Methods: Patients with genetically confirmed IBMPFD were identified at the Newcastle MRC Neuromuscular Centre and the clinical details, muscle biopsy findings and muscle MRI data were collected retrospectively.

Results: We estimate a point prevalence of the disease for the UK of 0.066/100 000 population. Muscle weakness was the leading symptom in 92.3% of the patients, either with a limb-girdle pattern and/or distal weakness. One patient presented with Paget disease of the bone and 3 muscle carriers were asymptomatic at the time of investigation. The mean age at onset was 42.8 years and the mean time to loss of ambulation 13.37 years. Parkinson's disease, bladder, anal, and erectile dysfunction were additional features. Two patients required assisted ventilation and four patients developed cardiomyopathy. Dementia or mild cognitive impairment was observed in 48.2% and Paget disease of the bone was present in 20.5% patients. All muscle biopsies showed myopathic changes, 61% had rimmed vacuoles and 33.3% small inflammatory infiltrates. We have identified four previously described missense mutations (p.R155C, p.R155H, p.R191Q, and p.R93C) and 2 novel mutations (p.G202W and p.A439G).

Conclusions: IBMPFD is a rare disorder probably under diagnosed due to the variable phenotype. Our study provides strong evidence that IBMPFD should also be considered in patients presenting with distal muscle wasting and weakness which is uncommon in other myopathies. Larger cohorts are needed to better clarify the phenotype and to establish phenotype/genotype correlations in order to produce clear guidelines for the diagnosis and management of patients with IBMPFD.

IBMPFD, Inclusion Body Myopathy with Early-Onset Paget Disease and Frontotemporal Dementia, valosin-containing protein, VCP gene mutations, prevalence

Myofibrillar myopathies- #3016

P07-103- Extensive muscle biopsy retrospective analysis in a large cohort of Myofibrillar myopathies (MMF) patients
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Myofibrillar myopathies (MFM) are clinically and genetically heterogeneous conditions characterized by the presence of focal dissolution of the myofibrils associated with deposits of different proteins in skeletal muscle. Mutation in DES (desminopathy), CRYAB (?B-crystallinopathy), MYOT (myotilinopathy), LDB3 (zaspopathy), BAG3 and FLNC (filaminopathy) have been associated to these conditions. In this study we retrospectively analyzed clinical, morphological, immunocytochemical and ultrastructural findings of 39 MFM patients. 23 patients were male and 16 were female. Age at onset varied from 2 to 66. The most common clinical sign was distal weakness (66% of patients) followed by distal and proximal weakness (49%). CK level varied from normal to 1248 UI/L. Cardiac involvement was present in 20% of patients. Ten patients received a genetic diagnosis. DES was mutated in 5 patients, ZASP in 4, MYOT, BAG3 and FLNC in one patient respectively. A muscle biopsy performed in all patients between 28 and 71 years showed the presence of irregularly stained eosinophilic masses, darkly stained with the mGT and lacking oxidative activity, associated with variable unspecific histological findings. The aggregates were variably positive for desmin, myotilin and ?B-crystallin in 87% of biopsies. Ultrastructural analysis was performed in 19 patients and revealed: a) Inclusions or aggregates (rods, tubular filamentous aggregates were variably positive for desmin, myotilin and ?B-crystallin in 87% of biopsies.

We described a wide histopathological spectrum associated with MFM. Extensive immunohistochemical and immunofluorescence studies will help to better characterize our genetically undiagnosed MFM patients.

Protein aggregates myopathies, Myofibrillar myopathies, MFM, distal weakness

P08- Dystrophinopathies (Duchenne, Becker, others)- N° 104 to N° 156

Dystrophinopathies (Duchenne, Becker, others)- #2311

P08- 104- The natural history of Duchenne Muscular Dystrophy with corticosteroids using the Motor Function Measure
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Objective: Monitoring evolution of motor function in patients with Duchenne Muscular Dystrophy (DMD) treated by corticosteroids (CS) in comparison with untreated patients and to study the responsiveness of Motor Function Measure (MFM) as outcome measure in this population, and provide estimations of the number of patients with DMD needed for clinical trials to prove the effectiveness of a given drug.

Design: observational, retrospective, multicenter cohort study.

Setting: Eleven French departments of physical medicine and rehabilitation, neurology and/or consultations in a reference center for neuromuscular diseases.

Participants: A total of 74 patients with DMD, aged 5.9 to 11.8 years, with at least 6 months of follow up and 2 MFM were enrolled for a 24 months period, 29 in the CS treated group (8 ± 1.5y) and 45 in the untreated group (7.91 ± 1.50y).

Main Outcome Measures: the relationship between MFM scores (total score and its 3 subscores) and age was studied in two separated groups (CS treated patients and untreated patients). The evolution of these scores was compared between groups, on a 6, 12 and 24 months period by calculating slopes of change. Standardized response mean was used to study responsiveness of the MFM.

Results: At 6, 12 and 24 months, significant differences in the mean score change were found, for all MFM scores, between CS treated patients and untreated patients. For D1 subscore specifically, at 6 months, the increase is significant in the treated group (12.6 ± 15.5 %/y; SRM 0.8) while a decrease is observed in the untreated group (-17.8 ± 17.7 %/y; SRM 1). At 24 months, D1 subscore stabilized for treated patients (+4.8 ± 7.6%/y; SRM 0.6) but declined significantly for untreated boys (-18.8 ± 7.1 %/y; SRM 2.6). 23 patients lost the ability to walk during the study: 7 in the CS treated group (25% at 24 months, mean age: 10.62 ± 1.18y) and 16 in the untreated group (64.71% at 24 months, mean age: 9.20 ± 1.78y).

Conclusions: Patients with DMD treated by CS present a different course of the disease described in this paper using the MFM. Based on these results, an estimation of the number of patients needed for clinical trial could be done.

Dystrophinopathies (Duchenne, Becker, others)- #2390

P08-105- Human dystrophin expression in Rag/mdx mice muscles following the graft off genetically corrected dystrophic hiPSCs

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Human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs) have shown self-renewal capacity and can potentially differentiate into all types of lineages. They thus represent an unlimited source of cells for the therapy of degenerative diseases, such as Duchenne Muscular Dystrophy (DMD), a disease characterized by rapid progression of muscle degeneration that occurs early in life. Here, we developed a two-step procedure to differentiate hiPSCs in myogenic cells. Dystrophic hiPSCs were corrected or not with TALENs. The genetic correction was the insertion of 1 bp to restore the reading frame. We first utilized our own myogenic culture medium (called MB1) to promote differentiation of hiPSCs into mesenchymal-like precursors. We then transduced them with a lentivirus expressing MyoD transcription factor, under the control of the composite CAG promoter, in order to induce their differentiation into myoblasts. Those transduced cells were grafted in the Tibialis anterior of Rag/mdx mice where they fused with existing muscle fibers. The presence of human dystrophin was confirmed by immunohistofluorescence in muscles grafted with the genetically corrected cells and in the positive control grafted with myoblasts of a healthy donor. Cell therapy shows great promises for DMD patients since it cannot only introduce a normal dystrophin gene in the muscle fibers but it can also increase the regenerative capacity of the muscle and the muscle strength.

hiPSCs, DMD, Lentivirus, Myoblasts, Cell Therapy

Dystrophinopathies (Duchenne, Becker, others)- #2400

P08-106- Correction of dystrophin gene reading frame and of the protein structure using the CRISPR-induced deletion (CinDel) method

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In most Duchenne Muscular Dystrophy (DMD) patients, expression of dystrophin (DYS) protein is disrupted because exon deletions result in a frame shift. We present here the CRISPR-induced Deletion (CinDel), a new promising genome-editing technology to correct DYS gene reading frame and the protein structure. This strategy is based on the use of two gRNAs targeting specifically exons that precede and follow the patient deletion in the DYS gene. This pair of gRNAs induced a precise large additional deletion leading to fusion of the targeted exons. Using an adequate pair of gRNAs, the deletion of parts of these exons and the intron separating them restored the DYS reading frame of the hybrid exon in DMD myoblasts and in hMDM mice. Moreover, selected pairs of gRNAs also permit to produce a dystrophin protein containing correct spectrin-like repeats. The
expression of an internally deleted DYS protein was detected following the formation of myotubes by the unselected treated DMD myoblasts. Given that CinDel is a permanent reparation of the DYS gene this treatment would not have to be repeated as it is the case for exon skipping induced by antisense oligonucleotides.

**CRISPR, Cas9, gRNA, Duchenne Muscular Dystrophy, dystrophin, gene therapy, protein structure**

Dystrophinopathies (Duchenne, Becker, others)- #2409

**P08-107- Cholesterol increases the anchorage of human dystrophin repeats 16 to 21 in model membrane at physiological surface pressure**

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Duchenne and Becker muscular dystrophy (DMD and BMD respectively) are caused by mutations of the dystrophin's gene coding for a skeletal muscles protein included in the dystrophin-glycoprotein sarcolemmal complex. Dystrophin connects the cytoskeleton and the extracellular matrix via the sarcolemma, conferring resistance to muscular cells. Whereas complete deficit in dystrophin leads to the severe DMD, expression of truncated or mutated forms lead mainly to the milder BMD phenotype. In this case, great majority of mutations consists of ?in frame? exons deletions leading to lack of part of the central domain's spectrin-like repeats. About one third of the mutations leading to (BMD) are located in the region coding for repeat R16 to R21.

Interactions between the recombinant DYS R16-21 fragment and lipids were examined in vitro using monolayers of anionic and zwitterionic lipids. The film fluidity was modified by the addition of 15% cholesterol. Whatever the lipid mixture examined, at low surface pressure (20 mN/m) few differences appeared on the protein insertion and the presence of cholesterol did not affect the protein/lipid interactions. At high surface pressure (30 mN/m), the protein insertion was very low and occurred only in the liquid-expanded phase of zwitterionic films. In anionic films, electrostatic interactions prevented the protein insertion outright, and caused accumulation of the protein on the hydrophilic part of the monolayer. Addition of cholesterol to both lipid mixtures drastically modified the protein-lipid interactions: the DYS R16-21 insertion increased and its organization in the monolayer appeared to be more homogeneous. Two accessible cholesterol recognition amino-acid consensus (CRAC) sequences were found in this fragment. Their presence may enhance the protein/membrane binding at physiological lateral pressure. These results suggest that the anchorage of dystrophin to the membrane in vivo may be stabilized by cholesterol-rich nano-domains in the inner leaflet of sarcolemma.

**muscular dystrophy, spectrin superfamily, protein lipid interaction, CRAC sequence, Atomic force microscopy**

Dystrophinopathies (Duchenne, Becker, others)- #2453

**P08-108- Digital PCR quantification of miR-30c and miR-181a as serum biomarkers for diagnosis and prognosis of muscular dystrophies**

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MicroRNAs (miRNAs) are involved in several physiological and pathological processes such as skeletal muscle development, regeneration and fibrosis. In addition, they are potential non-invasive biomarkers for diagnosis and for monitoring disease progression and the efficiency of clinical trials. Therefore, measuring miRNA expression levels in serum can provide a potential approach for the diagnosis and prognosis of Duchenne muscular dystrophy (DMD). Using gene network analysis, we have identified two miRNAs, miR-30c and miR-181a, that appear to be key regulators of muscular dystrophy and which we found increased in tissue and serum from patients with collagen VI defects. We hypothesized that they could represent novel biomarkers of muscular dystrophy.

To exploit miRNAs potential in a clinical setting it is necessary to improve and standardize methodologies to quantify them accurately and reproducibly in human samples. Digital PCR has been raised as a robust technique for precise, direct, and absolute quantification of nucleic acids in scarce biological samples.

The objective of this small pilot study was to assess the absolute level of miR-30c and miR-181a in serum using digital PCR for diagnosis and prognosis of DMD.

We used digital PCR to quantify copy number of miR-30c and miR-181a, one well-studied miRNA in DMD (the dystromir miR-206), and one synthetic spiked-in miRNA (cel-miR-39p-3p) in a small group of DMD patients (n=5, 7-11 years, ambulants) and healthy controls (n=5, 4-10 years).

We have optimized the use of digital PCR to quantify miRNAs in serum from patients with DMD. The serum levels of miR-30c and miR-181a increased 10- and 8-fold respectively in DMD patients compared to healthy controls (p=0.0003 and p=0.003, respectively). The muscle specific miR-206 increased 1000-fold in serum of DMD patients (p=0.002). There was no significant difference in the level of the spiked-in cel-miR-39p-3p.
We demonstrate that digital PCR is an affordable and powerful technique for precise and sensitive quantification of miRNA in serum of DMD patients. Although the analysis of a larger number of patients is necessary, we propose miR-30c and miR-181a as valuable biomarkers for DMD diagnosis and disease progression.

digital PCR, microRNAs, biomarkers, serum, diagnosis

Dystrophinopathies (Duchenne, Becker, others)- #2465

P08-109  ?The influence of water immersion on vital capacity in Duchenne Muscular Dystrophy?
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Introduction The Duchenne muscular dystrophy (DMD) leads to progressive degeneration of respiratory musculature, followed by reduction and coughs ineffective ventilation, leading to respiratory failure. The Aquatic Therapy is beneficial for children as it provides functional activities. The ventilatory response to immersion in water is a result of the hydrostatic pressure in the body immersed causing reduction in forced vital capacity. Objective: Compare the values of forced vital capacity (FVC) obtained in land and immersion.

Material and methods The FVC was collected with spirometer in land, in the seated position (SS) and lying position (LP) and sitting with immersion level in the seventh cervical vertebra (IC7) and xiphoid process (IPX).

Results The study included 27 patients with DMD with a mean age of 15.03 ± 3.78 years, 21 wheelchairs and walkers. 8 of these were using non-invasive ventilation (NIV). The total sample was no statistically significant difference in FVC compared SS [2100 (1570-2685)] to LP [1900 (1425 to 25154)] (p >0.01), SS with IC7 [1650 (1450-2400) (p >0.01) and IC7 with IPX [1850 (1400-2570)] (p >0.05). By dividing the sample was statistically significant difference in FVC (ml) in patients not using NIV compared SS [2550 (1935 to 2915)] with LP [2200 (1825-2650)] (p >0.01), LS with IC7 [2150 (1650-2830)] (p >0.002), IPX [2280 (1825-2650)] to SP (p >0.025); IPX with IC7 (p >0.01). In the group that used NIV was no difference when compared postures in SS [1525 (1280-1825)] to LP [1350 (1050-1470)] (p >0.012), SS with IC7 [1150 (1000-1350)] (p >0.012), SS IPX [1325 (1055 to 1550)] (p >0.012); LP with IC7 (p >0.046). In ambulating patients was statistically significant difference of FVC (ml) when compared to SS postures [2460 (1820-2600)] and LP [2110 (1750-2350)] (p >0.046), SS and IC7 [2065 (1700-2250)] (p >0.028), SS and IPX [2065 (1750-2400)] (p >0.028), SP and IC7 (p >0.028). In wheelchair was no difference in FVC (ml) when compared to SS postures [2050 (1500-2750)] and LP [1750 (1250-2580)] (p >0.003), SS and IC7 [1600 (1250-2550)] (p >0.01), SS and IPX [1800 (1350-2690)] (p >0.017), IPX, and LP (p >0.019) and IC7 and IPX (p >0.004). Conclusion The FVC of patients with DMD differ between land and immersion with the lowest value noticed in IC7, demonstrating that the physical principles of water act in these ventilation parameter. This aspect should be valued in the aquatic physical therapy care in this population.

Duchenne muscular dystrophy, Aquatic Physical Therapy and Respiratory Assessment

Dystrophinopathies (Duchenne, Becker, others)- #2472

P08-110  Identification of miRNAs to improve myogenic differentiation of mesenchymal stem cells as regenerative therapy 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. The absence of the protein caused fibrotic tissue deposition and adipose infiltration into the muscle until complete replacement of original tissue at later stages of the disease and DMD patients die from heart and respiratory failure. Unravelling the precise cellular origin and molecular mechanism of fibrotic and adipogenic tissue within a degenerating human DMD muscle is crucial to understand the influence of dystrophic muscle environment could influence the outcome of stem cells based therapy and to improve future treatments. Recently, we isolated through FACS sorting from dissociated muscular biopsies two MSC populations that express or not the CD133 antigen. These populations could be responsible for muscle regeneration exhaustion and adipogenic tissue deposition in DMD. We identified miRNAs involved in in-vitro differentiation process and in DMD muscle degeneration in the isolated CD133+ and CD133- hmMSCs and unravelled gene regulatory networks that miRNAs control in DMD. In a clinical prospective, we also tested the therapeutic value of targeting miRNAs to enhance transduction efficiency of hmMSCs into muscle using a dystrophic animal model (scid-mdx mice).

miRNAs, mesenchymal stem cells, Duchenne muscular dystrophy
Dystrophinopathies (Duchenne, Becker, others)- #2538

P08- 111- A new antisense oligonucleotide composed of RNA/ENA chimera (AO85) against dystrophin exon 45 significantly increased six-minute walk distance in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is the most common inherited muscle disease and characterized by skeletal muscle dystrophin deficiency due to mutations in the dystrophin gene. Expression of dystrophin is a fundamental treatment for DMD. Antisense oligonucleotide (AO)-mediated exon skipping that convert out-of-frame mRNA into in-frame mRNA, thereby enabling semifunctional dystrophin production, is recognized as the most promising treatment for DMD. Two clinical trials that utilize different AOs using either 2′-O-methyl RNA or morpholino as monomers have been conducted worldwide. Recently, a new modified nucleotide of 2′-O, 4′-C-ethylene-bridged nucleic acid (ENA) has been invented and shown to have high binding affinity for the complementary RNA strand and nuclease resistance. We have identified an 18-mer antisense RNA/ENA chimera against dystrophin exon 45 (AO85) that is able to induce skipping of dystrophin exon 45. Here, we report that intravenous administration of AO85 induced skipping of exon 45 and increased the 6-minute walk distance (6MWD). The protocol of this study was approved by our ethics committees and the informed consent was obtained from the patient's parents. A 7-year-old Japanese DMD boy with dystrophin exon 44 deletion was enrolled. During one administration session AO85 at a dose of 0.5 mg/kg was infused intravenously at one-week interval for 4 weeks. And the session was repeated six times during three years. After 4-week administration muscle sample was obtained and dystrophin mRNA analysis by RT-PCR disclosed partial skipping of exon 45. 6MWD test was done before and after every session. In the first session at 7.5 years old 6MWD increased to 434 m from 360 m at baseline (net 74 m increase). In the last session at 10.7 years old 6MWD at the baseline was 217 m by the progression of the disease but it increased again to 267 m (net 50 m increase). The increase was observed at all 6 sessions. As a result 6MWD increased significantly from 319±56 m to 351±59 m (mean±SD) (p<0.05). Our results show for the first time that intravenous administration of AO85 provides the significant increase of 6MWD in DMD. Ambulation improvement by AO85 administration encourages continuing the clinical study.

AO85, ENA, exon skipping, antisense oligonucleotide, dystrophin

Dystrophinopathies (Duchenne, Becker, others)- #2549

P08- 112- FUBP1 modulates several alternative splicing events in the DMD transcript

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While the modulation of exon skipping in the DMD transcript is a promising therapeutic approach, the DMD splicing code is still far from being elucidated. By focusing on the splicing defects induced by a nonsense mutation, we recently uncovered the enhancing role of FUBP1 in the splicing regulation of DMD exon 39. We showed that FUBP1, whose splicing properties were largely unknown, binds to an UG-rich Intronic Splicing Enhancer upstream from exon 39. We investigated the involvement of FUBP1 in the splicing regulation of 2.2 Mb DMD pre-mRNA. The C25si48 normal human muscular cell line was depleted from endogenous FUBP1 using siRNA treatment and maintained in differentiation medium for 3 days prior to transcripts analysis by RNA-sequencing. A DMD-targeted approach was chosen to obtain uniform coverage and appropriate sequencing depth. The 11.3 kb DMD cDNA was specifically amplified by Long-Range PCR, and sequenced using the 454 GS junior sequencer. The Alternative Splicing Events (ASEs) detected in cells either knocked down for FUBP1 or treated with a control siRNA, were compared by differential splicing analysis. The first sequencing experiments highlighted the role of FUBP1 as either an enhancer or a repressor in several mono- or multi-exon skipping events. Notably, its involvement for exon 39 inclusion was also confirmed. These data are being experimentally validated by semi-quantitative RT-PCR.

By showing that FUBP1 modulates several ASEs in the DMD gene, these results further assess the splicing properties of FUBP1. We are now verifying by gene expression arrays that the depletion of FUBP1 does not modify the expression of other crucial splicing factors. Then, using biochemical approaches, we will look for FUBP1 binding sites to precisely describe the FUBP1-related cis-regulatory sequences in the DMD pre-mRNA.

DMD, splicing, FUBP1, RNA-seq

Dystrophinopathies (Duchenne, Becker, others)- #2555

P08- 113- The short isoform of dystrophin, Dp116, owns an extracellular matrix organization function in Drosophila melanogaster

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Dystrophin (dys) loss leads to the progressive muscle-wasting disease Duchenne Muscular Dystrophy (DMD). Dys creates a link between the actin cytoskeleton and the extracellular matrix (ECM) giving it a mechanical function that has been largely described. However, expression in muscle of Dp116, a short isoform of dys unable to interact with actin, in dys-deficient mice
We have identified that dys is required for the formation of ECM fibers in the basement membrane of follicular epithelial cells. Strikingly, the absence of ECM fibers in dys mutants is rescued by expressing human Dp116. Deeper exploration of this function in the ovarian follicle model is in progress. Furthermore, in Drosophila, as in mammals, dys deficiency causes cardiomyopathies and shortens the life expectancy. These defects are also rescued in fly by expressing Dp116 (Taghli-Lamalle et al., 2008). Thus, observation of the ECM in Drosophila heart is also planned to see whether cardiomyopathy might be linked to an ECM defect.

Dystrophin, Dp116, Extracellular matrix

Dystrophinopathies (Duchenne, Becker, others)- #2558

P08-114- In frame exon 45-47 deletion in the DMD gene leads to dramatic structural modification of the tortuous structure of the central domain of dystrophin and loss of nNOS binding

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Duchenne and Becker muscular dystrophies are caused by mutations in the dystrophin DMD gene. Dystrophin is an elongated scaffolding protein critical for maintaining plasma membrane integrity during muscle and heart contraction. The structural bases of its scaffolding function and the effects of mutations have remained elusive owing to a lack of structural data for dystrophin. We determined the three-dimensional structure of the spectrin-like repeat-containing dystrophin central domain using small-angle X-ray scattering and molecular modeling. Eight fragments covering the entire dystrophin central domain were cloned, produced and characterized by SAXS. We show that specifically placed kinks angles within dystrophin result in a tortuous filamentous structure that is far from a rod-like structure by contrast from all previous hypotheses (Figure below). These kinks modify the linear topology of the molecule and thus allow presenting specific interfaces for the interactions of dystrophin. This topology is profoundly modified by the most frequent in-phase mutation found in Becker muscular dystrophy, the deletion of exons 45 to 47. The new junction created by the deletion is highly flexible and is just at the point of the nNOS binding site. By docking the PDZ domain of nNOS on the deletion mutant structure, we show that the binding is lost due to the high flexibility of the new junction. This is in line with the absence of immuno-localization of nNOS that we observed in 5/6 patients with this deletion. This explains why the interaction with nNOS is not possible even though a large part of the nNOS binding site is maintained in the deletion mutant. This is in line with the rather severe phenotype observed for this deletion. This work shows for the first time the structure of the dystrophin central domain and gives the structural basis for understanding why BMD deletions are not equivalent. This is important for the design of mini- or micro-dystrophins and for therapies by exon skipping which aim to transform Duchenne patients in Becker patients.

Dystrophin, Dp116, Extracellular matrix

Dystrophinopathies (Duchenne, Becker, others)- #2561

P08-115- Becker muscular dystrophy severity is linked to the structure of the mutated dystrophin

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In-frame exon deletions of the DMD gene produce internally truncated proteins that typically lead to Becker muscular dystrophy (BMD), a milder allelic disorder of Duchenne muscular dystrophy (DMD). We hypothesized that differences in the structure of mutant dystrophin may be responsible for the clinical heterogeneity observed in Becker patients. We therefore studied six prevalent in-frame exon deletions, i.e. ?45-47, ?45-48, ?45-49, ?45-51, ?45-53 and ?45-55. All these deletions could be obtained by skipping exon 45 in DMD patients. Even though there is no known BMD patient, we studied also the variant ?44-54 which could be the result of skipping of the exon 44 for the DMD deletion of exons 45-54. Molecular homology modelling
revealed that the shortened proteins corresponding to deletions ?45-48, ?45-51, ?45-53 and ?45-55 displayed a regular succession of canonical coiled coil repeats similar to the wild type central domain of dystrophin, whereas deletions ?45-47 and ?45-49 lead to proteins with an unrelated structure (fractional repeats). All proteins were expressed in vitro in fragments encoding repeats 16 to 21 or 16 to 24 for the longest and exhibited an alpha helical folded state. We then characterized the biochemical properties, temperature stability and refolding rates, of all the proteins. The proteins ?45-47, ?45-48, ?45-49, ?45-51 and ?44-54 remained highly stable compared to the very stable wild type proteins and compared to the two ?45-53 and ?45-55 which were less stable. Refolding dynamics were similar for ?45-51 and the wild type while the ?45-53 and ?45-55 showed faster refolding kinetics and ?45-47, ?45-48 and ?45-49 refolded slowly. We retrospectively collected data for French BMD patients and reported the age of dilated cardiomyopathy onset and age of wheelchair dependency, classifying the severity of these deletions from the less to the highest severe: ?45-51, ?45-55, ?45-53, ?45-48, ?45-47 = ?45-49. Disease progression in BMD patients appears to be at least partly dependent on the deletion itself and associated with a specific structure of dystrophin at the deletion site. This work shows that all BMD deletions are not equivalent by their severity and by the characteristics of the dystrophin produced. It is an important element to consider for exon skipping strategies and for design of therapeutic mini-dystrophins.

Dystrophinopathies (Duchenne, Becker, others)- #2571

**P08- 116- The Cmah-/-mdx mouse: an early onset model of cardiomyopathy for Duchenne muscular dystrophy**

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Duchenne muscular dystrophy (DMD) is a devastating muscle wasting disorder caused by the lack of an integral structural protein called dystrophin. DMD boys develop severe cardiomyopathy by the second decade of life, and cardiopulmonary complications are a major cause of death amongst patients. The mdx mouse is the most widely used model for DMD, however it does not accurately recapitulate skeletal muscle pathology or the cardiac disease progression observed in patients. As such the CMAH mouse model, with a mutation in the Cmah gene on the mdx background, was developed and reported to show a more severe phenotype than the mdx mouse. However, an extensive comparison of cardiac function, metabolic profile and pathology was not described between the two models. Therefore, we set out to determine whether the CMAH mouse model is a more appropriate cardiac model of DMD. Cardiac function (Magnetic Resonance Imaging) and metabolism (Dynamic Nuclear Polarisation) were measured in CMAH and mdx mice at 12 and 24 weeks of age. Markers of cardiac damage were assessed by qPCR. CMAH mice exhibit pronounced left ventricular (LV) dysfunction, characterised by reduced LV end-diastolic volume, stroke volume and cardiac output. This was accompanied by raised pyruvate dehydrogenase flux which indicates a switch from fatty acid metabolism towards glucose oxidation for energy requirement. Additionally, markers of cardiac damage were significantly raised in CMAH mice at an earlier time-point than mdx mice. These events indicate that CMAH mice display earlier onset of cardiomyopathy, and may thus be a more appropriate model for assessing therapeutic benefit following treatment strategies for the disease.

cardiomyopathy, Duchenne Muscular Dystrophy, MRI, DNP

Dystrophinopathies (Duchenne, Becker, others)- #2576

**P08- 117- Analysis of the dystrophin interactome**

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The aim of the project is to systematically identify the local interactome of dystrophin in differentiated muscle cells, and to understand how the global interactome is affected by dystrophin knock-down. This may subsequently suggest new therapeutic targets, and give new insights into DMD disease processes.

Dystrophin has a well understood role in protecting muscle cell membranes from contraction-induced tearing. Other roles include the organization of the microtubule network, regulation of muscle blood perfusion, lipid binding and vesicle trafficking. Many binding domains of dystrophin have already been identified and implicated in pathologies. Despite the significance of dystrophin’s interactions, a systematic review of dystrophin’s interactome has yet to be carried out.

Using the ‘QUICK’ method for protein interaction screening by quantitative immunoprecipitation combined with knockdown (Selbach, 2008) upon stably immortalised human-derived myoblast cell lines will allow for such a systematic review.

Cells will be cultured in the presence (labelled) or absence (unlabelled) of heavy isotope amino acids. Labelled cells will be treated using siRNA to knock-down expression of dystrophin. Immunoprecipitation using anti-dystrophin antibody will then be applied to both labelled and unlabelled cell samples. Differences in the labelled vs. unlabelled interactome will be determined by mass spectrometry. Labelling will allow the identification of non-specific antibody-binding proteins by comparison of the knock-down with the dystrophin-expressing samples. This method has the advantages of not requiring protein tagging or overexpression, not being prone to false positive or false negative results and in being systematic, is not reliant upon searching for specific partners. By harvesting cells at an early time point when dystrophin is first expressed in the cell, and avoiding protein modifications and overexpression, cells will be as close to there in vivo environment as possible.

Preliminary mass spectrometry results of dystrophin immunoprecipitations identified 2694 distinct proteins, with an increased representation of proteins involved in known dystrophin functions such as muscle contraction, calcium regulation and vesicle trafficking. Additionally incorporating SILAC and protein knock-down, we are in the process of obtaining data with which we can reliably identify all individual proteins pulled down by dystrophin and hence deduce all its functional roles.

dystrophin, interactome, SILAC, QUICK, knock-down

Dystrophinopathies (Duchenne, Becker, others)- #2577
Therapeutic trials using splice switching Antisense oligonucleotides (AON) are under way in Duchenne Muscular Dystrophy (DMD). Depending on the specific dystrophin out of frame deletion, it is possible to synthesize target AONs to induce exon skipping of neighboring exons and induce a restoration of the reading frame, with the production of an internally deleted dystrophin protein (mimicking what occurs in Becker muscular dystrophy, BMD), that should prevent or delay muscle fiber degeneration and ameliorate disease progression. The primary biological endpoint of these AON clinical trials is therefore to induce functional dystrophin in DMD patients. A reliable and reproducible method for quantification of dystrophin protein is crucial to monitor biochemical outcome of such treatments. Our laboratory published the first method for quantifying dystrophin expression by semi-quantitative immunohistochemistry (Arechavala-Gomeza et al., 2010) and we are now optimizing the method to improve its efficiency and coverage by automated scanning of the entire section. To do this, we have analyzed different control muscle samples, both paediatric and adult, to test reproducibility and variability across the sections using a panel of selected antibodies against sarcolemma components (to verify sarcolemma integrity) as well as dystrophin antibodies recognizing different protein epitopes. We have also quantified dystrophin expression in DMD and BMD muscle samples with variable levels of dystrophin expression by acquiring images of the whole transverse section, giving information of the overall muscle sample. The accurate and unbiased quantification of dystrophin expression will help to have a robust pharmacodynamics endpoint in dystrophin restoration therapies. Future correlations with clinical response will eventually determine if dystrophin restoration could also be considered a surrogate endpoint in clinical trials. Arechavala Gomez V. et al. Immunohistological intensity measurements as a tool to assess sarcolemma-associated protein expression. Neuropathol Appl Neurobiol. (2010) 36, 265-274

Dystrophinopathies (Duchenne, Becker, others)-#2593

**P08- 118- Dystrophin as a biochemical outcome measure in Duchenne muscular dystrophy clinical trials**

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Dystrophinopathies (Duchenne, Becker, others)-#2593

**P08- 119- Plasma miRNA profiling of a large DMD cohort providing novel insight into disease molecular mechanisms**

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In 2008, it was reported that primary muscle diseases were associated by modification of miRNA profile in muscle biopsies (Eisenberg et al 2008). Subsequently, extracellular miRNAs and in particular serum/plasma miRNA, were used as indicators (biomarkers) for diagnosis and monitoring in a variety of normal physiological and pathological situations. In a previous study we identified serum miRNA profile dysregulation in a collection of mouse models for muscular pathologies, and detected disease specific and disease-overlapping miRNA profiles. The dystromiRs, miR-1, miR-133, miR-206 as well as miR-378 were shown to be commonly dysregulated in the serum/muscle model for a number of degenerative muscular dystrophies, and in DMD patients (Vignier et al 2013). In a continuation study, focused on the GRMD (DMD dog model), we have identified the dysregulation of additional serum miRNAs, including the cardiomyopathy related miR-208a, miR-208b, miR-499, a large number of the DLK1-DIO3 Genomic imprinted locus clustered miRNA, and miR-95 (Jeanson-Leh et al, 2014).

We are now reporting on the profiling of plasma miRNAs in a large age-stratified cohort of DMD patients, using a non-biased miRNA sequencing technology. The studied cohort was composed of 9 subgroups, including glucocorticoid treated and untreated DMD patients distributed in three age groups, thus providing for the first time comprehensive description of plasma miRNAs expression over time and under treatment in DMD disease. We identified dysregulation of over 90 miRNAs (pFDR > 0.1), between DMD and healthy controls. We identified massive miRNA dysregulation in the 3-8 and 8-12 years old patients, and a drastically reduced overall dysregulation level in older patients. We confirmed the dysregulation of most previously identified dysregulated miRNA in DMD animal models. In addition, we found many new dysregulated miRNAs, providing novel suggestions for molecular mechanisms in DMD disease. Expression of dysregulated miRNAs identified in DMD plasma is currently evaluated in muscle biopsies of mdx mice and GRMD dogs. Commonly dysregulated miRNAs for the plasma on DMD patients and muscle biopsies on DMD animal models may represent attractive therapeutic targets in DMD disease.

**P08- 120- Suramine treatment reduces basal autophagy levels and muscle damage in dystrophic mdx mice**

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Duchenne Muscular Dystrophy (DMD) is a recessive X-linked genetic disease, caused by mutations of the dystrophin gene in humans and mdx mice. We have previously described the role of extracellular ATP on muscle damage characteristic of DMD. Due to the increasing interest in the study of autophagy for the treatment of skeletal muscle diseases, we evaluated the contribution of purinergic receptors to regulate basal autophagy levels in mdx mice. Normal and mdx mice were treated with suramine, a purinergic receptor inhibitor, for one week (60 mg/Kg, daily intraperitoneal injections). After this time mice performed strength tests and basal autophagy levels were measured in different skeletal muscles. Mdx mice increased their strength performance after suramine treatment assessed by the inverted grip-hanging test and exercise tolerance measured with forced swimming and treadmill tests. No changes were observed in normal mice. In addition, the basal level of autophagy was increased after the treatment in flexor digitorium brevis, soleus and diaphragm mdx muscles. This result was in agreement with a decreased level of mRNA expression of autophagy genes such as LC3 and p62, observed after suramine treatment. We also observed a reduced number of central nuclei that correlated with lower levels of serum creatine kinase. This data suggests that suramine ameliorates the muscle damage observed in mdx muscles. We hypothesize that purinergic receptors regulate the expression of autophagy proteins in mdx skeletal muscle cells, probably via IP3 generation and slow Ca2+ waves.

**Dystrophinopathies (Duchenne, Becker, others): #2600**

**P08-121-** Mini-dystrophins for gene therapy of Duchenne Muscular Dystrophy: is the C-terminus important for the function of the protein?  
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Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene, which generally disrupt the translational reading frame. In the milder, allelic form of the disease, Becker muscular dystrophy (BMD), the reading frame tends to remain intact. Gene therapy is a promising treatment option, however the whole gene transcript is too large to be packaged into viral vectors for systemic delivery. This has led to the design of mini-dystrophins and micro-dystrophins, truncated yet functional versions of the gene based on deletions observed in mildly affected BMD patients. Preliminary data from gene transfer experiments suggests that when present, the C-terminus of dystrophin confers additional benefit to mini- and micro-dystrophins in skeletal muscle however the C-terminus has routinely been removed from these constructs to reduce the size of the transcript. Mini-dystrophins were created with identical internal deletions but differing deletions of the C-terminus to investigate the impact of the loss of this domain on the function of the resulting protein. Lentivirus containing the mini-dystrophins or green fluorescent protein (GFP) was produced and used to transduce primary cardiomyocytes isolated from mdx mice. Area measurements of cells were taken to measure the hypertrophic response of the treated and untreated cells. No significant reduction in hypertrophy was observed in cells transduced with mini-dystrophin-containing lentivirus. Unexpectedly, a significant increase in hypertrophy was observed in cells transduced with control GFP-containing virus suggesting that viral transduction itself increases the hypertrophic response of mdx cardiomyocytes. Comparison of the different mini-dystrophin-containing lentivirus effects on the hypertrophic response of mdx cardiomyocytes revealed that the mini-dystrophin maintaining the entire C-terminus caused a greater reduction in size than the mini-dystrophin completely missing this domain. This data suggests that the C-terminus of dystrophin may be important in the optimal functioning of the protein and further studies should be carried out to determine the mechanism behind this apparent benefit.

**Duchenne muscular dystrophy, cardiomyopathy, gene therapy, mini-dystrophins**

**P08-122-** Serum and urine proteomic profiling reveals biomarkers suitable for monitoring the outcome of therapeutic interventions in muscular dystrophies  
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Therapy-responsive biomarkers are an important and unmet need in the muscular dystrophy field where new treatments are currently in clinical trials. By using a comprehensive high-resolution mass spectrometry approach, and antibody arrays and western blot validation, we found that several proteins and protein fragments are abnormally present in sera and urines of Duchenne muscular dystrophy (DMD) patients, limb-girdle muscular dystrophy type 2D (LGMD2D) and their respective animal models. Levels of one the found biomarker, fragments of the myofibrillar structural protein myomesin-3 (MYOM3) were assayed in therapeutic model systems where stable restoration of ?-sarcoglycan expression in KO-SGCA mice was achieved by systemic injection of a viral vector. Following administration of the therapeutic agents MYOM3 was restored toward wild-type levels in a dose-dependent manner. MYOM3 fragments showed lower inter-individual variability compared with the commonly used creatine kinase assay, and correlated better with the restoration of the dystrophin-associated protein complex and muscle force. These data suggest that the MYOM3 fragments hold promise for minimally invasive assessment of experimental...
Dystrophinopathies (Duchenne, Becker, others)- #2603

P08-123- Identification of splicing regulators of DMD pre-mRNA by RNAi-based functional screen and targeted RNA-seq
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Global analyses led to new insights into splicing programs in various tissues including differentiating, developing and adult muscle. However these approaches have provided little information about pre-mRNA splicing of the DMD gene, most likely due to its low expression level in muscle and its complex structure organization (2.2 Mb, 79 exons). Pre-mRNA splicing is a highly complex process. In higher eukaryotes the core splicing signals are partially degenerated and many additional exonic and intronic Splicing Regulatory Elements (SREs) assist the splicing machinery to recognize the appropriate exon-intron junctions. The SREs recruit RNA Binding Proteins that can either facilitate or prevent spliceosome assembly at particular splice sites.

First, we took advantage of advances in RNA-seq technologies to set up a targeted RNA-seq protocol to establish for the first time a comprehensive repertoire of the alternative splicing events (ASEs) occuring in the muscular DMD transcript. Most of the 79 DMD exons were found to be constitutively included in the mature transcript suggesting the presence of an elaborate regulatory network to coordinate splicing in this huge gene. The three major events (alternative splicing of exon 71, exon 78 and inclusion of cryptic exon 1a) were present at least at 5%

Our second objective is to identify the trans-acting splicing factors (SFs) that participate to the definition of the 79 exons of the human DMD gene. The experimental procedure being currently developed relies on the RNAi-based silencing of a set of general and tissue-specific SFs in a human muscle cell line combined with 454-based targeted RNA-seq of the full-length 11.3 kb cDNA sequence. A dedicated bioinformatics pipeline has been set up for the differential analysis of resulting DMD splicing patterns. This functional screen is expected to identify major splicing regulators of human DMD pre-mRNA.

A better knowledge of the regulatory elements that participate to proper recognition of DMD exons is timely and of particular relevance for the current approaches that aim to manipulate splicing for therapy of Duchenne Muscular Dystrophy.

DMD, splicing, RNA-seq, RNAi

Dystrophinopathies (Duchenne, Becker, others)- #2604

P08-124- Outcome measures for Duchenne muscular dystrophy from ambulant to non-ambulant: implications for clinical trials
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Introduction: Novel emerging therapies for Duchenne Muscular Dystrophy (DMD), such as antisense oligomer (AO) mediated exon skipping, have generated the need of understanding the natural history study of the targeted genotype subgroups. Most natural history studies are focused on ambulant subjects, therefore very little data exists on non-ambulant DMD. Specifically targeting skippable deletions, we aim to assess the natural history of DMD through a composite assessment tool capable of capturing disease progression linking ambulant and non-ambulant phases of the condition.

Methods: With a recruitment target of 80 DMD with AO-skippable-mutations across 5 centres (London, Newcastle, Paris, Leiden and Nijmegen), subjects are assessed 6 monthly for 3 years according to an internationally agreed shared protocol, including the 6-minute walk distance (6MWD) the NorthStar Ambulatory Assessment (NSAA) as well as the Performance of Upper Limb (PUL) and the MyoSet (i.e. MyoGrip MyoPinch and MoviPlate). Both ambulant and non-ambulant subjects undergo upper limb evaluation and respiratory function test. We explored relationships between measures across the ambulant and non-ambulant stages. Exploratory biomarkers are also being evaluated.

Results: To date we analysed 60 DMD patients, ranging from 5 to 19 years old, 37 ambulant and 23 non-ambulant, half with 24-month follow-up data. The ambulant boys >7 years of age declined 20 meters/year (p<0.01) in relation to the 6MWD and 1.7 NSAA points/year (p<0.01). The non-ambulant boys over 1 year lost 4.3 PUL scores (p<0.001) and 6% in FVC% (p<0.01). In relation to MyoGrip strength, the yearly loss was 340gr in the non-ambulant population (p<0.01). We further compared MyoGrip strength with age/gender matched healthy controls; the percentage difference from expected mean was -45% for ambulant and -81% for non-ambulant boys. With this initial analysis we observed a correlation between the shoulder domain of the PUL and NSAA (r=0.58, p<0.001) and the 6MWD (r=0.55, p<0.01); and between MyoGrip strength and total PUL score (r=0.57, p<0.001). Analysis of 2 and 3 year follow up data is on-going.

Conclusion: Our on-going study offers a comprehensive concurrent natural history data including the non-ambulant DMD population with skippable mutations amenable to AO therapies.
Acknowledgments: L'Association française contre les myopathies (AFM) is greatly acknowledged for funding this study.

Performance of upper limb, Myoset, exon skipping, NorthStar Ambulatory Assessment, 6-minute walk distance

Dystrophinopathies (Duchenne, Becker, others)- #2823

P08-126- Structural model of the complex formed by Dystrophin R16-17 and nNOS-PDZ revealed the importance of R17 helix C and nNOS-PDZ beta-finger for the interaction

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Duchenne and Becker muscular dystrophies (DMD and BMD) are caused by mutations in the dystrophin DMD gene. The dystrophin protein connect the muscle membrane to cytoskeleton filaments through several specific binding domains, including two Actin-binding domains and a b-dystroglycan domain. Recently, it has been shown that dystrophin repeats 16-17 (R16-17) are involved in the binding of the nitric oxide synthase µ through specific recognition of its PDZ subdomain (nNOS-PDZ). However, the understanding of the association between PDZ nNOS and dystrophin R16-17 at a molecular level seems elusive regarding the lack of structural information about the dystrophin central domain. In this study, we focused on the characterization of the molecular regions involved in the binding of nNOSµ by the dystrophin R16-17. Alanine scanning experiments turning charged amino acids of R16-17 region into alanine generated a series of 30 dystrophin mutants. Biolayer interferometry was used to measure the affinity of these mutants with nNOS PDZ domain, leading to the precise determination of the dystrophin amino acids involved in the binding of nNOSµ. In accordance to these measurements, and by a combination of flexible fitting experiments based on Small-Angle Scattering Data and docking simulations, we propose the first high-resolution model of the dystrophin R16-17?nNOS-PDZ complex. The structural model suggest that the nNOS-PDZ domain binds with R16-17 through an extended molecular surface comprising its beta-finger subdomain, specific of the nNOS isoform µ. Dystrophin regions involved in the nNOS binding are the linker region located between repeat R16 and R17, and previously identified in vivo as the nNOS primary binding site, but also a loop of repeat R16 and the terminal helix of the repeat R17. Finally, our structural model of dystrophin R16-17?nNOS-PDZ complex also bring some clues about the origin of the various phenotype profiles encountered in the BMD patients despite their conservation of the nNOS primary binding site.

Duchenne Muscular Dystrophy, Becker Muscular Dystrophy, Dystrophin, nNOS

Dystrophinopathies (Duchenne, Becker, others)- #2849

P08-127- The anti-apoptotic CED-9/Bcl-2 protein protects against muscle degeneration in Caenorhabditis elegans models for muscular dystrophies

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Muscular dystrophies are characterized by muscle weakness and degeneration induced by genetic mutations affecting muscle structure or signaling components. The subcellular mechanisms of muscular dystrophies are poorly understood and the development of treatments is challenging, in part due to great variety of primary genetic defects.

Using different Caenorhabditis elegans models that mimic human muscular dystrophies such as Duchenne and Becker dystrophies, Limb-Girdle, Emery- Dreifuss or congenital muscular dystrophy, we found that a gain of function mutation of the anti-apoptotic protein CED-9, ortholog of mammalian B cell lymphoma 2 (Bcl-2), strongly reduced muscle degeneration. To investigate the molecular pathways, we analyzed the impact of mutations affecting key effectors of programmed cell death (PCD) mechanisms such as CED-3/caspase-3, CED-4/Apaf-1, CED-9/Bcl-2, CED-13 and EGL-1 (two orthologs of pro-apoptotic Bcl-2 protein family) on muscle degeneration in the Duchenne Muscular Dystrophy (DMD) C. elegans model. We evaluated different features of muscle cell death: destruction of actin filament network, nuclei loss, mitochondrial stress by measuring ROS production with Hyper transgene; and the state of mitochondrial network. Using CRISPR mediated genome engineering we currently determine domains of protein interactions between CED-9 and the different PCD effectors so as to reveal new PCD actors involved in muscle degeneration through the investigation of CED-9 partners.

Finally, by deciphering the role of the PCD machinery and the anti-apoptotic CED-9 protein in different C. elegans models for muscular dystrophies, we aim at identifying common molecular pathways to reduce, stop or reverse muscle degeneration in muscular dystrophies.

CED-9/BCL-2, muscle degeneration, Programmed cell death
Dystrophinopathies (Duchenne, Becker, others)- #2921

P08-128- Development and implementation of serum biomarkers in Duchenne Muscular Dystrophy.

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Introduction and Objectives:
Promising therapeutic strategies for Duchenne Muscular Dystrophy (DMD) are increasingly under development, with many now entering clinical trials. However, moving forward with clinical trials, using these new generation therapies, has been slow owing to the lack of reliable outcome measures to monitor treatment efficacy. The aim of this study is to define serum protein biomarkers that can reliably predict disease progression and response to therapy in DMD patients. Such biomarkers can be used to aid future drug development programs in DMD.

Methods:
Serum samples were collected from both mouse models for Duchenne and from DMD patients at different ages, disease stages and treatment conditions. Two complementary proteome profiling approaches were used for discovery and monitoring of levels of DMD associated biomarkers. Mass spectrometry based proteome profiling using SILAC and label free strategies, and SomaScan aptamer technology. ELISA assay was used as technical validation.

Results:
Using mass spectrometry and SomaScan proteome profiling approaches we completed a full ‘biomarker discovery’ phase for DMD. A total 67 protein biomarkers were identified in DMD sera samples and more than 50 % of these were concordant between DMD patients and dystrophin deficient mdx mouse models. These biomarkers can be classified into four major groups including myofibrillar biomarkers reflecting muscle fiber leakage, inflammation biomarkers, growth factor and fibrotic biomarkers. Levels of these biomarkers changed with age and disease progression. A set of these biomarkers responded to corticosteroids and to dystrophin replacement therapies in specific manner and their levels correlated with treatment outcome.

Conclusion:
Reliable biomarkers sensitive to DMD disease progression and response to treatments were identified using both mdx mouse model and DMD patients. These biomarkers could prove valuable to implement in present and future clinical trials.

Duchenne muscular dystrophy, proteomics, biomarkers, morpholino, corticosteroid

Dystrophinopathies (Duchenne, Becker, others)- #2926

P08-129- Second generation utrophin modulator for the therapy of Duchenne muscular dystrophy


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Duchenne muscular dystrophy (DMD) is a devastating, X-linked muscle-wasting disease caused by lack of the cytoskeletal protein dystrophin. There is currently no cure for DMD although various promising approaches (e.g. stop codon readthrough, exon skipping, gene therapy) are progressing through human clinical trials. By pharmacologically modulating the dystrophin-related protein utrophin, we aim to develop a therapy applicable to all DMD patients, regardless of the underlying genetic fault in the dystrophin gene, by targeting the primary defect and restoring sarcolemmal stability. In partnership with Summit Therapeutics, we previously developed SMT C1100, an oral utrophin modulator that reduces dystrophic symptoms in the mdx mouse and successfully completed a Phase 1b trial with an excellent safety profile in DMD patients.

The successful progression of SMT C1100 to date provides validation for the strategy we developed. We are now optimising second generation utrophin modulators with improved physicochemical properties and a more robust metabolism profile. Pre-clinical in vivo studies in the mdx mouse demonstrate that daily oral administration of these compounds increases utrophin expression in target muscle groups, including the diaphragm and heart. This results in improved sarcolemmal stability and prevents dystrophic pathology through a significant reduction of regeneration, necrosis and fibrosis with no change in fibre type composition. These improvements combine to provide wide ranging benefits, including protection from muscle fibre leakage, prevention of regeneration and necrosis, and protection of muscle function from contraction induced damage. The small molecule modulation of utrophin targeting skeletal, respiratory and cardiac muscles emphasises the potential of utrophin modulation as a disease-modifying therapeutic strategy for all DMD patients, irrespective of their mutation in dystrophin.

Duchenne, mdx, utrophin, treatment, pharmacology

Dystrophinopathies (Duchenne, Becker, others)- #2931

P08-130- Remudy, Japanese national registry for neuromuscular diseases, as a clinical utility model for various rare disease registries with international harmonization.

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Background

Recently, rare disease registries are recognized as an important tool of clinical research. One example is the Global dystrophinopathy patient registry, a harmonized registry from over 30 national registries (http://www.umd.be/TREAT_DMD/). An achievement made possible through collaboration with TREAT-NMD, which was established to accelerate the international clinical developments in neuromuscular diseases.

Objective

To provide an exemplar on the clinical utility of national patient registries in the rare neuromuscular disease field and share our knowledge to manage these registries with international collaboration.

Methods

Remudy was established in collaboration with national neuromuscular experts, patient advocacy groups, and the National Center of Neurology and Psychiatry in 2009. The launch featured the Japanese national Dystrophinopathy registry which was followed by a national GNE myopathy registry in 2012 and national Myotonic Dystrophy (DM) registry in 2014. Remudy uniquely took 1) Patient-reported system, 2) Genetic and Clinical curators, 3) Information committee to judge enquiry from third parties, 4) Following the Charter for TREAT-NMD Patient Database/Registry, and 5) Co-working with MDCTN (Muscular Dystrophy Clinical Trial Network in Japan).

Results

By September 2015, total registrants were 1,935 (dystrophinopathy: 1,446, GNE myopathy: 170, and DM: 319). Remudy provides information regarding clinical research and medicines for registrants; detailed information about natural history, epidemiology, and clinical care. It has contributed to feasibility studies and trial recruitments with TREAT-NMD or domestic groups nearly 20 times. Furthermore, Remudy shared management know-how with the Chinese national neuromuscular disease registry launched in 2012.

Conclusion

Remudy has demonstrated a clinical utility model to inform new web-based patient registry system in other rare diseases with not only in Japan but other nations.

Remudy, TREAT?NMD global registry, Dystrophinopathy, GNE myopathy, Myotonic dystrophy, clinical trial

Dystrophinopathies (Duchenne, Becker, others)- #2936

P08-131-Functional and NMR variables give a complementary picture of upper limb in Duchenne Muscular Dystrophy

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As therapeutic strategies are developed in Duchenne muscular dystrophy (DMD), the need for robust outcome measures to assess the effects of these interventions through the different stages of the disease is increasingly crucial. Most clinical drug studies are conducted in ambulatory patients. The extension of efficacy data to nonambulatory patients, in whom muscular tissue is more damaged, remains challenging. Given the potential side effects and the very high cost of innovative therapies, evaluation of efficacy in nonambulatory patients is essential. The main aim of this study was to explore the value of nuclear magnetic resonance (NMR) and functional assessments for the follow-up of ambulatory and nonambulatory patients with Duchenne muscular dystrophy (DMD).

Twenty-five 53-skippable DMD patients were included in this study; 15 were nonambulatory at baseline. All patients underwent clinical and functional assessments every 6 months using the Motor Function Measure (MFM), hand grip and key pinch strength, MoviPlate and NMR spectroscopy and imaging studies.

Upper limb distal strength decreased in nonambulatory patients over the period of one year; ambulatory patients showed improvement during the same period. The same applied for several NMRS indices, such as PCR/ATP, which decreased in older patients but increased in younger ambulatory patients. Fat infiltration in the upper limbs increased linearly with age. Almost all NMR and functional muscle assessment results correlated. The different outcome measures are often presented as competing whereas the current work shows the value of their complementarity. Sensitivity to change of various indices may differ according to disease stage. Investigators should consider complementary outcomes to not miss changes that cannot be detected by a given variable but that can be perceived by another. Moreover the variables used in the present study can be used independently of the ambulatory status of the patient.
Duchenne Muscular Dystrophy, outcome measures, upper limb

Dystrophinopathies (Duchenne, Becker, others)- #2953
P08-132- Rimeporide, an oral agent targeting NHE-1 in development for treatment of Duchenne Muscular Dystrophy: a Translational development
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Blocking NHE-1 activity, has been shown to decrease intracellular Na+ and Ca2+ overload and pH and rimeporide represents a promising therapeutic agent for DMD. Positive effects on skeletal, respiratory and cardiac muscles were shown in preclinical models. In vitro, Rimeporide restored resting pH and decreased store operated calcium entry in dystrophic myotubes of mdx mice. In the cardiomyopathic hamsters, rimeporide prevented hypertrophy, thrombosis and necrosis in the heart. Its cardio-protective effect being most clearly demonstrated by a significant increase in overall survival of treated animals. When tested in mdx mice, rimeporide significantly and consistently prevented both inflammation and the accumulation of fibrosis in skeletal muscle, in the diaphragm and in the heart. It also had positive effects on myofiber size and oedema. Based on robust and consistent preclinical testing in multiple species and based on its good safety and tolerability profile in healthy adults, Rimeporide is now tested in young ambulant DMD boys to assess the safety and PK and also to explore non-invasive biomarkers. Consultations with key clinicians in Europe, regulatory advice from EMA as well as Treat NMD Advisory Board helped to design this ongoing phase I multiple ascending dose study in young patients with DMD.

Rimeporide is available as an oral treatment which is expected to be muscle-sparing. It is mutation independent and was shown to have a safety profile that enables early intervention and could therefore be used to treat a broad population of patients with DMD. Given the complexity of DMD, it is unlikely a single agent will be effective over the entire course of disease and in all patients. Rimeporide’s potential to address skeletal muscle inflammation and fibrosis and cardiomyopathy makes it an ideal complement to other treatments designed to augment or replace dystrophin/utrophin.

Rimeporide has obtained an orphan drug designation in Europe. The ongoing translational development in patients with DMD as well as in GRMD Dogs will enable to uncover novel biomarkers that will help to design the pivotal clinical development of rimeporide.

Rimeporide, Duchenne Muscular Dystrophy, Oral, NHE-1, Anti fibrotic, anti inflammatory, Cardioprotection

Dystrophinopathies (Duchenne, Becker, others)- #2954
P08-133- Protein kinase C theta as a novel molecular target to counteract inflammation in muscular dystrophy
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The aim of this study is to establish, using mdx mice as a model of Duchenne Muscular Dystrophy (DMD), whether targeting Protein kinase C theta (PKCtheta) can be proposed as a novel anti-inflammatory approach in the treatment of muscular dystrophy. PKCtheta is a member of the PKC family of kinases, and it is predominantly expressed in skeletal muscle where it is involved in several intracellular signalling pathways that regulate muscle homeostasis. In addition, PKCtheta acts at the level of the immunological synapse as a critical regulator of T cell activation and proliferation. Indeed, previous studies have shown that inhibition of PKCtheta is associated with a beneficial effect on chronic inflammation and autoimmunity without compromising the immune response against viruses.

Inflammation is now recognized as an important player in the onset and progression of DMD. However, the exact role of the innate and adaptive immune response in disease progression and regulation of muscle fiber regeneration is still unclear. We recently observed that lack of PKCtheta in our bi-genetic mouse model, MDX/PKCtheta-/-, greatly improved muscle fiber integrity, regeneration and strength, and decreased inflammation and wasting. The observed improvement was primarily due to lack of PKCtheta in bone marrow-derived cells. Indeed, our preliminary data suggest that lack of PKCtheta is associated with a significant reduction in the frequency of CD45+ leukocytes (CD3+ lymphocytes and F4/80+CD11b+ macrophages) in dystrophic...
muscle during the early stages of the disease. This reduction in inflammatory cell infiltration was associated with a reduction in muscle fiber damage and increased regeneration potential. Furthermore, treatment of mdx mice with a PKCtheta specific inhibitor led to a similar improvement of the dystrophic phenotype, highlighting the potential of PKC theta as a novel therapeutic target in the treatment of muscular dystrophy.

**Duchenne Muscular Dystrophy, Inflammation, Protein Kinase C theta.**

Dystrophinopathies (Duchenne, Becker, others)- #2961

**P08-134- Isolation and characterization of human urinary cells from healthy donors and DMD patients as in vitro cell model for functional studies and drug testing**

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Duchenne muscular dystrophy (DMD) is a rare hereditary disease characterized by muscle weakness, cardiomyopathy with a severe disease course. The disease is due to mutations in the dystrophin gene. Novel therapeutic opportunities based on gene and molecular therapy include autologous and heterologous stem cells transplant, gene therapy using various dystrophin constructs, PTC124 correcting nonsense mutations, various formulations of antisense oligoribonucleotides to reframe the protein, and novel drugs (utrophin up-regulation, myostatin, others) that address some dystrophin related pathways. It should not be forgotten the corticosteroid treatment that remains, together with the now approved orphan drug Translarna? (Ataluren), the only treatment provisionally approved as orphan drug by Regulatory Authorities. These novel therapies would greatly benefit of an enhanced efficiency in terms of dystrophin rescue amount. Therefore there is the need to identify novel molecules able to enhance efficacy but with no toxicity and biomarkers to be used as surrogate endpoint of the trial efficacy.

Patient-derived human induced pluripotent stem cells (hiPSCs) are a promising and ideal cell source for drug discovery, and urinary stem cells can be accessed via an easy and non-invasive approach by low-cost procedure.

We have isolated and characterized the urinary cells provided by 4 healthy volunteers and 4 DMD patients. The cells found in human urine displayed a mesenchymal stem cell morphology: type I colonies show a regular smooth-edged surface, whereas type II cells appear with the typical elongated shapes in both healthy donors and DMD patients (figure 1).

We also evaluated the expression of cell surface markers for mesenchymal and stem cells (CD105, CD117), hematopoietic and lymphocyte line (CD3, CD4, CD8). The quantification of Oct4 and nanog reprogramming factors was performed by RT-PCR analysis.

The results of this study may provide the scientific and technological know-how for a personalized strategy for the utilization of urine-derived iPS in DMD patients, leading to the development and banking of therapeutic bioproducts suitable for autologous clinical application, as in vitro model for drug efficiency tests, pre-screening studies and biomarkers identification.

**Duchenne muscular dystrophy, urine stem cells, in vitro cell model, biomarkers**

Dystrophinopathies (Duchenne, Becker, others)- #2986

**P08-135- EPIGENETIC ANALYSIS OF THE DMD LOCUS REVEALS NOVEL CIS-ACTING DNA ELEMENTS THAT GOVERN MUSCLE DYSTROPHIN EXPRESSION**

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The Dystrophin gene (DMD) is the largest gene in the human genome, mapping on Xp21, spanning 2.2 Mb and accounting for approximately 1% of the entire human genome. Mutations in this gene cause Duchenne and Becker muscular dystrophy, X-linked dilated cardiomyopathy, and other milder muscle phenotypes. Beside the remarkable number of reports describing DMD gene expression and the pathogenic consequences of the gene mutations in dystrophinopathies, a definition of the full scenario of the DMD transcription dynamics remains poorly understood. Considering that the full transcription of the DMD gene requires about 16 hours, we have investigated the activity of RNA Polymerase II along the entire DMD locus in the context of chromatin modifications using different chromatin-based techniques.

Our results unveil a surprisingly powerful processivity of the RNA pol II along the DMD locus with just one site of pausing around intron 59. More importantly, epigenetic marks highlighted the existence of four novel cis-DNA elements, two of which, located within intron 34 and exon 45, appear to govern the architecture of the DMD chromatin with implications on the expression levels of the muscle dystrophin mRNA.
Our findings provide a global view on how the DMD locus is dynamically transcribed by the RNA pol II and shed light on the mechanisms involved in dystrophin gene expression control, which can positively impact on the optimization of the novel therapeutic strategies for dystrophinopathies.

**Dystrophin gene, DMD chromatin modifications, RNA pol II**

Dystrophinopathies (Duchenne, Becker, others)- #2988

**P08-136- Preliminary results of magneto-inertial motion analysis in Duchenne muscular dystrophy ambulant patients**

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Clinical trials in Duchenne muscular dystrophy (DMD) face challenges with the variability of the most commonly used primary outcome measure, the six-minute walk test (6MWT). Indeed, since a 15% variability at baseline (+/- 60 meters) is commonly considered as acceptable, this variability overpasses by far the commonly accepted clinically significant one-year change of 30 meters.

The primary objective of our study is the validation of continuous real-life motion actimetry as determined by a new leading-age magneto-inertial sensor (ActiMyo®) as an outcome measure in trials for ambulant DMD children. The choice of variables that are clinically pertinent and computable from measurements is of main importance.

Firstly, we successfully measured the distance walked by a cohort of 17 DMD patients as measured by the ActiMyo® worn on the ankle during the 6MWT in controlled setting. Each step was detected automatically, as well as the step length and the moving speed (advanced variables calculated in several stages from the inertial sensor measurements).

Secondly, in order to assess variability in a non-controlled setting, we used the SRP 4053-101 protocol (SKIP-NMD FP7 project) baseline data of ten patients.

A new algorithm was developed to calculate the variability of selected variables, by comparing adjacent intervals of data and estimating the variability as a function of the recording duration. This algorithm was applied to the first 4 weeks recorded by the ActiMyo® worn on the ankle during the 6MWT in controlled setting. Each step was detected automatically, as well as the step length and the moving speed (advanced variables calculated in several stages from the inertial sensor measurements).

Secondly, in order to assess variability in a non-controlled setting, we used the SRP 4053-101 protocol (SKIP-NMD FP7 project) baseline data of ten patients.

Afterwards, we found good correlations between the distance of the 6MWT at baseline and the mean step speed, mean step length, and other variables from the ActiMyo® home recording.

With our study, we showed that Actimy® precisely measures variables such as step speed and step length. Our method to assess variability indicated that a standard deviation of ActiMyo® variables under 2% can be achieved in a 2-week period of home recording. The ?real life? variables from the home recording were correlated with the 6MWT.

Longitudinal data are required to assess sensitivity to change (positive and negative) as benchmarked by the 6MWT.

**Duchenne muscular dystrophy, ambulant, magneto-inertial**

Dystrophinopathies (Duchenne, Becker, others)- #2989

**P08-137- RNA profiling discloses a link between circadian genes and muscle damage in Duchenne Muscular Dystrophy**

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The muscular dystrophies (MD) are a group of inherited genetic conditions that cause progressive weakness and loss of muscle mass. Mutations occurring in critical structural proteins such as dystrophin cause changes in the mechanical stability of muscle fibers that interfere with the muscles’ ability to function. Over time, this causes increasing disability. Circadian rhythm coordinates biological processes with the predictable 24 h cycle of day and night. It is known the role of circadian rhythm genes in maintaining the regular muscle functions both in animal models and in humans. However, the role of circadian genes in muscular dystrophy is still undefined.

The aim of this study was to define the global circadian transcriptional profile in mdx, unexercised mdx and exercised mdx mice treated with different types of drugs, including: resveratrol, apocynin, taurine, nandrolone, prednisolone, enalapril, calpain inhibitor, pentoxifylline and antisense oligoribonucleotides.

We designed an ad-hoc Micro Fluidic Card TaqMan based assay, transcription profile including 30 genes (FLUID-CIRC) related to circadian rhythms and muscle regeneration. We tested Gastrocnemius (GC) and tibialis anterior (TA) muscles from both unexercised and exercised mdx mice (the exercise leads to increased muscle damage).
The vast majority of analyzed circadian genes is strongly upregulated in both exercised and unexercised mdx mice. Statistical analysis prioritized seven most deregulated genes (CSNK1E, SIRT1, MYOG, MYOD1, CRY1, CRY2 and ARNTL) in both TA and GC tissues. We therefore further evaluated these selected genes in both exercised mdx mice treated with different types of drugs, which are able to ameliorate the dystrophic muscle phenotype. We demonstrated that drug exposures also induce modification of the expression profile of circadian genes. Finally we identified that MYOG, CSNK1E, and SIRT1 genes are constantly upregulated in DMD patients.

Our data demonstrate that the circadian genes are affected in both DMD patients and mdx mice supporting a correlation between circadian circuit and DMD pathology. This may lead to novel interesting therapeutic options, since many clinically used drugs are able to modulate circadian rhythm.

**DMD, circadian genes, muscle damage**

Dystrophinopathies (Duchenne, Becker, others)- #2992

**P08- 138- DMD in the clinical setting: Which assessment when?**

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Background: Duchenne Muscular Dystrophy (DMD) is the most common form of muscular dystrophy seen in children. In DMD the protein dystrophin, which is essential for muscle fibre stability, is missing resulting in muscular damage, progressive muscle wasting and weakness.

In the UK, the NorthStar Ambulatory Assessment (NSAA) is the standard clinical assessment for ambulatory DMD boys. The Egen classification scale (Ek2) subjectively assesses functional activities in the non-ambulant population however relies heavily on patient/parent recall and interpretation.

DMD boys are subject to fatigue and reduced concentration during prolonged assessments; therefore introducing correct assessments at optimal time will provide best outcomes with minimal fatigue and concentration effect. An effective, efficient, timely assessment would have great benefit in all DMD patients, especially those with learning or concentration difficulties.

**Aim:** This study aims to assess the optimal disease stage of introducing the Performance of the Upper Limb (PUL) assessment within the clinical and research setting.

**Method:** This is an ongoing study with a recruitment target of 80 DMD patients across 3 sites (London, Newcastle, Paris) between 5 to 18 years. Longitudinal data is collected for the NSAA, PUL and Ek2 scales 6 monthly for 3 years.

**Results:** To date 60 DMD subjects have been recruited, ranging from 5 to 20 years, ambulant to non-ambulant, half with 24 month follow up data. Preliminary results show a decrease in NSAA score prior to any detectable decline in the PUL total score or Ek2 data. Further analysis will investigate the relationship between NSAA, PUL and Ek2 to ascertain if there is a natural continuum of decline detectable with these three assessment measures.

**Conclusion:** Data collected for NSAA, PUL and Ek capture the functional ability of the DMD population through ambulation to non-ambulation. With adequate data available, the relationship between the above assessments can be analysed, leading to the possibility of a smooth transition between assessments depending on functional status.

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PI's: Francesco Muntoni, Volker Straub, Thomas Voit, Imelda de Groot, Erik Niks

**DMD, Function, Assessments**

Dystrophinopathies (Duchenne, Becker, others)- #3010

**P08- 139- The effect of low dystrophin levels on the function of the mouse neuromuscular synapse**

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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. In addition dystrophin is enriched at the postsynaptic membrane of the neuromuscular junction (NMJ) and its absence leads to morphological and functional synaptic changes. It is unknown which dystrophin levels are sufficient to prevent these NMJ abnormalities. To study this, we compared NMJ morphology and function of the mdx-Xist?hs mice, expressing low dystrophin levels (1-20%) as a consequence of non-random X-inactivation with mdx mice, which lack dystrophin, and two control strains, C57BL/10ScSnJ and Xist?hs. When tested in vivo the mdx and mdx-Xi?hs mice both showed muscle weakness compared to controls. In vivo repetitive nerve stimulation electromyography showed a modest decrement of the compound muscle action potential amplitude in mdx mice, suggesting mild NMJ dysfunction. This decrement was partially restored in mdx-Xi?hs mice, though not to wild type levels. Miniature endplate potentials (MEPPs) and endplate potentials (EPPs) were measured in micro-electrode studies. As compared to controls, mdx and mdx-Xi?hs NMJs showed smaller MEPP amplitudes, while having similar EPP amplitudes. Consequently, calculated quantal content (i.e. the number of acetylcholine quanta released per nerve stimulus) was considerably increased. High rate nerve stimulation induced a more pronounced EPP rundown in both mdx and mdx-Xi?hsNMJs. The latter showed a trend towards wild type level as a result of the dystrophin level. NMJ morphology study revealed fragmented acetylcholine receptor area in mdx mice, which was partially resolved in mdx-Xi?hs mice, depending on the level of dystrophin. Overall, our
results indicate that low dystrophin levels (>20% of normal) seem to partially prevents morphological aberrations at the NMJ, but are not sufficient to completely prevent the functional NMJ abnormalities associated with dystrophin absence.

Dystrophinopathies (Duchenne, Becker, others)- #3033

P08-140-Suramine treatment reduces basal autophagy levels and muscle damage in dystrophic mdx mice

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Duchenne Muscular Dystrophy (DMD) is a recessive X-linked genetic disease, caused by mutations of the dystrophin gene in humans and mdx mice. We have previously described the role of extracellular ATP on muscle damage characteristic of DMD. Due to the increasing interest in the study of autophagy for the treatment of skeletal muscle diseases, we evaluated the contribution of purinergic receptors to regulate basal autophagy levels in mdx mice. Normal and mdx mice were treated with suramine, a purinergic receptor inhibitor, for one week (60 mg/Kg, daily intraperitoneal injections). After this time mice performed strength tests and basal autophagy levels were measured in different skeletal muscles. Mdx mice increased their strength performance after suramine treatment assessed by the inverted grip-hanging test and exercise tolerance measured with forced swimming and treadmill tests. No changes were observed in normal mice. First, we determined that the decrease in basal autophagy levels in mdx was due to an increase in basal flux. Second, we have shown that the basal level of autophagy was diminished after suramine treatment in flexor digitorium brevis, soleus and diaphragm mdx muscles. This result was in agreement with a decreased level of mRNA expression of autophagy genes such as Lc3 and p62, observed after suramine treatment. In mdx diaphragm muscle, the more affected muscle in DMD, lci3II formation decreased about 60% (p>0.05) after suramine treatment but no change was observed for the expression of p62. Moreover in suramine treated mdx diaphragm the mRNA content of both lci3 and p62 decreased about 50% and 60% respectively (p>0.05). We also observed a reduced number of central nuclei that correlated with lower levels of serum creatine kinase. This data suggests that suramine ameliorates the muscle damage observed in mdx muscles. We hypothesize that purinergic receptors regulate the expression of autophagy proteins in mdx skeletal muscle cells, probably via IP3 generation and slow Ca2+ waves.

autophagy flux, gene expression, ATP signalling, Purinergic receptor

Dystrophinopathies (Duchenne, Becker, others)- #3060

P08-141-Ambulatory capacity and disease progression in Duchenne muscular dystrophy: comparison of subjects enrolled in the drisapersen study PRO051-02 with a matched natural history cohort

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Duchenne muscular dystrophy (DMD) is a rare life-limiting genetic disorder caused by mutations in the dystrophin gene. Drisapersen (DRIS) is an antisense oligonucleotide that induces exon-51 skipping to produce dystrophin protein. PRO051-02 (NCT01910649) was an open-label extension (N=12) of the dose-escalation DRIS study; the median (mean [standard deviation; range] change from baseline to Wk 177 in 6-minute walking distance (6MWD) was 8 m(-24.5 [161; -263, 163] for subjects (n=10) able to complete the test at baseline. A natural history (NH) population, treated with daily corticosteroids, was evaluated and baseline characteristics matched with DRIS-treated subjects (from PRO051-02) to provide context for the study results as an indirect comparator arm. Subjects were matched by age (+6 months) and 6MWD (+30 m) and data from a 188 week data cut were compared in the primary analysis. Data were plotted over time (the first data-matched time point for the NH cohort was the control baseline). This analysis included 75 NH subjects matched with 11 DRIS-treated subjects. In ambulatory subjects 6MWD declined more rapidly in the NH vs DRIS-treated cohort. Six subjects DRIS-treated showed improvement or maintenance of functional abilities compared with the NH-matched cohort. The 6MWD mean change from baseline between the two groups at last assessment was ?30 m difference. None of the DRIS-treated subjects with baseline 6MWD >330m loss ambulation as compared to 25% of the NH-matched cohort; 66% of DRIS-treated subjects with baseline 6MWD ?330m loss ambulation as compared to 75% of the NH-matched cohort. In conclusion, for DMD subjects with ambulatory capacity, a comparison of DRIS-treated and matched NH subjects showed a difference in functional trajectories over a three year time window in favour of DRIS treatment.

neuromuscular junction, mdx-Xist?hs mice, electrophysiology, acetylcholine receptor, endplate potentials

autophagy flux, gene expression, ATP signalling, Purinergic receptor
**Dystrophinopathies (Duchenne, Becker, others)- #3126**

**P08-142- Study of pathogenesis of cognitive disorders in patients with Duchenne Muscular Dystrophy in organotypic tissue culture**

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Duchenne Muscular Dystrophy is one of the most frequent forms of hereditary neuromuscular pathology which in some cases is accompanied by cognitive disorders which pathogenesis is not studied in full.

**Material and methods.** There were 15 patients with Duchenne Muscular Dystrophy at the age of 9-14 years. Blood serum of the patients was analyzed in organotypic tissue culture. We have analyzed 600 explants of sensory ganglions of 10-12-day chicken embryos cultivated in ?O2-incubator (Sanyo) for 3 days on collagen membranes in Petri dishes at 36,5°? and 5% ?O2. The culture medium contained 45% of Hanks solution, 40% of Eagle medium with insulin (0.5 units/ml), glucose (0.6 %), glutamine (2 ml), gentamicin (100 U/ml), 5 % of chicken embryo extract and 10 % of bovine fetal serum. Control explants were cultivated in culture medium conditions. In experimental dishes blood serum of patients with Duchenne Muscular Dystrophy was added to the cultural medium. Visualization of the objects was made using «AxiostarPlus» microscope («CarlZeiss», Germany). The received images were analyzed using ImageJ software. Quantitative assessment of explants growth was made using morphometry method. Area index (AI) was calculates as a ratio of explant growth zone area and its initial area. AI reference value was 100 %. Was using STATISTICA 6.0.

**Results.** The study using confocal microscope was carried out on third day of cultivation in the reference and experimental explants. Neurite growth was prevailing in the sensory ganglion explants growth zone. Blood serum of 15 patients was analyzed in a wide range of dilutions (1:100-1:2). In 1:2, 1:10, 1:50 dilutions patients' serum has completely blocked the growth of sensory ganglion neurites. After addition of blood serum to the cultural medium with 1:70 dilution we have observed authentic neurite-inhibitory influence (?>0,05) in 70% of cases. AI of studied explants was below the control values on the average by 25%. Further dilutions of blood serum had no influence on neurite growth. In 30% of cases explants of sensory ganglions of 10-12-day chicken embryos in the culture medium have shown growth of sensory ganglion neurites corresponding to control explants.

**Conclusions:** blood serum of patients has different influence on growth of sensory ganglion neurites, in 70% of cases it was dose-dependent inhibitory influence of blood serum of patients on neurite growth, in 30% of cases the growth of neurites corresponded to reference values.

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**Dystrophinopathies (Duchenne, Becker, others)- #3184**

**P08-143- Long term, high dose PMO treatment for restores dystrophin and improves function in skeletal muscle in mdx mice.**

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Phosphorodiamidate morpholino oligomer (PMO) mediated exon skipping gene correction is currently being used in human Duchenne muscular dystrophy (DMD) clinical trials. This therapy creates a truncated dystrophin protein similar to Becker's muscular dystrophy patients where disease progression and phenotype is less severe. Despite progression to human clinical trials, very little is known about optimal dosing and timing of PMO treatment in DMD and whether dystrophin restoration leads to significant improvement in muscle function. The goal of this study was to optimize dose and duration of PMO treatment in mdx mice. To test this hypothesis, five week old mdx mice were injected with PMO (800mg/kg) or saline as a control intravenously once a month for one or six months. Comprehensive behavioral, functional, histological, biochemical and imaging studies were performed at the end of each trial. We found significant improvements in the maximal and specific force of the EDL muscle (p>0.05) when comparing PMO-injected mdx mice to saline injected controls after only the long term treatment. PMO treated mice also had a significant decrease in inflammatory activity (p>0.05) in the forelimbs when tested using in vivo imaging and was validated using both histological and further molecular analysis (qRT-PCR). Further evaluation of skeletal muscle on the molecular level revealed dystrophin protein expression was restored in all skeletal muscles evaluated including gastrocnemius, triceps, quadriceps, diaphragm, tibialis anterior, EDL and to a small extent the heart. This restoration is evident in all the samples from the long term study. Overall, high doses of PMO are safe and for the long term effectively restore measurable levels of dystrophin in the skeletal muscles of mdx mice. This increase in dystrophin is associated with a decrease in inflammation and increase in function in the PMO-treated mice. This study indicates that a higher dose of PMO might be necessary in clinical trials to achieve functional improvement in patients with DMD and that further time course measurements should be performed to evaluate optimal dosing of exon-skipping therapies.

**PMO, Dystrophin, Duchenne muscular dystrophy, gene therapy, exon-skipping**

**Dystrophinopathies (Duchenne, Becker, others)- #3189**

**P08-144 - Effect of exercise on skeletal muscle and cardiac function and integrity in aged mice with low dystrophin levels**

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Duchenne muscular dystrophy (DMD) is an X-linked myopathy caused by a lack of dystrophin resulting in severe muscle weakness and cardiomyopathy. There are several therapeutic approaches resulting in dystrophin restoration in skeletal muscle, while cardiac myocytes appear more difficult to target. The dystrophin levels required to preserve skeletal muscle and cardiac function and integrity during exposure to low intensity exercise in aged mice are unknown.

To study this, we subjected 15.5 month old female mdx-Xist?hs mice expressing low dystrophin levels based on non-random X-inactivation (quadriceps: 3-40%, heart: 3-14%), mdx (0% dystrophin), and two wild type strains (C57BL/10ScSnJ and Xist?hs) to voluntary wheel running or no exercise for a period of two months. Thereafter, muscle function was assessed in a two-week functional test regime consisting of grid and wire hanging tests. At the age of 18 months, a cardiac MRI scan was acquired to assess cardiac function.

We observed a dystrophin level dependent increase in total distance ran per night and improvement in performance in both hanging tests in the mdx-Xist?hs mice. Notably, voluntary wheel exercise further improved muscle function in all strains. Creatine kinase levels assessed at 18 months were only elevated in mdx mice (non-exercised: 600U/L, voluntary wheel exercise: 976 U/L). Fibrosis of the quadriceps was pronounced in mdx mice, while it partly normalized to wild type levels in mdx-Xist?hs mice. Voluntary wheel exercise did not influence histopathology.

Regardless of the exercise regime, mdx mice but not mdx-Xist?hs or wild type mice, developed cardiac hypertrophy as determined by heart/body mass ratio. We observed a dystrophin level dependent increase in cardiac function. While mdx mice had decreased ejection fraction and cardiac output, this was partly normalized in mdx-Xist?hs mice. Interestingly, voluntary wheel exercise not only improved skeletal muscle function it also benefitted cardiac function. While ejection fraction was not altered by exercise, cardiac output and stroke volume of the left ventricle was increased in all strains. Collagen infiltration was partly prevented in mdx-Xist?hs mice and did not increase upon exercise in any of the strains.

These results show that low dystrophin levels improve skeletal muscle and cardiac function and suggest that low intensity exercise is beneficial for both cardiac and muscle function for both dystrophic and wild type mice.

**Duchenne muscular dystrophy, cardiomyopathy, mdx mouse, muscle function, voluntary exercise**

**Dystrophinopathies (Duchenne, Becker, others)- #3192**

**P08-145 - The first mature human primary in vitro muscle model: A new paradigm to accelerate drug discovery for muscle disorders**

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To date, a wild range of muscle disorders suffers from the lack of treatment opportunities. This is mainly due to the absence of relevant in vitro models recapitulating muscle physiology and because animal models poorly phenocopy most of human muscular disorders. Moreover, the vast majority of screens performed so far have been conducted on myoblasts or patient-
derived fibroblasts but not on myotubes which are in the in vitro muscle fiber counterparts and are more relevant to the physiopathology.

In this context, we developed the first in vitro human primary skeletal muscle model compatible with High Content Screening (HCS), MyoScreenTM. Based on a tight guidance of myogenesis on micropatterns, human primary myotubes achieve a higher level of functional maturity compared to standard culture together with a highly standardized morphology. Myotubes formed on micropatterns present striated sarcomeres, AChR pretzel-like structures, and contract after ACh stimulation. Combined with High Content Analysis, such model allows the detection of drug effects and the characterization of their mode of action by measuring impacts on myogenesis, maturation, morphology, and cell viability. Interestingly, normalization of myotubes improved the identification of hypertrophic compounds with a robust detection of IGF-1 and trichostatin A effects compatible with screening standards (Z’ factor > 0.5). This constitutes per se a new opportunity to identify drug candidates for several muscle disorders like muscle wasting pathologies or genetic diseases. To further demonstrate the model's versatility for drug discovery applications, DMD primary myoblasts were cultivated following the MyoScreenTM protocol. Remarkably relevant aspects of the in vivo human physiopathology were recapitulated like myoblasts lower differentiation and absence of dystrophin, and will be exploited as relevant HCS readouts for screening campaign purpose.

Altogether, MyoScreenTM is the first in vitro human muscle model compatible with HCS that can be applied for the detection of hypertrophic compounds and that can be adapted to primary cultures of patient derived myoblasts. As phenotypic screening delivers drug candidates with higher translational potential, MyoScreenTM truly pushes beyond the current limitations of in vitro muscle models, offering the dual impact of significantly enhancing existing approaches while at the same time opening up new "assay development space? for multiple neuromuscular disorders.

High Content Screening, Drug Discovery, DMD, Cell-based Assay, in vitro model

Dystrophinopathies (Duchenne, Becker, others)- #3195

P08-146- Ataluren Confirmatory Trial in DMD: Effect of ataluren on activities of daily living in nonsense mutation Duchenne muscular dystrophy
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Objective: Evaluate the effect of ataluren on ambulatory decline in patients with nonsense mutation Duchenne muscular dystrophy (nmDMD). Change in activities of daily living (ADL) was also investigated as a secondary endpoint.

Background: Ataluren is the first drug to target the underlying cause of nmDMD, by promoting readthrough of a premature stop codon to produce full-length functional dystrophin.

Design/Methods: ACT DMD was a randomized (1:1), double-blind, placebo-controlled Phase 3 study that evaluated ataluren 40 mg/kg/day, administered over 3 doses, vs placebo over 48 weeks. Inclusion/exclusion criteria were designed to enrich for a population with the greatest opportunity to detect a clinical benefit over a 48-week study, while being inclusive enough to enroll an appropriate number of subjects over a feasible time period for an orphan disease. Participants were males 7-16 years of age with a screening six-minute walk distance (6MWD) ≥150m and ≥80%-predicted. Patients or parents/caregivers reported changes from baseline in physical functioning, general energy level, cognition/school function, emotional/social functioning, and sleep, rated on a Likert scale from 1 (much worse) to 5 (much better), using a DMD-specific survey developed for this study.

Results: 230 patients were randomized to receive ataluren (n=115) or placebo (n=115). Changes in ADL/disease symptoms trended in favor of ataluren versus placebo across all physical functioning categories, including lower- and upper-extremity muscle function. At Week 48, more ataluren- than placebo-treated patients reported improvement (22.2% vs 16.1%, respectively) or lack of progression (55.6% vs 50.9%) in walking and fewer ataluren-treated patients reported worsening in walking (22.2% vs 33.0%). These effects did not reach statistical significance. The same pattern of changes (with smaller group differences) was observed for stair-climbing and upper extremity activities of self-care.

Conclusions: Results indicate a positive clinical effect of ataluren on ADL in boys with nmDMD, expanding the benefit beyond ambulation.

Study Supported By: PTC Therapeutics Inc.

ataluren, ACT DMD, nonsense mutation Duchenne muscular dystrophy, phase 3, activities of daily living

Dystrophinopathies (Duchenne, Becker, others)- #3198

P08-147- ACT DMD: Effect of ataluren on timed function tests in nonsense mutation Duchenne muscular dystrophy
**Background:** Ataluren is the first drug to treat the underlying cause of nmDMD by promoting readthrough of a premature stop codon to produce full-length functional dystrophin. It is approved in Europe for the treatment of nmDMD in ambulatory patients aged 5 years and older.

**Design/Methods:** ACT DMD (Ataluren Confirmatory Trial in Duchenne Muscular Dystrophy) is a Phase 3, randomized, double-blind, placebo-controlled study. Males 7-16 years of age with nmDMD and a screening six-minute walk distance (6MWD) ≥150m and ≥80%-predicted were randomized 1:1 to ataluren 40 mg/kg/day or placebo for 48 weeks. A pre-specified subgroup included patients whose baseline 6MWD was 300-400m. Secondary endpoints included TFTs: 10-meter walk/run; 4-stair climb; 4-stair descend. A meta-analysis of the overall ACT DMD population and the ‘ambulatory decline phase’ subgroup of the Phase 2b study (ie, those patients meeting ACT DMD entry criteria) was pre-specified in the ACT DMD statistical plan.

**Results:** In the overall ACT DMD population (N=228), changes in the three TFTs presented below favored ataluren over placebo: 10-meter walk/run, -1.2s (p=0.117); 4-stair climb, -1.8s (p=0.058); 4-stair descend, -1.8s (p=0.012). In the pre-specified subgroup (n=99), these differences increased to -2.1s, -3.6s, and -4.3s, respectively, and were statistically significant for the 4-stair climb (p=0.003) and 4-stair descend (p=0.001), and approached significance for 10-meter walk/run (p=0.066). Results are supported by the meta-analysis (N=291), which demonstrated significant differences in all three TFTs: 10-meter walk/run, -1.4s (p=0.025); 4-stair climb, -1.6s (p=0.018); 4-stair descend, -2.0s (p=0.004).

**Conclusions:** TFT results showed a benefit for ataluren in ACT DMD, and a larger treatment effect in the pre-specified baseline 6MWD 300-400m subgroup as well as the pre-specified meta-analysis of ACT DMD and the Phase 2b study decline subgroup.

**Study Supported By:** PTC Therapeutics Inc.

**ataluren, ACT DMD, nonsense mutation Duchenne muscular dystrophy (nmDMD) using timed function tests (TFTs)**

**Objective:** Determine the effects of ataluren on motor function in patients with nonsense mutation Duchenne muscular dystrophy (nmDMD) using timed function tests (TFTs).

**Background:** 6MWD is a validated clinical outcome for ambulatory DMD studies and the primary endpoint in trials of ataluren, drisapersen, taladafil, and eteplirsen. Decline in 6MWD is predictive of disease progression, time to loss of ambulation, and subsequent onset of disease milestones. Over time, there has been an effort to tighten 6MWT inclusion criteria, thereby excluding ambulatory patients with near-normal walking ability and those with severely impaired ambulation.

**Design/Methods:** Recent DMD studies were reviewed to determine how the 6MWT has evolved as a sensitive clinical endpoint. Results: 6MWT inclusion criteria for DMD clinical trials have evolved since 2008 from the original criteria of 75 meters with no ceiling value for the first two trials which used the 6MWT as a clinical endpoint. These baseline 6MWT criteria have narrowed to a floor value as high as 300 meters for the eteplirsen open-label phase 3 study (Sarepta 4658-301 initiated 2015) and ceiling values as low as 400 meters in the taladafil phase 3 trial (Eli Lilly H6D-MC-LVJJ initiated 2013). The Phase 3 ataluren study (ACT DMD; initiated 2013) included patients with a baseline ≥150m and ≥80%-predicted, with a pre-specified subgroup of 300-400m baseline. In the ACT DMD study, the benefit of ataluren over placebo observed in the overall population (48-week difference=15m; p=0.213) was enhanced in the pre-specified 300-400m subgroup (47m; p=0.007). Sensitivity analyses confirmed an ataluren effect with 6MWD ≥250 to >400m (29.5m; p=0.035); and ≥400m (16.5m; p=0.001).

**Conclusions:** When evaluating drugs expected to slow progression in DMD, narrower 6MWD ranges are being used as inclusion criteria. For ataluren, a pre-specified range of 300-400 m demonstrated the greatest treatment effect. Meaningful effects were seen with 6MWD from 200-450 m.

**Study Supported By:** PTC Therapeutics Inc.
**P08-149- Ataluren: an overview of clinical trial results in nonsense mutation Duchenne muscular dystrophy**

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**Objective:** Provide an overview of ataluren clinical trial results in patients with nonsense mutation Duchenne muscular dystrophy (nmDMD).

**Background:** Ataluren is the first drug to treat the underlying cause of nmDMD. It enables ribosomal readthrough of a premature stop codon to produce full-length functional dystrophin, without affecting normal stop codons.

**Design/Methods:** Phase 2 and 3 clinical trials of ataluren in nmDMD were reviewed, with efficacy and safety/tolerability findings summarized.

**Results:** Clinical trials of ataluren in nmDMD include: a Phase 2a proof-of-concept study (N=38) whose primary endpoint was muscle dystrophin expression following 28 days of treatment; a Phase 2b randomized controlled trial (RCT) (N=174), whose primary endpoint was change in six-minute walk distance (6MWD) over 48 weeks; an ongoing US-based open-label extension study (N=108) evaluating long-term safety; an ongoing non-US-based open-label extension study (N=94) evaluating long-term safety and efficacy; and a Phase 3 RCT, ACT DMD (N=228), whose primary endpoint was change in 6MWD over 48 weeks.

The proof-of-concept study demonstrated increases in dystrophin production in post-treatment muscle biopsies from ataluren-treated patients with nmDMD. The Phase 2b results demonstrated an ataluren treatment effect in 6MWD, timed function tests, and other measures of physical functioning, with larger treatment effects observed in patients at higher risk of ambulatory decline. This study was the basis for ataluren's approval in the European Union. The Phase 3 ACT DMD results demonstrated an ataluren treatment effect in patients with nmDMD in both primary and secondary endpoints, particularly in those with a baseline 6MWD of 300-400m. Ataluren was consistently well-tolerated in all three trials, as well as in the ongoing extension studies. Trial findings will be presented in detail.

**Conclusions:** The totality of the results demonstrates that ataluren enables nonsense mutation readthrough in the dystrophin mRNA, producing functional dystrophin and slowing disease progression in patients with nmDMD.

**Study Supported By:** PTC Therapeutics Inc.

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**P08-150- Safety and tolerability of ataluren in a phase 3 study of patients with nonsense mutation Duchenne muscular dystrophy**

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**Objective:** Examine the safety and tolerability of ataluren in the Ataluren Confirmatory Trial in Duchenne Muscular Dystrophy (ACT DMD), a randomized, double-blind, placebo-controlled Phase 3 study of patients with nonsense mutation DMD (nmDMD).

**Background:** A randomized, double-blind, placebo-controlled Phase 2b study of ataluren in patients with nmDMD reported that ataluren was generally well tolerated.

**Design/Methods:** In this Phase 3, ACT DMD, multicenter study, males aged 7-16 years with nmDMD, baseline six-minute walk distance (6MWD) ≥150m and ≥80% of predicted, and steroid use ≥6 months were randomized 1:1 to ataluren 10, 10, 20 mg/kg or placebo orally 3 times daily for 48 weeks.

**Results:** Overall, 230 patients were randomized (ataluren, n=115; placebo, n=115). Patient demographics were well balanced across both treatment arms. Overall, 96.1% of patients completed the 48-week trial, and 97% of those chose to continue in the extension study. 103 (89.6%) patients on ataluren and 100 (87.0%) patients on placebo experienced treatment-emergent adverse events (TEAEs). The most common TEAEs in the ataluren and placebo arms, respectively, were vomiting (22.6% and
18.3%), nasopharyngitis (20.9% and 19.1%), fall (19.1% and 17.4%), headache (18.3% for both), and cough (16.5% and 11.3%). Four patients in each arm had at least 1 serious adverse event (SAEs). In the ataluren arm, these SAEs were pneumonia and bronchiolitis in 1 patient, pneumonia and post-traumatic pain in 1 patient, tendon disorder in 1 patient, and adenoidal and nasal turbinate hypertrophy in 1 patient; none were considered ataluren-related by the investigator. Only 1 patient in each arm discontinued treatment due to TEAEs: 1 patient discontinued ataluren due to Grade 2 constipation considered possibly related to treatment, and 1 patient discontinued placebo due to loss of ambulation.

Conclusions: Ataluren was generally well-tolerated by patients with nmDMD, and the spectrum and severity of adverse events possibly related to treatment, and 1 patient discontinued placebo due to loss of ambulation.

Study Supported By: PTC Therapeutics Inc.

**ataluren, ACT DMD, nonsense mutation Duchenne muscular dystrophy, phase 3, safety/tolerability**

**Dystrophinopathies (Duchenne, Becker, others)**

### P08-151 Results of North Star Ambulatory Assessments in the Phase 3 Ataluren Confirmatory Trial in Patients with Nonsense Mutation Duchenne Muscular Dystrophy (ACT DMD)

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Objectives: Examine the efficacy of ataluren in patients with nonsense mutation Duchenne muscular dystrophy (nmDMD) as assessed by the North Star Ambulatory Assessment (NSAA).

Background: Results of the Phase 3, randomized, double-blind, placebo-controlled ACT DMD trial have been reported. The NSAA is a validated functional scale to measure disease progression specifically in ambulant boys with DMD.

Design/Methods: ACT DMD enrolled males aged 7-16 years with nmDMD and baseline six-minute walk distance (6MWD) >150m and >80%-predicted. Eligible patients were randomized 1:1 to receive ataluren 10, 10, 20 mg/kg or placebo orally three times daily for 48 weeks. A subgroup analysis of patients with baseline 6MWD of 300-400m was pre-specified. The NSAA consists of 17 activities ranging from standing from a chair to jumping. Each activity is scored as 0, 1, or 2; the sum of these 17 scores forms the total score, which is linearized to a 0 (worst)-100 (best) score.

Results: The intent-to-treat population of ACT DMD consisted of 228 patients (ataluren, n=114; placebo, n=114). Overall, patients who received ataluren gained a 1.5-point advantage in NSAA observed score compared with patients who received placebo (mean NSAA scores, ataluren: -7.0; placebo: -8.5; p=0.270). In the pre-specified subgroup of 99 patients with baseline 6MWD 300-400m, the advantage conferred by ataluren over placebo increased to 4.5 points (mean observed NSAA scores, ataluren: -5.7; placebo: -10.2; p=0.030).

Conclusions: Ataluren is the first drug to demonstrate a benefit to patients with nmDMD compared with placebo as assessed by NSAA scores; this benefit was especially pronounced in the subgroup of patients with baseline 6MWD 300-400m. NSAA results when combined with 6MWD results, provide complementary information on different aspects of motor function in nmDMD patients and further demonstrate the efficacy of ataluren in this patient population. More detailed analysis of NSAA domains will be presented.

Study Supported By: PTC Therapeutics Inc.

**ataluren, ACT DMD, nonsense mutation Duchenne muscular dystrophy, phase 3, North Star Ambulatory Assessment**

**Dystrophinopathies (Duchenne, Becker, others)**

### P08-152 Muscle proteomics reveals novel insights into the pathophysiological mechanisms of DMD and BMD dystrophynopathies

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Dystrophinopathies are X-linked recessive allelic disorders caused by mutations of the dystrophin gene, including a severe and fatal form, Duchenne Muscular Dystrophy (DMD), and a milder form, Becker Muscular Dystrophy (BMD). DMD is caused by mutations disrupting the open reading frame and preventing the full translation of dystrophin. BMD is caused by in-frame dystrophin mutations, typically involving part of the central rod domain, in particular deletions encompassing exons 44-51, that preserve the reading frame permitting the translation of an internally deleted dystrophin protein.

To date, no effective treatments are available for DMD and BMD patients. Corticosteroids are used in DMD patients only, leading to a delay in loss of ambulation and in progression of the disease. Despite recent progress, molecular mechanisms involved in DMD and BMD still remain unravelled and further studies are needed.

To elucidate the role of muscle metabolism in BMD and DMD patients, a proteome analysis was carried out on human muscle biopsies. Qualitative and quantitative differences were assessed by in-gel differential proteomics (2D-DIGE) coupled to MALDI-
ToF/ToF and by immunoblotting. Proteomics results conducted on 15 DMD, 15 BMD and 15 healthy control subjects, revealed a different behaviour between BMD and DMD patients in response to a common glucose shortage due to glycolytic enzymes decrement. In DMD muscles TCA cycle enzymes decreased whereas in BMD increased, opening the question of the use of an alternative energy source to sustain muscle function. To this point, proteomic data indicate the presence of a metabolic rewiring that can lead to a shortage of glutamate-derived molecules with protective functions triggering lipogenesis, causing lipotoxicity over time. Moreover, results highlight the link between glucose depletion and ER stress. Since glucose is essential for protein glycosylation, if intracellular glucose levels decrease, the carbohydrate chain, that is used to glycosylate proteins, cannot be assembled and an improper protein glycosylation, protein misfolding, activation of the UPR, and increased ER stress is observed.

By the present study we identify specific metabolic nodes to be targeted with the possibility to ameliorate the course of the disease in DMD patients.

Dystrophinopathies (Duchenne, Becker, others)- #3221

P08- 153- Diagnosis of myopathies in Burkina Faso.

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Introduction
The Neurology Department of Yalgado Ouedraogo University Hospital of Ouagadougou and FITIMA foundation cooperate since 2004 for the medical and psychosocial following of the patients affected by myopathies in Burkina.

Objective
The objective is to present the results of the medical following of patients affected by myopathies in Yalgado Ouedraogo University Hospital of Ouagadougou.

Materials and methods:
In a ten years (from 2004 to 2014) retrospective study, authors describe clinical review of 17 patients followed for myopathy at Neurology Department.

Results
The mean age of the patients (16 males and one female) was 19.82 years old with extremes from 6 to 40 years old. Onset was at 6-10 years old in 52.94% and after 20 years old in 11.76%. In medical history, consanguineous parents are found in two patients and six familial myopathies cases. The first symptom was walking retardation (57%). Neurologic examination noted a dandy walk and Gower’s sign in 88.25%. Cardiac lesions are found in 35.29% of cases. The mean CK was elevated (3016.64 IU/L). Genetic test performed on 58.82% of patients, identified two cases of Duchenne Muscular Dystrohy (DMD), two cases of Becker Dystrophy and one case of dysferlinopathy.

Conclusion
Myopathies are found in Burkina Faso, as in some regions in the world. Duchene disease was the most frequent neuromuscular disorder but we identified one case of dysferlinopathy. Genetic test is rare and needs North

Myopathies, Burkina

Dystrophinopathies (Duchenne, Becker, others)- #3224

P08- 154- Muscle proteomics reveals novel insights into the pathophysiological mechanisms of DMD and BMD dystrophinopathies

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6. Department of Medical Sciences, University of Ferrara, ferrara, Italy

Dystrophinopathies are X-linked recessive allelic disorders caused by mutations of the dystrophin gene, including a severe and fatal form, Duchenne Muscular Dystrophy (DMD), and a milder form, Becker Muscular Dystrophy (BMD). DMD is caused by mutations disrupting the open reading frame and preventing the full translation of dystrophin. BMD is caused by in-frame dystrophin mutations, typically involving part of the central rod domain, in particular deletions encompassing exons 44-51, that preserve the reading frame permitting the translation of an internally deleted dystrophin protein.

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alternative energy source to sustain muscle function. To this point, proteomic data indicate the presence of a metabolic rewiring that can lead to a shortage of glutamate-derived molecules with protective functions triggering lipogenesis, causing lipotoxicity over time. Moreover, results highlight the link between glucose depletion and ER stress. Since glucose is essential for protein glycosylation, if intracellular glucose levels decrease, the carbohydrate chain, that is used to glycosylate proteins, cannot be assembled and an improper protein glycosylation, protein misfolding, activation of the UPR, and increased ER stress is observed.

By the present study we identify specific metabolic nodes to be targeted with the possibility to ameliorate the course of the disease in DMD patients.

Dystrophinopathies (Duchenne, Becker, others)- #3271

**P08-155**- Loss of Dp140 dystrophin isoform and neuropsychological impairment in Tunisian boys with Duchenne muscular dystrophy

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**Background**

Mutation of the dystrophin gene has long been recognized as a cause of intellectual impairment in Duchenne muscular dystrophy (DMD). However, underlying etiopathologic mechanisms remain unclear.

**Objective**

To analyze neuropsychological profiles in a cohort of Tunisian boys with DMD and their correlation with causal mutations.

**Patients and methods**

Over 11 years (2004-2015), 20 boys were followed up in our department for a genetically confirmed diagnosis of DMD. Neuropsychological evaluation including full-scale intelligence quotient (IQ), memory assessment, verbal performances, attention processes, executive functions, mood and behavioral patterns. Correlation between dystrophin gene mutation and neuropsychological profiles were analyzed.

**Results**

Mean age at neuropsychological evaluation was 6.7 years. Causal mutations predicted to lead to a loss of the Dp140 isoform have been found in 15 (75%) patients. General intelligence assessments showed a mean IQ of 82 (range 50-110). Impairment in working memory was noted in 12 (60%) patients. Verbal performances and attention processes were altered in respectively 7 (35%) and 5 (25%) patients. Executive dysfunction was noted in 5 (25%) patients. Autism spectrum disorders were noted in 3 (15%) patients. We found that patients lacking Dp140 performed more poorly on all neuropsychological tests compared to those with preserved Dp140. There was no evidence of cognitive declining with the progression of muscular deterioration.

**Conclusion**

Our findings support emerging evidence of central nervous system involvement resulting in neuropsychological disorders in DMD. The loss of Dp 140 seems to be involved in the pathological mechanisms underlying neuropsychological disturbances and associated with a higher risk of cognitive impairment among patients with DMD. Neuropsychological deficits should be systematically detected in these patients in order to improve their quality of life.

Cognitive impairment, Duchenne muscular dystrophy (DMD), Dp140 isoform

Dystrophinopathies (Duchenne, Becker, others)- #4469

**P08-156**- Grip strength in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is characterized by a progressive loss of muscle fibers, which are gradually replaced by fat and connective tissue. The disease typically develops from proximal to more distal muscles. Muscles of the hands are thus less affected throughout the disease course. Few studies have focused on the upper limb of DMD patients, despite the importance of maintaining independent function as long as possible for young men with DMD.

The results of grip strength of 157 DMD boys were gathered from several natural history protocols. 46 patients were ambulant and 111 were non-ambulant. They were aged from 5 to 30 years. Each patient was followed during at least one year. As a global picture, grip strength increases in ambulant boys while it decreases in non-ambulant patients when expressed in absolute value. However when expressed relatively to age or height using predictive models, grip strength decreases in both groups of patients.

These results confirm that growth and maturation partly compensate for disease progression in ambulant boys. Our study also suggests that stature, better than chronological age, is a major predictor of muscle strength. Height or other stature parameters (like hand circumference or ulna length for instance) must then be measured during clinical trials in order to express muscle strength in percentage of predicted values estimated for stature. Normalized variables should be used in future to provide markers of disease evolution, independently of growth and maturation.

Duchenne Muscular Dystrophy, grip strength, outcome measure

**P09**- Facioscapulohumeral dystrophy (FSHD1, FSHD2)- N° 157 to N° 173

Facioscapulohumeral dystrophy (FSHD1, FSHD2)- #2551