4.3 f/mm, and distal IENFD in 4/5 at 3.8 ± 1.9 f/mm. Neurophysiological investigation showed abnormal LEP (n = 4), QST(n = 1), SSR (n = 2), and HRV (n = 4). Finally, congo stain disclosed amyloid deposits in 5/7 patients and 1/5 carriers in skin biopsy.

Conclusion: This pilot study showed that a combined approach based on IENFD quantification and a battery of neurophysiological tests are appropriate tools for the evaluation of SFN in the context of TTR-FAP. Such a combined approach may detect TTR-FAP at a presymptomatic stage and therefore identify potential candidates for innovative therapeutic strategies. In addition, skin biopsy can evidence amyloid deposits associated with TTR-FAP.

PS3-360 / #270

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Brain MRI findings in adults and children with Fabry disease

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Objective: To evaluate the frequency of ischemic and hemorraghic lesions and the pulvinar sign in brain MRI of patients with Fabry disease.

Methods: Brain MRI studies in 86consecutives patients without history of stroke or TIA were evaluated using classic sequences as well as GRE-weighted images, for ischemic lesions, chronic microbleeds and the pulvinar sign detection.

7 patients were excluded due to dyalisis. Of the 79 remaining patients, 16 were children (mean age 11.8 years , range 10 to 17) and 63 were adults (27 males: mean age 33.4 years, range 20 to 60; and 36 females: mean age 40 years, range 18 to 78).

Results: Thirty-two adults (50.8%) and 4 children (25%)had brain MRI evidence of small vessel disease in the basal ganglia, corona radiata, thalamus or brainstem, as well as in the periventricular white matter.

Adults with MRI abnormalities were older (51.3 vs 30.6 years old $p < 0.01$).

Three women and one man (7% mean age 61 years) presented chronic microbleeds identified by GRE, in the pallidum and thalamus. Moreover, Flair and T2-weighted images also showed evidence of white matter disease and deep grey matter involvement.

Only one patient presented the thalamic abnormality known as the pulvinar sign

Conclusion: Subclinical evidence of ischemic brain lesions is seen in half of adults with FD without history of CVA or end stage renal disease Moreovver these lesions start in childhood in 25% of patients.

FD is a treatable disorder that should be included routinely in the differential diagnosis of symptomatic and asymptomatic ischemic and microhemorrhagic lesions in young adults.

PS3-361 / #273

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Developmental hip abnormalities in paediatric Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth disease (CMT) is the most common inherited neuropathy, with an estimated prevalence of 1/2,500. Common orthopaedic complications of CMT include ankle, foot and spinal deformities. An association with developmental hip abnormalities is increasingly recognized, although the incidence and pathogenesis of these abnormalities is poorly understood.

A retrospective study of all children with CMT under care of the neuromuscular team at The Royal Children's Hospital Melbourne since 2000. Clinical notes were reviewed for:

-Type of CMT: demyelinating (CMT1), axonal (CMT2),intermediate (CMT-I) or X-linked (CMTX)
-Symptoms of hip disease
-Results of hip radiographs
-Treatment received

Hip dysplasia was defined radiologically as a Reimer's index >50%, and/or lateral centre edge angle <25 degrees. Coxa valga, coxa anteverta, pelvic tilt and/or lateral femoral head uncovering <30% were defined as mild hip abnormalities.

Of 116 children (70 male) with an inherited neuropathy, 87 had one or more hip radiographs between 2000-2013. They had CMT1A (49/87), other CMT1 (9/87), CMT2 (19/87), CMTX (6/87) and CMT-I (4/87). 77.7% (63/81) had radiographs for asymptomatic screening. 1/9 radiographs requested for hip pain revealed hip dysplasia.

25/87 patients had serial radiographs. Seven had hip dysplasia and three mild hip abnormalities. 2/25 patients with an initial normal radiograph at 6 and 7.5 years of age later developed hip dysplasia at 9 and 12.5 years of age. Three other patients had evidence of hip dysplasia that progressed over time. Those with mild hip abnormalities did not progress over time, but radiographs were not repeated after age 8 years.

11.5% (10/87) of children with CMT had hip dysplasia (7 CMT1A, 2 Déjerine-Sottas disease, 1 CMT2), for which five had osteotomies and one had bracing. 3/10 were dependent on a walker or wheelchair for ambulation prior to hip disease treatment. Median age at which radiologic abnormalities were identified was 6.7 years (range 0.3-11.6).

Developmental hip abnormalities are a common but generally asymptomatic finding in paediatric CMT, particularly CMT1. Baseline hips X-rays are indicated in all children with CMT at the time of diagnosis. Non-ambulation is a risk factor for severe hip dysplasia requiring orthopaedic intervention. Further hip screening and more longitudinal data in our cohort will guide routine screening in other types of CMT.

PS3-362 / #317

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Severe early onset Charcot-Marie-Tooth neuropathy caused by concomitant mutations in the MFN2 and GDAP1 revealed by Whole Exome Sequencing

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Background: Mutations in the mitofusin 2 gene (MFN2) are the most common cause of autosomal dominant axonal Charcot-Marie-Tooth disease (CMT2) and the screening of the MFN2 gene is recommended as the first step in molecular diagnosis in such cases.

Objective: To characterize electrophysiologically and explain genetic cause of severe CMT in a 3.5-year-old with asymptomatic parents and maternal grandfather with history of mild adult-onset axonal neuropathy.

Methods: Neuropathy severity was assessed with Charcot-Marie-Tooth neuropathy score (CMTNS). Whole exome sequencing (WES) was performed with Illumina TruSeq Exome Enrichment Kit on the HiSeq 1500 with results followed up by Sanger sequencing on ABI Prism 3500XL. Paternity was confirmed using a panel of 15 hypervariable markers.

Results: Electrophysiological studies demonstrated severe axonal sensory-motor neuropathy in the proband, mild motor neuropathy in his mother and mild sensory-motor neuropathy in his grandfather. CMTNS in the proband was 21 (severe), while his mother and grandfather had mild to moderate severity (CMTNS 1 and 12, respectively). On genetic analysis the boy was found to carry both a heterozygous dominant MFN2 T236M mutation transmitted via maternal line and de novo GDAP1 H123R mutation.

Conclusion: Our findings emphasize the need to search for more than one causative mutation when significant intrafamilial variability of CMT phenotype occurs.

PS3-363 / #345

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Diagnostic neurosonography in demyelinating Charcot-Marie-Tooth Disease type 1A

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Background: Ultrasonography is a useful tool for evaluating conditions of the peripheral nerve because of high-resolution anatomic information of nerves to complement standard electrodiagnostic studies. There have been several studies in which sonography revealed the enlargement of peripheral nerves in patients with demyelinating Charcot-Marie-Tooth disease (CMT).

Objective: In this study, nerve cross-sectional area in individuals with CMT disease type 1A (CMT1A) are compared with normal, healthy control subjects to describe the ultrasonographic changes that occur in the nerves of hereditary neuropathic patients.

Methods: Seventy CMT1A patients and 60 control subjects underwent ultrasonography of peripheral nerves. Median and ulnar nerve cross-sectional areas in the wrist, mid-forearm, elbow, and mid-arm regions were also measured. Ultrasound examinations were performed using an imaging system with an L12-5 linear array probe. Statistical analysis was performed using the Mann-Whitney U test.

Results: Patients with CMT1A have larger median and ulnar nerves than healthy control subjects ($p < 0.001$). The authors found length-dependent proximal-to-distal relative reduction of nerve size and age-dependent swelling of nerve cross-sectional area in CMT1A patients. For patients in their 20s, the nerve size of those with CMT1A increased rapidly, and, for patients in their 50s, the nerve size decreased to levels of teenagers. No similar findings in normal control subjects.

Conclusion: The study showed increased cross-sectional area of the median and ulnar nerves in the upper extremities in patients with CMT1A when compared with control subjects. These findings might suggest the usefulness of neurosonography for evaluation of the natural history of CMT1A. Further studies of greater numbers of other CMT subjects and more nerves are needed to confirm ultrasonography as a useful diagnostic tool in CMT.

PS3-364 / #427

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Hereditary axonal neuropathy with neuromyotonia - mutation p.R37P in the HINT1 gene is surprisingly frequent cause of HMN and HMSN II in Czech patients and neuromyotonia was frequently overlooked.

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Mutations in the HINT1 gene were recently discovered as the cause of autosomal recessive axonal neuropathy with neuromyotonia.

We aimed to establish the importance of HINT1 mutations as the cause of HMSN II and HMN among Czech patients and clinically characterize patients with biallelic mutations.

Initially we used Sanger sequencing of all three coding exons of HINT1 gene among in total 166 unrelated patients with inherited neuropathy of yet unknown cause. One hundred and fifty patients were sporadic and 16 had one affected sibling and were classified as autosomal recessive (AR). From the sporadic patients 122 were classified as HMSN II and additional 28 patients were classified as HMN. Additional 750 samples from unclassified patients all without dominant inheritance were screened. Both pathogenic mutations in HINT1 were found in total in 11 unrelated patients (13 individuals). Only two different mutations were found in this cohort. Ten patients were homozygotes for the previously reported and highly prevalent mutation p.Arg37Pro and one patient was compound heterozygote for p.Arg37Pro and a novel mutation p.Gln106Stop. Therefore in further 12 HMSN II patients only exon 1 was sequenced and in 3 of them p.R37P was found in homozygous state. Finally 750 patients with unknown cause of neuropathy were screened by a real time PCR assay for detection of p.R37P and further 5 patients with biallelic mutations were detected. Four were homozygotes and one compound heterozygote with p. Gln106Stop. In total biallelic mutations in HINT1 were detected in 21 patients from 19 families. All patients with HINT1 mutations we have reexamined experienced symptoms before the age of 9 years. Neuromyotonic discharges were noticed in original electrophysiological reports only in one out of 13 patients with both pathogenic mutations despite highly experienced neurologists and electrophysiologists. In general lower limbs were much more affected by distal weakness then upper limbs. HINT1 mutations seem to be a surprisingly frequent cause of hereditary neuropathy (comparable to MFN2) among Czech patients even in patients without manifest neuromyotonia. All patients with axonal HMSN or HMN of Czech origin, without clear dominant inheritance should be tested for the prevalent mutation p.R37P in the HINT1. Occurrence and frequency of this type of hereditary neuropathy should be tested also in other populations.

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PS3-365 / #449

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Autosomal dominant spinal muscular atrophy (ADSMA) with brisk tendon reflexes in a Czech patient with a de-novo complex mutation in the SETX gene

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Mutations in Senataxin gene (SETX) are associated with at least three genetic conditions and these are: 1. autosomal dominant form of juvenile amyotrophic lateral sclerosis (ALS4); 2. autosomal recessive ataxia

with oculomotor apraxia type 2 with (AOA2); and 3.autosomal dominant proximal spinal muscular atrophy (ADSMA).

ADSMA is a very rare genetic condition characterized by proximal and distal muscular weakness. The cause of the disease remains unknown in most patients. Here we present a patient with hereditary motor neuropathy with prominent proximal weakness and de-novo mutation in the SETX gene.

The patient is a sporadic case, no other family members are affected. Muscle weakness, mainly on lower limbs, was noticeable from the preschool age. The patient was first examined at the age of 9 years because of lower limb weakness and slow running. Proximal weakness was noticed, but he was able to rise from a squatting position, tendon reflexes were increased, but pyramidal signs were not present.

At the age of 13 years, he had problem with running and climbing stairs and at examination he had pronounced proximal and distal muscle weakness, muscle atrophies and brisk reflexes. He was unable to rise from a squatting position. Parents reported slow progression of his weakness. Electromygraphy examination revealed predominantly motor axonal polyneuropathy.

Recently we used next generation sequencing (custom designed HaloPlex kit, Agilent) method - testing with panel of known CMT genes (59 genes were included). A mutation in the SETX gene was identified and confirmed by Sanger sequencing.

The mutation c.[1656G>T(;)1658C>T] in SETX gene was detected. This mutation was not detected in patient`s parents, probably arose de-novo. We concluded the mutation causes change NM_015046.5>c.1656_1658delCTGinsTTT on one allele of the SETX gene. The indel mutation causes the loss of 2 residues and the insertion of HisPhe (p.Gln552_Ser553delinsHisPhe). This is an in-frame mutation, however due to the change of two aminoacids the mutation is probably causal for ADSMA in this patient. De-novo origin and patient`s phenotype further support this hypothesis. No variants have been reported at this position of the SETX gene in various databases (EVS, dbSNPbuild137, ESP, dbSNP ShortVariants/Swiss Prot Variants).

Our report expands the spectrum of phenotypes associated with SETX mutations and supports the findings by Rudnik-Schoneborn on the topic of ADSMA.

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PS3-366 / #455

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Pseudodominant inheritance in HINT1 neuropathy

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Introduction: Loss-of-function mutations in HINT1 cause axonal neuropathy with neuromyotonia (ARAN-NM) (Zimon M et al, 2012). Purpose: To present multigeneration transmission of the homozygotic HINT1 mutation. Methods: Neurological examination was performed by the same neurologist (VMR) Conventional ENMG technique was applied (Premier, Medelec apparatus) according to standard protocol. Molecular genetics studies were done in VIB Department of Molecular Genetics, Antwerpen and in PCR lab in Belgrade. Results: All 17 patients from 10 families expressed unique phenotype: severe neuropathy, dominantly motor, with active myotonia. ENMG was typical for axonal neuropathies. Spontaneous activity was neuromyotonic, frequently found in distal muscles. Type of inheritance was multigeneration in one family and recessive in others. Homozygote, founder R37P mutation in HINT1 gene was detected in all patients. Discussion and Conclusion: Our population expressed founder mutation in HINT1 gene and possible pseudodominant transmission of the disease. Neurophysological testing of nerves would be important in detecting neuromyotonia and directing genetic analyses to recessive neuropathies.

PS3-367 / #466

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Familial Amyloid Polineuropathy: Efficacy of liver transplant versus tafamidis in nerve fiber function

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Background: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is an inherited amyloidosis that presents as a progressive sensorimotor and autonomic polyneuropathy. Orthopic liver transplant and tafamidis meglumine was, to date, the only disease-modifying treatments proved to delay disease progression and preserve nerve fiber function. Neurophysiologic parameters, including sensory and motor nerve amplitudes and motor nerve velocities, have been used to assess TTR-FAP nerve function.

Objective: To compare effect of Tafamidis versus liver transplant in nerve conduction studies of TTR-FAP patients over 12 months.

Methods: Nerve conduction data from 53 stage I TTR-FAP patients was analysed retrospectively for 12 months after treatment. Twenty nine TTR-FAP patients, mean age 37,21 years (±8,94) , submitted to liver transplant were compared to 23 TTR-FAP patients on Tafamidis, mean age 38,38 years (±6,73).

A large fiber neurophysiological score composed by seven attributes of NCS as summated normal deviate score was used. Small fibers was assessed by a composed score of sympathetic skin response (SSR) obtained by the summing of hand and plantar SSR amplitudes. Disease duration to baseline and Karnofskyindex were also evaluated.

Paired-samples t-tests were used; a p value<0,05 was considered significant.

Results: We found no statistically significant differences in neurophysiological and SSR scores progression between the two groups over 12 months of treatment.

Disease duration at baseline and Karnofsky index were not significantly different between groups.

Conclusion: Efficacy of Tafamidis on nerve fiber function, of TTR-FAP patients, seems to be similar to liver transplant. As reported in the literature both treatments seems to stabilize disease progression and preserve nerve function.

PS3-368 / #492

Theme: *5.2 - Peripheral neuropathy of genetic origin*

MFN2 deletion founder mutation in the UK population

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Mitofusion 2 (MFN2) mutations are the most common cause of axonal Charcot-Marie-Tooth disease (CMT2). The majority are inherited in an autosomal dominant manner but recessive and semi-dominant kindreds have also been described. We previously reported this deletion resulting in nonsense mediated decay, segregating with disease when present in trans with another pathogenic MFN2 mutation.

Detailed clinical and electrophysiological data on five affected patients and, when available, their parents and relatives was collected. MFN2 sequencing was performed followed by multiplex ligation probe amplification (MPLA assay) to identify large deletions when a heterozygous mutation was found. Haplotype analysis was also carried out.

A severe early-onset CMT phenotype was seen in all cases: progressive distal weakness, wasting and sensory loss from infancy or early childhood. Optic

atrophy (3/5) and wheelchair dependency by age 20 were common (4/5). All were compound heterozygous for a deletion of exon 7 and 8 in MFN2 with another previously reported pathogenic mutation (Phe216Ser, Thr362Met and Arg707Trp). Carrier parents and relatives were unaffected (age range: 32-65 years). Haplotype analysis confirmed that the deletion had a common founder in all families.

Here we present five patients with severe, early-onset CMT2 compound heterozygous for a deletion of exon 7 and 8 in MFN2 with haplotype analysis confirming this deletion as a founder mutation in the UK population.

PS3-369 / #493

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Neuropathy phenotype in Hereditary Transthyretin Amyloidosis

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Hereditary transthyretin amyloidosis (ATTR) is associated with progressive peripheral neuropathy, cardiac, gastrointestinal and autonomic failure due to dominantly inherited transthyretin mutations causing accelerated amyloid deposition. The neuropathy phenotype is less well described than cardiac manifestations.

A cross-sectional study of ATTR patients attending the National Hospital Inherited Neuropathy Clinic. Detailed clinical neurological and electrophysiological data were collected on all patients alongside correlating autonomic and cardiac assessments. Follow-up data was available on a subset.

Thirty four patients (mean age at presentation$n=$ 62 years; 70.6% male) were assessed at least once; 19 cases (55.8%) had serial examinations, mean follow-up: 1.9 years. The genetic breakdown was 38.2% T60 A, 14.7% V30M, 8.8% I107F, 5.9% V122I, 32.4% individual mutations; 76.5% UK and/or Irish ancestry. 44.1% were treated (Diflunasil or liver transplant). 26.5% presented with neuropathy; 70.6% had neuropathy on follow-up. Positive and negative sensory phenomena were equally prevalent at presentation; with negative symptoms slightly more common in T60A patients ($p=0.22$). A length-dependant, axonal, sensory followed by motor neuropathy was typical; 6.7% had patchy onset and 10% had demyelinating features. Mean MRC score at first examination was 62.5 with a mean reduction of 2.7 points/year and a trend to slower deterioration in the treated group (2.4 versus 3.0 points/year; $p=0.77$).

The detection of measurable annual changes in MRC score is encouraging but monitoring of sensory deficits should also be quantifiable. This small but representative study mirrors difficulties observed in recent treatment trials regarding sensitivity of current outcome measures.

PS3-370 / #494

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Transthyretin cardiac amyloidosis (V122I) with clinical and histological evidence of amyloid neuropathy and myopathy

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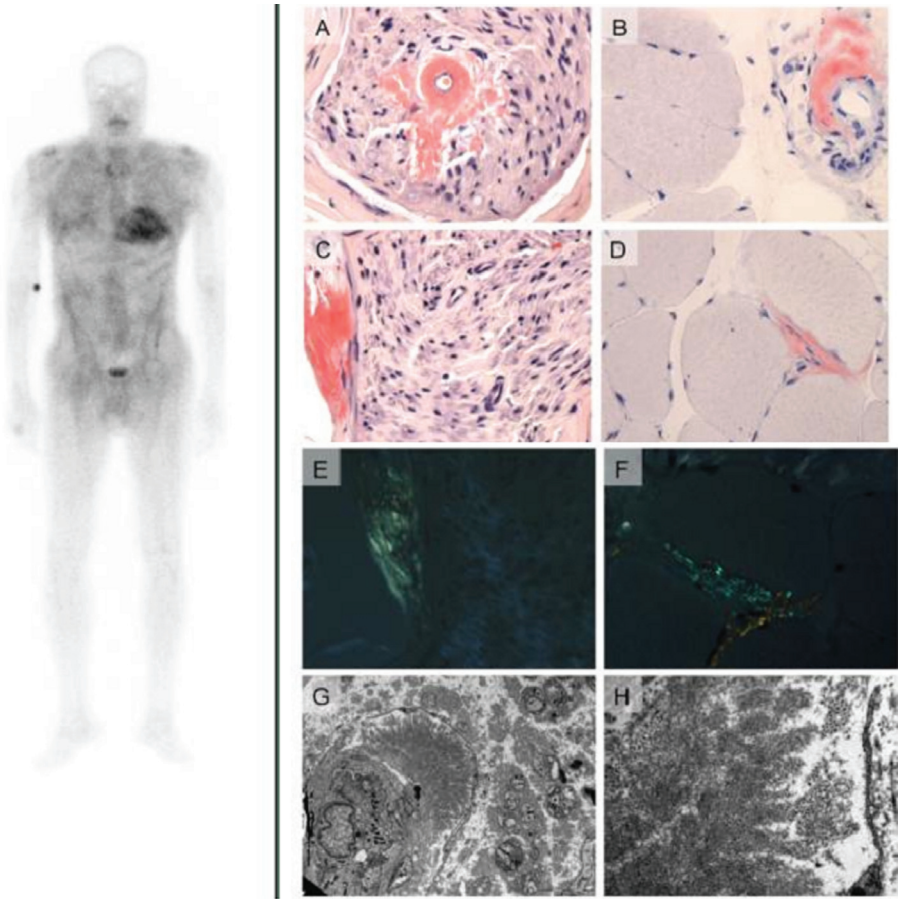
Hereditary transthyretin-related amyloidosis (ATTR) is a genetically heterogenous disease which usually presents with a predominant peripheral and autonomic neuropathy, a cardiac myopathy, or with both neurological and cardiac involvement. The V122I variant is the most common mutation associated with a predominantly cardiac phenotype.

Here we present the clinical, electrophysiological and histological findings of an unusual case of V122I ATTR with ^{99m}Tc-DPD scintigraphic evidence suggesting muscle amyloid deposition and with histological confirmation of both amyloid neuropathy and myopathy.

A 64 year old Jamaican man presented with cardiac failure. Cardiac MR revealed an infiltrative cardiomyopathy; abdominal fat aspirate confirmed the presence of amyloid and a homozygous V122I mutation in the transthyretin gene were identified. Serum amyloid P scintigraphy showed no evidence of systemic amyloid and a diagnosis of ATTR was made. Whole body ^{99m}Tc-3,3-Diphosphono-1,2-Propanodicarboxylic acid (DPD) scintigraphy showed uptake of tracer into in the heart and skeletal muscle. He also described general, non-specific weakness and EMG demonstrated some myopathic features. The sural nerve biopsy showed TTR amyloid deposits in the perineurium, endoneurium and circumferentially in the walls of endoneurial blood vessels. Amyloid deposits in the perimysium, endomysium and in the walls of small-sized blood vessels were also identified in the vastus lateralis muscle. The patient is being treated with diflunasil, an oral TTR stabilizing agent.

V122I has an estimated frequency of 3-4% in African Americans and a well described cardiac amyloidosis phenotype. Symptomatic neuropathy or myopathy with morphological confirmation of tissue amyloid deposition has not been previously described. The presence of extracardiac amyloidosis in these cases has implications for diagnosis and treatment.



PS3-371 / #552

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Search for genetic modifiers of CMT1A and HNPP by evaluating the extremes of the clinical spectrum

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Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP) are autosomal dominant peripheral neuropathies caused by copy number variation of the PMP22 gene. Considerable intrafamilial and interfamilial phenotypic variation is known for both disorders, suggesting the presence of modifiers. We previously identified cases of "double trouble" (the presence of two mutations in two different CMT-related genes) in severely affected patients, indicating that CMT-related genes indeed can act as modifiers. We undertook a search for genetic modifiers of PMP22 related neuropathies by selecting the extremes of the phenotypic spectrum of CMT1A and HNPP patients, based on disability assessed by the Overall Neuropathy Limitation Scale (ONLS). The ONLS has a minimum score of 0, meaning no disability and a maximum score of 12, meaning not being able to make purposeful movements with arms and legs. The ONLS was taken by telephone interview in 224 CMT1A and 114 HNPP patients and showed a Gaussian distribution for both disorders. The median score for CMT1A patients was 4 and for HNPP patients 3. Twenty-one mild CMT1A patients (ONLS <2), 26 severe CMT1A patients (ONLS >5), 26 mild HNPP patients (ONLS <2) and 24 severe HNPP patients (ONLS >4) were clinically evaluated to further characterize disease severity. Clinical evaluation included neurological examination, including extensive sensory testing, dynamometry of foot dorsiflexion and three point grip, hand function test, walk tests, and Charcot-Marie-Tooth Neuropathy Score (CMTNS). A sequence capture including 50 genes for inherited neuropathies was developed to screen all mild and severe patients' DNA for sequence variants, followed by next generation sequencing. Association between the DNA variants and the extremes of the phenotypic spectrum of CMT1A and HNPP are pending.

PS3-372 / #554

Theme: *5.2 - Peripheral neuropathy of genetic origin*

Rasch-built overall disability scale for Charcot-Marie-Tooth disease (CMT-RODS)

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Charcot Marie Tooth disease (CMT), also called hereditary motor and sensory neuropathy (HMSN), is the most common inherited neuromuscular disorder, characterized by slowly progressive predominantly distal wasting, weakness, and sensory loss, legs more than arms. Measuring activities and participation in this disorder is challenging. The Charcot-Marie-Tooth disease neuropathy score (CMTNS) was used by the CMT community for measuring disability in natural history studies and treatment trials in CMT patients, but has limitations being a composite ordinal-based metric.

We aim to construct an outcome measure at activities and participation level to assess outcome of patients with CMT using the Rasch method. A 149-item questionnaire was completed by 120 cases of CMT1A, an autosomal dominantly inherited form of CMT caused by copy number variation of the PMP22 gene. The cohort is currently being expanded by including patients of the Inherited Neuropathy Consortium (INC).

Data will be subsequently subjected to Rasch analyses in order to create a proper interval metric, fulfilling all model requirements. Through systematic investigation of response category ordering, model fit, item bias, and local response dependency, an easily applicable unidimensional scale will be constructed (CMT-RODS). Internal consistency and test-retest reliability values of item difficulty hierarchy and patient's ability of patient location (degree of functional deficit on the created ruler) will be also analysed.

The final specifically developed CMT-RODS will subsequently be tested in future studies to examine its responsiveness in these indolent illnesses.

PS3-373 / #57

Theme: *5.3 - Peripheral neuropathy: metabolic / toxic / paraneoplastic*

The role of insulin resistance in diabetic neuropathy in Koreans with type 2 diabetes mellitus: A 6-year follow-up study

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Purpose : We previously reported that insulin resistance, low HDL cholesterol, and glycaemic exposure (GE) Index are independently associated with peripheral neuropathy in Korean patients with type 2 diabetes mellitus. We followed the patients who participated in that study in 2006 for another 6 years to determine the relationship between insulin resistance and neuropathy.

Materials and Methods: This study involved 48 of the original 86 Korean patients with type 2 diabetes mellitus who were referred to the Neurology clinic for the assessment of diabetic neuropathy from January 2006 to December 2006. These 48 patients received management for glycaemic control and prevention of diabetic complications in the outpatient clinic up to 2012. We reviewed blood test results and the nerve conduction study findings of these patients taken over a 6-year period.

Results: Low HDL cholesterol and high triglycerides significantly influence the development of diabetic neuropathy. Kitt value (1/insulin resistance) in the previous study affected the occurrence of neuropathy, despite adequate glycaemic control with HbA1C <7%. Insulin resistance affected the development of diabetic neuropathy after 6 years: Insulin resistance in 2006 showed a positive correlation with a change in sural sensory nerve action potential (SNAP) in 2012.

Conclusion: Diabetic neuropathy can be affected by previous insulin resistance despite regular glycaemic control. Dyslipidaemia should be controlled in patients who show high insulin resistance because HDL cholesterol and triglycerides are strongly correlated with later development of diabetic neuropathy.

PS3-374 / #86

Theme: *5.3 - Peripheral neuropathy: metabolic / toxic / paraneoplastic*

Carpal tunnel syndrome in pediatric mucopolysaccharidoses

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Background: Carpal tunnel syndrome (CTS) is rare in children but is a recognised complication of the mucopolysaccharidoses (MPS). Clinicians should have a low threshold of suspicion for carpal tunnel syndrome in this group as symptoms may be atypical or minimal, especially in those with intellectual disabilities secondary to mucopolysaccharidoses. If untreated, CTS can cause significant, potentially permanent loss of hand function. We present findings in 11 children with MPS and suspected CTS, and propose guidelines for screening for carpal tunnel syndrome in children with these disorders.

Methods: Clinical and electrodiagnostic data of 11 children with confirmed MPS by enzymatic +/- molecular testing, who were suspected on clinical grounds to have carpal tunnel syndrome, was reviewed. All subjects underwent motor and sensory conduction studies of bilateral median and ulnar nerves. The presence of CTS and its severity was determined. Subsequent details of intervention(s) and recurrence were noted.

Results: Three children had Hurler syndrome (MPS I), five had Hunter syndrome (MPS II), one had Sanfilippo syndrome (MPS III) and two had Morquio syndrome (MPS IV). Seven had motor and three sensory features referable to median nerve compression. Nine of the eleven children (2/3 with MPS I, 5/5 with MPS II, 0/1 with MPS III, 2/2 with MPS IV) had median neuropathies at the wrist, (eight bilateral, one unilateral) which were mild in three, moderate in five, and severe in one. Three children presented with symptoms at age five years age. Six underwent median nerve decompression. Four of these had recurrent symptoms several years after surgery. Recurrent carpal tunnel syndrome was confirmed on nerve conduction studies in two cases. To the best of our knowledge, this is the first report of carpal tunnel syndrome in MPS IV.

Conclusion: Some children with MPS experience early development of at least moderately severe CTS. We recommend screening for CTS from age 5 years for children with MPS, particularly types I, II and IV, irrespective of symptoms or specific treatment received for the storage disorder. Those with no evidence of CTS and those having undergone surgery should be assessed annually by a neurologist and at regular intervals for evolution of compressive median neuropathy.

PS3-375 / #88

Theme: *5.3 - Peripheral neuropathy: metabolic / toxic / paraneoplastic*

Paediatric podophyllin neurotoxicity with neurological sequelae

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Podophyllin is a crude plant extract used in the cutaneous treatment of anogenital warts. Toxicity in adultpatients is well documented. Although rare,accidental ingestion and suicidal poisoning is associated with high morbidity and mortality.The toxic effects are multisystemic.There are few reported paediatric cases and this is the first in South Africa.

This is a case of accidental Podophyllin ingestion and neuroregression of a 3 year old boy of African ethnicity with peripheral axonal and autonomic neuropathy, anterior horn cell involvement and encephalopathy with cerebral atrophy.

History

15h00 Accidental ingestion.Premorbid normal development

18h00 ataxia,drooling,vomiting,diarrhoea

19h00 confusion

22h00 quadriplegia,cool to touch

Clinically

Encephalopathy E1 V1 M3

Pupils 4mm and sluggish light-reaction

Spinal shock:flaccid quadriplegia and areflexia

Maintained ventilation on nasal prong oxygen

Course

Seizures within 48 hours of admission aborted with Lorazepam

Encephalopathy resolved day 5

Weaned off oxygen on day 6

Paralytic ileus on day 16

Bulbar palsy

aphasia

Tongue fasiculations and polyminimyoclonus

Incontinence

Diaphoresis, tachycardia

4 months

Dysphonia,absent gag reflex,comprehends

quadriparesis

upper limbs: Hypertonia,brisk bicep reflex.Power:0 in all except finger flexion 2

lower limbs: Hypotonia,bilateral foot drop, tight TA.Power: hip flexion 2, dorsiflexion 0

6 months

Expressive aphasia,weak gag reflex,normal pain sensation. Muscle atrophy,lower limb hypertonia. Power: shoulder abduction 2, hip flexion 3, dorsiflexion 2.

Investigations

HIV negative, MRI spine, EEG, VCU normal

MRI brain: cerebral atrophy

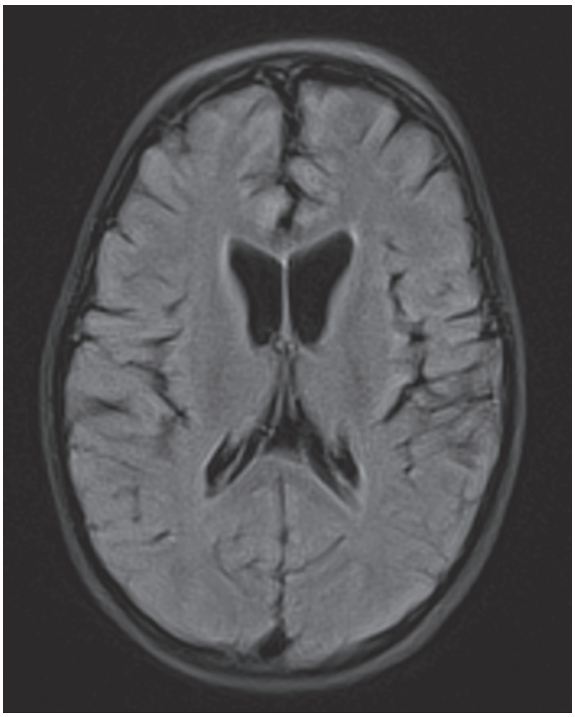
NCS/EMG 4 months:AMSAN. Fibrillations

Repeat NCS/EMG 6 months:normal Median and Ulnar. Other responses absent.Quadriceps: Fibrillation and fasiculations. Biceps Brachhii: PSW, fasiculations and polyphasia

Discussion: The patient presented with rapid onset mixed signs and features of myeloneuropathy and encephalopathy. Clinical fasiculations was an early and persistent feature. Although encephalopathy and seizures resolved early, significant neurodisability persisted at 6 months. Podophyllin neurotoxicity

spectrum includes myeloneuropathy, autonomic and axonal sensory neuropathy, sensory ataxia, necrotising myopathy, encephalopathy and seizures. Seizures and cerebral atrophy may herald a poor prognosis. Early haemofiltration may improve outcome. This case illustrates both peripheral and central nervous system toxicity with residual sequelae

Podophyllin toxicity cerebral atrophy



PS3-376 / #162

Theme: *5.3 - Peripheral neuropathy: metabolic / toxic / paraneoplastic*

Steroid treatment in diabetic lumbosacral radiculoplexus neuropathy: an observational study

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Introduction: Diabetic lumbosacral radiculoplexus neuropathy (DLRPN), also known as diabetic amyotrophy or Bruns-Garland syndrome,consists in an acute neuropathy that may appear as complication of diabetes mellitus. Although the pathophysiologic mechanism is not well established, recent studies point to an immune-mediated vasculitis of the vasa nervorum. Randomized controlled studies failed to prove the effectiveness of intravenous immunoglobulins or corticosteroids as treatment, so therapeutical recommendations are mainly based on isolated cases and retrospective case series.

Methods: We describe the characteristics, treatment and outcome of a cohort of DLRPN patients diagnosed in our unit between 2011-2013.

Results: 7 patients were diagnosed and 6 of them were male. Median age was 67 (range 51-78).In 4 cases the onset of the neuropathy coincided with the diagnosis of the diabetes mellitus. Median glycated hemoglobin was 7.6 (range 6.2-9.5). 6 patients presented bilateral (motor and sensitive) symptoms. 5 patients exhibited albuminocytologic dissociation in the CSF. Severity of symptoms was measured according the Neuropathy Impairment Score in the Lower Limbs (NIS-LL). Median NIS-LL at nadir was 24 (range 13.5-39.5). Decision to treat was left at the discretion of the neurologist who initially attended the patient, so 4 of 7 patients were treated and all 4 received oral corticosteroids.

We did not identify any statistically significant differences existed in terms of demographic, clinical characteristics and severity between the treated and untreated patients, but those treated exhibited a trend to higher recovery at first month (median improvement measured as the difference between NIS-LL at nadir and NIS-LL 1 month later was 17.5 vs 4, $p = 0.08$).

Conclusions: The characteristics and outcome of a cohort of 7 DLRPN patients are described. A trend to a greater and earlier improvement in the corticosteroid-treated group was noted.

PS3-377 / #202

Theme: *5.3 - Peripheral neuropathy: metabolic / toxic / paraneoplastic*

He-Ne Laser Therapy for Painful Diabetic Polyneuropathy Patients

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Diabetic polyneuropathy is one of the most common complications in the progression of diabetes mellitus. The effect of He-Ne laser therapy on static and dynamic planter pressure distribution, pain intensity level and electrophysiological function of peroneal and sural nerves in painful diabetic polyneuropathy patients was evaluated. Purposive sampleof 30 male and female patients with painful diabetic neuropathy and abnormal results of nerve conduction studies were selected . Their ages ranged from 45 to 60 years with a mean of 52.1 ± SD 4.7 years. In a design of pre test -post test control group design, patients randomly assigned into two equal groups of 15; an active laser group and a placebo laser group (control group). The laser group received scanning He-Ne infrared laser with 850 nm wavelength and density of 5.7 J/cm^2, applied to the lumbosacral area and the plantar surface of the foot for 15 min each site/session three times per week for four weeks (i.e. 12 sessions). Peak static and dynamic planter pressure was measured under three functional areas of the feet: big toe, little toe and mid heel using foot scan plate system ,pain intensity level via visual analogue scale and bilateral peroneal motor nerves and sural sensory nerves conduction velocity and amplitude were measured pre- and post-treatment for both groups. Peak static and dynamic foot planter pressure and pain intensity were significantly decreased ($p \leq 0.05$) and electrophysiological parameters were significantly improved ($p \leq 0.05$) in the laser group, while no significant change was obtained in the control group. The significant results of laser group could be due to bio stimulating effects of laser on nervous tissue that relief pain and improve conduction through peripheral nerves. Laser improve circulation that reflect on redistribution of pressure on planter foot surface. He - Ne laser therapy within applied parameters and technique could be an effective therapeutic modality in reducing foot plantar pressures and pain intensity and improving neurophysiological function in patients with painful diabetic polyneuropathy.

PS3-378 / #307

Theme: *5.3 - Peripheral neuropathy: metabolic / toxic / paraneoplastic*

Unusual association between myasthenia gravis, chronic inflammatory demyelinating polyneuropathy (CIDP) and Kaposi sarcoma

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The association of CIDP refractory to treatmment, Kaposi sarcoma and Myasthenia Gravis is extremely unusual, and the physicians should consider the possibility of an occult neoplasm.

A 74- year old man had ACRA (+) Myasthenia Gravis (MG) with bulbar onset since 2011 on clinical remission using pyridostigmine and meprednisone 20mg /d .

He later developed generalized weakness and lost 10 kg of weight over 3 months. A relapse of MG was considered and he received both IVIG and plasmapheresis without benefit. Moreover he developed red-wine cutaneous lesions in his legs. Skin biopsy confirmed Kaposi sarcoma (KS) and he was transferred to our hospital.

His neurological examination revealed, symmetric flaccid quadriparesis, generalized arreflexia, marked distal loss of pin prick, light touch, vibration and position sense in 4 limbs.

His CSF showed 2 lymphocytes, 0,95 gr/dl protein, 0,69 glucose,with normal cultures and no malignant cells. Anti Hu, anti Ri and anti Yo antibodies HIV and antigen P24 were negative.

EMG revealed a sensory motor demyelinating polyneuropathy and received a second course of IVIG 2g/kg

Chest CT showed a nodular lesion on the right lung whose resection revealed a non small cell lung adenocarcinoma. The patient died 1 month after admission.

Conclusion: CIDP is only rarely encountered as a paraneoplastic disorder outside the association with plasma cell dyscrasias or lymphomas. Moreover there

are no associations reported between CIDP, Kaposi and MG.

In patients with CIDP refractory to treatment combined with Kaposi sarcoma the search for an occult neoplasia is mandatory.

PS3-379 / #433

Theme: *5.3 - Peripheral neuropathy: metabolic / toxic / paraneoplastic*

Nav 1.8 nociceptive neurons excitability and inflammation in Painful Diabetic Neuropathy

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Neuropathic pain in diabetes or Painful Diabetic Neuropathy (PDN) is a debilitating affliction present in 26% of diabetic patients with substantial impact on their quality of life. Despite this significant prevalence and impact, current therapies for PDN are only partially effective. Moreover, the electrophysiological mechanisms underlying PDN are not well understood. Therefore, given the magnitude of PDN and the absence of effective therapies, the research object of this proposal is to elucidate the molecular and electrophysiological mechanisms responsible for PDN as a critical step towards more effective therapies.

Neuropathic pain is caused by sustained excitability in sensory neurons which reduces the pain threshold, so that pain is produced in the absence of appropriate stimuli. Sensory neurons display sustained and enhanced excitability in response to different molecules including chemokines.

We demonstrated that chemokine CXCR4/SDF-1 signaling is necessary for the induction of PDN in animal models of type II diabetes. Indeed, the specific CXCR4 antagonist, AMD3100, reverses PDN in animal models of type-II diabetes. Additionally, we demonstrated that application of SDF-1 also increased intracellular calcium concentration, in acutely isolated diabetic DRG neurons. Furthermore, electrophysiological current clamp studies demonstrated CXCR4/SDF-1 mediated hyper-excitability in diabetic Nav 1.8 nociceptive neurons.

We are now investigating the communication between Nav 1.8 nociceptive neurons hyper-excitability and inflammation using sophisticated chemo-genetic silencing of DRG neuron subtypes using mutated hM4D receptor (DREADD) receptors. The DREADD receptor can be selectively activated by the drug clozapine-N-oxide (CNO) allowing the subpopulation of neurons in which it is expressed to be specifically silenced during the time the drug is on board.

Overall, these observations establish chemokine signaling as a new candidate responsible for hyper-excitability in a distinct subpopulation of DRG neurons and inflammation underlying PDN, and will open up a completely new field of molecular investigation in this disease entity. Additionally, these experiments will add to our understanding of how changes in the excitability of sensory neurons contribute to the progression of small fiber neuropathy in PDN, which is a critical barrier to progression for effective treatment of this currently intractable and widespread affliction.

PS3-380 / #478

Theme: *5.3 - Peripheral neuropathy: metabolic / toxic / paraneoplastic*

Risk factors for ulnar nerve neuropathy - toxic and metabolic influences play a role

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Focal neuropathy of an ulnar nerve at the elbow is the second most common mononeuropathy. Diagnosis and monitoring is based on history, neurological examination and electromyography. However, the etiology of this neuropathy is not often exactly evaluated and a therapy is generally focused on rehabilitation, reduction of physical activity and surgical treatment.

The aim of this study is to evaluate the laboratory tests of patients with neuropathy of the ulnar nerve in the elbow and determine whether toxic and/or metabolic influences might play a role. The group included 26 patients with mild neuropathy of the ulnar nerve, which was characterized by mild atrophy, paralysis, paresthesia and hypoesthesia in the area of the nerve. An average (median) age was 46 ± 19 (44) years. The diagnosis was made clinically and electromyographically. All patients had undergone detailed blood tests. 23% of patients had hepatopathy (elevation of CDT and GGT), 15% of patients had defect of sugar metabolism and 8 % of patients had thyreopathy. 15% of patients had a combination of the diseases mentioned before. These percentage significantly exceed the average finding of general population in Czech Republic. Only a minority of our patients were without laboratory abnormalities. Conclusion: In patients with neuropathy of the ulnar nerve in the elbow, although without disease history, is needed to eliminate this toxic and metabolic diseases: alcoholism, diabetes mellitus and thyreopathy.

PS3-381 / #96

Theme: *5.4 - Peripheral neuropathy: others*

A case of tarsal tunnel syndrome secondary to varicose veins

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Tarsal tunnel syndrome (TTS) is a compression neuropathy of the posterior tibial nerve or its branches within its fibro-osseous tunnel located behind and below the medial malleolus. Numerous etiologies have been described explaining this entrapment, including trauma, space-occupying lesions, foot deformities, etc. We report a case of TTS secondary to varicose veins.

A 52-year-old man was admitted to our hospital due to burning pain and tingling sensation in the right sole for 4 months. Pain occurred after wearing shoes with an uncomfortable heel for last winter and was worse with weight bearing such as walking or standing. He also experienced pain of the same type in the left sole 2 months ago. He was in good physical health previously and had no traumatic history. On physical examination, there was no weakness or atrophy of the intrinsic foot muscles. Sensory disturbance and Tinel's sign were absent. Routine nerve conduction studies (NCS) of the right peroneal, posterior tibial, sural and superficial peroneal nerves were normal. Sensory NCS of bilateral median and lateral plantar nerves also were normal. Needle electromyography (EMG) showed denervated patterns in the right flexor digitorum accessorius and abductor hallucis, suggestive of partial injury in the right plantar nerve. MRI of both foot and ankle revealed high signal intensity in the quadratus plantae muscle, indicating denervation myopathy. MRI also showed engorgement of posterior tibial vein from lower leg to foot. Based on the clinical, electrophysiological and MR findings, a diagnosis of tarsal tunnel syndrome secondary to varicose veins occupying the tarsal tunnel was made. He was referred to our orthopedist and then underwent resection of varicose vein in both ankles to release tarsal tunnel. His symptoms have improved gradually after the surgery.

Tarsal tunnel syndrome is a rare but important condition which is regularly under diagnosed leading to a range of symptoms affecting the plantar aspect of the foot. Causes of TTS are numerous and false negative electrophysiological studies are not uncommon. Therefore, detailed history and clinical examination are essential for accurate diagnosis of TTS. In addition, appropriate use of electrodiagnostic and radiographic tests is necessary to confirm the diagnosis.

PS3-382 / #114

Theme: *5.4 - Peripheral neuropathy: others*

Euglycemic therapy restores endothelial and autonomic function in diabetic rats

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Background: Diabetes-associated endothelial and autonomic dysfunctions are major causes of mortality and morbidity in diabetic patients. In this study, the impact of blood sugar on endothelial and autonomic functions at acute and chronic stage was assessed in streptozotocin (STZ)-induced diabetic rats.

Methods: Five eight-week-old male Winstar Kyoto rats received intraperitoneal STZ and nicotinamide (NA), followed by weekly check of blood sugar. Endothelial and autonomic functions were respectively quantified using reactive hyperemia-induced dilatation index (DI) and standard deviation SD1/SD2 ratio (SSR) from Poinecaré method at age of 8 weeks, 24 weeks, and 32 weeks. Five age-matched controls receiving intraperitoneal physiological saline only followed the same protocol. Effect of euglycemic therapy was assessed after subcutaneous insulin at age of 32 weeks.

Results: Diabetes mellitus (blood sugar >250 mg/dL) occurred 16 weeks after STZ-NA administration. Blood sugar of STZ-treated rats was much higher than age-matched littermates at age of 24 weeks (450.8±62.2 mg/dL vs. 88.4±15.0 mg/dL, $P<0.001$). There were also significant decreases in DIs (1.97±0.67 vs. 3.03±0.35, $P<0.05$) and SSRs (0.29±0.11 vs. 0.99±0.14, $P<0.001$) between these two groups. After insulin treatment at the second month of diabetes, diabetic rats attained euglycemic status with similar blood sugar compared to normal controls (119.6±43.4 mg/dL vs. 96.4±24.9 mg/dL). There is no significant difference in DIs (2.28±0.96 vs. 2.89±1.31) and SSRs (1.07±0.23 vs. 0.91±0.32) between these two groups.

Conclusions: We demonstrated that hyperglycemia caused vascular endothelial and autonomic dysfunctions by measuring DIs and SSRs, respectively. Resuming an euglycemic status significantly reversed these dysfunctions even after chronic hyperglycemia.

PS3-383 / #126

Theme: *5.4 - Peripheral neuropathy: others*

How much electrical injuries contribute to peripheral neuropathy?

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Background: Electrical injury is well known to be associated with diverse neurological sequelae.Median neuropathy is known to be as most frequently damaged nerve. But there are possibilities asymptomatic carpal tunnel syndrome(CTS) has existed before injury because most victims are physical workerssuch aselectricity repairman. So we did nerve conduction velocity study on the first damaged day. Our aim is to reveal how much electrical damage truly hurts peripheral neuropathy.

Method: 20 patient who are damaged by electric injury were enrolled. We enrolled the patients who are arrived at hospital the day of accidents. We excluded diabetes, heavy alcohol user and any other disease that is known to be associated with peripheral neuropathy. They all were alert to express their symptom. All of them complained no numbness or weakness before. Some of them complained numbness or weakness on hands the day of exam. Others did not complain any neurological symptom on hand.

Result: 4 patient has CTS initially And 6 patient has CTS at 2 months later. 1 patient who had previous CTS were aggravated in terminal latency and SVC compared to before examination. But 1 patient who had CTS showed the same results.

Conclusion: Electrical injury definitely damaged the peripheral nerve. But not all median neuropathy were not due to electrical injury. These study had a limitation to conclude because of small study number. There is need to elucidate in future study how electrical injury damage to the peripheral nerve.

PS3-384 / #145

Theme: *5.4 - Peripheral neuropathy: others*

A comparison of magnetic resonance imaging and electrodiagnostic studies in the evaluation of idiopathic brachial plexitis

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Background: Idiopathic brachial plexitis (IBP) is characterized by acute attacks of pain and subsequent paresis in the upper extremities. The MRI of brachial plexus has been recently introduced as a useful tool to diagnose a brachial plexus lesion and evaluate its etiology. However, until recently, there have been few studies on the correlations of MRI and electrodiagnostic testing (EDX) in the assessment of IBP.

Objective: We compared MRI and EDX findings in patients with IBP to evaluate the agreement of two methods. Methods: We enrolled 32 patients (25 men; mean age ± SD of onset, 48.2±13.8 years) who were diagnosed with IBP between January 2000 and January 2013. All patients underwent both EDX and brachial plexus MRI. We analyzed the clinical characteristics, EDX results, and MRI features in patients with IBP and compared their findings.

Results: The mean interval between onset of symptoms and diagnosis of IBP was 3.9 months. Antecedent illness was identifiable in 7 patients (21.9%) and recurrence was seen in one patient. Initialmanifestations were pain ($n=15$), motor weakness ($n=10$), and sensory symptoms ($n=7$). Mean MRC grade of the affected muscles was 3.5±0.9. Based on the results of EDX, the upper trunk was most frequently affected ($n=12$, 37.5%), followed by the lower trunk ($n=10$, 31.3%), middle and lower trunks ($n=4$, 12.5%), and medial cord ($n=2$, 6.3%). According to MRI findings, involvement of upper and middle trunks was most common ($n=5$, 15.6%), followed by entire trunks ($n=4$, 12.5%), upper trunk ($n=2$, 6.3%), lower trunk ($n=2$, 6.3%) and entire plexus ($n=2$, 6.3%). Sixteen patients (50.0%) had only EDX abnormalities with no definite lesions on MRI, whereas only MRI abnormalities were found with normal EDX in one patient (3.1%). In 15 patients (46.9%), there were abnormal findings in both EDX and MRI. While six out of fifteen patients showed anatomically concordant spatial localization on both tests, nine patients showed more extensive lesions on MRI.

Discussion: Based on our results, EDX was more sensitive test than MRI in diagnosing IBP. However, MRI was particularly useful to evaluate patients with normal EDX and, in addition, to exclude other causes of brachial plexopathy. Furthermore, it identified more extensive lesions of the brachial plexus.

Conclusion: By the acquisition of clinical, electrophysiological and MRI findings, clinicians can make an accurate diagnosis of IBP and determine its precise location and extent.

PS3-385 / #198

Theme: *5.4 - Peripheral neuropathy: others*

Sensory-evoked potential study for evaluation of alcohol-related peripheral neuropathy

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Background and Objectives: Alcohol is one of the main causes for polyneuropathy and only total abstinence from alcohol can give a chance of regeneration or halt of the disease. Therefore, early diagnosis and treatment are essential for public health problem. Nerve conduction studies are commonly used to evaluate the function of large myelinated fibers, and are often found to be within normal limits in an early state of polyneuropathy. We assessed somatosensory evoked potentials to determine usefulness in detecting electrophysiologic abnormalities in the early state of polyneuropathy in patients with chronic alcohol abuse.

Methods: We performed nerve conduction studies and somatosensory evoked potentials from posterior tibial nerve stimulation in alcoholic patients having clinical symptoms or signs of polyneuropathy. Among them, we recruited 15 alcoholics who had normal values of nerve conduction studies. Control subjects were age and height matched 18 healthy volunteers.

Results: The mean latency of spinal evoked potentials was 22.4 msec for the control subjects and 21.5 msec in for the alcoholics. The mean latency of cortical evoked potentials was 39.3 msec and 41.3 msec respectively. The cortical evoked potentials were significantly prolonged in alcoholics compared to control subjects. The mean central conduction time was not different between groups.

Conclusions: This study demonstrate that the latency of cortical evoked potentials is prolonged in the alcoholics with clinically suspected polyneuropathy and the somatosensory evoked potentials can be a useful diagnostic test for early detection of alcoholic polyneuropathy.

PS3-386 / #235

Theme: *5.4 - Peripheral neuropathy: others*

T-Cell-Lymphoma first presenting as radial nerve lesion

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Although "Saturday night palsy" is the most common cause of spontaneous radial palsy, other differential diagnoses need to be considered in atypical presentations.

Here we present a 45-years-old man who developed pain and a skin rash in his left arm in April 2013. He was diagnosed with herpes zoster and received treatment with famciclovir followed by gabapentin and oxcarbazepine. Pain persisted and in May he developed a left-sided hand drop suggestive of a radial nerve lesion. Because of unchanged symptoms he was referred to our clinic in June.

He had a left-sided wrist and finger drop and weakness of the brachioradialis muscle, while elbow extension was unremarkable. The left brachioradialis reflex was absent with otherwise normal deep tendon reflexes. There was a sensory loss in distribution of the superficial radial nerve. NCS and needle EMG revealed an axonal lesion of the main trunk of left radial nerve with pathological spontaneous activity in M. extensor dig. comm., M. extensor indicis proprius, M.brachioradialis and no pathologies in M. triceps brachii indicating a possible lesion in the midarm around the spiral groove.

Because of headache he received a cranial MRI showing a mass in the right nasal cavity. Medical history revealed that the patient had developed a skin rush affecting his legs six months earlier suggestive of an unspecific lymphoid proliferation. We prompted a biopsy of the nasal mass showing a T-Cell-Lymphoma. An MRI of the left arm showed a tumour in the distal third of the arm infiltrating M. brachialis and affecting the main trunk of radial nerve. The patient was referred to the haematological department for further management.

In conclusion, this case demonstrates that although the initial history was suggestive of a zoster radiculitis, careful history taking, clinical examination, and electrodiagnostic assessment finally prompted the appropriate workup of this patient.

PS3-387 / #277

Theme: *5.4 - Peripheral neuropathy: others*

Rational therapy of painful diabetic peripheral neuropathy associated with depressive symptoms

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Chronic painful diabetic peripheralneuropathy (PDPN) can cause symptoms that last for years and severely impair quality of life. The long-term complications of diabetes are associated with depressive symptoms.

The relationship between diabetic neuropathy specifically and depressive symptoms is less clear. Our main objective was, therefore, to investigate the association between diabetic neuropathy severity and depressive symptoms and to find out the principles of rational therapy.

Fifty-eight males (mean age 34±9.2) with PDPN were observed during 2009-2012 in Tbilisi Medical University Clinic. Thirty patients (I group) were undergoing generally accepted therapy (anticonvulsants and antidepressants); second group (28 patients) additionally to drug therapy were treated by acupuncture (a 20 day course per person). Neurological examination were performed using the Toronto Clinical Scor-

ing System (TCSS). Depressive symptoms were assessed with the seven-item subscale from the Hospital Anxiety and Depression Scale (HADS). The influence of acupuncture on brainstem systems was regarded by data of EEG, which were produced by digital EEG system - "Neurovisor" BMM before the process of inserting needles into the auricular points (AP 100, AP 104, AP 107, AP 130) and 20 minutes later, after removal of the needles. Corporal additional stimulation of erectile function was performed by two meridian points - U.B. 25 and U.B. 31. Statistical analysis was performed by Statistica 6.0.

Analysis of our data show that influencing "inhibitory neurons" of brainstem like a nucleus tractus solitari (located in a bulbar area and common for n. accessorius, sensitive parts of n. glossopharyngeus and n. vagus, whose some peripherial branches ended on the ear) by auriculo- and acupuncture was successful (two auricle and two meridian points) - response rate (RR 0.78 to 2.10, 95% CI) and remission rate (RR 0.57 to 2.95; 95% CI).

Influencing brainstem systems in patients of second group was effective for reducing the degree of pain ($p < 0.001$) as well as for improving patient's HADS (67% oppose to 21% in I group). Thus, it identifies potential targets for interventions to eliminate chronic pain associated with depression in patients affected by diabetic peripheral neuropathy, but more high-quality studies are required to prove this view point.

PS3-388 / #325

Theme: *5.4 - Peripheral neuropathy: others*

Amyloid neuropathy with respiratory failure – an unusual clinical presentation

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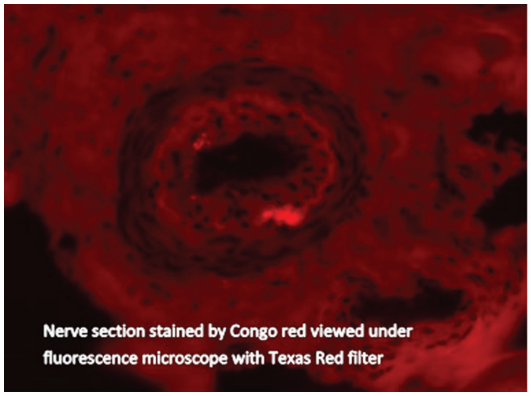
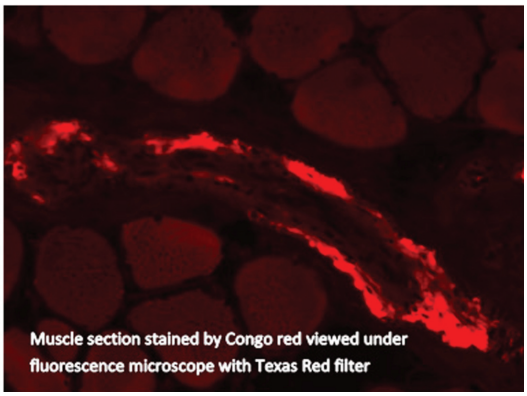
Amyloid neuropathy typically presents with a painful sensory and or autonomic neuropathy often with systemic manifestations including cardiomyopathy, myopathy, myelopathy or renal failure. However in recent years it has been increasingly recognized that the phenotypic presentation of amyloid neuropathy is much broader with presentations mimicking chronic inflammatory demyelinating neuropathy for example. Here we present a case of amyloid neuropathy presenting with respiratory failure and a clinical picture suggestive of motor neuron disease.

A 76 year old man presented to the ED of a large teaching hospital with hypoxic hypercapnic respiratory failure. He reported increasing shortness of breath for 10 months with a background history of hypertension, hyperlipidaemia, ischaemic heart disease, COPD, pulmonary embolism (twice in the past 2 years, warfarin ceased) and chronic cognitive decline. Nerve conduction study revealed markedly reduced or absent phrenic nerve motor responses. The initial clinical impression was of either late onset Pompe's disease or motor neuron disease. Sural nerve and muscle biopsies were performed in order to clarify the diagnosis.

The nerve biopsy showed almost normal nerve fibre density with no inflammation or demyelination. Muscle biopsy did not show any evidence of neurogenic atrophy or features of Pompe's disease. The only striking finding in both biopsies was amyloid deposition in the vessel walls. The diagnosis of amyloid neuropathy affecting mainly phrenic nerve was then established.





Discussion: The clinical presentation of amyloid neuropathy, particularly late onset TTR related familial amyloid neuropathy is now known to much less uniform than previously recognized. In recent years it has become clear that amyloid neuropathy should be considered in any progressive or disabling neuropathy especially in older patients. In late onset amyloid neuropathy autonomic symptoms are less likely to be evident at initial presentation and the diagnosis may not be thought of early. The case reported here, with predominant respiratory muscle weakness adds to the widening spectrum of amyloid neuropathy presentations.

PS3-389 / #503

Theme: *5.4 - Peripheral neuropathy: others*

Acquired idiopathic generalized anhidrosis – clinical, neurophysiological, pathological findings and treatment response on thirteen patients

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Background & Objectives: Acquired idiopathic generalized anhidrosis (AIGA) is a rare clinical syndrome characterized by generalized absence of sweating without other autonomic dysfunction. Most cases of AIGA have been reported in young Japanese males. Also there are only a few previous reports addressing their courses of treatment for AIGA. We report the clinical and neurophysiologic features of thirteen patients with generalized anhidrosis with different response to treatment depending on their pathological findings.

Method: Thirteen patients were diagnosed to generealized anhidrosis between September 2009 and July 2013. All the patients underwent comprehensive assessment such as clinical history, physical and neurologic examinations, neurophysiologic tests including nerve conduction study, heart rate variability to deep breathing and Valsalva maneuver, Q-SWEAT test, skin biopsy, and other focused laboratory studies. Thermoregulartory sweating test or digital infrared thermography, methacholine and heat provocation test were performed in selected patients.

Results: All 13 patients were experienced generalized anhidrosis and cholinergic urticaria or sharp pain under hot condition or after exercise. They were all young Korean male, aged from 18 to 41 years old. Ten of them have been suffered from anhidrosis for a few months (from 2 to 9 months), while three patients have a long history since childhood. Among the 13 patients, three patients showed subclinical postural tachycardia syndrome, which was revealed in autonomic function tests. Eight patients who accepted immunotherapy got IV or PO steroid therapy. Six of them improved clinical symptoms and Q-SWEAT results, while two patients whose biopsies were normal had not relieved.

Conclusion: AIGA is a very uncommon but potentially treatable autonomic disorder of unknown etiology. Our study has significance in that this is the first case series report on the Korean patients with AIGA and treatment response based on their pathologic findings.

PS3-390 / #534

Theme: *5.4 - Peripheral neuropathy: others*

Serial measurement of intraepidermal nerve fiber loss in critically ill patients

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Background: Small fiber pathology based on cross-sectional assessment of intraepidermal nerve fiber density (IENFD) as a hallmark of small fiber neuropathy has recently been shown in acute phase of neurocritical illness in patients without previous neuropathy or neuropathic risk factors and may explain pain and chronic sensory involvement in critical care survivors. The aim of the study was to verifythe decline in IENFD on serial measurement, that is stil lacking, and to correlate the small fiber loss with the large fiber neuropathy, myopathy and encephalopathy.

Patients and methods: We enrolled 11 adult neurocritical care patients with no previous history or risk factors for neuromuscular disease who underwent serial skin biopsy together with evaluation of consciousness (including daily assessment of the Confusion Assessment Method for the Intensive Care Unit), sensory functions, muscle strength usinm MRC score (in cooperative patients), nerve conduction study and needle electromyography, and autonomic dysfunction using spectral analysis of heart rate variability. Development of infection, sepsis and multiple organ failure was recorded throughout the ICU stay. IENFD was assessed at the onset of critical illness, mostly within 24 hours (up to 3 days after the onset, median: 1st day) at the distal site of the right leg, and compared with values obtained from the left leg within 10-14 days (median: 13 days) after the first examination.

Results: Of the 11 patients recruited, 9 (82%) had sepsis or multiple organ failure. Initially, 2 patients (18 %) had abnormal IENFD (median: 5.05 fibers/mm) and showed significant decrease with abnormal IENFD in 8 patients (73%) on the second evaluation (median: 2.18 fibers/mm; $p < 0.001$). Signs of critical illness myopathy and/or neuropathy was found in 6 patients (55%), signs of autonomic dysfunction in 3 patients (2%) and signs of septic encephalopathy manifested as an episode of delirium in 7 cases (64%).

Conclusions: Serial IENFD measurement showed the evidence ofvery frequent sensory small-fiber involvement in the acute phase of critical illness. Loss of sensory small fibers is an inseparable part of multiple involvement of neuromuscular system in critically ill patients representingan integral part of multiple organ failure in critically illpatients.

★PF4

PS4-391 / #128

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Neuroprotective effects of JGK-263 in transgenic SOD1-G93A mice of amyotrophic lateral sclerosis

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Background: Glycogen synthase kinase-3β (GSK-3β) activity plays a central role in motor neuron degeneration. GSK-3β inhibitors have been shown to prolong motor neuron survival and suppress disease progression in amyotrophic lateral sclerosis (ALS). In this study, we evaluated the therapeutic effects of a new GSK-3b inhibitor, JGK-263, on ALS in G93A SOD1 transgenic mice.

Methods: Previously, biochemical efficacy of JGK-263 was observed in normal and mutant (G93A) hSOD1-transfected motor neuronal cell lines (NSC34). Based on these previous results, we administered JGK-263 orally to 93 transgenic mice with the human G93A-mutated SOD1 gene. The mice were divided into three groups: a group administered 20 mg/kg JGK-263, a group administered 50 mg/kg JGK-263, and a control group not administered with JGK-263. Clinical status, rotarod test, and survival rates of transgenic mice with ALS were evaluated. Sixteen mice from each group were selected for further biochemical study that involved examination of motor neuron count, apoptosis, and cell survival signals.

Results: JGK-263 administration remarkably improved motor function and prolonged the time until symptom onset, rotarod failure, and death in transgenic mice with ALS compared to control mice. In JGK-263 groups, choline acetyltransferase (ChAT)

staining in the ventral horn of the lower lumbar spinal cord showed a large number of motor neurons, suggesting normal morphology. The neuroprotective effects of JGK-263 in ALS mice were also suggested by western blot analysis of spinal cord tissues in transgenic mice.

Conclusion: These results suggest that JGK-263, an oral GSK-3β inhibitor, is promising as a novel therapeutic agent for ALS. Still, further biochemical studies on the underlying mechanisms and safety of JGK-263 are necessary.

PS4-392 / #134

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

The blood-spinal cord barrier is temporarily impaired in TDP-43 conditional knockout mice

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Background: The loss-of-function of TDP-43 protein seems to affect the pathogenesis of ALS, and in this context it remains unclear whether the loss of TDP-43 protein is associated with the changes observed in the blood-spinal cord barrier (BSCB).

Objectives: To clarify if the loss of TDP-43 protein causes the changes in the BSCB in TDP-43 conditional knockout (CKO) mice.

Methods: We immunohistochemically and electron-microscopically studied the lumbar spinal cord in 8 TDP CKO mice and 8 controls. TDP CKO mice were divided into 4 groups: the early presymptomatic (aged 20 wks), late presymptomatic (36 wks), early symptomatic (50 wks), and late symptomatic (100 wks) stages ($n=2$, respectively). TDP-$43^{flox/flox}$ mice served as age-matched controls in each group ($n=2$). The cervical spinal cord was immunostained for MAC-2. Western blot analysis was performed on the wild-type and TDP CKO mice at the age of 20, 50, and 70 wks, using the anti-claudin-5, occludin, and ZO-1 antibodies.

Results: Immunohistochemical analysis: TDP-43 CKO mice showed macrophages/microglia proliferation exclusively in the anterior horn after the age of 50 wks.

Western blot analysis: No difference was detected between the wild-type and TDP CKO mice.

Electron microscopic analysis

Controls: The capillaries consisted of endothelial cells characterized by tight junctions, and pericytes which were completely surrounded by a basal lamina and shared by the end-feet of the astrocyte. Most of the capillaries were surrounded by a narrow perivascular space.

TDP CKO mice:At the early symptomatic stage (50 wks), the cytoplasm of most endothelial cells was injured, frequently causing vacuoles. The endothelium occasionally exhibited thickened cytoplasm by edema with the intact tight junction, and prominent protrusions of some parts of the cytoplasm into the lumen. The cytoplasm of pericytes was occasionally swollen. Perivascular spaces were frequently edematous and vacuolated. At the other stages, the capillaries were well-preserved.

Conclusions: The temporary and reversible breakdown of the BSCB with leakage or increased permeability at the early symptomatic stage could be a direct consequence of the loss of TDP-43 protein in motor neurons. This temporal impairment of the BSCB seems to contribute to the degeneration of motor neurons in TDP CKO mice.

✶PF1

PS4-393 / #146

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Direct conversion of patient fibroblasts demonstrates non-cell autonomous toxicity of astrocytes to motor neurons in familial and sporadic ALS

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Amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease is a devastating disorder affecting upper and lower motor neurons (MNs) in the motor cortex, brain stem and spinal cord. Due to the mostly sporadic and unknown cause of ALS, it is extremely difficult to model this disease *in vitro*. Models are urgently needed for studying disease mechanisms and to screen potential therapeutics. We have used the new and fast method of direct conversion to generate tripotent induced neuronal progenitor cells (iNPCs) from adult human skin fibroblasts of familial and sporadic ALS patients as well as non-ALS controls. Briefly, we transduced the fibroblasts with retroviral vectors encoding the reprogramming factors c-Myc, Klf4, Sox2 and Oct3/4. After 3 days of recovery, the culture conditions were changed to allow NPC formation in the presence of FGF2 and EGF. Within 2 weeks, iNPCs could be collected and expanded. The direct conversion is a much faster way to generate NPCs than the regular process of reprogramming to induced pluripotent stem cells (iPS) and their consecutive re-differentiation. In addition, it does not require any clonal selection. The iNPCs can then be differentiated into oligodendrocytes, neurons and astrocytes to study cell interactions and intrinsic properties. With this new protocol, it is possible to generate astrocytes within 1 month after taking the skin biopsy of a patient. When we made astrocytes from ALS patients with this method and co-cultured them with mouse motor neurons, we observed similar toxic characteristics as previously reported with spinal cord derived astrocytes by our group. In the meantime, i-astrocytes from four age matched healthy controls did not convey any toxicity towards the co-cultured motor neurons. We also included three patients carrying the newly discovered C9orf72 mutation in this study and demonstrate for the first time that these astrocytes also convey toxicity towards motor neurons. Excitingly, our findings emphasize that skin fibroblasts contain a toxic memory that can be activated upon somatic reprogramming. This new method to generate astrocyte-like cells can therefore be used to model the disease while the patients are still alive. In addition, we report for the first time non-cell autonomous toxicity of C9orf72 mutation carrying astrocytes towards motor neurons and created the first *in vitro* system to study the mechanism behind this killing process.

PS4-394 / #174

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Satellite cell activation in muscles of terminal ALS patients

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Amyotrophic lateral sclerosis (ALS) is an incurable, neurodegenerative disorder characterized by loss of motor neurons, progressive paralysis and muscle wasting. We have shown that the extraocular muscles (EOMs) are distinctively less affected than the limb muscles at the end-stage of ALS and usually they remain clinically functional. The basis for this relative sparing is currently unknown. The EOMs have been suggested to have a higher regenerative capacity than other muscle allotypes, which may explain why they remain unaffected in muscle dystrophies.

We have investigated whether there are differences in satellite cell (SC) numbers and activation between the EOMs and limb muscles of terminal ALS patients and in comparison with age-matched controls.

The mid-portions of EOMs and limb muscles samples from 8 terminal ALS patients, as well as corresponding EOMs from 4 age-matched controls were investigated with immunohistochemistry. A multiple labelling method, visualizing basement membrane and two SC markers (Pax7 and NCAM) was used to quantify SCs. Immuno labeling for MyoD, myogenin and Ki-67, together with markers for basement membrane and/or the plasma membrane/Pax7 was used to detect SC activation.

Preliminary data suggest that control EOMs have lower numbers of SCs than previously reported in the literature, and that activation markers (MyoD, myogenin and Ki-67) are only sparsely present. The EOMs of terminal ALS patients did not differ from controls. In limb muscles of terminal ALS patients, SC numbers varied but were generally within ranges previously reported for aged, healthy controls. However, in the limb muscles of two patients with long disease duration, SC numbers were unusually high. The degree of SC activation varied among the limb specimens, but most specimens showed little SC activation, similar to that found in EOMs of controls and ALS patients.

PS4-395 / #266

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Human pluripotent stem cells: Potential for neuro muscular diseases modeling?

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The lack of existing models of pathologic tissues has rendered many important questions in disease pathogenesis inaccessible. Humanpluripotent stem cells, from embryonic origin or obtained by genetic conversion of somatic cells, offer the unique opportunity to have access to a large spectrum of disease-specific cell models. Disease-specific pluripotent stem cells capable of differentiation into the various tissues affected in each condition could undoubtedly provide new insights into the pathological mechanisms by permitting analysis in a human system. These new disease-specific cell models are applicable for a wide systemic mechanistic analysis ranging from functional studies at the cellular level to a large-scale functional genomics screening.

As a proof of principle, we demonstrated that hES cells and derivatives which, express the causal mutation implicated in the Myotonic Dystrophy type 1 (DM1), may mimic molecular defects associated to the pathology, such as the nuclear aggregation of mutant RNA. By taking advantage of this pertinent cellular model, we identified, through a genome-wide analysis, two early developmental defects in genes involved both in myogenesis as well as in neurite formation and establishment of neuromuscular connections. These neuropathological mechanisms may bear clinical significance as related to the functional alteration of neuromuscular connections associated with DM1.

In parallel to these functional pathological studies, we developed two different approaches to identify new therapeutic strategies. The first one was based on a high content screening approach. A pilot drug screening experiment has been successfully conducted in order to identify new molecules which, due to their ability to disrupt the nuclear mutant RNA aggregation, might represent new therapeutic strategies. The second strategy used a genomics screening based on gene knockdown approach. This analysis allowed the identification of a potentially druggable target protein, inhibition of which tends to normalize molecular defects associated to DM1.

Altogether, our results indicate that disease-specific hES cell lines could be used for resources driven large-scale analysis, in complement to classical approach based on the analysis of candidate gene, that could highlight the development of new therapeutic strategies. Our goal is now to extend our expertise towards other neuromuscular diseases.

PS4-396 / #297

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Adult neurogenesis in Amyotrophic Lateral Sclerosis patients with or without dementia

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease. It was previously considered as exclusive of motor neurone. However in the last years it has been described as affecting other cells and can be associated with dementia. Dementia most characteristically associated with ALS is frontotemporal dementia (FTD). In human adult brain two neurogenic niches has been described: subventricular zone (SVZ) and dentatus girus in hippocampus (GD). These niches changes under pathological conditions. In this work we describe both niches affectation in 8 ALS patients (2 with FTD, and one SOD associated form) Eight necropsies from ALS patients (2 with FTD, and one SOD associated form) and 3 controls were studied, using pathological conventional study, confocal immunohistochemistry and electron microscopy. ALS pathology was confirmed by classical lesions and cystatin, TDP-43 and peripherin immunology. SVZ and GD niches were studied for proliferation markers (Ki67, histone3), neuroblasts (PSA-NCAM, DCX,

TUJ-1) stem cells (Nestin, dGFAP), glia and microglia (IBA-1 and GFAP) We found an increased proliferation in SVZ in all ALS patients, more important in those with FTD associated. In GD we found a reduced of proliferation, significative in those with DFTL ALS modifies adult neurogenesis by increasing proliferation in SVZ, more importantly in cases with DFTL. These is in contrast with GD affectation, where we found a reduction of proliferation with significance in DFTL cases. These results supports both niches have different functional significance. However their specific functional significance is not yet known.

PS4-397 / #321

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Role of beta – Amyloid oligomeric (AbO) fraction in ALS disease

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In amyotrophic lateral sclerosis (ALS), the progressive loss of motor neurons is accompanied by extensive muscle denervation, resulting in paralysis and ultimately death.

It has been shown in SOD mouse model as well as in ALS patients, an up-regulation of amyloid beta precursor protein (APP) in muscle fibres coinciding with symptom onset. Additionally, motor neuron axon defects and motor neuron death have recently been observed in murine models of familial Alzheimer's disease (AD) that produce elevated levels of b-amyloid oligomers (AbO), indicating susceptibility of motor neurons to this neurotoxic peptide. Morover, it has been recently shown that neuromuscular junction (NMJ) loss and motor neuron degeneration are substantially reduced in SOD1G93A mice when APP is genetically ablated, which indicates that endogenous APP actively contributes to pathology in this model of ALS.

Here, we have further investigated the role of AbO on an *in vitro* model of functional NMJ (rat nerve / human muscle co-cultures, Braun et al. 1996, Askanas et al., 1987) in order to better understand its involvement in the NMJ death and the neurodegenerative process on motor neurons. We showed that AbO application on functional co-culture induced a progressive loss of NMJ and motor neurons degeneration. Additionally we proved that low concentrations of AbO (at a nontoxic level) induced a large glutamate release. The glutamate release was dependent of time of application as well as concentration dependent. This release seems to be involved in the process of degeneration induced by AbO suggesting that AbO could be an important element triggering the massive excitotoxicity event occurring in ALS pathology.

Together with previous findings, therefore, our results suggest that targeting AbO may be helpful in the design of a disease-modifying approach for ALS.

PS4-398 / #339

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Diffusion magnetic resonance imaging: A promising novel tool for early detection and monitoring of motor neuron degeneration in ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by selective degeneration of motor neurons in the motor cortex, brainstem and spinal cord. Its diagnosis is based on clinical and electrophysiological criteria. Diffusion magnetic resonance imaging (MRI), and in particular diffusion tensor imaging (DTI), might be helpful to directly assess motor neuron degeneration in patients and in stratifying ALS patients.

In this study, we examined the potential of the *in vivo* DTI-derived parameters, such as fractional anisotropy (FA), longitudinal diffusivity (DL), radial diffusivity (DR) and apparent diffusion coefficient (ADC), to measure white and grey matter degeneration in the lumbar spinal cord of the ALS animal

model G93A-SOD1 mice, to verify whether DTI abnormalities are correlated with disease severity and progression.

Diffusion MRI experiments were carried out by 7T MRI scanner in G93A-SOD1 and control mice at different time of age (from 7 to 18 weeks of age). Single-shot echo planar imaging diffusion MRI acquisition protocols were performed providing a good anatomical view of the examined lumbar spinal cord region, in terms of spatial resolution and image contrast, and showing a good reproducibility of the measures in a non-invasive manner. The diffusion MRI analysis showed a reduction in FA and DL parameters, reflecting an altered cellular disorganization in the ventral gray and white matter of 12-week-old G93A-SOD1 spinal cords, a time in which G93A-SOD1 mice present the first clinical signs of the disease. The reduction of the DTI-derived parameters became more evident with disease progression. Histological analysis of the spinal cord tissues confirmed a significant reduction of the number of motor neurons in G93A-SOD1 mice at week 12 of age compared to controls. Moreover, an electron microscopy analysis showed structural alterations of motor neurons in spinal cord of G93A-SOD1 at week 12 compared to control mice.

The overall results of our diffusion MRI analysis identified the earliest pathological changes of ALS mouse model G93A-SOD1, suggesting FA and DL parameters as possible biomarkers of motor neuron degeneration.

PS4-399 / #382

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

The missing factors influencing spinal and bulbar muscular atrophy phenotype: Evaluation of genetic polymorphisms

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Background: Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is an adult-onset lower motor neuron disorder, characterized by proximal limb and bulbar muscle atrophy and weakness. The disease is caused by a pathological expansion over 38 of a CAG repeat in the first exon of the androgen receptor (AR) gene on chromosome X, coding for a polyQ tract (La Spada et al, 1991). SBMA is an androgen-dependent disorder, with males with full disease manifestations, and females showing only mild symptoms even if homozygous for the mutation. While a correlation between expansion size of polyQ tract and disease severity has been reported, patients with the same number of CAG repeats have different age at onset and disease progression even if relatives. Human AR exon 1 encodes further short aminoacidic stretches. The effect of these sequences on SBMA phenotype has not been studied yet.

Aim and methods: In order to further characterize the effect of AR coding repeated sequences on SBMA phenotype, we genotyped the AR exon 1 of 132 molecularly defined SBMA patients, referring to the Motor Neuron Clinic of the University of Padua and to Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan.

Results: Our resultsfailed to estimate a correlation between the length of SBMA-causing polyQ tract and the age at disease onset. Among the other AR exon-1 trinucleotide stretches, a polymorphic GGN sequence coding polyG showed a tendency to be higher in patients with an earlier onset.

Discussion and conclusion: Our study confirms that the length of SBMA-causing polyQ tract does not fully explain the disease phenotype. Conversely, we could point to the presence of a polyG variant within AR exon 1 that is a potential disease modifier in SBMA

Reference:

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature. 1991;352:77-9.

PS4-400 / #386

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Human induced pluripotent stem cells as an *in vitro* model for investigating infantile-onset ascending hereditary spastic paralysis pathogenesis

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Infantile-onset ascending hereditary spastic paralysis (IAHSP) is a rare, early-onset autosomal recessive motor neuron disease associated with mutations in ALS2 gene. At present there is no effective treatment for IAHSP and the pathogenesis is still unclear. The possibility to reprogram fibroblasts from IAHSP patients into pluripotent stem cells could offer an *in vitro* model to study the molecular and cellular mechanisms underlying IAHSP pathogenesis.

The aims of the study were: 1) to obtain and characterize induced human pluripotent stem cells (hiPS) from fibroblasts of a IAHSP patient and a healthy donor; 2) to differentiate hiPS into motor neurons, and to demonstrate whether IAHSP hiPS derived-motor neurons are altered compared to control cells; 3) to verify whether microRNA (miRNA) expression profile is altered upon motor neuron differentiation of IAHSP hiPS compared to control cells.

To address these aims, we obtained hiPS from IAHSP and control fibroblasts, and demonstrated that they were positive to pluripotent markers, as Oct4, Nanog, Sox2 and Lin28, by immunohistochemistry and molecular analysis. From motor neuron differentiation of IAHSP and control hiPS, we obtained motor neurons positive to HB9, MAP2 and beta-tubulin III, three neuronal markers. However, IAHSP hiPS-derived neurons showed in the cytoplasm aggregates positive to alsin that co-localized with endosomal markers, as mannose-6-phosphate receptor (late endosome) and early endosome antigen 1. This observation might be indicative of an altered vesicular transport in diseased neuronal cells reflecting a pathogenic feature of IAHSP.

To investigate the possible involvement of miRNAs upon motor neuron differentiation, we analyzed miRNA expression profile in fibroblasts, embryoid bodies (EBs), neural stem cells and neuronal cells derived from IAHSP and control hiPS. miRNAome profiling showed an altered miRNA expression, mostly of those miRNAs implicated in vesicular transport pathways, in IAHSP neuronal cells compared to control.

Our findings suggest that hiPS from IAHSP patients might represent a valuable *in vitro* model to study the molecular and cellular mechanisms implicated in the disease pathogenesis. The extraordinary versatility of these hiPS could represent a valuable approach to identify potential patient-specific targets for IAHSP therapy.

PS4-401 / #468

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Stem cell-derived motor neurons from spinal and bulbar muscular atrophy patients

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Spinal and bulbar muscular atrophy (SBMA, Kennedy's disease), is a motor neuron disease caused by polyglutamine repeat expansion in the androgen receptor. Although degeneration occurs in the spinal cord and muscle, the exact mechanism is not clear. Induced pluripotent stem cells from spinal and bulbar muscular atrophy patients provide a useful model for understanding the disease mechanism and designing effective therapy. Stem cells were generated from six patients and compared to control lines from three healthy individuals. Motor neurons were differentiated from the stem cells and characterized to understand disease-relevant phenotypes. Stem cells created from patient fibroblasts express less androgen receptor than control cells, but show androgen-dependent stabilization and nuclear translocation. The expanded repeat in several stem cell clones was unstable, with either expansion or contraction. Patient stem cell clones produced a similar number of motor neurons compared to controls, with or without androgen treatment. The stem cell-derived motor neurons had immunoreactivity for HB9, Isl1, ChAT, and SMI-32, and those with

the largest repeat expansions were found to have increased acetylated a-tubulin and reduced HDAC6. Reduced HDAC6 was also found in motor neuron cultures from two other patients with shorter repeats. Evaluation of stably transfected mouse cells and SBMA spinal cord showed similar changes in acetylated a-tubulin and HDAC6. SBMA stem cells present new insights into the disease, and the observations of reduced androgen receptor levels, repeat instability, and reduced HDAC6 provide avenues for further investigation of the disease mechanism and development of effective therapy.

PS4-402 / #475

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Wnt expression in extraocular and limb muscles from mouse models with ALS

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Amyotrophic lateral sclerosis is a late-onset progressive neurodegenerative disorder affecting both upper and lower motor neurons. ALS is characterized by increasing muscle weakness, paresis and paralysis. In contrast to limb skeletal muscle, extraocular muscles (EOMs) are relatively spared in ALS. Recent studies suggest that the initial degeneration begins at the neuromuscular junctions. If these degenerative changes could be delayed, motor function could be improved in these patients. The Wnt pathway is known to modulate the formation and function of synapses. Our aim was to examine the expression of 4 members of Wnt family at neuromuscular junctions (NMJs) in EOMs and limb muscles from $SOD1^{G93A}$ transgenic mice at early and terminal stages. Age-matched mice were used as controls.

All muscle specimens were collected with ethical approval. Muscle sections were immunostained for Wnt1, Wnt3a, Wnt5a, or Wnt7a. NMJs were identified by labelling with alpha-bungarotoxin. Double labelling with neurofilament and alpha-bungarotoxin were also used to evaluate innervation in EOMs and limb muscles.

Expression of Wnt1, Wnt3a, Wnt5a, and Wnt7a was unaffected in the NMJs of both limb muscles and EOMs in the young transgenic mice. However, in $SOD1^{G93A}$ mice at the terminal stage, Wnt3a and Wnt5a expression was dramatically down-regulated whereas Wnt1 and Wnt7a expression was slightly decreased in NMJs of limb muscles. In contrast, all 4 Wnts were relatively unchanged in NMJs of EOMs. There was no sign of loss of axon contacts at NMJs in both EOMs and limb muscles at early stage of ALS. However, the vast majority of NMJs were denervated in limb muscles from terminal $SOD1^{G93A}$ mice, whereas the NMJs in the EOMs of terminal animals were maintained.

We hypothesize that differences in the levels of specific Wnt molecules at NMJs in EOMs allow maintenance of functional muscle-nerve connections in ALS. Wnt3a and 5a have been particularly implicated in modulating neuromuscular junction assembly at the muscle membrane.

The down-regulation of Wnt3a and Wnt5a at NMJs was correlated with denervation of NMJs in limb muscles whereas the persistence of Wnts expression may maintain the well-preserved NMJs in EOMs. We hypothesize that similar differential Wnt expression patterns could play important roles in maintaining EOM function in the ALS patients.

PS4-403 / #477

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Importance of peripheral AAV9 mediated SMN expression for gene therapy of spinal muscular atrophy mice

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Spinal muscular atrophy (SMA), the first genetic cause of mortality in chilhood, is an incurable disease characterized by motor neuron death, muscle weakness, and progressive loss of movement. In 95% of cases, SMA is caused by disruption of the Survival of motor neuron 1 gene (SMN1), which encodes for

SMN, a protein which has been involved in snRNP assembly, transcriptional regulation, axogenesis, cell trafficking, and cell differentiation.

We have recently demonstrated that self-complementary Adeno-Associated Virus vector serotype 9 (scAAV9) is highly efficient for gene delivery to motor neurons after intravenous (IV) injection in both neonatal and adult animals (Barkats 2007, PCT/EP2008/063297; Duqué et al, 2009). We and others successfully translated this approach to spinal muscular atrophy (SMA) gene therapy by using an scAAV9 vector encoding the human "Survival of Motor Neuron 1" in SMA mouse models. We found that IV injection of optimized vectors encoding SMN1 under control of the ubiquitous PGK promoter (scAAV9-PGK-SMNopti) significantly improved the survival and phenotype of SMNΔ7 mice (Dominguez et al. 2011).

We investigated whether the alternative intracerebroventricular (ICV) route of scAAV9 injection could improve therapeutic benefit in SMA mice by increasing SMN release close to the motor neurons. The therapeutic results surpassed those reported after IV injection, that confirmed the crucial role of SMN in motor neurons. However we found that, unexpectedly, a single injection of scAAV9-SMN into the brain lateral ventricle mediated widespread SMN gene transfer in the central nervous system, but also at the periphery.

(e.g. the heart and the liver). Further IV/ICV co-injections of scAAV9-PGK-SMNopti vectors, and ICV injection of AAV9 vectors expressing SMN under control of the neurospecific Synapsin promoter further revealed the importance of peripheral SMN expression in the rescue of SMA mice.

Together, these studies highlight the considerable potential of scAAV9-mediated SMN gene delivery for the treatment of SMA and provide IV/ICV co-delivery as the optimal route for SMN gene therapy.

PS4-404 / #526

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

Homozygous mutation in Atlastin GTPase 1 causes recessive hereditary spastic paraplegia

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Hereditary spastic paraplegia is a genetically highly heterogeneous disorder and currently at least 20 recessive disease genes have been identified. Despite this progress 30% of the cases remain unsolved. We have now using whole exome sequencing identified a co-segregating homozygous stop mutation in ATL1 NM_015915; c.649C>T; (p.R217*) in a highly consanguineous Arabic family from Emirates with three affected sibling suffering from hereditary spastic paraplegia. The spasticity started at 2 years of age in all three affected male siblings. Due to progressive spasticity an operative release of several tendons was performed at around 8 years of age to enable standing. On examination, severe spasticity was noted in the lower limbs and also a reduction of distal muscle force, affecting also the hands. Vibration sense was reduced in lower limbs. Patients also reported problems with bladder control. At latest clinical follow-up of the oldest patient at 18 years of age, he was able to stand and walk with crutches. The heterozygous carrier parents, currently aged 40 years, had no neurological symptoms so far. The discovery of homozygous mutations in ATL1 was surprising because ATL1 usually causes autosomal dominant SPG3A. Our finding of a homozygous mutation supports the hypothesis that ATL1 missense mutations have usually dominant negative effect on the tetrameric GTPase domain. This would explain that the heterozygous carrier parents with a heterozygous Loss-of-function (LOF) mutation have no phenotype and the affected siblings with homozygous LOF mutation have a spastic paraplegia phenotype. This discovery expands the clinical phenotype and inheritance patters of ATL1 associated diseases and cases with recessive HSP should be investigated for ATL1 mutations.

✶PF2

PS4-405 / #558

Theme: *6.1 - Motor neuron diseases: pathophysiology, genetics*

ALS astrocytes kill motor neurons via ligation of death receptor 6 by a fragment of N-APP/APLP1

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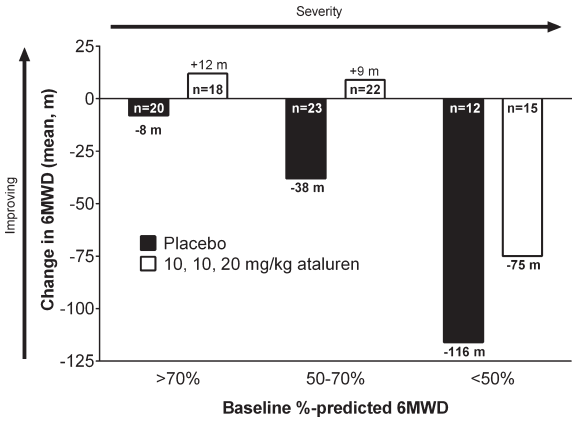
Mutations in superoxide dismutase-1 (SOD1) cause a form of amyotrophic lateral sclerosis (ALS). Previously, we have shown that primary or embryonic stem cell-derived (ES-) motor neurons (MNs) are killed by mutated SOD1-expressing astrocytes or their conditioned medium (CM) (Nagai et al., Nat Neurosci. 2007). Here we show that the deleterious effect of mutant astrocytes is due to a toxic activity and not to lack of beneficial effects on MNs and that it is mediated by a negatively charged protein of ~5-30 kDa, which, we surmised, might be a ligand to MN cell surface transduction protein. Based on this premise, we found by liquid chromatography-mass spectrometry analysis a list of 121 potential ligands selectively enriched in, or unique to, the anionic fraction of the toxic mutant astrocyte CM. To identify within this list the extracellular ligand mediating the astrocyte dead signal and its associated cell surface transducer, we decided to adopt a non-bias genome-wide approach. For this, purified ES-MNs were exposed for 72 hours to the ALS astrocyte CM and analyzed by RNA-seq. From these gene expression profiling data, differential protein activity were inferred through a regulatory network-based approach that we have recently developed (Alvarez et.al., 2014, submitted). We found significant changes in activity for 84 membrane proteins (FDR < 0.01). To narrow-down this list of candidate receptors we combined the inferred activity with previous knowledge on protein-protein interaction cataloged in the STRING V9.1 database. Specifically, we selected the putative cell surface transduction proteins for which interaction with any of the 121 mutant SOD1 astrocytes released proteins have been reported with high confidence (STRING score > 850). We ended up with 5 couples of putative receptor/ligand (FDR < 0.2) that we have systematically tested in our co-culture models by a combination or immunological and genetic strategies. We found that the neutralization or genetic ablation of the second most activated receptor, death receptor 6 (DR6) in the MN compartment was fully protective. In agreement, the mirror neutralization or ablation in the astrocytes of amyloid beta precursor protein (APP) or of amyloid precursor-like protein 1 (APLP1), two potential ligands of this orphan receptor, also completely reversed MN death. We also found that inhibitors of beta-secretase (BACE1) were protective whereas a recombinant of the E1 domain of N-terminal APP and APLP1 was toxic to MNs in a DR6-dependent manner. Supporting the relevance of these findings to ALS is our observation that astrocytes from sporadic ALS patients also kill MN by a DR6/APP/APLP1-dependent mechanism. Thus, the present study not only reports on a new disease mechanism that rests on a deleterious molecular interaction between MNs and glial cells, but also on new promising therapeutic avenues for this common, incurable disease.



Change in 6MWD over 48 weeks stratified by baseline %-predicted 6MWD

PS4-406 / #48

Theme: *6.2 - Motor neuron diseases: epidemiology, clinic, treatment*

Immunohistochemical studies of hepatocyte growth factor in the skin of the patients with amyotrophic lateral sclerosis

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Background: Studies of amyotrophic lateral sclerosis (ALS) skin have shown unique pathological and biochemical abnormalities in collagen, elastic fibers, and the ground substance. The lack of bedsore formation even in the terminal stages in ALS patients is considered characteristic. Hepatocyte growth factor (HGF) is one of the most potent survival-promoting factors for motor neurons, comparable to glial cell line-derived neurotrophic factor *in vitro*. In addition, overexpression of neuronal HGF has been shown to result in the attenuation of neuronal cell death and progression of disease in a familial ALS transgenic mouse model. Therefore, HGF might be beneficial for motor neuron survival. It is unknown, however, whether HGF-positive structures are present in the skin from patients with sporadic ALS (SALS).

Objective: We made immunohistochemical studies of HGF in the skin of ALS patients (59.7±9.2 years). Methods: Skin biopsy samples were taken from the left biceps from 16 SALS patients and 16 control subjects with other neurologic disorders (59.3±9.2 years). Routine formalin-fixed paraffin-embedded 6 μm sections were immunostained according to standard techniques. A densitometric analysis was performed using an image analysis system.

Results: HGF immunoreactivity was positive in the epidermis and dermal blood vessels and glands in ALS patients. The HGF immunopositive (HGF+) structures of the epidermis were the nucleus and the cytoplasm. These findings became more conspicuous as ALS progressed. The optical density for HGF immunoreactivity of the nucleus in the epidermal cells in patients with ALS was significantly higher ($p<0.001$) than in controls. The optical density of the cytoplasm in the epidermal cells in patients with ALS was also significantly higher ($p<0.001$) than in controls. The density of HGF immunoreactivity in ALS patients showed a progressive increase in relation to duration of illness. This positive correlation was highly significant ($r=0.63$, $p<0.01$ and $r=0.76$, $p<0.001$, respectively) in the nucleus and the cytoplasm in the epidermal cells, but there was no such relationship in control subjects.

Conclusion: These findings suggest that changes of HGF in ALS skin are related to the disease process and that metabolic alterations of HGF may take place in the skin of patients with ALS.

PS4-407 / #136

Theme: *6.2 - Motor neuron diseases: epidemiology, clinic, treatment*

Detection of LRP4 antibodies in serum and CSF from amyotrophic lateral sclerosis patients

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Amyotrophic lateral sclerosis (ALS) and myasthenia gravis (MG) are caused, respectively, by motor neuron degeneration and neuromuscular junction dysfunction. The membrane protein LRP4 is crucial in the development and function of neuromuscular junctions and motor neurons and anti-LRP4 autoantibodies have recently being detected in some MG patients, usually seronegative for anti-AChR and anti-MuSK antibodies. Because of the critical role in motor neuron function we searched for LRP4 antibodies in ALS patients. We developed a cell-based assay and a radioimmunoassay and with these we studied the sera from 104 ALS patients. LRP4 autoantibodies were detected in sera from 24/104 (23.4%) ALS patients from Greece (12/51) and Italy (12/53), but only in 5/138 (3.6%) sera from patients with other neurological diseases and 0/40 sera from healthy controls. The presence of LRP4 antibodies in 5 of 6 tested patients was persistent for at least 10 months. Cerebrospinal fluid samples from six of seven tested LRP4 antibody-seropositive ALS patients were also positive. No autoantibodies to other MG autoantigens (AChR and MuSK) were detected in ALS patients. No differences in clinical pattern were seen between ALS patients with or without LRP4 antibodies. Differences were seen in the female/male ratio between ALS patients and MG patients with these antibodies (~1/1 versus ~3/1, respectively) and in the IgG subclass of the LRP4 antibodies (IgG1 in most ALS samples and IgG1/IgG2 in most MG samples). In conclusion, we infer that LRP4 autoantibodies are involved in patients with neurological manifestations affecting LRP4-containing tissues and are found more frequently in ALS patients than MG patients. LRP4 antibodies may have a direct pathogenic activity in ALS by participating in the denervation process, a critical step causing the characteristic muscle symptoms and neurophysiological changes. The identification of anti-LRP4 autoantibodies in several ALS patients offers a potentially useful biomarker for diagnostic purposes, but much work is needed to investigate the pathogenic potential of these antibodies.

PS4-408 / #160

Theme: *6.2 - Motor neuron diseases: epidemiology, clinic, treatment*

Relevance of the disease progression pattern in amyotrophic lateral sclerosis

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Introduction: Although the majority of amyotrophic lateral sclerosis (ALS) patients exhibit a clinical contiguous pattern of progression (CP) consisting in a spreading of symptoms from the initial localization affected to adjacent regions, a non-contiguous pattern (NCP) has been described. The aim of our study is to describe clinical and prognosis features among ALS patients according to their progression disease pattern.

Methods: From our ALS cohort we identified patients with clinically contiguous pattern of disease progression (CCP) and patients with clinically non-contiguous pattern (CNCP). The non-contiguity was defined as having lower limbs or bulbar symptoms at onset, and the next region affected was bulbar or lumbar region respectively, without involvement of the upper limbs. Among this last group, we defined a subgroup of patients as "definite non-contiguous pattern" (DNCP) when there was evidence of electromyographical indemnity of intermediate regions between the clinical affected localizations.

Results: In total, from 119 patients analyzed, 17.6% (21/119) presented CNCP of progression. Compared with CCP patients, CNCP patients showed a bulbar onset predominance (26.5% vs 66.6% $p < 0.001$). Five of these 21 patients followed a DNCP progression; all of them were bulbar onset forms and were older than CNCP patients (72 vs 63, $p = 0.009$). When we compared CCP bulbar onset forms with DNCP, patients with a "definite non-contiguous pattern" of progression tended to have a higher median age at onset (72 vs 66, $p = 0.088$) and a shorter survival time ($p = 0.094$).

Conclusions: A minority of ALS patients exhibits a "definite non-contiguous pattern" of disease progression and, compared with ALS patients with a contiguous pattern, are older at onset of symptoms and have a shorter survival time.

PS4-409 / #341

Theme: *6.2 - Motor neuron diseases: epidemiology, clinic, treatment*

ALS onset and propagation: Insight from pulmonary function test

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Background: Amyotrophic lateral sclerosis (ALS) was thought to originate from a single focal onset site and propagate in rostrocaudal direction. But some group suggest multifocal hit and propagation in ALS. Maximal inspiratory pressure (MIP) reflects the strength of the diaphragm, main inspiratory muscle, which is innervated by cervical spinal roots (C3, C4, C5).

Objective: To investigate single or multifocal hit in ALS we compare inspiratory muscle and expiratory muscle strength in patients who clinically and electrophysiologically fulfill El Escorial criteria of definite and probable ALS. According to focal hit theory, MIP and maximal expiratory pressure (MEP) ratio will be bigger in caudal region onset patients than rostal region onset patients at the early stage of ALS without significant respiratory dysfunction.

Method: Retrospective study was performed in 114 ALS patients who visited Seoul National University Hospital between March, 2012- Febrary, 2014. Patients received spirometry, forced vital capacity(FVC), forced expiratory volume in one second(FEV1), MIP, MEP, stiff nasal inspiratory pressure (SNIP). MIP per MEP ration and SNIP per MEP ratio were compared among the patients with different region.

Result: There was 25 bulbar onset(male 14, female 11), 65 cervical onset(male 42, female 23), 23 lumbosacral onset(male 17, female 6) ALS patients. Mean time from symptom onset to exam was 20.6months(2-74months). 47 patients showed FVC >70%; 7 bulbar onset (7/25), 28 cervical onset(28/65), 12 lumboscaral onset(12/23). MIP and MIP/MEP ratio was lower in bulbar onset patients compared with cervical and lumbosacral onset patients.($p=0.02$, $p=0.32$) But SNIP and SNIP/MEP ratio showed no difference between groups ($p=0.825$, $p=0.352$) FVC was lower in bulbar onset patients but showed no significance.($p=0.052$)

Conclusion: MIP and MIP/MEP was lower in bulbar lesion compared with limb onset ALS. MIP might be influenced with bulbar dysfunction because there was no difference in MEP, SNIP, SNIP/MEP ratio between ALS patients. These findings might be explained by multifocal hit and propagation hypothesis.

PS4-410 / #380

Theme: *6.2 - Motor neuron diseases: epidemiology, clinic, treatment*

Home-based multidisciplinary care for ALS/MND in Moscow and Russia

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Background: According to statistics there are 3585 cases of MND in Russia annually. This article presents the experience of an outpatient program for MND patients in Russia.

Methods:This program is a result of collaboration of 11 doctors, 1 psychologist, 2 social workers, 3 nurses, 3 coordinators and 20 volunteers. Our organization provides multidisciplinary care at home, hospitals and with online service consultations. We regularly work with our patients in the end-of-life decision making process. Our team holds a monthly outpatient clinic. We provide patients with NIV and IV machines and consult on their installation and use. We also organize hospitalizations in local hospitals to perform gastrostomy and tracheostomy. We provide information support through mndfund.ru.

Results: 250 patients applied to our organization from 20 regions. 82 of patients died during the studies period. Average patient's age is 58±13 years. 28,4% of patients or their family members applied to outpatient clinics. Approximately 15% of patients take Riluzole. Such low Riluzole prevalence rate exists because this drug is not registered in the Russia. The first neurologist consultation was provided after 9 months of symptom's onset in average. ALS diagnosis was confirmed in 14 months after disease manifestation. Degenerative spine disorders, lower limbs vessels atherosclerosis, stroke, polyneuropathy, peronael nerve neuropathy, encephalopathy, ENT diseases, multifocal motor neuropathy were misdiagnosed by local neurologists. Only 30% patients have been informed about their diagnosis. 34% patients had bulbar form of ALS. Mean ALSFRS-R score was 33±6. The large majority of our patients (78%) apply to our service when they have the 4stage of MND, 31% patients are on 4? stage, 47% 4B. 30% patients use PEG or NG tube feeding and get enteral nutrition. Since 2011 73 patients received NIV machines and 6 received IV. Currently we observe over 37 NIV patients and 7 IV patients. 66% had dyspnea during exertion, daytime sleepiness or morning headaches. Mean desaturation index was 16±5; Mean FVC was 49±20% of predicted,38%patients had pain complaints.

Conclusions: Common feature of Russia ALS patients is low level of disease knowledge. Main problem is inability to perform and highly cost of NIV and IV machines in Russia. Russian patients don' t have full access to pain and anxiety medicationAll data concludes that the care for ALS patients in Russia should be reorganized.

PS4-411 / #428

Theme: *6.2 - Motor neuron diseases: epidemiology, clinic, treatment*

Novel data from BENEFIT-ALS: Blinded Evaluation of Neuromuscular Effects and Functional Improvement with Tirasemtiv in patients with Amyotrophic Lateral Sclerosis

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Introduction: Tirasemtiv is a novel fast skeletal muscle activator that sensitizes the sarcomere to calcium and leads to an increase in the force of muscle contraction at submaximal contraction rates. In previous studies, it was well tolerated in patients with Amyotrophic Lateral Sclerosis (ALS), and dose dependent improvements on measures of muscle strength and patient function were noted.

Methods: 711 patients with ALS, with or without exposure to Riluzole, were recruited from 73 centers in North America and Europe. Slow vital capacity was > 50% of predicted, at least one handgrip was moderately weak, and ≥ 4 ALSFRS-R items scored 2 or 3. Before randomization, patients received 1 week of open-label tirasemtiv 125 mg BID to ensure this dose was well tolerated. Patients who tolerated open-label tirasemtiv were then randomized 1:1 to double-blind placebo or tirasemtiv beginning at 125 mg BID and escalating weekly based on tolerability to a maximum of 250 mg BID for a total of 12 weeks of treatment. ALSFRS-R and quantitative measures of respiratory and extremity muscle strength and endurance were assessed at baseline, after 4, 8, and 12 weeks of treatment, and at 1 and 4 weeks after the last dose. Plasma concentrations of tirasemtiv were measured during double-blind treatment.

Results: The last patient was enrolled on November 27, 2013 and the final study visit occurred in March, 2014. Safety and efficacy results related to the dose and plasma concentration of tirasemtiv, and the effects of withdrawing tirasemtiv after the 12 weeks of treatment, will be presented.

Conclusions: BENEFIT-ALS tests the hypothesis that tirasemtiv increases skeletal muscle performance leading to an improvement in function in patients with ALS.

PS4-412 / #432

Theme: *6.2 - Motor neuron diseases: epidemiology, clinic, treatment*

Does concurrent medication affect survival in amyotrophic lateral sclerosis?

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An increasing number of disease-modifying factors have been reported to have an impact on the disease course of amyotrophic lateral sclerosis (ALS), among which concurrent medication could be of great relevance. The use of statins has been proposed to impair survival and selective serotonin reuptake inhibitors (SSRI) could influence ALS prognosis since serotonergic mechanisms are involved in ALS pathophysiology. Moreover, since exposure to many toxic agents have been implicated in ALS developement, proton-pump inhibitors (PPI) could affect detoxification and thus influence survival.

The aim of the study was to explore the concurrent medication of ALS patients and the influence of SSRI, statins and PPI on survival.

This survey is based on invoice data of the regional sickness funds of Austria from January 1, 2008, to June 30, 2012. The registries comprise each insurant's demographic details, all hospital discharge diagnoses and all prescription data. ALS patients have been identified by the discharge diagnosis of ALS or by riluzole prescription. Any concurrent medication was evaluated by descriptive statistics and Kaplan-Meier analysis was performed to assess survival according to the use of SSRI, statins or PPI.

A total of 908 ALS patients were identified with a male-to-female ratio of 1.15. The median age at diagnosis was 66.0 years (IQR 56.8-73.2). 523 patients (57.6%) used riluzole with a median treatment duration of 314 days (IQR 129-590). The use of statins ($n=163$) and SSRI ($n=253$) had no clear significant impact on overall survival. In contrast, the use of PPI ($n=350$) was associated with a shorter median survival (560 versus 841 days, $p=0.002$). No such effect was seen when evaluating other antacids ($n=47$). A subanalysis of the PPI-group showed that significantly more patients were women ($p=0.04$), used riluzole ($p<0.001$) and were older at disease onset ($p=0.02$).

We show for the first time that PPI may have an intrinsic detrimental effect on the disease course in ALS. This is supported by the fact that there was no significant association when testing other antacids than PPI, though the lower case number might limit the power of this analysis. Interestingly, the effect was seen despite the higher rate of riluzole use in the PPI-group.As a limitation of the study we were not able to collect clinical data of the patients and, thus, could not exclude any potential clinical heterogeneity, e.g. patients treated with PPI might be affected more severly.

✶PF4

PS4-413 / #490

Theme: *6.2 - Motor neuron diseases: epidemiology, clinic, treatment*

Intraspinal stem cell transplantation in ALS: Results of a phase 1/2 clinical trial

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The FDA-approved trial, "A Phase 1, Open-label, First-in-human, Feasibility and Safety Study of Human Spinal Cord-derived Neural Stem Cell Trans-

plantation for the Treatment of Amyotrophic Lateral Sclerosis, Protocol Number: NS2008-1," has been completed in 15 patients with amyotrophic lateral sclerosis (ALS). Our overall objective was to assess the safety and feasibility of stem cell transplantation into lumbar and/or cervical spinal cord regions in ALS. Patient cohorts consisting of 3 ALS patients each followed a "risk escalation" paradigm progressing from non-ambulatory to ambulatory patients receiving unilateral ($n=5$) or bilateral ($n=10$ total) lumbar or cervical injections. The final cohort of 3 patients, Group E, received cervical injections and had previously received bilateral lumbar injections. All injections delivered 100,000 cells in a 10 µl volume, for a dosing range between 500,000 to 1.5 million cells over the 18 surgeries. The procedure was well-tolerated by all patients with minimal perioperative or postoperative complications. Although this was a safety trial, clinical progression was monitored and will be reported. Advanced analyses on Group E outcome data revealed preliminary insight into potential windows of stem cell biological activity and identified assessment measures that closely correlate with disease progression. Overall, results demonstrate that lumbar, cervical and dual-targeted intraspinal transplantation of stem cells in ALS patients is feasible and well-tolerated, supporting future trial phases examining therapeutic dosing and efficacy. Phase 2 of the trial commenced September 2013 with anticipated completion by June 2014; initial results from this trial will also be reported.

PS4-414 / #508

Theme: *6.2 - Motor neuron diseases: epidemiology, clinic, treatment*

What is the difference between brachial amyotrophic diplegia and upper limb onset ALS? Clinical and neurophysiological manifestations

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ALS is a fatal neurodegenerative disease of motor neurons and has various subtypes and shows a markedly heterogeneous clinical presentation and course. Atypical ALS presentations include the flail arm and flail leg variants. The Flail arm variant of ALS, also known as brachial amyotrophic diplegia (BAD) or 'man in a barrel' syndrome, is characterized by progressive, predominantly proximal, symmetric weakness and wasting of upper limbs, with no significant lower limb or bulbar muscle involvement. There are few reports about this phenotype. Studies investigating the clinical features and electromyographic findings of BAD are lacking. When clinicians encounter a patient who remains largely restricted to the upper limbs over time, they should be concerned that the possible diagnosis includes BAD or upper limb onset ALS (UL-ALS). Few study reported that BAD has male predominance and proper prognosis. Therefore, it could be difficulty in differentiation BAD and UL-ALS. The aim of the present study is to compare the clinical and electromyographic findings of patients defined as BAD with that of patients defined as UL-ALS. In the period 2006~2010 in our ALS center, we selected all patients diagnosed with motor neuron disease who had initial complaints of bilateral upper limb weakness, and excluded patients with evidence of bulbar dysfunction secondary to brainstem lower motor neuron involvement and lower extremities weakness to lumbar spinal motor neuron involvement. EMG/NCS were performed on all patients at the initial visit only. We set the time of onset of the disease as the date when patients first noticed their symptoms. During the follow-up visits, we established the progression to ALS when they had evidence of bulbar, respiratory muscles, and lower limb weakness.

Of the total 578 patients diagnosed with motor neuron disease, 103 patients had symmetrical upper limb weakness as initial presentation, 68 (54%) patients were classified as UL-ALS, and 18 (17%) as BAD. The mean observation period was 25 months (range 17-51) in UL-ALS, and 24 months in BAD (range 13-50). The mean age at symptom onset was 57 years (range 29-76) in the UL-ALS and 59 year (range 48-76) in BAD. The male to female ratio was 1.85:1 in UL-ALS, and 5:1 in BAD. BAD showed male predominance but this trend was not statistically significant ($p=0.129$). Fasciculation was present in 39/68 (67%) patients with UL-ALS and in only 3/18 (15%) patients with BAD ($p=0.001$). Proximal dominant weakness showed 70% (7/10) in BAD and 18% (6.34) in UL-ALS, but distal dominant weakness 30% (3/10) in BAD and 82% (28/34) in UL-ALS ($p=0.003$). Medical Research Council grade of the weakest arm

muscle was lower score in BAD than that in UL-ALS (2.8 vs 3.4, $p=0.047$). The proportion of UMN signs in bulbar, cervical, lumbar and two or more of these three regions, there was no difference between two groups. Five UL-ALS patients died on observation period.

Electromyographic studies of the total 4 regions including bulbar, cervical, thoracic, and lumbar spinal segments, was performed in 52 patients with UL-ALS and in 12 patients with BAD. Between two groups, there was no difference in lower motor neuron signs in EMG such as fibrillations, positive sharp waves, fasciculation, and giant MUPs.

Our findings underline the several clinical features (dominant affected site at initial onset, MRC grade, the rate of clinical fasciculation) that differentiate between BAD and U-ALS.

On the other hand, upper motor neuron signs on neurologic examination and two or more regions with lower motor neuron signs on electromyography showed no significant difference.

✶PF1

PS4-415 / #59

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

The pathogenic contribution of astrocytic and muscular TWEAK to ALS pathology

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ALS is a neurodegenerative disease for which the primary target is undeniably the motoneuron. However, reactive glial cells also contribute to motoneuron loss in ALS. ALS astrocytes release IFNgamma, thus triggering a motoneuron-specific death. IFNgamma stimulates TWEAK, which binds Fn14 and CD163 receptors. Importantly, TWEAK is expressed in astrocytes and promotes muscle atrophy, an ALS pathological hallmark that results from the inherent motoneuron loss. Our hypothesis is that TWEAK contributes to ALS via pathogenic roles in astrocytes and skeletal muscle. We have used the SOD1^{G93A} ALS mouse model and show a specific increased TWEAK expression in spinal cord astrocytes of endstage ALS mice. In muscle, TWEAK expression remains unchanged throughout disease progression, while there is a significant increase in Fn14 expression. Interestingly, treatment of primary motoneurons with TWEAK results in a caspase-3-dependent, Fn14-independent and CD163-dependent motoneuron death. We also demonstrate that ALS astrocytes express more Fn14, suggesting increased sensitivity to TWEAK. We therefore modulated TWEAK expression in SOD1^{G93A} mice via pharmacological and genetic approaches. Our preliminary results suggest that the genetic deletion of TWEAK in ALS mice improves lifespan and weight, specifically of males. TWEAK deletion in SOD1^{G93A} mice also increased neuromuscular junction (NMJ) endplate size, pointing to a pathogenic role for TWEAK in ALS skeletal muscle. Importantly, the injection of symptomatic ALS mice with an antagonistic TWEAK antibody also improves muscle parameters. We are presently completing our evaluation of the effect of genetic and pharmacological TWEAK depletion on motoneuron loss, astrogliosis, microgliosis, muscle atrophy and NMJ morphology. In summary, our uncovering of pathogenic roles for astrocytic and muscular TWEAK in ALS identifies a novel contributor to ALS pathology and thus an additional potential therapeutic target.

PS4-416 / #61

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Muscle-intrinsic defects and atrophy both contribute to the reduction in skeletal muscle size in mouse models of spinal muscular atrophy

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Spinal muscular atrophy (SMA) is a leading genetic killer of infants under two years of age. This neuromuscular disease is characterized by selective loss of motor neurons in the spinal cord and muscular atrophy due to survival motor neuron (SMN) protein depletion. Most research to date has focused on understanding why motor neurons are preferentially

affected in the disease. In contrast, the contribution of muscle defects to SMA pathogenesis has been mainly overlooked. Regardless, emerging evidence is demonstrating that muscle cell-autonomous defects are also implicated in the overall clinical picture of SMA. We therefore investigated the molecular pathways implicated in the reduction of muscle fiber size in mouse models of SMA. To achieve this goal, we assessed multiple measures in both myogenesis and atrophy. We provide evidence that the myogenic program is misregulated and delayed in two different mouse models of SMA. Additionally, we show that atrophy in these mice is mediated through FoxO3 transcriptional regulation, which induces important target genes involved in both proteosomal and autophagic degradation. Taken together, these results strongly reinforce the idea that intrinsic muscle defects, in addition to atrophy, leads to the SMA muscle phenotype. However, the specific role of SMN in skeletal muscle remains unclear. Future research will emphasize on filling the gap between the various roles of SMN, and the muscle defects reported in this study.

✶PF3

PS4-417 / #124

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Mutations in CNTNAP1 and ADCY6 are responsible for severe arthrogryposis multiplex congenita with axoglial defects

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Non-syndromic arthrogryposis multiplex congenita (AMC) is characterized by multiple congenital contractures resulting from reduced fetal mobility. Genetic mapping and whole exome sequencing were performed in 31 multiplex and/or consanguineous undiagnosed AMC families. Although this approach identified known AMC genes, we here report pathogenic mutations in two new genes. Homozygous frameshift mutations in CNTNAP1 were found in four unrelated families. Patients showed a marked reduction in motor nerve conduction velocity (<10m/sec) and transmission electron microscopy (TEM) of sciatic nerve in the index cases revealed severe abnormalities of both nodes of Ranvier width and myelinated axons. CNTNAP1 encodes CASPR, an essential component of node of Ranvier domains which underly saltatory conduction of action potentials along myelinated axons, an important process for neuronal function. A homozygous missense mutation in Adenylate Cyclase 6 gene (ADCY6) was found in another family characterized by a lack of myelin in the Peripheral Nervous System (PNS) as determined by TEM. Morpholino knockdown of thezebrafish orthologs led to severe and specific defects in peripheral myelin in spite of the presence of Schwann cells. ADCY6 encodes a protein that belongs to adenylate cyclase family responsible for the synthesis of cAMP.Elevation of cAMP can mimic axonal contact *in vitro* and upregulates myelinating signals. Our data indicate an essential and so far unknown role of ADCY6 in PNS myelination likely through the cAMP pathway. Mutations of genes encoding proteins of Ranvier domains or involved in myelination of Schwann cells are responsible for novel and severe human axoglial diseases.

PS4-418 / #137

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Amelioration of spinal muscular atrophy using RNA therapy to increase SMN level and modulate other secondary therapeutic targets

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Spinal Muscular Atrophy (SMA) is a genetic disorder caused by mutations of the survival motor neuron gene (SMN1) and leading to the degeneration of motor neurons, causing paralysis and death. No effective treatment is available so far; however, antisense therapy to increase SMN levels is a promising strategy. We generated induced pluripotent stem cell (iPSC) lines, derived from human skin fibroblasts, using lentiviral constructs and with a non-viral, non-integrating method, based on the expression of reprogramming factors with episomal vectors. We differentiated iPSCs using a protocol to promote motor neuronal phenotype. The phenotype of these cells was analyzed by morphological, functional, gene expression and protein analysis. An RNA strategy, based on antisense morpholino, shRNA and siRNA, and aimed at increasing SMN levels or inhibiting Fas activation, was tested. We show here that SMA iPSC-motor neurons recapitulate the disease phenotype with significantly fewer and smaller motor neurons, and at later time periods in culture, compared with wild-type subject iPSC lines. These features were ameliorated in SMA motor neurons treated with antisense morpholino or U1 shRNA, which increase SMN levels. During motor neuron development, SMA lines showed an increase in Fas ligand-mediated apoptosis and increased caspase-8 activation. Importantly, this could be mitigated by Fas silencing with siRNA.Our data support the utility of SMA iPSCs as *in vitro* disease model, suggesting that RNA therapy can be a possible therapeutic strategy for SMA through SMN up-regulation and modulation of disease pathways that can be achieved with different RNA therapeutic strategies.

PS4-419 / #139

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Improvement of SMARD1 phenotype using iPSC-derived neural stem cells transplantation

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Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is an infantile autosomal-recessive motor neuron disease caused by mutations in the IGHMBP2 gene. We previously demonstrated that primary neural stem cells (NSCs) can ameliorate the SMARD1 phenotype in mice. However, the clinical translation of primary NSCs is subject to many restrictions. The reprogramming of adult somatic cells into induced pluripotent stem cells (iPSCs) can provide an unlimited source of NSCs for therapeutic use. Here, we demonstrate that NSCs from human induced pluripotent stem cells (iPSCs) have therapeutic potential in the context of SMARD1 motor neuron disease. We generated iPS cell lines, derived from human skin fibroblasts, using a non-viral, non-integrating method, based on the expression of reprogramming factors with episomal vectors. We differentiated the iPSCs using a protocol to promote neuronal stem cell fate. The phenotype of these cells was analyzed by morphological, gene expression and protein analysis. Finally, iPSC-purified NSCs were transplanted by intraspinal cord injection into nmd mice, an animal model used for SMARD1. NSCs from iPSCs are self-renewing and multipotent, and can differentiate *in vitro* into neurons and glia as well as in motor neurons. We demonstrate that iPSC-derived NSCs can engraft

into the spinal cord of SMARD1 animals, ameliorating their neuromuscular phenotype and significantly improving their survival. iPSC-derived NSCs integrate appropriately into the parenchyma, differentiate into the three neuroectodermal lineages, and exert a neuroprotective effect on endogenous motor neurons. Our data support the translational potential of pluripotent cells for cell-mediated therapies in motor neuron disorders.

✶PF2

PS4-420 / #172

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Muscle mitochondrial dysfunction in a large cohort of genetically-determined SMA patients

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder caused by mutations in the SMN1 gene and characterized by degeneration of motor neurons with skeletal muscle weakness. Based on age of onset and clinical course, patients can be divided into three main groups (type I, II and III).

Severe depletion of mitochondrial DNA (mtDNA) had been reported in patients with SMA as a consequence of the severe fiber atrophy. Also, mtDNA depletion was found in patients with TK2 mutations and a SMA-like phonotype. In addition, TK2 null mice showed a COX deficiency in the anterior horn.

This prompted us to study muscle samples from 24 genetically proven SMA patients (type I: 9, type II: 8, type III: 7) using histochemical, biochemical and molecular techniques.

In all patients, skeletal muscle biopsy showed a chronic neurogenic pattern, with groups of atrophic fibers and fiber type grouping.

In addition, variable, but unequivocal COX deficiency was evident in most samples and was very severe in SMA I and SMA II subjects, where the enzyme stain was totally lacking. In all specimens, the enzyme defect was evident in both atrophic and normal/hypertrophic fibers. No histochemical defect was found in healthy control samples and in muscles from patients with chronic neurogenic disorders.

In 8 SMA patients compared to 7 age-matched controls, respiratory chain complexes were severely impaired, with 41.3 % residual activity for complex I, 26.6% for complex II and 30.7% for complex IV.

Also, in one SMA I patient we documented severe depletion of mtDNA (mean residual value <30%) in different muscles (autopsy)

Using custom array gene expression studies, we linked these alterations to the down-regulation of PGC1-alpha (13% of control levels) and of its downstream targets, including transcription factors NRF1 (37%), NRF2 (43%) and TFAM (35%).

We noted that SMA severity directly correlated with the extent of these abnormalities.

The repression of mitochondrial biogenesis has been postulated to be a major mechanism underlining neurodegenerative disorders such as Huntington's disease and amyotrophic lateral sclerosis.

In conclusion, our findings show that the oxidative defect was not restricted to atrophic fibers but it was also found in normal and hypertrophic fibers in samples obtained from all SMA subtypes.

MtDNA and PGC-1α expression are significantly reduced in SMA muscles.

We propose that muscle mitochondrial dysfunction plays a key role in the pathogenesis of the disease.

PS4-421 / #180

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Efficient SMN rescue following tricyclo-DNA antisense oligonucleotides treatment

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Spinal muscular atrophy is a recessive disease caused by mutations in the SMN1 gene, which encodes a protein (SMN) involved in RNA processing whose absence dramatically affects the survival of motor neurons. In Man, the severity of the disease correlates with the SMN2 gene copy number, which varies from individual to individual. SMN2 encodes the same SMN protein as SMN1. However, a single nucleotide change affects the definition of exon 7 during splicing such that about 90% of SMN2 mRNAs lack this exon. One of the most promising therapeutic strategy for SMA aims at reincluding exon 7 using antisense oligonucleotides. In this study, we investigate the therapeutic potential of a new class of conformationally constrained DNA analogues: the tricyclo-DNAs (Tc-DNA). We show that SMN activity can be restored in SMA cells by using Tc-DNA antisense oligonucleotides annealing a nearby intron 7 splice silencer (ISS) of the SMN2 pre-mRNA. RT-PCR showed approximately 60% of SMN2 mRNAs rescued after treatment with Tc-DNA analogues targeting the ISS, leading to nearly normal levels of SMN, detected by Western blot. Immunostaining also confirmed that rescued SMN was correctly located in nuclear gems.More importantly, weekly subcutaneous injections of Tc-DNA[ISS] in SMA type III mice revealed efficient inclusion of exon 7 in all tissues analyzed, including in brain and spinal cord. Tc-DNA treatment rescued the phenotype of SMN type III mice and prevented necrosis of tails, ears and toes in treated mice compared to controls.Altogether, these results suggest the therapeutic potential of Tc-DNA for the systemic treatment of SMA.

PS4-422 / #228

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Bone Health Determinants in Spinal Muscular Atrophy (SMA) type II/III

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Purpose: Severe osteopenia and fractures are reported in spinal muscular atrophy (SMA). Aim of our study was to evaluate determinants of bone status in SMAII/SMAIII patients.

Methods: DXA measurements of total body less head bone mineral density (TB-BMD,g/cm^2 and Z-score), bone mineral content (TB-BMC, g), fat mass (FM%, kg) and fat free mass (FFM kg) were obtained in 17 SMA subjects at baseline; 14 patients below 20 yrs of age ($n=9$ SMAII, 4 females, 5 males; $n=5$ SMAIII, 4 females, 1 male) and 19 controls (9 females and 10 males) were followed longitudinally at time T0 (9,6±4,0 yrs of age), T12 and T24 months. All patients underwent height (HT SDS), body mass index (BMI SDS), FMI (FM, kg/m^2) measurements. Five subjects ($n=3$ males with SMAII, and 1 female and 1 male with SMAIII) reported fragility fractures.

Results: SMAII and SMAIII subjects did not differ at T0 for age, HT SDS, BMI, FM, FFM and FMI although SMAII tended to be shorter and more muscularly atrophic than SMAIII subjects at all time points. Control subjects were significantly taller compared to both SMA groups. TB-BMC and BMD-Z-score values were significantly reduced in SMAII compared to SMAIII and controls at all time points, while bone mass parameters did not differ significantly between control subjects and SMAIII patients. Controls, SMAII and SMAIII showed a significant but highly different increase of BMD over 2 years (0,082g/cm^2, $P<0,001$; 0,038g/cm^2, $P=0.03$ and 0,116g/cm^2, $P=0.01$, respectively), with an absolute TB-BMC increase of 260g, 75.4g and 96,3g during the observation period. TB-BMD Z-score was inversely related

to age in SMAII and fell below normal values for age and sex (<-2 Z-score) in 70% of SMAII subjects by the age of 15 yrs; only 1 SMAIII showed low bone mass. DXA parameters did not discriminate between fractured and not fractured SMA patients. In contrast BMI, FM and FMI were significantly higher and FFM significantly reduced in SMAII subjects with fractures compared to SMAII without bone events (Ps<0,05).

Conclusions: SMAII patients present a profoundly compromised bone status, although bone mass accrual is reduced also in SMAIII subjects compared to controls. Body composition may be a major determinant of skeletal fragility in SMAII patients.

PS4-423 / #312

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Functional consequences of spinal muscular atrophy at the neuromuscular junction

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of alpha motor neurons in the ventral horn of the spinal cord. The main cause of SMA is the low levels of functional Survival of Motor Neuron (SMN) protein. This protein plays an important role in diverse cellular processes and is ubiquitously expressed. Several animal models have been generated to understand SMA related physiological changes and studies performed on these suggest that the earliest detectable pathology is at the neuromuscular junction (NMJ). Thus, the aim of this study is to determine the causes underlying NMJ degeneration in the severe SMA mouse model. We have already published the results of an ultra structural study performed on the NMJs of the diaphragm, intercostal and calf muscles of prenatal (E21) and post natal (P0 and P4) mice (Voigt, Neve, Schuemperli 2013). The characteristic ultrastructural changes observed in the disease mice were abnormal swelling of the mitochondria and vacuole like electron translucent profiles in the condensed cytoplasm of the perisynaptic Schwann cells (PSCs). These defects were more pronounced in the diaphragm muscle, which also appeared to be more advanced with respect to muscle fibre fusion and differentiation, in comparison to the intercostals and calf muscles. This led us to speculate that SMA related alterations appear only when the muscles have reached a certain level of maturity. Furthermore, immunohistochemistry analysis revealed a significant partial denervation in the diaphragm muscle of 4 day old SMA mice. Also, a significant reduction in the active zone density was observed, suggesting probable defects in synaptic transmission. We are now investigating the importance of glial cells in the pathophysiology of SMA, accompanied by a study of excitatory/inhibitory signalling at the level of the alpha motor neurons in the ventral horn of the spinal cord.

✶PF4

PS4-424 / #318

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

A Phase II study to assess safety and efficacy of olesoxime (TRO19622) in 3-25 year old Spinal Muscular Atrophy (SMA) patients

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Background and objectives: The experimental drug olesoxime (TRO19622) has beneficial effects both in *in vitro* as well as in animal neurodegeneration models, showing its capacity to promote neuron survival, increase neurite outgrowth, promote recovery after nerve crush injury, rescue motor neuron cell bodies from axotomy-induced cell death, accelerate myelination and promote repair in models of demyelination.

Maintaining survival of motor neurons is a mechanism highly relevant to SMA, an autosomal recessive disease associated with progressive motor neuron compromise mainly affecting proximal neuromuscular function.

To evaluate the benefit of this compound in patients with motor neuron diseases we designed and executed a pivotal phase II study to assess the efficacy and the safety of olesoxime in SMA patients.

Results: A multicentre, multinational, clinical trial was conducted from November 2010 to November 2013; final results are expected in Q1 2014. This double-blind, randomized, parallel groups, placebo-controlled trial incorporating a 3-stage adaptive study design was conducted in type 2 or non-ambulant type 3 SMA patients, aged 3-25 years old. 165 patients were recruited in less than one year from 22 sites in 7 European countries. Patients were randomized to olesoxime or placebo in a 2:1 ratio and treatment duration was for 104 weeks.

The primary endpoint is the mean change from baseline in motor function, assessed through the MFM scale, a neuromuscular disease-specific motor function measure. The secondary outcome measures include HFMS (Hammersmith Functional Motor Scale for Spinal Muscular Atrophy), electromyography measures (CMAP/MUNE), pulmonary function as measured by forced vital capacity (FVC), clinical global impression, quality of life measures (PedsQl) and safety.

Conclusions: Results from this clinical trial will be used to evaluate the potential benefit of olesoxime in type 2 and type 3 non-ambulant SMA patients as well as provide an unprecedented and valuable source of longitudinal data for motor function and electrophysiological outcome measures in a broad range of SMA patients.

Acknowledgements: AFM-Téléthon, the patients/families and all the Investigators involved in the clinical trial.

PS4-425 / #355

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Identifying small molecules targeting an RNA stem-loop involved in the alternative splicing of the SMN2 gene: A therapeutic target in SMA

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of motor neurons in the spinal cord, with a prevalence of 1 in 6000 births. SMA is caused by homozygousmutations in the SMN1 gene that disrupt the synthesis of SMN protein. SMN2, a gene 99% identical to SMN1 but with a different splicing pattern, can also produce SMN protein, although at lower levels. Manipulating the splicing of SMN2 to boost SMN protein production can compensate for the lack of SMN1. A number of cis- and trans-acting factors are known to regulate SMN2 splicing. Of these, experimental evidence supports the key inhibitory role of a local 19-nt RNA terminal stem-loop (TSL) on the splicing of SMN transcripts, thus making it an attractive therapeutic target for the treatment of SMA. In particular, we have found that single-nucleotide substitutions that disrupt the formation of this RNA hairpin in human cells can promote SMN2 splicing patterns similar to SMN1. *In vitro*, we have used the base stacking sensor 2-amino purine (2AP) and circular dichroism to confirm that these mutations induce different degrees of TSL relaxation that can be associated with distinct splicing efficiencies *in vivo*. Based on this, we have started a small-scale screening campaign using compounds with privileged RNA-binding scaffolds in order to identify TSL-binding molecules that would affect the formation of this RNA structure, modify the splicing of SMN2 transcripts, and ultimately boost SMN protein levels. To date, ~60 binding candidates have been recovered, which show different TSL-binding abilities, specificity and de-structuring effects. The activity of these molecules on the splicing of SMN2 is currently being investigated in human cells, and will also be presented at this meeting. In summary, this work confirms the key role of RNA structure in SMN2 splicing regulation and establishes a new line of work for the identification of drugs against SMA.

PS4-426 / #389

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Muscle-resident stem cells in Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a common inherited disease of childhood characterized by degeneration of motoneurons (MN) and muscle atrophy leading to severe paralysis and death in most severe cases. In 95% of cases, this pathology is due to mutations in the Survival of Motor Neuron gene (SMN1) which codes for SMN an ubiquitously expressed protein. SMN has been involved in various cellular processes including cytoplasmic assembly of snRNP into the spliceosome, pre-RNA splicing, and axonal trafficking of mRNAs. More recently, a possible cell-autonomous role of SMN in the regulation of stem cell function has been raised in brain, testis and skeletal muscle.

We and others have recently demonstrated that a single intravenous injection of self-complementary AAV9-SMN1 vectors (scAAV9-SMN1) mediates central and peripheral SMN expression, leading to a tremendous rescue of SMA mouse model. As growing evidences suggest that peripheral tissues, such as skeletal muscle are affected by low levels of SMN, it appears of particular importance to study the role of these tissues in the physiopathology of SMA for the design of future therapeutic strategies.

Thus, in order to investigate a potential dysfunction of muscle progenitors in SMA and thereby identify potential new therapeutic targets, we studied two populations of muscle-resident progenitors(MRPs): satellite cells and PW1+ interstitial progenitors (called PICs), in two mouse models of SMA. Our preliminary data strongly support a possible pathogenic role of these progenitor cells in SMA mice. In this context, we performed a pilot study to assess the capacity of AAV9 vectors to transduce MRPs in post-natal and adult muscles. We injected intramuscularly sc-AAV9-PGK-GFP vectors in wildtype mice and analysed the kinetic of GFP expression in satellite cells and interstitial progenitors. Our results provide AAV9 as a suitable vector for gene transfer in MRPs, which could be useful for therapeutic or physiopathological studies.

PS4-427 / #393

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

Correlation between genotype and phenotype in Algerian patients with spinal muscular atrophy

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Introduction: Spinal muscular atrophy (SMA) is one of the most common autosomal recessive disorders, characterized by degeneration of anterior horn cells in the spinal cord, and leads to progressive muscular weakness and atrophy. SMA is clinically divided into four subtypes depending on age at onset and clinical course. Genetic linkage studies have mapped responsible genes for all clinical types of SMA to chromosome 5q13, homozygous deletion in SMN1 gene causes the disease but the clinical severity may be modified by copy number of homologous gene SMN2 as well as the extent of deletion at SMN locus. In the present study, to elucidate the correlation between genotype and clinical severity in SMA patients, we analyzed the molecular genetics features of 92 Algerian patients with SMA, from 57 unrelated families.

Patients and methods: All patients fulfilled the diagnostic criteria of SMA as defined by the International SMA Consortium. Genomic DNA was extracted from peripheral blood following the conventional procedures. Deletions of exons 7 and 8 of the SMN gene were analyzed by an enzyme digestion assay. NAIP gene analysis was performed by PCR amplification of exon 4 and 5. SMN2 gene copy number analysis was carried out by the use of a quantitative PCR-based assay.

Results and Discussion: The patients were classified into type I SMA (20 patients), type II (16 patients), Type III (53 patients) and type IV (3 patients). 43 of the 57 SMA families (75.43%) were homozygous for SMN1 deletion of exon7 and 8 (type I, type II, type III and type IV).NAIP exon 4 and 5 were deleted in 15 cases (4/14 type I, 2/10 type II, 9/31 type III, 0 type IV), of 57 SMA families. In all patients with a NAIP deletion of exon 4 and 5, there was also a SMN1 deletion of exon 7. Frequency of NAIP deletions were significantly higher in type I patients than in type II or III patients. Also SMN2 copy numbers were higherinSMA type IV and SMA type III than in type I and type II. This increase in copy number of SMN2 gene may be responsible for the less severe form of SMA with late onset of symptoms.

Conclusion: Our results are compatible with the data of the literature.

PS4-428 / #472

Theme: *6.3 - Motor neuron diseases: Spinal muscular atrophy / Neuronopathies*

A novel lysophospholipid inborn error of metabolism underlying a cause of a complex distal spinal muscle atrophy: Neuropathy target esterase gene and its connections with organophosphorus-induced neuropathy

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Background: Neuropathy target esterase (NTE)is a serine hydrolase that was first identified as the initial target for organophosphorus compounds (OPs) causing organophosphorus-induced delayed polyneuropathy. Being an integral membrane protein located on the endoplasmic reticulum and showing phospholipase activity, NTE is involved in the hydrolysis of lysophosphatidylcholine to yield glycerophosphocholine. system. Distal hereditary motor neuropathies (DHMN) comprise a growing group of disorders that mainly affects nerve cells in the spinal cord, although more complex phenotypes with central nervous system involvement have been described.

Objective: To report a new phospholipid disorder in a Brazilian family caused by mutations in the neuropathy target esterase gene (Patatin-like phospholipase domain containing 6, PNPLA6) and its role in the metabolism of lysophospholipids.

Methods: Biochemical and molecular investigations were undertook in patients from a Brazilian kindred affected by a complex form of distal spinal muscle atrophy. Besides the motor neuropathy, patients showed signs of cerebellar atrophy with hypogonadism and early vision loss. After molecular analysis of genes associated with spinocerebellar ataxia and exclusion of other inborn errors of metabolism (IEMs) associated with cerebellar disease, whole exome sequencing (WES) was performed.

Results: Mutations in the PNPLA6 gene (in the SPG39 locus) were identified in all affected patients in the family. Patients affected by this disease have early onset visual loss (chorioretinal dystrophy) accompanied by progressive axonal motor neuropathy (with distal predominance), cerebelar ataxia and primary hypogonadism. Brain MRI showed cerebellar and pons atrophy.

Discussion/Conclusions: Mutant mice with mutations in PNPLA6 gene have shown a relentless neurological disease; lethality has been seen in knockout mice models for this gene, showing a severe dysfunction in neural tube development. Since lysophospholipids appear in different tissues and cell types and are involved in numerous physiological processes, such as vascular development, reproduction, myelination, neuronal development, NTE deficiency has profound biological consequences in the central and peripheral nervous system, expanding the connection between the organophosphorus neuropathy and a genetic form of DHMN.

PS4-429 / #52

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Through myotubes normalization, CYTOO 2D+ increases sensitivity of Muscle Damage HCS assay

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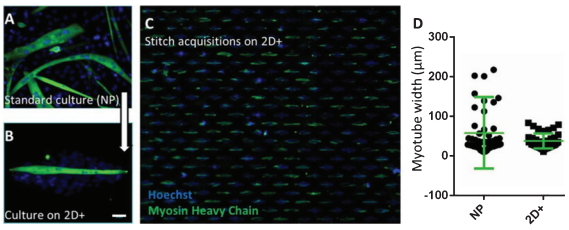
Since classical ADME/Tox tests failed to detect statin drugs family as toxicant by inducing chronic muscle damages such as myositis, rhabdomyolysis, muscle pain; which leaded to a considerable economic burden for pharmaceutical industry, e.g. cerivastatin withdrawal from the market, companies need to develop more relevant *in vitro* models dedicated to muscle damage drug discovery. In this context, CYTOO developed a physiological muscle model improving the sensitivity of myotoxic drug detection. When cultured on 2D+ technology, primary human myoblasts faster differentiate in myotubes containing a higher level of sarcomere striation and nuclei alignment compared to standard culture condition. Moreover, 2D+ technology standardizes myotubes formation and enables accessing new parameters for myotubes characterization upon drug treatment (see figure). Thanks to the development of new image analysis algorithms and the reduced variability of myotubes morphology, we gain access to finer and more relevant readouts. To further demonstrate the benefits of our model, we tested reference drugs inducing hypertrophy or atrophy on both standard culture condition and 2D+ platforms. Our results showed that this model is robust and compatible with High Content Screening with increased Z' factors compared to standard assays. Altogether, the normalization of myoblast differentiation in highly mature myotubes, coupled to enhanced image analysis capacities, demonstrated a higher predictivity over standard assays and will allow the detection of myotoxic drugs during the early phases of preclinical studies for compound development.



Myotubes adopt a standardized morphology when cultured on 2D+ technology. (**A**) Representative shape of myotubes formed on classic culture device compared to (**B**) 2D+ micropatterns. Contrary to standard culture devices, myotubes grown on 2D+ micropatterns don't exhibit artifactual branching and (**C**) their morphology appears highly standardized between structures. (**D**) This allows a reduced variability of the myotubes morphological parameters, *i.e.* width, and provides a new tool to robustly characterize hypertrophy and atrophy in a cell based assay format (scale bar: 50 µm).

PS4-430 / #204

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Serum biomarkers for Duchenne Muscular Dystrophy and muscular dystrophy animal models

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Duchenne muscular dystrophy (DMD) is a rare incurable disease affecting one in 3500 - 5000 boys. Several clinical trials are underway for DMD and there is an urgent need for the valuable biomarkers to follow up the short and long time effects of the treatments. In the present study, we used serum as a source of biomarkers for DMD diagnostic. Using complementary approaches: a bottom-up proteomic (permitting to identify proteins in the range 10^{-3} to 10^{-9} g/ml) and antibody arrays (with the sensitivity 10^{-9} to 10^{-12} g/ml) we analyzed samples from DMD patients ($n=94$, aged 3 - 20 years) and their respective age-matched controls ($n=53$) collected in the frame of ADNA program (Advanced Diagnostic for New therapeutic Approaches). These techniques allowed us to define new serum proteins differentially expressed between healthy and DMD patients. Importantly, some of these proteins were also found in the serum of animal models of DMD: mdx mice and GRMD dogs permitting to test and confirm the utility of some of these markers for the follow up of gene therapy approaches in preclinical animal studies. These markers could provide a new important monitoring tool of DMD treatment.

★PF3

PS4-431 / #214

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Skeletal muscle fatty degenerative changes can be evaluated both qualitatively and quantitatively from whole-body Dixon NMR images with an important gain in acquisition time

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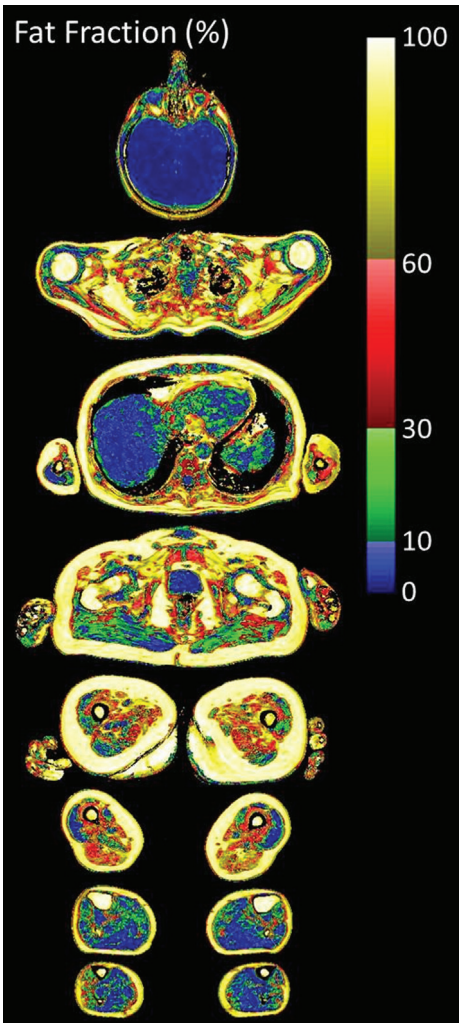
Fatty infiltration of muscles is a marker of disease progression in many neuromuscular disorders. Muscle MRI is capable of revealing patterns of muscles involvement that are disease specific and facilitates the diagnostic workup of patients. Although routine T1-weighted (T1w) imaging can give an indication of the presence or absence of muscular fat infiltration, it is difficult to extract quantitative data from these images. On the contrary, 3-points Dixon method can give a quantitative measure of both water and fat fraction. Generally, whole-body exams consist in the acquisition of the T1w sequence, followed by Dixon acquisitions on targeted regions to quantitatively assess fat infiltration. Our goal was to demonstrate that a well calibrated sequence allows replacing whole-body T1w imaging by a whole-body Dixon imaging.

One patient exhibiting severe fat infiltration was scanned on a 3T whole-body scanner. Whole-body T1w images were acquired with a 2D TSE sequence (in plane resolution = $1.1 \times 1.1 mm^2$, slice thickness = 6mm, T_{acq} = 5min40s). Whole-body Dixon acquisition consisted in a 3D VIBE sequence with 3 echo times (spatial resolution = $1 \times 1 \times 5\,mm^3$, T_{acq} = 14 min 5s). Quantitative fat fraction maps were derived from this sequence using a standard 3-points Dixon reconstruction method. A customed lookup table was embedded in the DICOM file to provide a clear lecture of fat fraction maps corresponding to the Mercuri's scale. The figure shows eight slices of the whole-body fat fraction map. At the first glance, the radiologist can estimate the location and severity of fat infiltration in the entire musculature of the patient.

Our results show that the acquisition of a high resolution whole-body Dixon imaging is possible in less than 15 minutes using our optimized 3-echos VIBE sequence. This provides quantitative data that are more suitable than T1w images for longitudinal natural history studies, or therapeutic clinical trials. Moreover, the color representation allows for a more accurate and instantaneous visual inspection of the fat infiltration. Although T1w images are considered by radiologists to be most appropriate for anatomical mapping and for determining muscle cross-sectional area or volume, natives images of the VIBE sequence can provide anatomical details that have already proved to be efficient for manual or automatic muscle segmentation. Whole-body Dixon might then overcome the use of whole-body T1w images for diagnostic of neuromuscular disorders.



PS4-432 / #233

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

A novel tool for fast, precise, interactive segmentation of skeletal muscle NMR images

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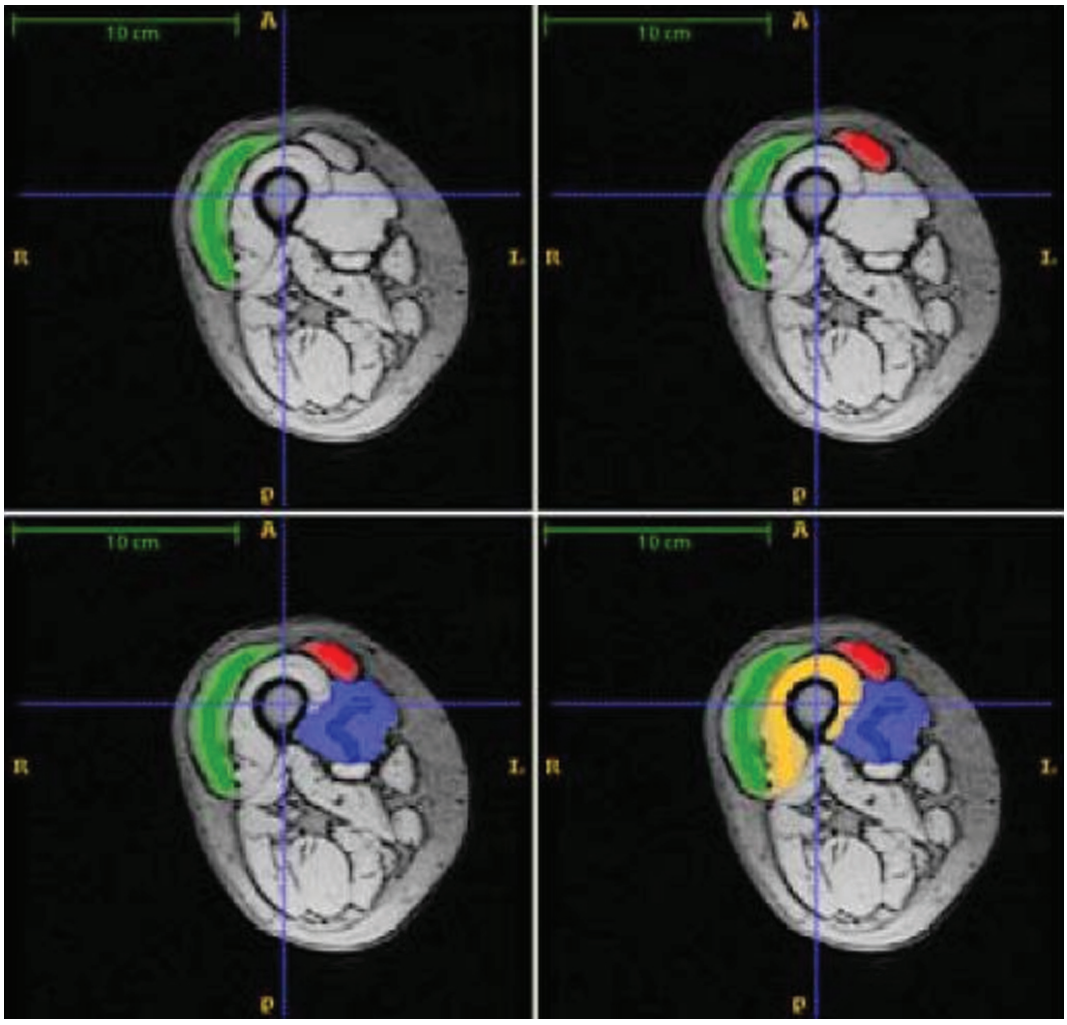
In the study of neuromuscular pathologies, NMR Imaging is a powerful and non-invasive method for monitoring evolution and comparing populations with respect to biomarkers such as: muscle atrophy, proportion of adipose tissue, etc. In order to perform such analysis on a per-muscle basis, it is necessary to segment the acquired volumes. While such task is easily achieved manually, segmenting entire muscles this way is extremely timely and tedious. Thus, it often happens that studies are delayed or cancelled due to the amount of work that segmenting dozens of volume represents. Automatic segmentation would seem an obvious solution to this problem. However, certain visual properties of the muscles in MRI, such as fuzzy boundaries or different regions with similar texture, make the development of automatic methods difficult. Typically, the results of fully automatic segmentation are unsatisfactory.

Thus, we directed towards an interactive image segmentation approach, with the idea that an algorithm could achieve accurate and fast results when it is guided by human supervision. As a result, we present



a segmentation tool that is based on the random walker segmentation algorithm that we integrated into the open-source ITK-SNAP software. Segmentation is performed thanks to a newly implemented intelligent brush: each time the user draws on the image, a large region around the newly colored region is automatically segmented. Fast computation is achieved thanks to the locality of the segmentation and to an efficient algorithm implementation. The proposed tool is intuitive and allows to segment the image progressively, muscle by muscle, as well as effective local correction.

We performed comparison tests on out-of-phase Dixon images, (TE/T*R* = 3.95/10ms) with spatial resolution = $1 \times 1 \times 5\,mm^3$ and grid size = $224 \times 224 \times 116$ voxels acquired on 3T whole body scanner (Tim Trio, Siemens Healthcare), selected for their good display of the muscle fasciae. Segmentation time was fixed to be less than 10 min. The resulting segmentations were compared to the manual segmentation for measuring the accuracy in a given time. On the quadriceps muscles of 10 subjects, we observed few differences between the fully manual approach and ours, both in terms of volume difference (4.49%) and Dice coefficient (0.96). These results show that it is possible to accurately segment the four muscles of the quadriceps under 10 min where fully manual segmentation normally requires more than 3 hours.

PS4-433 / #252

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Quantitative MRI in hypokalaemic periodic paralysis reveals age-dependent fat infiltration of lower limb muscles

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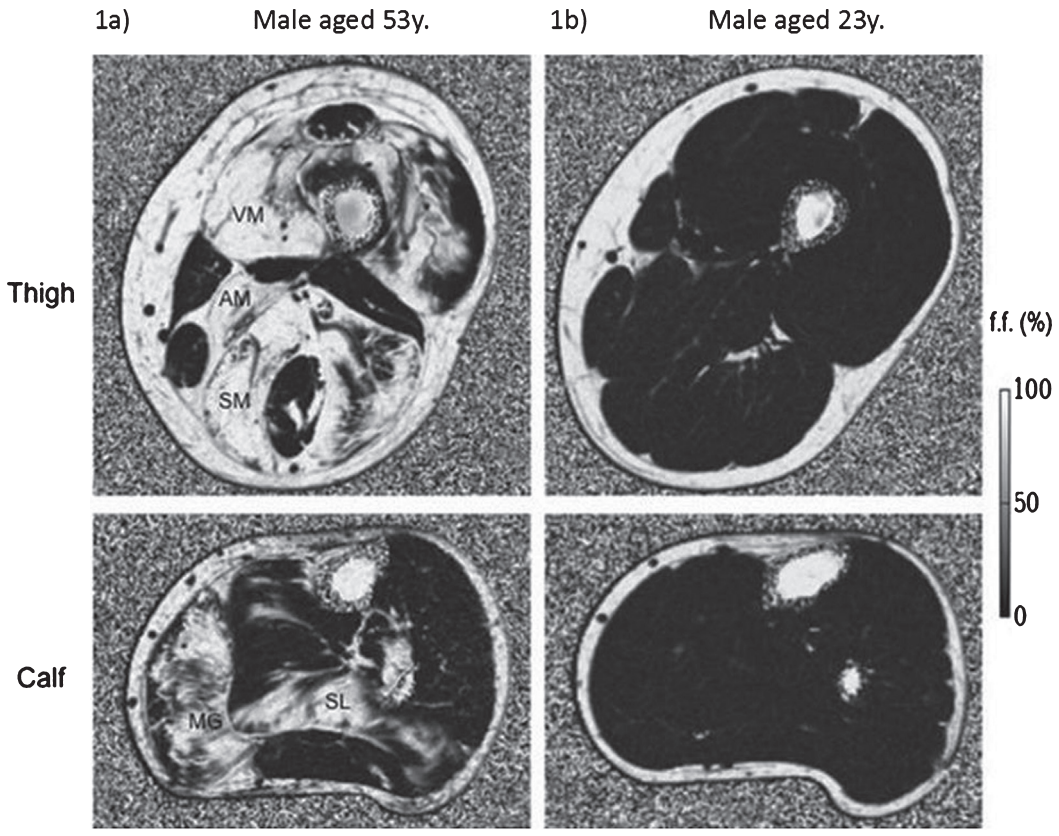

Background: Hypokalaemic periodic paralysis (HypoPP) is a muscle channelopathy characterised by episodic muscle weakness often related to low potassium levels. In addition to attacks of paralysis, a proportion of patients develop a fixed proximal myopathy which results in significant disability, though this has not previously been quantified. The aim of this study was to quantify chronic muscle pathology in HypoPP using MRI.

Methods: We performed lower limb muscle MRI at 3T in 12 patients with HypoPP (9M/3F, age 42 ± 12y) and 12 healthy controls (9M/3F, age 41 ± 10y) using the 3 point Dixon technique for fat-water quantification. Muscle fat fraction was measured in thigh and calf muscles using whole muscle regions of interest drawn by an observer blinded to diagnosis, on a single axial thigh and calf slice, respectively 20cm above and 15cm below the knee joint.

Results: Mean muscle fat fraction was significantly increased in HypoPP patients compared with controls at both thigh (patients: 10.2 ± 15.4%; controls 1.5 ± 0.5%) and calf (8.3 ± 10.5%; 1.7 ± 0.6%) level. Greatest mean fat fraction was in adductor magnus (17.8%) and vastus medialis (14.7%) in the thigh and in medial gastrocnemius (15.9%) in the calf. The least affected muscle was rectus femoris (5.4%) in the thigh and lateral gastrocnemius (5.1%) in the calf. Overall fat fraction correlated with age in patients (rho=0.76, $p<0.01$) but not controls (rho=0.17, $p=0.6$). The age-severity relationship appeared dichotomous with normal fat fraction in patients younger than 40 (1.8 ± 1.2%), but significantly increased in patients older than 40 (15.5 ± 14.2%). Example thigh and calf fat fraction maps for a male patient aged 53, and a male patient aged 23 are shown in figure 1a) and 1b) respectively.

Conclusions: Quantitative MRI reveals significant fat infiltration in selective thigh and calf muscles of HypoPP patients over 40, suggesting a period prior to this age during which effective treatment may avoid irreversible muscle damage.

PS4-434 / #255

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Lower limb muscle MRI findings in X-linked Charcot-Marie-Tooth disease

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Background: X-linked, dominant Charcot-Marie-Tooth disease (CMTX1) due to mutations in the gap junction beta-1 gene encoding connexin 32, is the second most common form of CMT. To date, there are no reports of lower limb MRI findings in patients with CMTX. Herein, we describe clinical and qualitative MRI findings in a 64 year old male with CMTX1 due to a p.Arg219Cys mutation in connexin 32.

Methods: We performed 3T MRI of both calves and thighs, using a standard clinical imaging protocol comprising T1-weighted (T1w) and STIR (short-tau inversion recovery) axial imaging with 5mm thickness and 1mm slice gap. T1w images were analysed according to the Mercuri scale.

Results: Lower limb examination revealed wasting distal to mid thighs. Knee strength was normal. There was severe bilateral foot drop with only mild ankle plantar flexion weakness. Pin sensation was decreased to the knees, while proprioception/vibration sense were normal bilaterally. Reflexes were absent.

T1w MR images revealed normal muscle bulk in thigh muscles, with marked atrophy of both calves. Vastus lateralis, biceps femoris, semitendinosus and semimembranosus were Mercuri grade 2a, while remaining thigh muscles were grade 1. In the calf, there was almost complete fatty replacement of gastrocnemius and soleus (grade 4) while tibialis anterior (TA), extensor hallucis longus (EHL), peroneus longus and tibialis posterior (TP) were Mercuri grade 3. There was distal accentuation of fat infiltration in all muscles. STIR images were normal in the thigh, but revealed marked symmetrical hyperintensity in TA,

EHL and TP bilaterally. Sciatic nerve size was normal. T1w thigh and T1w/STIR axial calf images are shown in figure 1.

Conclusion: QualitativeT1w MRI findings are in keeping with the clinical picture of a symmetric length-dependent neuropathy. MRI revealed chronic changes in distal thigh muscles which were normal strength clinically, suggesting superior sensitivity to early disease changes in the muscle. Unexpectedly given the slow progression observed in CMTX1, STIR imaging revealed changes of active denervation in anterior calf muscles, which may contribute to the observed severe ankle dorsiflexion weakness. The findings, including preferential involvement of posterior superficial calf muscles and normal sciatic nerve size, are different to that reported in CMT1A. Lower limb MRI is currently being undertaken in a larger series of CMTX1 patients to fully define the spectrum of MRI abnormalities.

PS4-435 / #274

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

MRI appearance of conduction block in vasculitic neuropathy

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Objective: To investigate the radiological features of conduction block in vasculitic neuropathy by magnetic resonance imaging (MRI).

Methodology: Case Report. A 70-year-old lady, with late-onset asthma, presented with fever, skin rash and bilateral foot drop and numbness for 2 months. She then developed right hand weakness and numbness for 3 days. Physical examination showed a vasculitic rash and signs of right ulnar neuropathy together with distal symmetrical sensorimotor polyneuropathy in the legs. Blood test showed eosinophilia and high p-ANCA titer. Skin biopsy confirmed necrotizing vasculitis with eosinophilic infiltrates. A diagnosis of Churg-Strauss syndrome was made. High dose prednisolone was started. Right ulnar nerve was studied by nerve conduction study (NCS) with inching technique and by MRI with gadolinium enhancement at 4 days, both being repeated at 11 days, after the onset of the hand symptoms.

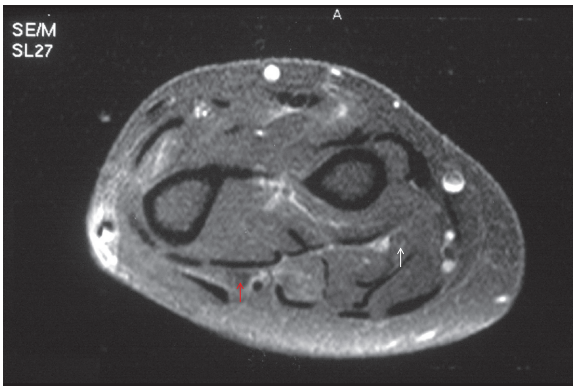

Figure 1. MRI forearm in T1-weight sequence with fat suppression and gadolinium enhancement. it show absent enhancement of ulnar nerve at site of conduction block (red arrow) and normal enhancement of median nerve (white arrow).

Results: NCS showed a conduction block in the right ulnar nerve at 8 cm above the wrist joint. MRI of the right forearm in T1-weighted sequence with fat saturation found a localized absent gadolinium enhancement of the right ulnar nerve at the same level. Follow-up MRI showed normal enhancement at the previous lesion.

Conclusion: This study reveals the MRI features of an acute conduction block in vasculitic neuropathy. The findings might be explained by the focal ischemic pathogenesis of the disease.

PS4-436 / #282

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Whole body MRI study in 28 genetically confirmed Chilean patients with dysferlinopathy

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To assess the natural course of dysferlinopathy, a cohort of 27 patients with genetically confirmed diag-

nosis underwent a whole body MRI scanning at distinct stages of disease. MRI scans were performed on a 1.5T Siemens equipment. Axial T1W and STIR images were obtained from the temporal regions to the ankles. Fatty replacement was ranked according to the scale of Kornblum et al., (2006). Two musculoskeletal radiologists, blinded to patients' clinical and genetic data, analysed the images. Patients were separated into five groups according to years of disease course (<5y $n=4$; 6-8y $n=5$; 9-12y $n=6$; 13-16y $n=6$ and >17y $n=6$). MRI infiltration score correlated positively with disease duration (ro= 0.53, $p=0.005$) and negatively with global Muscle Functional Measure scale score (ro= -0.70, $P<.001$). At early stages (i.e. <5 years group) grade 3-4 of fatty infiltration involved posterior compartments both legs and thighs as well as obturator externus, gluteus minimus and thigh adductors; lumbar erector spinae in the thoracoabdominal region, subscapularis on the shoulder girdle, along with relatively milder involvement of other muscle groups across the body. In the group of patients with a disease course from 9 to 12 years, fatty infiltration extended to the anterior compartments of legs and thighs, and progressed to the anterior compartment of the arm and forearm flexors. Interestingly, after 17 years of disease, masticatory and neck muscles, pectoralis minor, forearm extensors, transverse abdominal, piriformis and popliteus muscles were spared or showed mild compromise, while all the other muscles observed showed a grade 3-4 fatty replacement. Our results mainly agree with previous reports of MRI findings in lower limbs and their poor correlation with clinical phenotypes. Additionally we describe MRI findings in thoracoabdominal region, shoulder girdle and upper limb muscles from early to late stages of disease. FONDECYT#1110159 ANILLO ACT1121 (Conicyt, Chile).

PS4-437 / #296

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Development of a multifaceted biomarker strategy to support clinical development of utrophin modulators for Duchenne muscular dystrophy therapy

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Utrophin modulation i.e. the re-programming of utrophin transcription such that utrophin RNA and protein is continually expressed in mature fibres is expected to be a disease modifying treatment for Duchenne muscular dystrophy (DMD). SMT C1100 is a small molecule utrophin modulator demonstrating significant benefit on the muscular dystrophy in the dystrophin deficient mdx mouse. Plans for the first patient trials of SMT C1100 have been developed consisting of two components; a safety and dose finding study in DMD boys started in late 2013 to be followed by a proof of concept study starting in the second half of 2014.

In order to help confirm the benefit of utrophin modulators in DMD patients, a multicomponent exploratory biomarker strategy has been implemented that comprises of two modules; firstly the increase of utrophin levels and secondly, evidence of reduction in muscle regeneration. To demonstrate increased utrophin derived from drug treatment above that normally found in regenerating DMD muscle, we aim to quantify utrophin RNA, total utrophin protein and utrophin fibre localisation derived from pre- and post-dose biopsies. To determine a reduction in the rate of degeneration, i.e. increase in mature fibre survival, changes in the percentage of newly regenerating fibres as determined by the presence of neonatal and foetal myosin will be calculated from the biopsies. Using serum samples we will quantify the levels of specific miRNAs associated with fibre leakage and peptide markers of active fibrosis which characterises fibre damage and degeneration respectively.

We will present the data from candidate biomarkers tested both in DMD samples and dystrophin deficient animals. Data from these exploratory approaches may yield a set of predictive biomarkers to support clinical development of future utrophin modulators.

PS4-438 / #342

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Diagnostic algorithm for myoadenylate deaminase deficiency based on metabolic exercise testing parameters: a prospective study

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Background: The definitive diagnosis of metabolic myopathies requires an invasive muscle biopsy and subsequent highly specialised techniques for analysis. A non-invasive first line test could help the diagnostic approach.

Objective: Our aim was to evaluate the accuracy of metabolic exercise testing to provide a non-invasive algorithm to diagnose myoadenylate deaminase (MAD) deficiency, the most common metabolic myopathy.

Design: This observational and prospective study was performed at Brest university hospital from December 2008 to September 2012.

Setting: Academic referral center.

Participants: All the consecutive patients that both underwent a metabolic exercise testing and a muscle biopsy were prospectively enrolled.

Main Outcome Measure(s): Subjects performed an incremental and maximal exercise testing on a cycle ergometer. Lactate, pyruvate, and ammonia concentrations were determined from venous blood samples drawn at rest, during exercise (50% predicted maximal power output, peak exercise), and recovery (2, 5, 10, and 15 min). Absence, Decreased and Normal MAD activity were determined using p-nitro blue tetrazolium staining in cryostat sections from open muscle biopsy. The sensitivities and specificities of plasma ammonia, lactate, lactate/pyruvate and pyruvate/ammonia ratios to identify absent and decreased MAD activity were assessed using Receiver Operating Characteristic (ROC) curves analysis. A decision tree for MAD deficiency diagnosis was therefore generated using a stepwise approach.

Results: 51 patients were included. Omiting patients with glycolysis defects ($n=3$), MAD staining was absent in 5, decreased in 6, and normal in 37 subjects. Lactate/rest at the 10th minute of recovery provided the greatest area under the ROC curves (AUC, 0.981 ± 0.044) to differentiate Absent from Present MAD activity. The pyruvate/ammonium ratio at the 5th minute of recovery from exercise displayed the best AUC (0.871 ± 0.096) to discriminate between Decreased and Normal MAD activity. By combining the two biomarkers, the resulting decision tree achieved a diagnostic accuracy of 92.9%.

Conclusion and Relevance: The present algorithm provides a non-invasive test to accurately predict absent and decreased MAD activity, contributing to select patients for muscle biopsy and target appropriate histochemical analysis.

✶PF3

PS4-439 / #361

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Upper limb muscle fat-water quantification MRI and clinical functional evaluation in non-ambulant Duchenne muscular dystrophy

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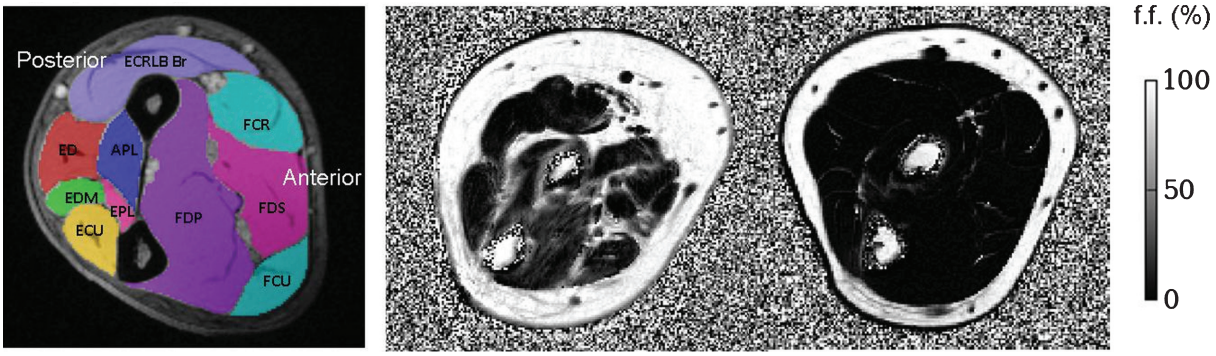
Background: Outcome measures in Duchenne Muscular Dystrophy (DMD) currently rely on invasive and insensitive functional tests. Muscle MRI could offer a valuable alternative. Furthermore, the opportunity to evaluate the upper limb permits inclusion of non-ambulant individuals not able to perform functional tests such as the 6-minute walk test.

Methods: In this on-going study, fat-water quantification was used to compare fat-infiltration in the forearm muscles of non-ambulant DMD patients and healthy controls. DMD individuals underwent 3T 3-point Dixon imaging of the dominant forearm to measure muscle fat-fraction (f.f.). Ten forearm muscles were segmented and mean f.f. and cross-sectional area recorded. Patients also underwent physiotherapy evaluation: Performance of Upper Limb (PUL) module; wrist extension myometry; and EK2 performance of tasks in daily life interview. Time to loss of ambulation (LOA) was recorded.

Fig 1a) Muscle segmentation b) DMD – 3-point Dixon f.f c) Control



Results: To date, 8 non-ambulant DMD patients (mean age: 13.6 years; mean duration of non-ambulation 20 months) and 10 volunteers (mean age:14.6 years) have been imaged. Example f.f. maps for a patient and control are shown in Fig. 1b&c. Overall mean f.f (±SD) in DMD was significantly higher than healthy controls: (13.4±11%vs 0.8±0.1%, $p=0.002$). Total mean area was reduced in DMD (1735±331mm^2) compared to healthy controls (2398±821mm^2, $p=0.04$). Overall f.f. correlated with LOA (Spearman $r=0.8$, $p=0.02$) and wrist extension myometry ($r=0.8$, $p=0.004$) with relationships also suggested between f.f and PUL ($r=-0.6$, $p=0.09$) and with EK2 ($r=0.6$, $p=0.09$).

Conclusion: Initial data support MRI fat quantification as a potential objective biomarker to monitor disease progression in the upper limb in DMD, showing significant correlation between putative MRI pathological indices and clinically meaningful endpoints.

PS4-440 / #375

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Automated tract based analysis of diffusion properties in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a clinically and genetically heterogeneous, fatal, progressive neurodegenerative disorder affecting the lower and upper motor neurons. Diffusion tensor imaging (DTI) has been suggested as a promising technique to assess disease-related microstructural tissue changes. Here we aimed to explore the utility of a fully automated technique for assessing differences in diffusion properties in the major fiber tracts of the brain between patients with ALS and healthy controls.

Structural images were acquired with an MPRAGE sequence and DTI data were collected using a two-dimensional diffusion weighted EPI sequence using a 3T-MRI. Diffusion properties (fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD)) were determined in 18 automatically segmented major white-matter tracts using TRACULA (TRActs Constrained by UnderLying Anatomy) which combines a global probabilistic approach with an automated seed region positioning.

We investigated 23 ALS patients (age range 34-82 years, mean ALS functional rating scale score = 37.9) and 18 age-matched healthy controls. TRACULA identified all major white-matter tracts in patients and controls. Differences between patients with ALS and controls were seen in the corticospinal tract (CST) for FA (0.489 in ALS vs. 0.506 in controls, $p=0.05$) and RD (0.510 in ALS vs. 0.489 in controls, $p=0.03$). No other significant differences between the groups were found.

Our data confirm the structural damage of the CST in ALS. The absence of significant group differences in other structures is in line with the dominating role of the CST in but may also indicate a limited sensitivity of the employed technique. However, in contrast to other group-based analysis approaches TRACULA yields tractographic measures on a subject level. This will therefore allow comparisons of microstructural changes with clinical data on an individual basis. Thus, TRACULA may develop into a valuable diagnostic and prognostic tool in future research.

PS4-441 / #482

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Effect size of quantitative muscle imaging in Duchenne muscular dystrophy exceeds the effect size of clinical scores of muscle function

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The aim of this ethics approved one year observational trial was to compare functional abilities and muscle imaging data in 20 patients with genetically confirmed Duchenne muscular dystrophy. Physical assessment was performed using the motor function measurement (MFM) scale. Quantitative magnet resonance imaging (qMRI) of thigh muscles was performed using the two-point Dixon method. One year functional and imaging changes were different according to the age and walking abilities at inclusion (group 1: below the age of seven; group 2: seven years and older and ambulant; group 3: non-ambulant). While patients of the first group still showed improvement of motor abilities, in group 2 the largest effect size (1.2) was found in the D1 subscore (standing and transfer function) of the MFM, and in group 3 the largest effect size was found for the total MFM score

Spalte1	Spalte12	Spalte2	Spalte3	Spalte4	Spalte5	Spalte6	Spalte7
Patient group	**Test**	**Score**	**Effect size**	**80% Power**		**90% Power**	
				50%	None	50%	None
Group 1	**MFM**	Total MFM	1,13	51	14	68	18
(< 7 years)		D1	1,00	65	17	86	23
		D2	-0,58	191	49	256	65
	MFF	All thigh muscles	1,63	25	7	33	9
		Extensors	1,50	29	9	39	11
		Flexors	1,36	35	10	47	13
		Adductors	1,35	36	10	47	13
	HMA	All thigh muscles	-0,86	86	23	114	30
		Extensors	-0,01	294633	73659	394429	98608
		Flexors	0,48	274	70	366	93
		Adductors	-0,90	78	21	105	27
Group 2	**MFM**	Total MFM	-0,73	118	31	158	41
(> 7 years, ambulant)		D1	-1,15	49	13	65	17
		D2	-0,12	4619	1156	6182	1547
	MFF	All thigh muscles	2,86	9	4	12	4
		Extensors	2,38	13	4	16	5
		Flexors	4,09	5	3	7	3
		Adductors	1,69	24	7	31	9
	HMA	All thigh muscles	-1,91	19	6	24	7
		Extensors	-1,86	20	6	26	8
		Flexors	-0,84	89	24	119	31
		Adductors	-1,69	23	7	31	9
Group 3	**MFM**	**Total MFM**	-0,66	145	37	194	50
(non ambulant)		**D1**	-0,35	515	130	689	173
		D2	-0,59	180	46	241	61
	MFF	**All thigh muscles**	2,11	16	5	20	6
		Extensors	2,33	13	5	17	6
		Flexors	2,05	17	5	22	7
		Adductors	1,91	19	6	25	7
	HMA	**All thigh muscles**	-1,76	22	7	28	8
		Extensors	-2,03	17	5	22	7
		Flexors	-0,60	174	45	233	59
		Adductors	-1,42	32	9	43	12

(0.66). In contrast, the effect sizes using qMRI was much larger consisting of 1.6 in group 1 (all thigh muscles), 4.1 in group 2 (hamstrings), and 2.3 in group 3 (knee extensors). These data suggest that qMRI has an added value compared to clinical outcome measures when designing therapeutic clinical trials in patients with DMD. Power analysis suggest that there are only a few needed patients to show an effect of a putative novel treatment when using quantitive muscle MRI in DMD.

PS4-442 / #536

Theme: *7.1 - Techniques of diagnostic in NMD: Imaging / Biomarkers*

Involvement of the brachial plexus - a combined diagnostic approach

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Diagnosis of brachial plexus lesions in patients with cancer has improved due to available imaging methods, which can confirm and enhance clinical and electrophysiological findings. A 50 year old patient was treated for breast cancer with lymph node resection and local RT 10 years ago. She had been under regular oncological treatment including surgery for recurrence several years ago. One month prior to the exam she noted painless sensory of all fingers of her left hand, weakness of the hand muscles, and a numbness in the distribution of the medial cutaneous antebrachial nerve. At the examination the small hand muscles except the thenar were atrophic, EMG confirmed denervated small hand muscles, median and ulnar motor NCV were absent and a small sensory potential could only be elicited at the thumb. There was no Horner`s syndrome. Lower brachial plexus lesion was suspected and an MR of the brachial plexus as well as a high resolution ultrasound (US) of the brachial plexus and the arm's nerve were conducted. MR Imaging showed a regional edema surrounding the brachial plexus and the medial brachial sulcus. Within this edema especially the caudal parts of the brachial plexus were thickened. US imaging showed a substantial thickening of the brachial plexus from inferior trunk's constitution to the medial fascicle. Additionally, the median nerve's fascicles were also extensively thickened ranging from its roots to the distal forearm. Surprisingly the ulnar nerve had no conspicuous alternation. The addition of imaging techniques in the detection of brachial plexus lesions has become an important tool. In this case the imaging techniques could demonstrate pathological thickening of the brachial plexus' inferior trunk and medial fascicle in US and MR, correlating with the site of the clinically and electrophysiologically suspected lesion. US and MR complement one another, as MR offers a good overview at the level of the brachial plexus and it's surrounding tissue. High resolution US in addition depicts details of the pathology and allows an easy correlation to the brachial plexus' different parts. At present the imaging techniques do not allow a distinction between inflammatory, neoplastic and late RT effects and surgical exploration is required.

PS4-443 / #100

Theme: *7.2 - Techniques of diagnostic in NMD: Histopathology / Immunopathology / Ultrastructural study*

Effect of mitochondrial changes on myopathies clinical course

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Background: Valuation methods of mitochondrial dysfunction led to the understanding that neuromuscular diseases may be accompanied by "secondary" changes in the mitochondria. The clinical significance of these changes requires consideration.

Objective: Determination of the clinical characteristics of mitochondrial myopathies and clinical features related to mitochondrial changes in manifestations of another types of myopathies.

Methods: Clinical and laboratory features of neuromuscular diseases were evaluated in 997 patients. There were 317 (32%) patients with mitochondrial myopathies and mitochondrial encephalomyopathies. Mitochondrial disorders have been found in 52% congenital myopathies, 27% congenital muscular dystrophy and in 39% progressive muscular dystrophy. Evaluation of mitochondrial functions was carried out with the help of biochemical and morphological methods.

Results and discussion: Analysis of the clinical presentation of mitochondrial myopathies compared with other myopathies revealed the following features: frequent multiorgan lesion, undulating course, the prevalence of fatigue is higher than muscular strength reduction, milder progress of myopathic syndrome, response to energotropic therapy. Related mitochondrial disorders have influence on the non-mitochondrial myopathies course. Muscle tissue assay identifies two types of changes: 1) mitochondrial destruction with or without RRF presence; 2) increase number of mitochondria in subsarkolemmal regions. The second option is more often shown in non-mitochondrial myopathies. The presence of such mitochondrial clusters has compensatory clinical effect. We suppose that 34% of patients with non-mitochondrial myopathies have delayed age debut due to this effect.

Conclusion: Determination of the above clinical features indicate mitochondrial disorders and the need for energotropic therapy. Subsarkolemmal mitochondria cluster assay is important to assess the compensatory capacity of the patient.

PS4-444 / #39

Theme: *7.3 - Techniques of diagnostic in NMD: Electrophysiological techniques*

Mirror movements in amyotrophic lateral sclerosis

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Background: Mirror movements (MMs) are involuntary movements suggest an UMN involvement. Objectives: Assessment of the MMs phenomena as an early sign of UMN involvement in amyotrophic lateral sclerosis (ALS).

Patients and methods: Fifty patients with ALS were subjected to full clinical neurological examination and identification of the MMs phenomena in both upper limbs(ULs) and lower limbs(LLs) if present. Detection of MM were done using surface electromyography (EMG) study from the abductor digiti minimi and tibialis anterior muscles and transcranial magnetic stimulation (TMS) and simultaneous recording of the EMG activity and motor evoked potentials(MEPs) from the ipislateral and contralateral sides.

Results: MMs had been detected by analyzing ipsilateral MEP and the EMG of the examined patients. MMs detected by EMG of the examined muscles were correlated well with the increased muscle tone, exaggerated reflexes and central motor conduction time in LLs. Specificity of the MM and the positive predictive value were higher when compared with the sensitivity and the negative predictive value. Subclinically, MMs had been detected by analysis of ipsilateral MEP of LLs(27%), ULs(45%) , by the EMG of the ULs (45%) and the LLs(45%)

Conclusions and recommendations: MMs are rarely detected clinically in ALS but we can detect them by using electrophysiological procedures like the MEP and the EMG. Detection of MM is a good specific with high positive predictive value in diagnosing the ALS.

PS4-445 / #97

Theme: *7.3 - Techniques of diagnostic in NMD: Electrophysiological techniques*

The reproducibility and usefulness of motor unit number index (MUNIX) using abductor digiti minimi and tibialis anterior muscles in ALS patients

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Introduction: Motor unit number index (MUNIX) is a novel diagnostic technique developed to quantify the axonal loss in nerve of the ALS patients. The

objective of this study was to establish the reproducibility and usefulness of the MUNIX using abductor digiti minimi (ADM) and tibialis anterior (TA) muscles in ALS patients and normal controls.

Methods: MUNIX was performed on bilateral ADM and TA muscles in 30 ALS patients and 27 normal controls. MUNIX, compound muscle action potential (CMAP), Medical Research Council (MRC) sum score, and ALS functional rating scale (ALSFRS) were evaluated and their correlation was calculated using Pearson correlation analysis. All muscles were recorded twice to assess reproducibility and coefficient of variation (COV) was calculated. The mean values of all 4 (bilateral ADM and TA) muscles were assessed and their correlations with functional status were analyzed.

Results: The mean values of CMAP amplitude and MUNIX showed no significant difference between the tests. The COVs for CMAP and MUNIX were within the acceptable range of less than 20%. There was a significant correlation between MUNIX and CMAP amplitude in both ADM ($r=0.918$, $P<0.01$) and TA ($r=0.850$, $P<0.01$) muscles in ALS patients. In normal controls, there was also significant correlation between MUNIX and CMAP amplitude in both ADM ($r=0.629$, $P<0.01$) and TA ($r=0.894$, $P<0.01$) muscles. In ALS patients, MUNIX was significantly correlated with the ALSFRS in ADM ($r=0.439$, $P=0.015$), but not in TA ($r=0.357$, $p=0.053$). There was a significant correlation between MUNIX and MRC sum score in TA ($r=0.474$, $P<0.01$), but not in ADM ($r=0.349$, $P=0.058$). Using the mean values of all 4 muscles, there was a more significant correlation between CMAP and MRS sum score ($r=0.480$, $P<0.01$), and CMAP and ALSFRS ($r=0.517$, $P<0.01$). There was also a more significant correlation between MUNIX and MRS sum score ($r=0.493$, $P<0.01$), and MUNIX and ALSFRS ($r=0.481$, $P<0.01$).

Conclusions: This study has shown a reproducibility of MUNIX using ADM and TA muscles. Both ADM and TA muscles showed good correlations, but the patterns of correlation of MUNIX were slightly different. The mean value of combination of 4 muscles was more useful to assess functional status and disease progression. Further prospective, follow-up study with more patients will be needed to confirm the result of this study.

PS4-446 / #127

Theme: *7.3 - Techniques of diagnostic in NMD: Electrophysiological techniques*

Motor unit number index (MUNIX) in the orbicularis oculi muscle of healthy subjects

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Introduction: The motor unit number index (MUNIX) refers to an electrophysiological method that measures the approximate number of motor units using the surface electromyographic interference pattern (SIP) recorded during graded muscle contractions. In spite of brief test, MUNIX can suggest valuable information about the number of motor units and would be a practical electrophysiological method for assessing the progression of diseases such as amyotrophic lateral sclerosis (ALS). So far, a few limb musclesmuscles have been investigated. However, MUNIX technique assessing bulbo-facial muscles has not been reported to our knowledge, even though most ALS patients present with cortico-bulbar symptoms, including dysarthria, dysphagia, decreased tongue mobility and facial weakness.

Methods: This study was designed to assess bilateral orbicularis oculi muscles innervated by facial nerve with using MUNIX, and the reference value and reproducibility of MUNIX and MUSIX were investigated in 41 healthy subjects. The facial nerve is stimulated supramaximally at anterior tragus directly in front of lower ear and, the CMAP of the orbicularis oculi muscle is recorded using flat disc electrodes with standard nerve conduction protocols. Then, the orbicularis oculi muscle is activated at five different levels of isometric force performed by eye closing.

Results: In this study, MUNIX was successfully applied to the orbicularis oculi muscles, and showed a

good reproducibility between two trials. The coefficient of variation was within the acceptable range of 20% in MUNIX compared with previous studies. The correlations between MUNIX values obtained from the different operators were significant. And also correlations between MUSIX and CMAP values obtained from the different operators were all significant.

Conclusion: In conclusion, the current study suggested a first clinical trial of MUNIX assessing the bulbo-facial muscles, and also showed a good reproducibility between operations in MUNIX of orbicularis oculi muscles. This would be a useful electrophysiological method for assessing the severity and progression of bulbar symptoms in ALS.

PS4-447 / #542

Theme: *7.3 - Techniques of diagnostic in NMD: Electrophysiological techniques*

Prognostic significance of A-waves as an isolated abnormity of nerve conduction studies

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Background: A-waves represent a frequent type of late responses. They are generally considered as a non-specific electrophysiological finding and their significance remains unclear, particularly in tibial nerves. The aim of our study was to examine, if the occurrence of A-waves as the only abnormality may predict increased probability of the development of other electrophysiological abnormalities in corresponding nerves in the future.

Patients and methods: The occurence of the A-waves was assessed in patients examined in the EMG laboratory of the University Hospital Brno. Only individuals, whose examination was assessed as normal (or with occurence of A-waves as the only acceptable abnormality) and who were re-examined in our laboratory in the same part of the body at least 12 months apart, were than included into the study. Altogether, 74 patients (38 men, 36 women, mean age 52.7 ± 9.1 years) fullfilled these criteria, with the mean interval between the examinations of 37 ± 24 months.

Results: At initial examination, A-waves were very rare in upper extremities (where they occured in 1 of 90 examined motor nerves only), and were significantly more frequent in lower extremities, where they'd been found in 4% of 69 examined peroneal nerves and in 25% of 72 examined tibial nerves ($p = 0.003$). At control examination 12 or more months apart, clearly defined EMG abnormality in a corresponding nerve was newly found in 100% individuals with A-waves in the ulnar nerve (ulnar nerve lesion in the cubital cannal) or in the peroneal nerve (polyneuropathy or L5 radiculopathy) in contrast to 11 and 24% of newly found abnormalities in the individuals without A-waves in these nerves at initial EMG examination ($p < 0.001$). In the tibial nerves, polyneuropathy or S1 radiculopathy developed in 62% and 21% of patients with and without A-waves at initial examination, respectively ($p = 0.006$).

Conclusions: Occurence of A-waves as the only electrophysiological abnormality significantly increases the probability of development of clearly defined neurophysiological abnormities in the future. Our findings thus confirmed the significance of A-waves as initial abnormality in any of the examined nerves including the tibial nerve.

PS4-448 / #108

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

Sequence capture and targeted resequencing in DNA diagnostics of neuromuscular diseases

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We present the first results of utilization of a new diagnostic approach established in the Czech Republic in diagnostic practice of neuromuscular diseases - Sequence capture and targeted resequencing. For identification of mutations associated with neuromuscular disorders, we introduced a solution-based capture method SeqCap EZ Choice Library (Roche NimbleGene) and targeted resequencing (TR) on the

GS Junior System (Roche) or MiSeq (Illumine). A custom capture array was designed to capture exons and adjacent intron sequences of 42 genes (1020 exons, 280 kb). The list of selected diseases and genes includes Duchenne muscular dystrophy (DMD), Emery-Dreifuss muscular dystrophy (EMD, FHL1, LMNA), limb-girdle muscular dystrophy (MYOT, LMNA, CAV3, CAPN3, DYSF, SGCG, SGCA, SGCB, SGCD, TCAP, TRIM32, FKRP, TTN, POMT1, ANO5, FKTN, POMT2, POMGNT1); and additionally genes associated with congenital muscular dystrophies (LAMA2, LARGE, SEPN1, COL6A1, COL6A2, COL6A3, ITGA7, DNM2); congenital myopathies, cistal myopathies, and other myopathies (NEB, TPM3, ACTA1, TPM2, TNNT1, CFL2, RYR, MTM1, BIN, CRYAB, DES, LAMP2, PABPN1) (http://www.musclegenetable.org/). Using this approach, 33 patients were analysed so far, in 15 of them mutations associated with a disease were detected (in some patients the analysis is not finished yet). Causal mutations were identified in the ACTA1, CAPN3, COL6A1, COL6A3, DES, DNM2, DYSF, LAMA2, RYR1, SGCB, and SEPN1 genes.

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PS4-449 / #130

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

A 7-gene signature allows the identification of altered muscle tissue

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Gene expression arrays were used to define molecular profiles of clinically relevant myopathy subtypes; however, a gene expression signature that distinguishes a diseased from a healthy muscle was not identified. Aim of the present work was to identifya sensitive, reproducible, objective molecular diagnostic tool able to differentiate myopathic from normal muscle tissue.A data mining pipeline consisting of feature selection, model building and model validation was applied. Feature selection used public and INNCB’s Affymetrix gene expression microarray data, which include muscle biopsies of 176 controls and 191 individuals diagnosed with myopathy, for selecting probe-sets useful to classify patients into normal and diseased. Seven genes (ANXA2, C1QB, CDKN1A, DDAH2, LGMN, MYH3andTPPP3) were identified and analyzed by real-time RT-qPCR in 38 muscle biopsies, 19 affected by one of the myopathies included in the microarray data set, and 19 not affected. The RT-qPCR data were then used in the model building to train different classifiers and a multilayer perceptron exploiting the 7-gene signature was selected as the optimal classifier. It achieved an average cross-validated sensitivity equal to 95.67%, specificity equal to 95.74% and accuracy equal to 95.67% using diagnoses based on the muscle biopsy as ‘gold standard’. We built a classifier based on the expression of 7 genes that is able to discriminate between normal and diseased muscle tissue. This signature is common to different types of myopathy and might represent a novel diagnostic tool, which may improve the management of myopathic patients.

This work was supported by Italian Ministry of Health, years 2013-2015 (annual research funding).

PS4-450 / #178

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

An amplicon-based massive parallel sequencing for diagnosis of Duchenne and Becker muscular dystrophies

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The dystrophin gene (DMD) is the largest human gene spanning 2,200 kb of genomic sequence and containing 79 exons. The mutational spectrum of dystrophinopathies includes exonic copy number variations (CNVs) which account for approximately 65% of DMD mutations, and 30 to 35% of point mutations (Single Nucleotide variations - SNVs) introducing a premature stop codon. Current assays for analysis of the 79 exons and exon-introns junctions of the full length muscle isoform involve a variety of combined methodologies which are time-consuming. The objective of our project is to implement the massively sequencing technology for diagnosis application in dystrophinopathies.

In the present study, we evaluated an amplicon-based method (Multiplicom) for the analysis of the DMD gene on the Roche 454 GS-FLX sequencer, to identify disease-causing mutations in Duchenne (DMD) or Becker (BMD) muscular dystrophy patients. We first compared data generated by this technology and the reference Sanger sequencing method for SNVs analysis to determine the sensibility, specificity and reproducibility of this assay.

We used two different software for performing alignment and data analyses. Variants were filtered on the basis of sequencing depth (>40X) and variant frequency -VF (>20% for heterozygous mutation and 70% for homo/hemizygous mutations). All known SNVs were detected by this amplicon based sequencing approach, corresponding to a sensibility of 100%. Evaluation of criteria for improving specificity (detection of false positive especially in homopolymeric regions) is currently in progress. We are also now focusing on the detection of CNVs in order to screen 100% of disease-causing mutations in the DMD gene. By combining the detection of CNVs and SNVs in a single technology, we could improve the efficiency, and reduce the cost and delay of DMD and BMD diagnoses.

PS4-451 / #230

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

Free radical oxidation in hereditary motor-sensory neuropathies and myotonic dystrophy

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Steinert's disease (myotonic dystrophytype 1; DM1) and hereditary motor and sensory neuropathies (HMSN) are different neuromuscular diseases. Although the pathogenic mechanisms underlying in DM1 and HMSN are still not very clear, a role of oxidative stress in these diseases has been suggested. Thirty six HMSN patients (13 patients with HMSN 1X, 13 patients with HMSN 1A, 10 patients with genetically unidentified variants of HMSN) and 16 DM1 patients were enrolled in the study to evaluate the generation of reactive oxygen species, the content of the products of lipid peroxidation, the total blood antioxidant activity and related them to clinical severity scores. Changes in the generation of reactive oxygen species were characterized by an increase in the spontaneous blood chemiluminescence in patients with HMSN compared to controls ($p=0.016$).The concentration of the products reacting with thiobarbituric acid was significantly higher in patients with DM1, than in controls ($p=0.010$). There were the high correlation coefficients between the levels of induced blood chemiluminescence in HMSN patients and aging ($r=0.47$; $p=0.004$), sensitive ataxia ($r=0.46$; $p=0.005$). In patients with DM1, there was no evidence of relationships between the parameters studied and clinical/genetic characteristics. On these bases it is postulated that the changes are individual and the administration of drugs with antioxidant activity to patients with HMSN and DM1 should be substantiated.

PS4-452 / #251

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

Developing diagnostic techniques for dysferlinopathy

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Dysferlinopathies are autosomal recessive diseases which include the clinical manifestations of Limb Girdle Muscular Dystrophy type 2B (LGMD2B) and Miyoshi Myopathy 1 (MMD1) and are caused by mutations in the dysferlin gene (DYSF) that lead to absent or dysfunctional dysferlin protein. Diagnosis of dysferlinopathies is often very difficult because of the large number of LMGDs with similar clinical manifestations and the high cost of analysis. Furthermore, 20% of patients who are suspected to have a dysferlinopathy have only one identified mutation. It is thought that individuals with only one identified mutation and absent dysferlin protein have unidentified mutations in other locations such as deep intronic or regulatory regions. To assist physicians in the diagnosis of dysferlinopathies, we have been developing several diagnostic techniques. Unlike most other muscular dystrophies where the protein is found only in muscle, dysferlin is expressed in both blood and muscle. Because there is a high correlation between the absence of dysferlin protein and the identification of mutations in the DYSF gene, analyzing the amount of dysferlin in monocytes is an excellent, minimally invasive way to predict who has a dysferlinopathy before moving onto sequencing. We have also recently developed a free online diagnostic algorithm called the Automated LGMD Diagnostic Assistant (ALDA) that can help physicians determine which LGMD or related muscular disease a patient has based on family history and medical record. Through the use of both the monocyte assay and ALDA, the overall cost of obtaining a diagnosis can be significantly reduced by decreasing the amount of hit-and-miss genetic analysis that is performed. In addition, given the large number of patients that have only one identified mutation in the DYSF gene but who we feel truly have a dysferlinopathy due to the lack dysferlin by monocyte analysis, we are using full genomic sequencing of the dysferlin gene to identify the second mutation and hope to identify new regions that can be analyzed in patients with incomplete diagnoses.

PS4-453 / #301

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

Urine biomarkers for Duchenne Muscular Dystrophy and dystrophin-deficient animal models

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Duchenne muscular dystrophy (DMD) is a rare incurable disease affecting one in 3500-5000 boys. Several clinical trials are underway for DMD and there is an urgent need for valuable biomarkers to follow up the short and long time effects of the treatments. In present study, we used urines as a source of biomarkers for DMD. Using complementary approaches: a bottom-up proteomic (permitting to identify proteins in the range 10^{-3} to 10^{-9} g/ml) and antibody arrays (with the sensitivity 10^{-9} to 10^{-12} g/ml) we analyzed samples from DMD patients ($n=94$, aged 3 - 20 years) and their respective age-matched controls ($n=53$) collected in the frame of ADNA program (Advanced Diagnostic for New therapeutic Approaches). These techniques allowed us to define new urine proteins differentially expressed between healthy and DMD patients. Importantly, titin N- and C-terminal fragments were the most abnormal protein detected in DMD urine, and never found in controls. Work on the confirmation of these biomarkers on another cohort of patients including 200 individuals is underway. Interestingly, some of these proteins were presented by specific fragments, indicating the highly organized protein catabolism in DMD patients. Importantly, some of these fragments were also found in the urines of animal models of DMD: mdx mice and GRMD dogs which allowed for their assessment during physical exercise. These markers could provide a new important non-invasive monitoring tool of DMD treatment.

PS4-454 / #306

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

Targeted NGS sequencing using HaloPlex - for analysis of highly heterogeneous CMT neuropathy patients detected the causing gene in 24% of examined patients

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Background: Mirror movements (MMs) are involuntary movements suggest an UMN involvement. Objectives: Assessment of the MMs phenomena as an early sign of UMN involvement in amyotrophic lateral sclerosis (ALS).Patients and methods: Fifty patients with ALS were subjected to full clinical neurological examination and identification of the MMs phenomena in both upper limbs(ULs) and lower limbs(LLs) if present. Detection of MM were done using surface electromyography (EMG) study from the abductor digiti minimi and tibialis anterior muscles and transcranial magnetic stimulation (TMS) and simultaneous recording of the EMG activity and motor evoked potentials(MEPs) from the ipislateral and contralateral sides Results: MMs had been detected by analyzing ipsilateral MEP and the EMG of the examined patients. MMs detected by EMG of the examined muscles were correlated well with the increased muscle tone, exaggerated reflexes and central motor conduction time in LLs. Specificity of the MM and the positive predictive value were higher when compared with the sensitivity and the negative predictive value. Subclinically, MMs had been detected by analysis of ipsilateral MEP of LLs(27%), ULs(45%) , by the EMG of the ULs (45%) and the LLs(45%)

Conclusions and recommendations: MMs are rarely detected clinically in ALS but we can detect them by using electrophysiological procedures like the MEP and the EMG. Detection of MM is a good specific with high positive predictive value in diagnosing the ALS.

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PS4-455 / #308

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

Targeted and exome sequencing for diagnosis and novel gene identification in congenital myopathies

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Congenital myopathies are severe muscle disorders affecting adults as well as children in all populations. The diagnosis of congenital myopathies is constrained by strong clinical and genetic heterogeneity. Moreover, the majority of patients present with unspecific histological features, precluding purposive molecular diagnosis and demonstrating the need for an alternative and more efficient diagnostic approach. In addition, about half of patients do not have a molecular diagnosis, supporting the implication of novel genes.

We are using targeted and exome sequencing complemented by histological and ultrastructural analysis of muscle biopsies to identify the causative mutations in homogeneous cohorts of patients affected by protein aggregate myopathy, centronuclear myopathy, cores and rods myopathies, vacuolar myopathies, structural myopathies (tubular aggregate, cylindrical spiral ...), and uncharacterized congenital myopathies. Part of this work is embedded in the French Myocapture project aiming to identify the genetic cause of the myopathy in 1000 individuals.

We detected mutations in previously linked genes and validated their impact by functional tests. We provide the evidence that an integrated strategy combining next generation sequencing with clinical and histopathological investigations overcomes the limitations of the individual approaches to allow a fast and efficient diagnosis, accelerating the patient's access to a better healthcare and disease management. This is of particular interest for the diagnosis of congenital myopathies, which involve very large genes like RYR1 and NEB.

In addition, exome sequencing highlighted potential novel genes mutated in several forms of congenital myopathies, that are under functional validation.

Overall, next generation sequencing strategies are revolutionizing the genetic diagnosis and speeding-up genes discovery and thus the identification of novel therapeutic targets.

PS4-456 / #315

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

PFKM gene defect and glycogen storage disease GSD VII with misleading histochemical activity result

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Objective: Tarui disease (GSDVII) is the second most common glycogen storage disease (GSD) after McArdle`s disease (GSDV), and is caused by various recessively inherited muscle phosphpfructokinase (PFKM) gene defects. Muscle biopsy is the golden standard in the diagnosis showing typically myopathic changes, subsarcolemmal vacuoles, and increased glycogen content. The findings are not pathognomonic for Tarui disease only. Muscle enzyme histochemical analyses are used in routine diagnostics, and normal findings of phosphofructokinase and myophosphorylase are considered to exclude GSDVII and GSDV, respectively.

Methods: Two siblings with disease suggestive of GSD underwent thorough clinical analysis including spiroergometry, muscle biopsy, muscle MRI, MR-spectroscopy, and laboratory examinations including whole-exome sequencing.

Results: Both siblings had recurrent myoglobinuria andexercise intolerance with muscle cramping and vomiting. Tarui disease was first thought to be excluded by normal skeletal muscle enzyme histochemical phosphofructokinase staining. Highly elevated blood ammonia and very low and late lactate level increase were found in exercise testing. Whole- exome sequencing, however, revealed a causative homozygous PFKM gene defect, R39Q in both siblings establishing the diagnosis of GSDVII. In addition, PFK enzyme was reduced to 3-4% of normal level in biochemical studies.

Conclusion: Final diagnosis of GSDVII should be based on muscle enzymatic PFK activity measurement and molecular identification of the gene defect. The conventional muscle enzyme histochemical findings should be interpreted with caution.

PS4-457 / #365

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

A large screening of myopathic patients by a targeted NGS approach reveals great genetic heterogeneity and "multiple troubles"

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The identification of causative mutations in muscular dystrophies and other myopathies is becoming a key issue for the future possibility of a differentiated treatment on a genetic basis. However, the great genetic heterogeneity of very similar disorders may make the molecular diagnosis a challenging issue. In addition, as evidenced in literature, other factors, such as "multiple troubles", effects of modifier genes and other low penetrant variations, must be considered for the clinical interpretation of molecular findings.

To gain a comprehensive view of all sequence variants in patients, we built a broad core panel of genes involved in myopathies. This panel comprises 93 muscular disease genes for which we have developed a Next Generation Sequencing-based workflow to sequence at high coverage 2,544 human exons. We designed the enrichment probes using a Haloplex custom platform (Motorplex) targeting 99.2% of exons. Motorplex has been demonstrated to be a reliable, sensitive and specific method to assess variants in the targeted genes with all the control mutations ($n=80$) correctly identified and 97% of variations confirmed by Sanger sequencing.

We studied 200 samples from myopathic patients with prevalent limb-girdle muscle involvement and 150 samples with congenital myopathies. The majority of these patients have been unsuccesfully screened for the most common disease genes. In addition, some patients presented non-specific signs and were isolated cases. About 20% of patients showed typical causative mutations, while an additional 30% other putative pathogenic variations. In addition to the causes of monogenic disorders, we also discovered more than 35% of patients showing damaging variations in other disease genes. These variants, if they had been detected alone in the context of a single gene testing, would have been considered as causative.

In conclusion, in a large cohort of patients with non-specific symptoms our strategy has been able to identify pathogenic mutations, despite of the heterogeneous conditions, improving significantly the diagnostic yield if compared to traditional diagnostic processes. Finally, intrafamilial and interfamilial phenotypic variability of patients sharing the same pathogenic mutations must be reevaluated considering the comprehensive view of all sequence variants.

PS4-458 / #384

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

Improving diagnostic cell-based assays for myasthenia gravis

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Acquired Myasthenia Gravis (MG) is an autoimmune channelopathy of the post-synaptic neuromuscular junction. Antibodies to the acetylcholine receptor (AChR), muscle specific kinase (MuSK), and low density lipoprotein related protein-4 (LRP4) can be detected by radioimmunoprecipitation assays (RIA) and/or cell-based assays (CBA).

Our aim was to test and improve the sensitivity and specificity of the MuSK CBA.

AChR and MuSK RIA(-) MG patients, positive controls, healthy controls, and other neurological disease controls were tested. Human Embryonic Kidney cells were transfected with MuSK and incubated with human sera (1:20). IgG binding to cell surface MuSK was detected using anti-human IgG (heavy and light chains) or anti-human IgG Fc (gamma) antibody. End point titrations were carried out on positive controls.

With anti-human IgG(H+L) MuSK positivity could be seen in 11/34 healthy controls and 3/17 other neurological disease controls. In some cases this coincided with the presence of IgM MuSK antibodies. The anti-human IgG Fc (gamma), which is specific for the

IgG class does not cross react with IgM antibodies, and was positive in 3/34 of the healthy controls. Both secondary antibodies detected positive controls but the anti-human IgG Fc (gamma) provided a slightly more sensitive as well as specific assay.

The light chains of IgG are shared between immunoglobulin isotypes. Other isotypes, in particular IgM may inadvertently be detected using anti-human IgG (H+L). An anti-IgG Fc(gamma) appears more specific for IgG. The functional role and specificity of IgM in seronegative MG remains unclear and will form part of future work.

PS4-459 / #458

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

Array CGH in the diagnosis of neuromuscular disorders – The NMD-Chip experience in Hungary

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Background: Inherited NeuroMuscular Disorders (NMD) form a large group of diseases. Most molecular diagnostic approaches correspond to successive, gene by gene analysis, starting with the most pertinent gene based on clinical reasoning and protein analysis. Novel genomics based technologies may represent an efficient alternative for molecular diagnosis, enabling quick and reliable simultaneous testing of numerous NMD genes for genomic rearrangements such as exonic deletions and/or amplifications and point mutations. Based on these requirements, an EU FP7 funded consortium, NMD-CHIP was established in 2009, which developed CGH and re-sequencing arrays for high-throughput diagnosis of NMD.

Materials and methods: Specifically designed oligonucleotide-based Roche/NimbleGene CGH arrays containing most genes involved in DMD, LGMD, CMD and CMT were used. These DNA-arrays were validated on diagnosed DMD/BMD and CMT1A/HNPP patient DNAs. Moreover, 72 patients with the clinical diagnosis of muscular dystrophy and 12 patients affected by unknown type of Charcot-Marie-Tooth disease were tested in order to detect possible pathogenic copy number variations in any of the analysed genes.

Results: The CGH array precisely detected all dystrophin deletions and duplications validated by traditional MLPA analysis. Also heterozygous carriers were identified. The CMT array was able to detect PMP22 duplications and deletions. In the patient cohort lacking genetic diagnosis several pathogenic mutations have been identified in known genes as follows: three patients of a large consanguineous family were classified as having a yet unknown homozygouse deletion in the SGCD gene; one patient had a heterozygouse deletion in the LARGE gene, one patient was unexpectedly identified as DMD patient, one patient had a heterozygouse deletion in the CAPN3 gene, one patient had a heterozygouse deletion in the TTN gene and one patient proved to be heterozygouse for a deletion in the NEB gene.

Discussion: The DNA CGH array increased the number of precisely diagnosed patients in our laboratory. This technology may have the potential to improve molecular genetic diagnosis and counselling with further implications for patient management, phenotype-genotype correlations, dedicated databases and clinical trials.

PS4-460 / #506

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

FGF21: A biomarker of neuromuscular diseases?

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Background: Human fibroblast growth factor 21 (FGF21) is a 181 amino acid protein belongs to the human FGF superfamily. The basic biological role of FGF21 is the regulation of the glucose and lipid metabolism.

Objective: Recently two observations were published where they showed elevated circulating FGF21 in human mitochondrial diseases, therefore it was suggested that FGF21 might be a biomarker of mitochondrial diseases.

Patients and Methods: The serum level of FGF21 was determined by ELISA in blood samples from 20 healthy subjects, 15 patients with myotonic dystrophy type 1 (MD1) and 25 patients with mitochondrial diseases.

Results: Among healthy subjects serum FGF21 correlated with body mass index (BMI).

Mean FGF21 level was significantly raised in MD1 compared to healthy subjects (424±328 and 207±165 pg/ml, respectively, p?0.05, Mann-Whitney U test). Among mitochondrial patients FGF21 was elevated only in PEO (progressive external ophthalmoplegia) group (589±496 pg/ml, p?0.05, Mann-Whitney U test), but was not significantly altered in MELAS and myopathy patients. FGF21 correlated with serum creatine kinase (CK) and lactate levels, with clinical severity score as well as some biopsy findings (e.g. ratio of ragged red fibers and mitochondrial inclusions).

Conclusion: Our study implicates that serum FGF21 might be a biomarker for neuromuscular disorders. In contrast to the previous findings our results showed that elevation of FGF21 is not specific and not restricted to mitochondrial disorders. Further research is necessary to find out what neuromuscular disease groups are associated with abnormal FGF metabolism and to investigate the molecular pathomechanism.

PS4-461 / #512

Theme: *7.4 - Techniques of diagnostic in NMD: Biochemical and molecular techniques*

Will the next generation sequencing strategy change deeply the diagnosis of Charcot-Marie-Tooth disease ?

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Charcot-Marie-Tooth disease (CMT) is one of the most common inherited neurological disorders. It comprises a group of diseases caused by mutations in genes involved in Schwann cells homeostasis and neuronal function that affect the peripheral nerves. So far mutations in more than 50 genes have been identified causing either the demyelinating form (CMT1) or the axonal form (CMT2). The relative uniform phenotypes in many patients with CMT make it difficult to decide which of the over 50 known CMT genes are affected in a given patient. Genetic testing decision trees are therefore broadly based on a small number of major subtypes (eg, CMT1, CMT2) and the observed mutation frequency for CMT genes. Since conventional genetic testing is expensive many rare genes are not being tested for at all.

In order to improve CMT diagnosis, we developed the alternative approach of the Next Generation Sequencing. We created an Ampliseq custom panel of 1402 amplicons able to screen 37 target genes (120 kb) involved in the motor and axonal forms of the disease and we started to perform NGS strategy in several undiagnosed families with CMT.

We successfully detected new variants in REEP1, AARS, SETX, IGHMBP2, DCTN1, INF2, TRPV4 and PLEKHG5 in these undiagnosed families. Sanger sequencing confirmed the presence of the mutations and cosegregation in these families are under investigation.

We have shown that the next generation sequencing strategy is a good alternative approach compared to genetics testing decision trees for CMT diagnosis of the axonal and motor forms. New mutations can be detected rapidly and with a constant declined price in the assumed “rare” genes that finally could appear not as so rare. We currently develop this strategy for demyelinating forms and we plane to develop whole exome sequencing in order to discover new genes involved in the CMT disease for the families in which no mutation would have been found using our target genes NGS strategy.

PS4-462 / #125

Theme: *8.1 - Miscellaneous: Home cares / Social programs in neuromuscular diseases*

Making a positive difference for families living with a neuromuscular condition - the Muscular Dystrophy Association of New Zealand's Fieldwork Practice Framework

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Background: The Muscular Dystrophy Association of New Zealand (MDA NZ) provides information, support and advice to families living with a neuromuscular condition. It achieves this using a variety of methods including the operation of a social work service known as the MDA Fieldwork Service. Prior to 2011 the MDA Fieldwork Service operated in a casual fashion, reliant on the attributes, skills and knowledge-base of each individual fieldworker with little cohesion across the service. In 2011 MDA NZ developed the person-centred fieldwork practice framework enabling families from across NZ living with a neuromuscular condition, fieldworkers and all stakeholders to have a shared understanding of Fieldwork practice. The Framework is intended to guide the daily practice of Fieldwork to achieve the outcomes defined. It is client-centred, strengths-based and family / whanau focused reflecting the requirements to maintain clear alignment with the vision, mission and values of MDA NZ and to respond to a wide range of people and diverse contexts.

Methods: The framework was developed by a process of review of current services, analysis and consultation.

Results: The MDA Fieldwork Practice Framework sets out the elements that make up Fieldwork Practice and describes how the integrated elements form a consistent approach to service delivery. The practice principles underpinning Fieldwork are defined along with the attitudes and beliefs that Fieldworkers are expected to bring to the role. The outcomes that Fieldwork aims to deliver, the core components of Fieldwork practice and the skills applied within Fieldwork practice are outlined. The various elements of the Framework are interdependent as it is intended to be applied as a whole. The service is measured by activity report and survey of family members receiving the service and a summary of survey results is also presented here.

Conclusion: By developing and implementing the fieldwork practice framework MDA NZ has moved from "tea and sympathy" to providing a professional social work service that is person-centred, strengths-based, culturally responsive, family / whanau focused and makes a positive difference for families living with a neuromuscular condition.

PS4-463 / #132

Theme: *8.1 - Miscellaneous: Home cares / Social programs in neuromuscular diseases*

Actual condition survey for solitudinous patients with subacutemyelo-optico-neuropathy in Japan

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Background: Subacutemyelo-optico-neuropathy (SMON) is caused by clioquinol intoxication. In Japan, there are a large number of SMON patients. Silvering or advancing in severity has been pointed out in medical treatment for SMON patients. Solitary life is paid attention on social problem for aging population. The aim of this study was to investigate the characteristic features in SMON patients who lived alone.

Methods: We analyzed data from 730 SMON patients that was obtained at medical check-ups carried out by Japanese SMON Research Committee from 2010 to 2012. Neurological and general symptoms classified by severity, psychiatric manifestation, activities of daily living (ADL), and care giving condition were surveyed. Influence of residential area was also studied.

Results: Twenty-four percent of SMON patients lived alone. The mean age of patients in solitude was 78 years (non-solitary patients; 77 years). Eighty-four

percent of patients in solitude were female (non-solitary female; 66%).Regarding the severity of neurological and general symptoms, there was no significant difference between solitude and non-solitude. For ADL, going out was less frequent in solitary patients, and solitary patients tended to feel life dissatisfaction. Twenty-seven percent of patients in solitude did not need care giving, 67% could have care giving when needed, and 5% had no caregiver despite of necessity. There was not so much difference in severity or activity of daily life between solitary patients lived in the metropolis and those in the countryside. Utilization rate of social work service was higher in patients lived in the metropolis, however patients had no caregiver notwithstanding necessity were less frequent in the countryside.

Conclusions: Not a few number of SMON patients lived by themselves in spite of aging or advancing. Particular application of treatment considered individual care environment should be required for SMON patients who live alone.

PS4-464 / #74

Theme: *8.2 - Miscellaneous: Psychological and neuropsychological approaches of neuromuscular diseases / Ethical aspects*

Parents' experience of having a child with spinal muscular atrophy type 1 - informing clinical practice

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Spinal muscular atrophy type 1 is a relatively common, untreatable and invariably fatal neuromuscular disorder of early childhood. Family support and genetic counselling form a vital part of the management of the families affected by this condition. There are few studies examining the impact of having a family member with a neuromuscular disorder, and none describing parents' experiences of having a child with SMA type I. This qualitative study undertook thematic analysis of eleven in-depth interviews with thirteen bereaved parents of children with SMA type 1. While individuals' experiences were unique, common themes emerging from the data included: feeling helpless, regaining control by being able to make decisions about their child's life and death, the unanticipated experiencing of multiple losses, and feeling well-supported. Health professionals can best support such families by offering grief-specific support at the time of diagnosis, participating in joint decision-making to increase parents' sense of control, and acknowledging the multiple types of losses parents may experience.

The aim of this study was to gain an in-depth understanding of parents' experiences of having a child with of SMA type 1, from thejourney of diagnosis to bereavement. We present thefindings exploring the meanings constructed by parents of the experience of having a child with SMA type 1 and some of the identified common emerging themes. In analysing themes we report aspects acknowledged by parents as having the most meaning. This study contributes perspectives of fathers,a group who are often under represented in the research community, and explores thecontribution of bereaved parents to research. The findings highlight roles in the pathway to diagnosis and explore the value of multidisciplinary management.

PS4-465 / #154

Theme: *8.2 - Miscellaneous: Psychological and neuropsychological approaches of neuromuscular diseases / Ethical aspects*

Construction of a disease-specific health-related quality of life scale for patients suffering from slowly progressive neuromuscular disease

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Medical care and treatments of patients suffering from slowly-progressive neuromuscular disease (NMD) need to take into account health-related quality of life (HRQOL). The use of generic tools to assess HRQOL (e.g. WHOQOL-BREF) in those patients do not allow capturing every important aspects of life potentially impaired by NMD and may include aspects of life that are irrelevant to NMD. Any study on HRQOL in patients suffering solely from slowly progressive NMD must use a specific questionnaire if available. To date there is no French HRQOL-measurement tool specifically designed for these patients. We developed a new questionnaire named "Quality of Life in Neuromuscular Diseases" (QoL-NMD). QoL-NMD will be the first freely distributed questionnaire designed to assess slowly progressive NMD patients' quality of life.

A previous work allowed building a bank of items short enough to be conveniently administered to patients (69 items) using focus groups and Delphi method. This bank of items and 2 validated questionnaires (H.A.D. and WHOQOL-BREF) were administered to 170 patients recruited in 7 tertiary hospitals dedicated to NMD. Statistical analysis included methods derived from both Items Response Theory and Classical Test Theory. The Classical Test Theory indicators used are validity (Factor analysis, Loevinger's coefficient), and reliability (Cronbach's Alpha, test-retest reliability).

Data analysis allowed selecting the most informative items from the bank to build QoL-NMD. The questionnaire structure was modified to respect both statistical results and clinical arguments. QoL-NMD showed adequate psychometric properties and was short enough to be used in clinical practice (26 items). QoL-NMD is structured in 3 main domains[1] "Impact of body symptoms",[2] "Self-perception" and[3] "Activities and social participation". Comparison to H.A.D. and WHOQOL-BREF allowed verifying concurrent validity and putting forward QoL-NMD psychometric superiority.

This study led to the construction of a new HRQOL questionnaire specifically designed for slowly progressive NMD patients. The next step will be to administer this questionnaire to a new sample of patients to validate it. Once its French version is validated QoL-NMD will be translated, culturally adapted and validated in other languages.

PS4-466 / #200

Theme: *8.2 - Miscellaneous: Psychological and neuropsychological approaches of neuromuscular diseases / Ethical aspects*

Experience of patients receiving their diagnosis of myotonic dystrophy as compared with Huntington's disease

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Receiving a genetic diagnosis has the potential to be a very traumatic experience especially when the disease is incurable, likely to cause significant morbidity and may also affect other family members as is the case for myotonic dystrophy (DM) and Huntingtons disease (HD). Patients' experience of the process of receiving a diagnosis for progressive neurogenetic disease has been described in small qualitative studies but no systematic survey has been undertaken before. The purpose of this study was to survey the whole experience of genetic testing: the patients' preparation for having the test, the time of receiving the result and the follow up after genetic testing. We predicted that factors affecting patients experience would be the staff involved, whether the test was predictive or diagnostic, whether the test had been performed a long time ago or more recently, and also demographic factors such as age and gender. 139 patients of a neurogenetic clinic with a positive diagnosis of either HD or DM were mailed the questionnaire. A similar response rate occurred in each group 26/69 (38%) in DM and 24/70 (34%) in HD. Patients diagnosed with DM had an inferior experience relative to patients diagnosed with HD. This is likely to be due to closer adherence to a standardised protocol for HD. Better results were obtained when the diagnosis was made through genetically trained practitioners. Greater flexibility around where the patients receive their results and attention to follow up are further aspects that could be improved.

PS4-467 / #412

Theme: *8.2 - Miscellaneous: Psychological and neuropsychological approaches of neuromuscular diseases / Ethical aspects*

Gender differences in predictors for social network and quality of life in DM1 patients

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Various symptoms, such as pain, muscle weakness and daytime sleepiness have been reported to be related to quality of life (QOL) in DM1 patients. In this study we investigate whether gender affect the relations between symptoms and reported QOL and amount of social contact (SC)

31 adult-onset DNA confirmed DM1 patients (Age 21-61; 18 male 13 female) included. All able to walk independently and within normal IQ range. No significant differences between the gender group on: CTG expansion, IQ, Age or years since first symptom.

A range of somatic and psychological variables were checked for relation to QOL and SC. QOL was measured with WHOQOL. SC was derived from sociograms and reflects the number of people encountered on a typical day (SC).

Males and females differed with respect to all variables associated with QOL (*: $p < .05$, **$p < .01$). In ?, QOL was related to: Borg fatigue after 6 min walking test (r -.61**), and number of pain areas (r -.59**). In ? QOL was highly related to: General fatigue (r -.92**) and Beck Depression Index (r -.71**)

The sexes also differed with respect to variables associated with SC.

In ?, SC was related to: Muscle strength in distal extremities (r .73**), Daytime sleepiness (r .63*) Muscle strength in trunk (r .54*). In ? SC was related to: Muscle strength in distal extremities (r .84**) and Age (r -.56)

The findings suggest caution about general conclusions about predictors for quality of life and amount of social contact in DM1 patients; the gender differences are substantial. It is also worth noting that there are no shared predictors for amount of social contact and reported quality of life in this group of DM1 patients.

PS4-468 / #241

Theme: *8.3 - Miscellaneous: Rehabilitation in neuromuscular diseases*

Effect of ankle-foot orthosis on the gait biomechanics of a patient with Duchenne muscular dystrophy

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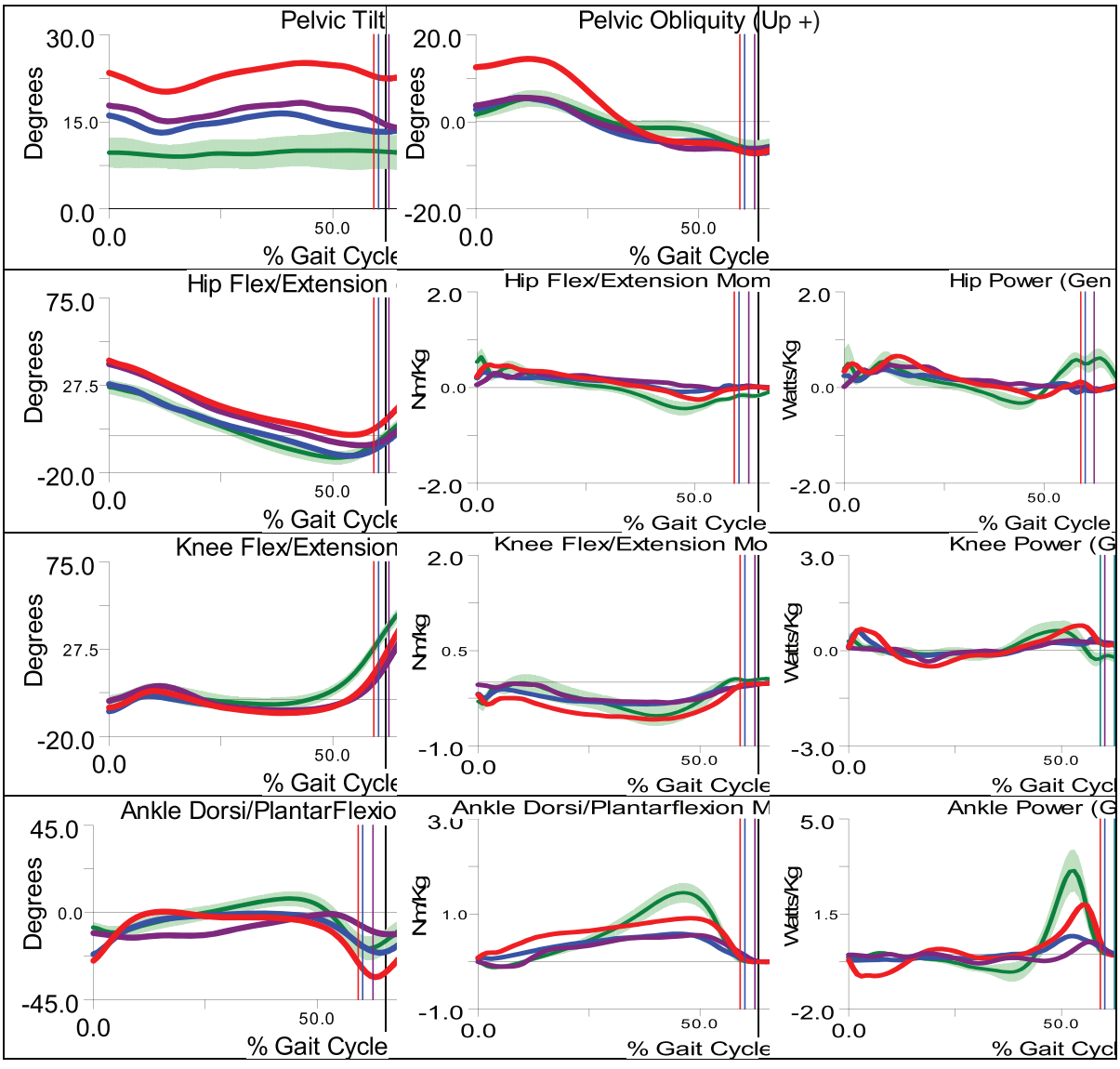
This case study aimed to assess the effect of ankle-foot orthosis (AFO) on the gait biomechanics of a patient with Duchenne muscular dystrophy (DMD) 6 months after therapeutic intervention. A 7-year-old eutrophic child was clinically evaluated getting data of the passive joint range of motion (ROM), Motor Function Measure (MFM) and biomechanical gait analysis. In the first evaluation (Ev1), the child was not receiving medical treatment or physiotherapy nor using orthosis. Six months after the treatment (use of corticosteroid drugs, nocturnal and diurnal articulated AFO orthosis, and physiotherapy twice a week) these parameters were reassessed (Ev2). The gait data were collected using the Helen Hayes marker set, and a Pro-Reflex Camera System (Qualisys), with the child walking at a normal speed, with and without orthosis. The

Table 1: Gait parameters

Spatiotemporal parameters	**Ev1**	**Ev2 - No orthosis**	**Ev2 - With orthosis**
Velocity (m/s)	0.79	0.78	0.62
Cadence (steps/s)	0.66	0.63	0.51
Step length (m)	0.84	0.85	0.86
Step width (m)	0.12	0.16	0.17
Double support phase (s)	0.17	0.19	0.28
Gait cycle (s)	1.0	1.0	1.3

kinematics and kinetics parameters	**Ev1**		**Ev2 - No orthosis**		**Ev2 - With orthosis**	
	Max peak	**Min peak**	**Max peak**	**Min peak**	**Max peak**	**Min peak**
Pelvic tilt angle (°)	25.1	20.1	17.3	13.1	18.2	13.9
Pelvic obliquity angle (°)	14.4	-7.1	5.5	-7.1	7.7	-5.1
Hip flexion/extension angle (°)	52.0	0	37	-10.8	41.7	-5.1
Hip flexor/extensor moment (Nm/Kg)	0.4	-0.2	0.3	0	0.3	0
Hip power (Watts/Kg)	0.6	-0.1	0.4	-0.1	0.5	0
Knee flexion/extension angle (°)	58.7	-6.3	50.2	-7.1	53.6	-5.9
Knee flexor/extensor moment (Nm/Kg)	0	-0.5	0	-0.2	0	-0.3
Knee power (Watts/Kg)	0.5	-0.7	0.5	-0.6	0.4	-0.2
Ankle plantar/dorsiflexion angle (°)	-5.6	-35.2	-0.5	-23.2	-0.7	-13.0
Ankle plantar/dorsiflexion moment (Nm/Kg)	0.9	0	0.5	0	0.5	-0.1
Ankle power (Watts/Kg)	1.8	-0.8	0.6	-0.2	0.4	-0.3

Ev: evaluation; max: maximum; min: minimum.



parameters were obtained with a Visual 3D system and used for the average curve of 3 trials, the maximum and minimum peak of each variable, considering the gait cycle normalized to 100%. The data presented here refer to a descriptive analysis of the right lower limb. The MFM total scores and in dimension 1 (standing position and transfers) were 91 and 36 (Ev1), 79 and 33 (Ev2), respectively, suggestive of a functional decline. The dorsiflexion ROM of the right ankle increased 2° (from 0 to 2°). The gait speed and cadence decreased 6 months later; it was lower when using AFO. The step width was larger in Ev2 and not modified by the orthosis. The use of AFO increased the double support time (Table 1). The spatial-temporal parameters of the patient's gait were negatively affected in Ev2 by using AFO. Among the biomechanical parameters, the pelvic tilt and pelvic obliquity in Ev2 were reduced by using AFO when compared to the Ev1. In the ankle, a reduction of the plantar flexion angle was observed, an increase of dorsiflexor moments and a decrease of power (Table 1, Figure 1) in Ev2 when compared to Ev1, regardless of AFO use. Considering the spatial-temporal parameters, the use of AFO negatively impaired the patient's gait. However, based on kinematic and kinetic parameters obtained by using AFO, it can be noted that the pelvis and the ankle presented minimal compensations. If gait in children with DMD is lost when there are no strategies to compensate for weakness and deformities, the recommendation of this device would require further studies to assess biomechanics aspects in a larger number of patients.

PS4-469 / #349

Theme: *8.3 - Miscellaneous: Rehabilitation in neuromuscular diseases*

Multidisciplinary respiratory care support team can reduce respiratory complications of neuromuscular disease inpatient

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Background: In our institute, about 40% of inpatients with neuromuscular disease have been using non-invasive positive pressure ventilation (NPPV) or tracheostomy positive pressure ventilation (TPPV). To improve patient's prognosis or to reduce respiratory complications, we started multidisciplinary respiratory care support team (RST) from 2007.

Objectives: We performed retrospective hospital-based study to clarify the clinical effectiveness of RST for hospitalized neuromuscular disease patients.

Methods: We retrospectively reviewed medical records of inpatients to elucidate the RST activity from 2007 to 2012. The prognosis after RST intervention of 26 patients who were followed more than one year was investigated at the time of year 2012 end. They contained 12 myopathy patients such as muscular dystrophy, 5 patients of amyotrophic lateral sclerosis, and 9 patients of other neurological illlnesses.

Results: RST intervention were performed for 291 cases. And we were able to pick up 398 conference records from 2007 to 2012. In 2012, five of 26 cases had ended RST intervention. One reason for having ended intervention was a death by pneumonia and respiratory failure. The other reasons were discharging from our hospital by having achieved the initial aim of RST intervention. Furthermore, pneumonia frequency reduction was observed by 16 of 21 patients who were continuing RST intervention.

Conclusion: From this retrospective study, the improvement of respiratory conditions like pneumonia frequency reduction etc. can expect for neuromuscular disease patients by systematic RST intervention. We concluded multidisciplinary RST intervention can be clinically effective.

PS4-470 / #501

Theme: *8.3 - Miscellaneous: Rehabilitation in neuromuscular diseases*

Early management intervention and parent empowerment in neuromuscular diseases: the clinical center NEMO experience

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Background: The clinical diagnosis of a neuromuscular disorder occurs in highly-specialized muscle clinics. Subsequent care of children and families may be hindered by limited data on early management and coaching of parents, schoolteachers and other caregivers.

Parents/ caregivers have new roles and responsibilities and must carry out elaborate health care regimens, face medical challenges together with schooling matters.

Aims: To design a rehabilitation and parents' coaching protocol, applied at NEMO Clinical Center for the early management of children with neuromuscular diseases (NMD's) and to verify its impact on quality of life perception of the affected child and his/her family.

Methods: An early rehabilitation and parents' coaching protocol has been designed following a three-phases process:1 review of previous literature data and guidelines on the care of children with NMD's; 2 administration of a structured interview to all parents of children followed for early diagnosis of NMD focused on procedures available within the National Health System in out-patient setting (n = 60 children < 15 years), 3 retrospective analysis of the early management of children with neuromuscular diseases during the 5-year experience at Nemo Clinical Center. (346 children under 18 years).

Our protocol includes weekly or monthly assessments and coaching to optimize posture, to manage bath and mealtimes; educational programs on interventions in possible critical situations.

In order to qualitatively define the effectiveness of the proposed protocol, a questionnaire on quality of life (PedsQL,family impact and information module) was administered to a subgroup of patients < 3 years that accessed NEMO with recent or suspected diagnosis of NMD.

Results: Our preliminary analysis indicate that adequacy of parent-child care directly correlates to the degree of educational support and empowerment process and varies from region to region. We observed improvement of quality of life and reinforcement of parents' empowerment expressed in ability to make choices, possibility to make changes in one's life, assertiveness and self-esteem

Conclusions: Early management improves quality of life of the affected child and family. Child physiotherapist support soon after diagnosis creates alliance and supports parents from the initial phases of diseases. The efficacy of the proposed protocol and its impact on child's psychomotor development will be determined during follow-up assessments.

PS4-471 / #510

Theme: *8.3 - Miscellaneous: Rehabilitation in neuromuscular diseases*

Hydrotherapy program in Duchenne Muscular Dystrophy : Motor functional evaluation and body self perception

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Background: Hydrotherapy has been implemented in rehabilitation programs to reduce spasticity and pain and to improve recovery of muscle damage after traumatic injury but there is still limited data on its use and efficacy in Duchenne Muscular Dystrophy DMD.

Objective: To verify the efficacy of an aquatic rehabilitation program in a DMD population. The study will consider both motor function and body self-perception

Material and methods: 10 DMD patients , both ambulant and wheel-chair bound, aged from 3 to 17 years were subjected to weekly hydrotherapy for 45 min for a 6 weeks period. MFM, North Star, PUL, 6MWT assessments and hip, knee and ankle angle measurement were performed at the beginning and the end of the study. Body perception was evaluated using pictures drawn by kids, representing their human figure and the Goodenough-Harris Draw-a-Person Test of body self-perception and body scheme knowledge.

Results: Preliminary results suggest that water physical therapy maintains motor function and improves passive range of motion. Initial analysis of drawings indicate that body self-awareness is also positively affected by this rehabilitation program.

Conclusions: Our preliminary results suggest that hydrotherapy may improve motor function and body self-perception in DMD. Confirmatory data in larger number of patients will create the rationale to include hydrotherapy in the standard treatment program for DMD.

PS4-472 / #69

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

Duchenne muscular dystrophy- a correlational study of upper limb performance with ambulatory performance

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Quality of life in young men with Duchenne muscular dystrophy (DMD) is largely dependent on their degree of independence, which to a significant degree reflects their upper limb function. In contrast to the extensive literature on lower extremity function in DMD, there is little data on the natural history of upper limb weakness and loss of function in this condition. Most previous studies on this aspect of DMD pre-date routine steroid therapy, which has changed its natural history, and older reports have described disease progression in relation to age rather than to more relevant measures of function. The true timing and nature of biomechanical dysfunction in the upper limbs in DMD are therefore poorly defined. This cross-sectional correlational study will define the natural history of changes in upper limb function in strong ambulant, weak ambulant and recently non-ambulant boys with DMD, using a number of valid, clinically relevant upper extremity outcome measures. These changes in upper limb function will be correlated with ambulatory performance, as determined using the North Star Ambulatory Assessment Scale (NSAA).

30 males with DMD aged 5-15 years have been recruited from the Neuromuscular Clinic of the Royal Children's Hospital, Melbourne. All are currently or previously steroid-treated. Participants have been assigned to one of three groups based on their ambulatory performance on the NSAA: NSAA score >28; NSAA score = 5-18; or non-ambulant for ≤ 3 years. Ambulatory performance will be compared via regression analysis with upper limb performance on the following parameters: range of motion; strength; unilateral and bimanual timed motor performance; and independence in daily activities. Regression analysis will be undertaken to describe the relationship between mobility and upper limb performance. Results of these studies will be presented and the natural history of loss of upper extremity function in steroid-treated boys with DMD examined in relation to the defined progression of lower extremity weakness in this condition.

PS4-473 / #89

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

Rasch analysis of the motor function measure in patients with congenital muscle dystrophy and congenital myopathy

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Objective: Valid outcome measures are necessary to monitor the treatment effects in patients with congenital disorders of muscle.

Patients and Methods: In nineteen departments in France, Belgium, and USA, 289 patients aged 5 to 77 years old were enrolled. A Rasch analysis examined the robustness of the Motor Function Measure across the disease spectrum. The three domains (standing position and transfers, axial and proximal motor function, and distal motor function) were examined using RUMM 2030 software with a partial credit model.

Results: The original 32-item MFM did not fit the Rasch model expectations enough in neither of its domains. Switching from a four- to a three-category response-scale in 18 items restored response order in 16. Various additional checks suggested the removal of seven items. The resulting 25-item MFM demonstrated a good fit to the Rasch model. Domain1 was well-targeted to the whole severity spectrum whereas Domains 2 and 3 were better targeted to severe cases. The reliability coefficients MFM-25 suggested sufficient ability for each summed score to distinguish between patient groups (0.9, 0.8, and 0.7 for Domains 1, 2, and 3, respectively).

Discussions: The Rasch-scaled MFM-25 can be assumed to be a linear scale in each of its three domains.

Keywords: congenital muscular dystrophy, congenital myopathy, motor function assessment, outcome measure, Rasch analysis

PS4-474 / #91

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

English cross-cultural translation and validation of the NM-Score: A system for motor function classification in patients with neuromuscular diseases

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Objective: To develop an English version of the Neuromuscular-Score (NM-Score).

Patients and methods: 42 patients aged 5 to 19 years old with a confirmed or suspected diagnosis of congenital muscular dystrophy were enrolled. An English version of the NM-Score in each of the three domains (D1: standing and transfers; D2: axial and proximal motor function; D3: distal motor function) was developed by a 9-expert panel. Its concurrent validity was tested against criterion standards (Brooke, Motor Function Measure, Activlim, Jebsen Test, and myometry). Informant agreement between patient-reported and clinician-reported NM-Score was measured by weighted Kappa.

Results: Significant correlation coefficients were found between NM-Score and criterion standards, the best correlations occurring with MFM D1 (r=-0.944, p<0.0001), Activlim (r=-0.895, p<0.0001) and Hip abduction (r=-0.811, p<0.0001). Informant agreement between clinician- and patient-reported NM scores was excellent for D1 (k=0.801, 95% CI 0.701-0.914) but moderate for D2 (k=0.592, 95% CI 0.412-0.773 and D3 (k=0.485, 95% CI 0.290-0.680). The correlation coefficients between NM-Score and criterion standards were not significantly different between clinician-reported NM-Score and patient-reported NM score.

Discussion: The English version is a reliable and valid instrument that can be used in clinical practice and research to describe the functional abilities of patients with NM diseases.

Keywords: Neuromuscular diseases, disability evaluation, rehabilitation, activities of daily living

PS4-475 / #209

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

1000 Norms Project: Clinical catalogue of human neuromuscular variation

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Table 1: Items and questionnaires administered in the 1000 Norms Project

Measures of physical function	Questionnaires to be administered
Anthropometric and general measures	Knee injury and Osteoarthritis Outcome Score
Foot Posture Index	Cumberland Ankle Instability Tool
Static and dynamic lower limb alignment	Nordic Musculoskeletal Questionnaire
Gait	International Physical Activity Questionnaire
Plantar pressure	Assessment of Quality of Life Questionnaire
Timed up and down stairs test	General Self-Efficacy Scale
Bruininks-Oseretsky Test of Motor Proficiency (BOT-2)	Workability
Star Excursion Balance Test	
Functional Dexterity and Nine Hole Peg Tests	
Six minute walk test	
Upper limb and lower limb active range of motion	
Choice Stepping Reaction Time	
Upper limb and lower limb isometric muscle strength	
Toe Flexor Strength	
30-second Chair Sit to Stand Test	
Countermovement Jump	
Standing Long Jump	

Caring for patients with neuromuscular disorders requires an understanding of normal variation, as decisions regarding diagnosis and management are frequently based on comparison with healthy or normal values. To make these decisions, researchers and clinicians need access to robust patient-centred outcome measures and appropriate reference values. Currently there is an urgent great need for comprehensive reference data representing the physical and functional capabilities of a healthy population. The aim of the 1000 Norms Project is to generate a freely accessible database of normative musculoskeletal and neurological reference values essential for developing outcome measures and conducting clinical trials of interventions for children and adults with neuromuscular disorders.

The 1000 Norms Project is recruiting 1000 healthy individuals between 3 and 100 years to provide reference values for >50 clinical and biomechanical measures within the constructs of: strength, dexterity, balance, ambulation, sensation, range of motion, endurance and fatigue. Strength of lower limb and upper limb muscle groups are assessed with fixed or hand held dynamometry, with vertical jump height and long jump reflecting power measures. Questionnaires will evaluate quality of life, physical activity, work capacity and pain. Saliva DNA will be analysed for the ACTN3 genotype - the 'gene for speed' which will contribute to a greater understanding of the influence epigenetic factors have on muscle phenotypes. The items have been selected based on the lack of normative reference values, the clinical significance and applicability for people living with a range of neuromuscular conditions.

The 1000 Norms Project reliability study was completed in November 2013. Inter-rater reliability was excellent (ICC>.75) for all items assessed manually. The release of the final database to the international healthcare community is anticipated to occur in March 2016.

The 1000 Norms Project will provide a substantial contribution to our understanding of the range of functional and physical variation in healthy individuals. The dataset is intended to aid all healthcare stakeholders: researchers, clinicians, caregivers and policymakers. The reference dataset will help develop and validate sensitive clinical trial outcome measures and provide a unique collection of healthy normative measures to facilitate the diagnosis of neuromuscular dysfunction and age-related pathological changes.

PS4-476 / #237

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

Oxygen uptake evaluated with two different methods using upper limbs of the non-ambulatory children

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Secondary clinical dysfunctions of the children with myelomeningocele (MMC) have been widely reported in the scientific literature. However, a lack of information in the literature related with the evaluation of aerobic capacity in non-ambulatory children (strictly wheelchair users) and their cardiorespiratory risks limit the choice of a secure protocol of aerobic training. Thus, this preliminary study aimed to verify if the absolute oxygen uptake (VO_2A) is compatible to the estimated value of VO_2 (VO_2E), an indirect test proposed by Franklin et al. (1990), in non-ambulatory children with MMC. Three (3) male volunteers (ages: 8, 11 and 14 years and BMI: 16.1, 22.5 e 23.2, respectively) with MMC and totally wheelchair users, participated of this study. The volunteers 2 and 3 were overweight for their ages. A cycle ergometer electromagnetic brake KHL Elektroanlagen GmbH adapted for upper limbs and ergospirometer Vmax SensorMedics was used to obtain the uptake of VO^2A, according to standardized protocol. The blood pressure (BP) was evaluated before and at peak of exercise and the heart rate (HR) variables were obtained throughout the test with 12-lead electrocardiogram. The protocol proposed by Franklin et al. (1990) and modified by Gorla et al. (2009) was used to obtain the VO^2E. The records of the HR were taken using a Polar S810i every 2 minutes and the BP was measured before and at peak of test. The results obtained in both tests (HR, BP and VO^2peak) were showed in the Table 1. The 3 volunteers presented superior VO^2A values than that ??estimated using the field test (VO^2E). The values ??of systolic and diastolic BP obtained at rest and at peak testing showed no pathological alterations. The 3 volunteers also presented increment of the HR peak values in the field test when compared with those obtained with the cardiopulmonary exercise test. Therefore, the volunteers showed different values to VO^2E and VO^2A showing that the indirect test seems not appropriated to estimate the VO^2peak values. This is a preliminary study and we expect to confirm these results evaluating more volunteers with MMC.

PS4-477 / #253

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

Cross-cultural adaptation, reliability, and validity of the Turkish version of Motor function measure (Mfm-Tr)

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Introduction: Neuromuscular diseases include a wide-range of diseases affecting the peripheral nervous system, which consists of all the motor and sensory nerves that connect the brain and spinal cord to the rest of the body. Evaluation of motor function is complementary to measurement of muscle strength. Different scales have been developed to quantify motor function in specific neuromuscular diseases.The Motor Function Measure (MFM) is a tool designed to monitor precisely the severity and progression of motor function in neuromuscular diseases.

Purpose :The aim of this study was to describe the cultural adaptation, validity, reliability of the Turkish version of MFM (MFM-TR) in a population with neuromuscular diseases.

Methods: The cultural adaptation committee (CAC) was responsible for producing a pre final version aimed at maintaining equivalence with the original scale while maximizing comprehension by the target population. Then, interviews were conducted with 51 participants, during which further modifications were implemented to overcome perceived difficulties in comprehension. The final version was drafted at a second meeting of the CAC. Independent back translation of the final version was done by a native English speaking professional translator. The translated version was examined by the principal author of the MFM and the members of the CAC to verify its equivalence with the original instrument. The final version was named the Turkish version MFM. 51 (12F/39M; 12.56±8.84yrs) patients with neuromuscular diseases (25 DMD, 9 Myopathy, 5 SMA, 5 BMD, 4 Polyneuropathy, 2 Friedreich ataxia, 1 Guillain-Barré) were evaluated with the MFM-TR. The results of the Vignos and Brooke assessments were used to compare the results and decide on the validity of MFM-TR that composed with 32 items rated on a 4-point likert scale, and grouped under three dimensions: as standing position-transfers (D1:13 items), axial-proximal motor function (D2:12 items), distal motor function (D3:7 items).

Results: Agreement coefficients for inter-rater reliability were excellent (0.72-0.93) for 10 items (D1:11,12,30,31,32-D2:1,2,9,13-D3:20), good (0.58-0.77) for 16 items (D1:6,24,25,26,27, 28, 29-D2:3,5,7,14,16,23-D3:4,21,22) and moderate (0.42-0.56) for 6 items (D1:8-D2:10,15-D3:17,18,19). The interobserver reliability varied from good to excellent, intraclass correlation coefficient (ICC) was 0.76-0.93. The MFM and Vignos and Brooke grades were highly positively correlated with the coefficients of 0.47-0.75, indicating concurrent validity of the MFM-TR.

Conclusions: The MFM can be used as an outcome measure for the assessment of motor function of people with neuromuscular diseases. We concluded that the Turkish version of the MFM is a reliable and valid assessment to measure the motor function measurement in our sociocultural context.

PS4-478 / #263

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

Foot deformity and stabilometric parameters in children with charcot-marie-tooth disease

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Foot deformities are present in Charcot-Marie Tooth neuropathy (CMT) and tend to be worse with disease severity. Search for the association between posture of foot and stabilometry may help in selecting measures to guide making decision in rehabilitation. This study verified if specific stabilometric parameters correlate to foot deformities in children and adolescents with CMT. Ten volunteers (V1-V10) with different subtypes of CMT (age 6 to 18; 4 male and 6 female) were enrolled. Postural Foot Index (PFI) was used to classify foot deformities. Force platform AMTI OR6-5-1(Advanced Mechanical Technology - Newton, MA, USA) measured quiet standing. Sway area (SA), frequency (MF) and velocity of the center of pressure (Vcp) displacement were studied in opened and closed eyes. Three trials of 30 s were collected for each condition and the last one was considered for analysis. BioDynamics Br software was used for analysis; associations between variables were ex-

Table 1. Values of Foot posture index, stabilometric parameters for each volunteer and coefficient of correlation (r)

volun-teers	FPI	FM lat	FM ap	AS	Vcp lat	Vcp ap	FM lat	FM ap	AS	Vcp lat	Vcp ap
		Hz	Hz	m^2	m/s	m/s	Hz	Hz	m^2	m/s	m/s
				EO					EC		
V1	12	-0,062	0,067	0,0036	0,066	0,053	1,093	0,268	0,0050	0,107	0,073
V2	12	0,756	0,031	0,0088	0,082	0,113	0,637	0,136	0,0375	0,272	0,227
V3	-2	0,450	0,105	0,0026	0,057	0,041	0,067	0,047	0,0007	0,023	0,029
V4	9	0,037	0,038	0,0005	0,032	0,029	0,043	0,051	0,0003	0,033	0,030
V5	8	0,224	0,018	0,0002	0,025	0,028	0,182	0,020	0,0005	0,027	0,038
V6	3	0,054	0,139	0,0011	0,026	0,027	0,028	0,026	0,0002	0,019	0,024
V7	-4	0,268	0,033	0,0057	0,049	0,046	0,689	0,068	0,0102	0,107	0,070
V8	9	0,323	0,049	0,0012	0,023	0,025	0,210	0,050	0,0004	0,019	0,029
V9	7	0,702	0,252	0,0005	0,022	0,020	1,368	0,188	0,0015	0,034	0,042
V10	-4	0,022	0,021	0,0002	0,025	0,027	0,032	0,039	0,0004	0,032	0,033
(r)	1,0	0,2	0,0	0,1	0,2	0,3	0,3	0,5	0,3	0,3	0,4

foot posture index (FPI); mean frequency of lateral displacement (MF lat); mean frequency of antero posterior displacement (MF ap); SA – sway area; mean velocity of center of pressure (Vcp lat); mean velocity of antero posterior center of pressure (Vcp ap); total velocity (V t) ; eyes open (EO); eyes closed (EC).

pressed by correlation coefficients. Ninety percent of volunteers presented foot deformities (3 supinated, 2 highly pronated; 4 pronated and 1 normal). When the complexity of the task increased (eyes closed), there were an increase of MF of lateral displacement (50% of the volunteers), MF of antero posterior displacement (40% of the volunteers), SA; Vcp for lateral displacement (70% of the volunteers); Vcp for antero posterior displacement (80% of the volunteers). There were correlations between FPI and the following variables measured in closed eyes conditions: MF ap (r=0,5); Vcp ap (r=0,4); SA (r=0,3) and MF lat (r<0,3). Based on FPI, there were well defined foot deformities. Stabilometric parameters measured with eyes closed showed the strongest relations to foot deformities, particularly MF ap. Velocity-related measures also correlated with foot deformities. These findings are coherent with the theorythat velocity-related measures are good indicators of postural instability in peripheral neuropathy. As quiet standing is not so challenging for CMT, and the amount of volunteers was small, correlations were moderated to weak. Tendencies can be confirmed in the following investigations including dynamic tasks. In summary, for children and adolescents with CMT, foot deformities are associated with increased displacement of the center of pressure in closed eyes condition. These stabilometric parameters can compose evaluations or guide exercise and orthotic prescriptions.

PS4-479 / #368

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

The NorthStar ambulatory assessment in Duchenne muscular dystrophy: Considerations for the design of clinical trials

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Objective:With the emergence of experimental therapies for Duchenne Muscular Dystrophy (DMD), it is fundamental to understand the natural history of this disorder to properly design clinical trials. The aims of this study were to describe the motor function decline of ambulant DMD boys treated according to the standards of care, describe this decline in the genetic subpopulations of different skippable deletions and explore the role that age plays in the evolution of the disorder.

Methods:Through the NorthStar Network and database, clinical data systematically collected from 2004-2012 on 405 DMD boys in 17 UK neuromuscular centres were included in the analysis. For the analysis of the genetic subpopulation we included data from 74DMD boys followed-up in Rome.

Results:On the linearized NorthStar Ambulatory Assessment (NSAA) we observed that after age 7 an average slope of decline is of 8 units per year, with a median age at loss of ambulation was 13 years (95% CI 12.1 - 13.5). Two years prior to loss of ambulation, the mean total linearized NSAA score is 42/100. We describe the evolution of the condition in the young DMD and the effect of starting glucocorticoids between 3 and 5 years, which results in gaining additional motor function (10 units, $p=0.003$) by age 7. The items where a difference between the 2 groups is observed includes: standing on heels, jumping, hopping, standing on heels, lifting head, standing to sit and the 10 meter run. Wedescribe the effect of age in determining the progression of the disorder. Finally, when compared with the whole cohort of DMD boys, individuals with deletions that can be corrected by exons 44 or 46 skipping, decline at a slower rate over 2 years (9 units, $p<0.001$), while cohorts skippable by exons 53 or 51 show an additional loss of 14 ($p<0.001$) and 5 ($p=0.02$) units respectively.

Conclusion:Our study provides helpful information on the current natural history of DMD. The analysis of motor function gain and decline in DMD boys of different ages and the fine evolution of genetic subpopulations will be instrumental for the design of clinical trials.

PS4-480 / #418

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

Transcutaneous capnography as early indicator of nocturnal hypoventilation in neuromuscular disorders

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Nocturnal hypoventilation (NH) is a common complication of respiratory muscle weakness which appears insidiously as restrictive respiratory deficiency in neuromuscular disorders. It is due to respiratory muscle involvement and altered alveolar gas exchange during sleep. Daytime assessment and pulmonary function tests are not fully reliable in identification of NH since symptoms can be deceptive (headache, daytime sleepiness, nocturnal awakening) and daytime hypercapnia can appears in latter phases. Untreated NH, indee, can lead to daytime hypercapnia. Early detection of NH is important to start noninvasive ventilation (NIV) although recent guidelines recommend to support with NIV children with symptomatic nocturnal hypoventilation or daytime hypercapnia. Our purpose is to find the best tool in early detection of chronic respiratory failure by nocturnal transcutaneous capnography in childhood neuromuscular disorders (ND).

We retrospectively analysed 43 ND patients (3,5-24 years): 12 Duchenne Muscular Distrophy (DMD), 6 Becker Muscular Dystrophy (BMD), 6 Spinal Muscular Atrophy type II (SMA II), 10 Congenital Myopathies (CM), 7 Congenital Muscular Dystrophy (CMD), 2 Congenital Myotonic Dystrophy (SD) at follow up in our Unit, with FVC<60% who have needed mechanical ventilation (MV) because of NH detection.Fourteen patients had started MV at birth or during acute upper respiratory tract infection and were excluded. In the remaining 29 patients (9 CM, 8 DMD, 6 DMB, 3 SMAII, 3 CMD) we performed evaluation of NH symptoms, daytime capillary blood gases, nocturnal recording of combined transcutaneous pulse oximetry and capnography by a combined PtcCO2/SpO2 monitor. 11 out of29 patients (37%) complained symptoms of NH. Nocturnal hypercap-

nia, defined as mean nocturnal PtcCO2>50mmHg but not hypoxemia (defined as SpO2<90%) was detected in all. Carbon dioxide in blood gases was above 45mmHg (defined as predictor of nocturnal hypoventilation) in 6 out of 29 patients (20%). Evaluation of clinical, daily and nocturnal parameters after six months of ventilatory support showed a significant reduction of nocturnal pCO2 ($p<0,005$) in all patients, while nocturnal SpO2 and carbon dioxide in daytime blood gas was not statistically significantly. Our data confirm that nocturnal hypercapnia is the earliest predictor of NH, whereas routinary assessment of daytime blood gases and nocturnal SpO2 are not sufficient to detect early respiratory deficiency in neuromuscular disorders.

PS4-481 / #518

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

Long-term follow-up of sporadic Inclusion Body Myositis using dynamometry

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Sporadic inclusion body myositis (sIBM) is the most common acquired inflammatory myopathy in patients over 50 years of age. Development of new therapeutic approaches needs sensitive and reproducible evaluation methods to assess their effect on the neuromuscular function. However natural histories are rather scarce and limited in the number of patients involved. The aim was to assess the motor function changes within a period of time of 9 months and then 4 years.

Twenty-two patients were initially recruited; 16 came back for a second visit 9 months after baseline and 13 came back 4 years after baseline. At each visit, a manual muscle testing of 32 muscle groups was performed. Patients were also assessed using two non-specific functional scales (Walton and Rivermead Mobility Index) and two specific scales (sIBM weakness composite index and IBM Functional Rating Scale). The walking ability of patients was assessed by a six minute walk test. Specific dynamometric measurements were performed to measure hand grip strength and extension and flexion torques for wrist, elbow, ankle and knee.

The weakest muscle functions were hand grip, wrist flexion and elbow flexion at the upper limbs and knee extension and ankle flexion at the lower limbs. The most spared muscle functions were wrist, elbow and ankle extensions. Muscle weakness was generally asymetric between sides, especially for upper limbs where all tested functions were significantly stronger at the dominant side. At lower limbs this dominance effect was not observed. Patient strength was correlated with the disease duration only for knee extension. After 9 months, only knee extension changed in a significant way, whereas a trend towards a decrease was clearly detectable for hand grip and elbow flexion. After 4 years, all scores and strengths were significantly decreased. The most important baseline-to-end point changes for single functions were observed for knee extension and ankle flexion and extension. The different outcomes were highly correlated, linearly or not, but were not equally affected across the different clinical conditions.

This study shows that knee extension strength is particularly relevant to follow the patients in this disease. Strength loss does not have linear consequences on motor ability involution, since motor adjustments are built in relation to the health status of each patient. However, strength and motor ability are complementing each other in the understanding of disease progression.

PS4-482 / #522

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

One-year follow-up of patients with Duchenne muscular dystrophy using high precision tools for upper limb assessment

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Patients with Duchenne muscular dystrophy (DMD) begin to experience upper limb weakness while still ambulant with a proximal to distal progression. When becoming non-ambulant, suitable outcome measures are rather scarce for these patients. In addition, longitudinal data demonstrating sensitivity are lacking. This study was designed to follow DMD patients over one year in order to identify possible reliable and sensitive functional outcome measures.

At baseline, 53 non-ambulant DMD patients were recruited in a multicenter natural history study. Clinical measures included vital capacity, left ventricular ejection fraction, intellectual disability, Brooke Upper Extremity Functional Rating Scale and other clinical features (genetics, age at ambulation loss...). Strength of the distal functions of the upper limbs was measured for grip, pinch and wrist flexion and extension using specially-designed high precision devices. Motor abilities were assessed using the MoviPlate (ability to reproduce as fast as possible flexion/extension of wrist/fingers), the Motor Function Measure (MFM) and a tapping test. Two baseline visits were performed in order to assess the reproducibility of the measurements. Among the initial population, 35 patients underwent further evaluations after 6 and 12 months. 53 healthy boys were recruited as controls. All the evaluators were trained to strict standardized operating procedures to ensure measurement consistency.

High reliability was assessed in all the measurements performed (Intraclass Correlation Coefficient higher than 0.88). Strength devices were able to detect strengths as low as 50 g for grip, 70 g for pinch and 0.15 Nm for wrist flexion and 0.02 Nm for wrist extension. Strength and functional tests were significantly correlated. After one year, grip strength was significantly decreased by about 300 grams on both sides, while pinch strength was significantly decreased by about 200 grams. Meanwhile, the MoviPlate score was not significantly changed. However, for patients having lost ambulation for more than 3 years, the MoviPlate score was significantly decreased. This observation may reveal than the relation between strength loss and function loss depends on the clinical status of the patients.

This study shows that strength and function decline can be followed even in very disabled patients using reliable outcome measures, opening the possibility of conducting therapeutic trials in these populations.

PS4-483 / #530

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

GNE Myopathy Functional Activity Scale (GNEM-FAS): Results from a Phase 2 study of extended release sialic acid (SA-ER)

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GNE myopathy is a rare autosomal recessive myopathy without an approved treatment. Symptoms of distal leg weakness typically present in early adulthood and progressive weakness results in greater dependence and disability over time. The GNEM-FAS is a disease-specific measure of functional activity and independence in ambulatory patients. Mobility (MOB), Upper Extremity (UE) and Self-Care (SC) domains are assessed. A Total Score (TS) is calculated and higher scores represent greater independence. The GNEM-FAS was administered by clinical interview to 47 ambulatory subjects enrolled in a randomized, placebo-controlled, 48 week, Phase 2 study of extended release sialic acid (SA-ER). Subjects were randomized to receive placebo, 3g or 6g of SA-ER/day and after 24 weeks, the placebo group crossed to 3g or 6g/day for the remaining 24 weeks. Strength and functional performance measures administered in the study included hand-held dynamometry to evaluate UE and lower extremity (LE) strength and a 6-minute walk test (6MWT). The GNEM-FAS was administered at 12 week intervals. At baseline, the mean TS was 69 out of 100 (range 22-94) with MOB scores indicating more limitations than in UE or SC function. Higher MOB scores were associated with greater LE strength ($r=0.85$) and longer 6MWT distances ($r=0.83$). A moderate association was seen between UE strength and the UE ($r=0.66$) and MOB ($r=0.62$) domains. At week 48, the differences between subjects treated with 6g and 3g doses are reported. Analysis of the GNEM-FAS TS demonstrated a 3.71 point difference favoring the high dose group ($p=0.08$). Further analysis revealed a statistically significant difference in the MOB score (2.14; $p=0.02$) although this effect was not evident in measures of LE strength or function. A positive trend in the UE domain score (1.60; $p=0.11$) was also observed and supported by a statistically significant difference in UE strength ($p=0.003$). No differences in SC domain scores were observed. The statistically significant differences observed in both the MOB domain and UE strength may reflect an increased reliance on UE use as the disease and weakness progresses. The GNEM-FAS shows promise in evaluating patient-reported change in functioning and for use in future studies of patients with GNE myopathy. Additional work is underway to further validate this version of the GNEM-FAS and expand it for use with more impaired patients.

PS4-484 / #545

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

Evaluation of dysphagia in GRMD dogs using respiratory inductance plethysmography

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The dystrophin-deficient dog is a clinically relevant model of Duchenne muscular dystrophy (DMD) to assess a functional therapeutic effect, because it shares

numerous similarities with patients in its disease course. Among them, the oro-pharyngeal dysphagia is a prominent feature of the canine disease and is a cause of death from insufficient water and food intake and/or aspiration pneumonias, in the absence of an adapted medical support. Despite the involvement of this vital function, no tool to quantify dysphagia has been developed in this model. We thought that a way to easily assess dysphagia would be to quantify the apnea phases induced by deglutition efforts. Seven GRMD (Golden retriever muscular dystrophy) and 3 healthy adult dogs were evaluated using respiratory inductance plethysmography (RIP). The dogs were wearing two elastic belts respectively around the rib cage and the abdomen allowing simultaneous measurement of their motion during spontaneous breathing. During the first part of the test, the dog underwent a respiratory evaluation including a calibration of the belts signal using a pneumotachometer attached to a facemask. During the second part of the test, the dog was given a 25 g piece of wet food highly palatable, and RIP measurement was made continuously after food intake. The mean minute ventilation (Tidal volume x Respiratory frequency) during the minute following food intake was normalized by the mean minute ventilation at rest (before food intake). This ratio (MVdeglut/MVrest) was assessed in GRMD versus healthy dogs in order to address its relevance to monitor dysphagia. The MVdeglut/MVrest ratio was found significantly lower in GRMD dogs, meaning that their respiratory exchanges were impacted by deglutition efforts. However the values of MVdeglut/MVrest were heterogeneous among GRMD dogs. Interestingly, low MVdeglut/MVrest values were correlated with low Body Mass Indexes, suggesting that this ratio is clinically relevant to evaluate dysphagia. First results on young GRMD dogs followed-up during their first months of life tend to show a decrease of the MVdeglut/MVrest ratio during this period, during which dysphagia occurs and progresses. To our knowledge, this study is the first aiming to quantitatively evaluate dysphagia in GRMD dogs, and appears successful in identifying an easy-to-obtain parameter able to monitor non-invasively a swallowing impairment. A translation of this tool to patients could be realistically envisioned.

PS4-485 / #546

Theme: *8.4 - Miscellaneous: Functional evaluation in neuromuscular diseases / Outcome measures in clinical trials*

Comparative respiratory function evaluation in two canine myopathies using respiratory inductance plethysmography

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Canine myopathies offer the opportunity to assess therapies in a relevant pathological context, because they closely reproduce the human disease course including respiratory impairment. A relevant tool to monitor this vital function is thus essential when evaluating a functional effect during a pre-clinical trial. This study aimed to assess respiratory inductance plethysmography (RIP) as a tool to evaluate respiratory function in two canine models of human myopathies: the centronuclear myopathy (CNM) due to HACD-1 deficiency and the Golden retriever muscular dystrophy (GRMD) due to dystrophin-deficiency. Seven healthy, 7 CNM, and 12 GRMD adult conscious dogs underwent a RIP test, consisting in recording motion of rib cage and abdomen during Tidal breathing. The stretching signal from thoracic and abdominal belts was calibrated by a concomitant spirometric recording. RIP trace analysis allowed the calculation of respiratory volumes and flows, thoraco-abdominal asynchrony indices, and contributions of thorax and abdomen. In both myopathies, the Tidal volume and the respiratory frequency remained normal. Both models had in common a decreased abdominal contribution to inspiration as a consequence of diaphragmatic weakness. The other abnormalities evidenced by RIP testing were disease-specific. CNM dogs exhibited a major thoraco-abdominal asynchrony, quantified by increased phase angle, shift time, and proportion of the respiratory cycle spent in opposite signs by the derivative signals from belts (%deriv opposite). These patterns evidence a respiratory muscle weakness. In GRMD dogs, an increase of the inspiratory/expiratory times and of the maximal expiratory/inspiratory flows ratios confirmed our previous results from a spirometry study and those published by De Vanna et al. No true thoraco-abdominal asynchrony was found in GRMD, but in some dogs a

second abdominal excursion was observed during expiration. This phenomenon, probably related to the recruitment of abdominal muscles to support expiration and diaphragm relaxation, has been quantified by an increased %deriv opposite. Finally the most striking abnormality measured in GRMD dogs was an increased respiratory pause maybe related to increased relaxation and contraction times of the diaphragm, already described in limb muscles. This study has shown that RIP testing provides relevant disease-specific indices and appears a suitable tool to non-invasively monitor respiratory function in canine myopathies.

PS4-486 / #288

Theme: *8.5 - Miscellaneous: Others*

THE New Zealand Neuromuscular Disease Registry – A review of diagnoses confirmed by molecular test

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The NZ Neuromuscular Disease Registry (NZ NMD Registry) is part of TREAT NMD, an international networkfor patients with neuromuscular diseases. Its main aim is to ensure that the most promising new therapies reach patients as quickly as possible. From the perspective of researchers interested in trialling treatments it is useful to have data on the pool of potential research participants. From a patient's perspective it is important to know what trials they can take part in. Both of these require a confirmed genetic diagnosis in the patient. Sometherapeutic strategies not only require knowledge of which gene is affected but are targeted at specific mutations within the gene. In reviewing data held in the NZ NMD Registry it was noted that, of those diagnosed with a genetic condition, only 51% have a confirmed molecular genetic diagnosis. This low rate of genetic diagnosis is a potential barrier to research participation but can be removed with improved genetic technology and with changes in knowledge about and attitudes towards genetic testing.

PS4-487 / #323

Theme: *8.5 - Miscellaneous: Others*

Serum proteins as predictive biomarkers of rAAV efficiency in translational studies

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Clinical relevance of gene therapy using the recombinant adeno-associated vectors (rAAV) often requires widespread distribution of the vector and in this case systemic delivery is the optimal route of administration. The success of future clinical trials depends much on the adequacy of the results obtained in animal models. We have previously demonstrated that blood proteins interact with rAAVs and impact their efficiency in a species specific manner. For instance, interaction of human and dog galectin 3 binding protein (G3BP) with rAAV-6 diminished its transduction efficiency, while interaction with mouse C-reactive protein (CRP) increased more than 10 times the transduction efficiency of rAAV-1 and rAAV-6 under systemic delivery (Denard et al., J Virol. 2012: 6620-31; Denard et al., J Virol. 2013: 10784-91). In the present work we carried out systematic studies of proteins interacting with different AAV serotypes in sera from mice, dog, cow, macaque and human. We demonstrate for the first time that the blood of each tested species contain specific set of proteins to interact with a given serotype. The role of these particular proteins in the transduction efficiency of rAAV vectors in animal models and human will be discussed.

PS4-488 / #343

Theme: *8.5 - Miscellaneous: Others*

Changes of deep paraspinal muscles in idiopathic scoliosis: A pilot electrophysiological and histochemical study

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Background: The pathogenesis of idiopathicscoliosis has been poorly understood. The potentially involved local changes in deep paraspinal muscles have remained unknown.

Objective: To characterize the electrophysiological and histological findings in deep paraspinal muscles (on the concave and the convex side of the curve) in patients with idiopathic scoliosis.

Methods: Six patients with idiopathic scoliosis (5 females, 1 male, 12 - 29 years) were enrolled into this study. Needle EMG of the paraspinal muscles at convexity and concavity of the curve was performed. The study also included 24 biopsy samples from different deep paraspinal muscles obtained from all the patients during correctivespinal surgery. The muscle tissue samples were frozen in isopentane pre-cooled in liquid nitrogen. Serial cross-sections were stained for myofibrillar ATPase for the classification of fibers types and the fibers were counted in each biopsy to establish the distribution of fiber types.

Results: Five patients had a right curve convexity and all of them showed increased amplitude of motor unit action potentials (MUP) on the convex side of the curve. One subject presented double curve and had higher amplitude of MUP on the left side. In all cases, histological analysis of paraspinal muscle biopsies revealed changes in the distribution of muscle fibers with numerical predominance of type I fibers and occasional fiber type grouping on the convex side of the curve.

Conclusion: Our pilot data demonstrate a significant asymmetry in fiber type distribution corresponding with an altered function in paraspinal muscles with predominance on the convexity of the curve. Thus, local neurogenic changes in the paraspinal muscles might play an important role in the development of idiopathicscoliosis.

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PS4-489 / #347

Theme: *8.5 - Miscellaneous: Others*

Clinical manifestation and disease course of the patient with HAM/TSP

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HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is characterized by slow progressive spastic paraparesis and positivity for anti-HTLV-1 antibodies in both serum and cerebrospinal fluid (CSF). The progression is slow throughout the course of the disease, but sometimes with periods of rapid deterioration. While factors influencing the disease activity are not known clearly, it has been suggested that a subset of older patients rapidly progress with worse prognosis. We reviewed the patients with HAM/TSP admitted to Kagoshima University Hospital in the last 10 years to clarify the clinical course and manifestation, especially about muscle weakness and laboratory findings of the subset with rapid disease progression. This study reviewed all patients with HAM/TSP, 124 in number, hospitalized to Kagoshima University Hospital from 2002 to 2012. The disease courses of the patients were evaluated with Osame's motor disability. As a result, 35 patients (28.2%) in total 124 rapidly progressed. The mean age of the onset of patients with rapid disease progression was about 15 years older than that of the patients with slowly progression (62.3 vs. 47.4 years, $P<0.001$), although there was no differences in sex and initial

symptoms between the two groups. The percentage of the cases with rapid progression has the tendency to increase with age. The patients with rapid progression needed a wheelchair in daily life in 1.5 years after onset, while the patients with slow progression did it in 14.4 years (1.5 vs. 14.4 years, $P<0.001$). The patients with slow progression often show proximal weakness of their extremities, especially of the deltoid and iliopsoas muscles. Cell numbers, protein levels, and anti-HTLV-1 antibody titers in the CSF were significantly higher in patients with rapid progression than in those with slow progression (11.6 vs. 3.2, $P<0.001$; 55.3 vs. 36.7 mg/dl, $P<0.001$; 1,251 vs. 416, $P<0.014$, respectively). Interestingly, HTLV-1 proviral loads in the peripheral blood mononuclear cells (PBMCs) were significantly lower in patients with rapid progression than in those with slow progression (370 vs. 1,245 copies, $P<0.001$). These suggest that factors other than HTLV-1 PVLs also contribute to the disease course of HAM/TSP.

PS4-490 / #373

Theme: *8.5 - Miscellaneous: Others*

Exploring the SOD1-G93A transgenic swine as a novel animal model for Amyotrophic Lateral Sclerosis (ALS)

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ALS is a fatal neurodegenerative disease that occurs in two forms: sporadic and familial, the latter linked to mutations in the SOD1 gene. The use of mice carrying the hSOD1^{G93A} mutation is currently widespread in ALS research, however a real improvement of patients prognosis has not yet been obtained (1). Another model, more closely related to human species, is strongly demanded by the scientific community that has already foreseen swine as an attractive alternative for modelling human neurodegenerative diseases (2). Recently we produced four hSOD1^{G93A} cloned boars (3), that were analysed to confirm the transgene integration and expression, while its long term effects are still under investigation. Thus in order to assess if this species may represent a suitable model in reproducing ALS features and in supporting clinical research, an extensive phenotypical characterization, adapting to pig currently employed human diagnostic devices, was applied to the transgenic swine.

Adapted clinical evaluation and neurological examination protocols were applied on living animals. We also performed monthly haematological and haematochemical analysis. Motor function and gait dynamics were evaluated using an integrated protocol of digital gait analysis (3D Motion Capture) and surface electromyography (EMG). Furthermore tissues from stillborn piglets and from animals that died soon after birth were analyzed by immunohistochemistry and double immunofluorescence.

One of the four transgenic cloned boars started to show lameness around twenty seven months of age. At twenty eight months of age, only a slight incoordination was detectable, which become clear and associated to hypermetria around the 29-30 months of age. However no other clinical or neurological signs were visible. Thus hSOD1^{G93A} transgenic swine showed motor dysfunction and symptoms resembling ALS. These findings might be consistent with the disease onset. However, since this is the first swine ALS model produced so far, further molecular and pathological investigations are required. Thus ongoing investigation will be important for further interpreting these data and determining disease onset and duration, till the defined end-points and the pathological features of this model.

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PS4-491 / #396

Theme: *8.5 - Miscellaneous: Others*

Neuromuscular complications of HTLV-I: Case Reports

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The human T lymphotropic virus type I (HTLV-I) is a human retrovirus that is known to cause ATL (adult T cell leukemia/lymphoma), a lymphoprolitrative cancer, and HAM/TSP (HTLV-I associated myelopathy/tropical spastic parapareis), a chronic progressive spastic paraparesis. HTLV-I that belongs to retroviridae family is an endemic viral infection in Mashhad, Iran.

HAM/TSP is the most common neurological complication of HTLV-I, however it develops in only 2-5% of infected individuals. Recently, a wide spectrum of HTLV-I associated neurological complications has been reported throughout the world. Among them are: dementia, chorea, Parkinsonism, encephalopathy, meningoencephalitis, acute transverse myelitis, amyotrophic lateral sclerosis (ALS), sensorimotor polyneuropathy, sensory ganglionopathy and inflammatory myopathies. Also multiple sclerosis like presentations of HTLV-I has been reported.

In this article we are presenting a few cases of neuromuscular complications of HTLV-I including: HTLV-I associated ALS-like syndrome, HTLV-I associated sensorimotor polyneurpathy, HTLV-I associated sensory ganglionopathy, HTLV-I associated IBM (inclusion body myositis), and HTLV-I associated non-specific myositis. In all these cases, PCR (polymerase chain reaction) showed that the tissues (cerebrospinal fluid, muscle and sensory nerve) were infected by HTLV-I. However it seems that the course of the diseases were different from classic forms.

PS4-492 / #397

Theme: *8.5 - Miscellaneous: Others*

Patterns on spinal magnetic resonance image in pediatric Guillain – Barré syndrome

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Objectives: describe the most frequents MRI findings in pediatric Guillain - Barré Syndrome.

Materials and Methods: we performed a retrospective chart review of 8 patients admitted to the Pediatric Neurology Service at J. P . Garrahan Children Hospital between January 2012 and December 2013 who were diagnosed with Guillain Barré syndrome and who had undergone complete spinal or lumbosacral spinal magnetic resonance imaging, with the administration of gadolinium.

All magnetic resonance imaging scans were performed on a 1.5 T scanner (Toshiba), and included precontrast T1 - weigthed and T2- weighted images and poscontrast T1- weighted images in axial and sagital planes.

The MRI scans were assessed independently by two different neuro-radiologist.

Results: Classic isolated anterior nerve root enhacement was evident in two patients (one of them with associated thickening); both anterior and posterior nerve root enhacement without thickening was evident in two patients; concurrent enhacement and thickening of both anterior and posterior nerve root was evident in two patients and also two patients evidenced anterior and posterior nerve root enhacement associated to posterior roots thickening. In all patients the lumbar root enhancement extension was proximal and distal.

Conclusions: There were different patterns of nerve roots anomalies on MRI scans in our serie of pediatric patients with Guillain-Barré syndrome. The most frequent finding being the anterior nerve root enhancement.

PS4-493 / #420

Theme: *8.5 - Miscellaneous: Others*

Systemic diseases diagnosed by muscle biopsy

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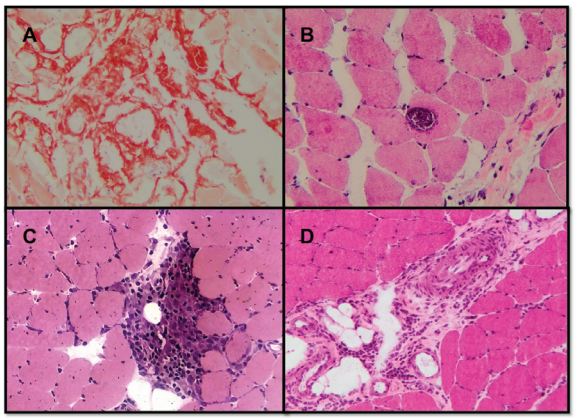
Although muscle biopsy represents the most important tool in the diagnosis of the majority of diseases of muscle, its role in helping to final diagnosis in diseases other the myopathies has not been systematically explored. Since our Muscle Research Unit belongs to the Internal Medicine Department and in addition many patients with suspected systemic diseases are currently been studied, we decide to conduct a study to check how and in how many patients, muscle biopsy contributed to final diagnosis.

We did a retrospective study, analyzing our database from October 2004 to October 2013 where 978 muscle biopsies for diagnostic purposes of either a true myopathy or a suspected systemic disease were performed. Myopathies were diagnosed in 316 cases (32%) while in 70 cases (7%) a systemic disease was the main diagnosis. Systemic vasculitis (both ANCA positive and negative) was diagnosed in 60 cases, amyloidosis in 5, sarcoidosis in 2 and Whipple's disease, Erdheim-Chester disease and Sarcocystis parasitation, one case for each condition. Muscle biopsies were performed by internal medicine residents tutorized by a senior specialist, who also did the pathologic study of the samples. Muscle biopsies were frozen in cooled isopenthane, cryostat sectioned and stained and reacted routinely (minimum 13 stainings). Additional reactions (myophosphorylase, adenosindeaminase and immunohystochemistry) were performed when it was considered necessary. No mortality or significant morbidity was observed in the present series.

Taking into account that many of the systemic diseases included in this series would require invasive and expensive procedures such as kidney and lung biopsies or angiography, muscle biopsy represents a safe and non-expensive technique that may be quite useful in this setting.



A. Rojo-Congo stained, amyloid deposits. B. H&E stained, *Sarcocystis*'s cyst into the sarcoplasm. C. H&E stained, sarcoid granuloma. D. H&E stained, medium-sized vessel with a perivascular inflammatory infiltrate.

PS4-494 / #476

Theme: *8.5 - Miscellaneous: Others*

Pronoctic Features of LGMD presenting as symptomatic or pacisymptomatic HyperCKemia

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Asymptomatic or paucisymptomatic hyperCKemia (A/P-HCK) is a syndrome which represents a preclinical or benign subclinical stage of a broad spectrum of muscular disorders. In many instances their prognosis in the long run is uncertain

The objective of the study is to search for data that may predict the outcome patientsof patientspresentingasA / P- HCK andreceived the diagnosis of limb-girdle muscular dystrophy (LGMD).

From a series of 250 cases referred as A/P-HCK we selected 13 patients diagnosed of LGMDthat had beensubmitted toat leasta7-year follow up. Allsubject underwnt MRI and muscle biopsy. Analyses include detection of proteins involved in the major forms of LGMD by immunohistochemistry (IQ), immunofluo-

rescence (IF) , Western blot (WB) and genetic sequencing. Thespecific LGMDdiagnosis included:4LGMD2A (2 males-M-and 2 females -F-), 6 LGMD2B (5 M and 1 F), 2 LGMD2C (both F) and 1 LGMD2L(M)

Patientoutcomesafter 7-yearfollow up were:asymptomatic (1 LGMD2A, 4 LGMD2B), mildly afected (2 LGMD2A, 2 LGMD2B, 1 LGMD2C, 1 LGMD2L) and mildly-severely affected (1 LGMDA, 1 LGMDC).Age at diagnosisand serum CK level were indicative of severity but with puntual exception. MRI was highly predictor of the evolution:absence or minimal muscle afectation predected the maintenece of the asymptomatic conditions.

Conclusion: Muscle MRI may be en useful tool to predict outcome to LGMD patients presenting as asymptomatic or pauci-symptomatic hyperCKemia

PS4-495 / #491

Theme: *8.5 - Miscellaneous: Others*

A Rare Reason For Paraplegias: Myositis Ossificans

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Purpose:To consider and draw attention to an important reason of paraplegias.

Introduction: This formation called Myositis Ossificans was defined for the first time in 1883.

Case: A 22 year old male patient was hospitalized for research purposes in the physiotherapy clinic due to loss of strength in both legs. He said that his complaints of swelling, pain, coldness, strain and weakness started on 26 December 2013 in his both legs, after he was exposed to cold for a week during military service, slept in a cold environment but did not have fever infection and trauma history. Brain MR, cervical, thoracal and lombar MR had been taken, and evaluated to be normal. Abdomen BT, lower extremity arterial venous Doppler USG were also taken, and determined to be normal. ENMG was taken in our clinic and was evaluated normal. The patient was transferred to the neurology clinic for advanced observation. His neurologic examination was normal except for loss of muscle strength in both legs. BOS findings were normal. In the femur MR: Appearances of fluid collections were observed, which exhibited peripheral contrast involvement after apparent hyperintense IVKM in clean contour T2A views in about 21×3 cm size within the muscle plans in the right femur anteromedial. There were signal alterations coherent with the prevalent edema in the soft tissues around these fluid collections. Numerous fluid collections were observed, exhibiting peripheral contrast involvement, the largest being in a size of 18×6 cm, in the anterior, medial and lateral in the left femur, and signal alterations were available, coherent with the prevalent edema in the soft tissues around these fluid collections. Biopsy was taken. The pathology result was reported as Myositis Ossificans.

Conclusion: Myositis Ossificans was discussed as a reason of paraplegias with this case.

PS4-496 / #496

Theme: *8.5 - Miscellaneous: Others*

Multisegmental anterior horn motor nueron disease due to a case

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Purpose: To draw attention to early childhood paralyses.

Introduction: The reason for early childhood paralyses is infection or autoimmune.

The aetiologic reason for this kind of paralyses should be researched in a wide range.

Case: A 6 year old male patient applied to our clinic on 12.03.2013 due to pain in the right side of the neck and loss of strength in the right arm. He said that he had a severe pain in the right shoulder; the right side of the neck about a month ago and he was not able to lift his right arm the next morning. Proximal muscle strength was 4/5 in the right arm, distal was 0/5, and sensory examination was normal. DTR reflexes were not obtained in the right upper extremity, but was obtained live in the lower extremities. Babinski bilateral was indifferent, and low with respect to IgA:65.6 mg/dl IgG2: 101 mg/dl reference range. CSF biochemistry was normal. Daily locomotor examination was made and he was taken to physiotherapy program. During monitoring, atrophy developed in distal muscles of the right upper extremity. A hyperintense lesion extending from C2 to T1 level was determined in

the cervical MR. The Cervical MR suggested transverse myelitis. ENMG was applied to the patient. In the right arm, motor transmission speed measurements and CMAP amplitudes could not be recorded. Sensory neural transmission speed measurements as well as motor and sensory neural transmission speed measurements of other extremities were normal. A single motor unit potential was available in the deltoid muscle in the right in the needle EMG. In conclusion, multisegmental spinal anterior horn motor neuron disease was considered with EMG. After one year of monitoring, clinics of the patient did not change and the patient was called again for control on 27.01.2014. Partial recovery was observed in the intrinsic hand muscles. ENMG was repeated. Transmission speed studies did not change. The cervical spinal MR was reported to be normal.

Conclusion: Autoimmune paralyses appear as an important reason of paralyses developing in early childhood.

PS4-497 / #499

Theme: *8.5 - Miscellaneous: Others*

AFM-Téléthon / Bpifrance Seed Fund dedicated to innovative biotherapies and rare diseases

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Bpifrance has been mandated by the French government to manage the FNA (Fonds National d'Amorçage) as part of the Investing in the Future Programme (Programme d'Investissements d'Avenir). It is in collaboration with AFM-Telethon (French muscular dystrophy association organizing French Telethon), an association of patients and parents of patients, to help introduce new biotherapies and provide access to groundbreaking treatments for people with rare diseases. The partners are announcing that a new €50 million fund has been set up and will be overseen by Bpifrance.

This is FNA's first fund dedicated to innovative biotherapies and rare diseases. It is designed to inject capital into new companies working to:

- Promote medical research projects than can give patients with rare diseases access to the latest treatment methods;
- Help bring treatments for common pathologies based on the therapeutics innovations developed for these rare diseases.

Its main focus is on innovative therapies for rare diseases, most of which do not currently have any cure. This includes new therapeutic approaches and some of them are mature enough to begin industrial development, such as gene therapy, cell therapy, pharmacological modulation of gene expression, monoclonal antibodies, therapeutics proteins and immunotherapies.

Most of the investments will be made in France but there is talk of also including notably other parts of Europe. It plans to target innovative SMEs with high growth potential that are under eight year old.

As such, it is ensuring the companies aim to expand their activities with a view to providing patients suffering from rare diseases medication at a "fair price". When companies practice "fair prices", medication is priced (accounting for the medical reimbursement system) so it does not become an obstacle for patients in obtaining treatment.