The continued collection of longitudinal data and ongoing recruitment of new participants over time will be imperative when assessing the progression of the condition and will assist in the design and feasibility of clinical studies in the future.

D39 Characterising the skeletal muscle histological phenotype of the DeltaE50-MD dog, a preclinical model of Duchenne muscular dystrophy

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Background: Canine models of Duchenne muscular dystrophy (DMD) are increasingly recognised as essential for the study of this incurable muscle-wasting condition: mouse models do not replicate the more severe pathological features of the human disease (muscle wasting, accumulation of fibrotic/fatty infiltrates). The deltaE50-MD dog is a novel canine model for DMD: bred onto the beagle background and carrying a mutation amenable to exon-skipping strategies that also lies within a common human hotspot, this dog represents a valuable experimental model for translation of therapies from laboratory to clinic.

Aims: The severity and time-course of DMD can vary both with the specific nature of the mutation and individual genetic background: as a novel model on a relatively outbred background, it is essential to characterise the nature of disease progression (and extent of individual variation) before the deltaE50-MD dog can be used in therapeutic trials. Here we investigate the histological phenotype of skeletal muscle in this model as the animals age.

Methods: 16 dogs (8 healthy, 8 DMD) were enrolled on an 18-month natural history trial: to characterise the skeletal muscle histological phenotype, repeated vastus lateralis biopsy samples from all animals were collected at 3-monthly intervals, with a wider panel of muscle samples collected at 18 months (following end-point euthanasia). Samples were used to conduct a range of histological and gene expression analyses, allowing assessment of muscle architecture, fibrotic/fatty accumulation, and extent of regeneration and inflammation.

Results: Muscles of deltaE50-MD dogs develop progressive fibrotic scarring, accumulate adipose deposits, and exhibit a marked variation in fibre size (characteristic of muscle regeneration and compensatory hypertrophy), especially between the ages of 6 and 12 months. Wider muscle analysis suggests postural and respiratory muscles experience more severe dystrophic remodelling; however, at 18 months of age, all muscles still robustly express transcriptional markers consistent with an ongoing process of degeneration and repair.

Conclusion: DeltaE50-MD skeletal muscle displays profound dystrophic progression and offers a variety of discrete metrics by which the efficacy of therapeutic intervention can be assessed.

D40 Analysis of premature mortality in a cohort of adult Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) survival has substantially improved in recent years with mean life expectancy at 29 years and a growing number surviving into fourth and fifth decades. However, despite this, premature deaths still occur, and the most common reported cause is cardiomyopathy.

Aim: The aim of this retrospective data collection study was to gain a better understanding of premature mortality in DMD and identify potential avoidable risk factors.

Methods: We reviewed causes of death in a cohort of 83 adults with DMD followed up at our centre (mean age 22.5 ± 3.6), and analysed the events leading to these deaths. Approval was given by our Hospital audit committee.

Results: Fourteen deaths were registered between 2012 and 2017. These deaths can be regarded as unexpected, if we consider the young age at death (mean 21.1 ± 2.5) and mean age at loss of ambulation 11.4 ± 1.9. Eleven patients (79%) were steroid naïve or had discontinued steroid treatment in childhood. Interestingly, 7/14 patients (50%) had autism or learning disability (LD). The cause of death was due to cardiomyopathy in 5 (36%) and respiratory failure in 3 (21%, all of whom had autism/LD and were non-compliant with NIV). One died from tracheostomy haemorrhage. Two (14%) died from cachexia.

Conclusion: These findings are in keeping with the current medical literature on mortality in DMD, but the frequency of autism/LD in our sample of patients who died prematurely was overrepresented when compared to the frequency in DMD reported in the literature (1/3). Our data raise concerns over the nutritional status and NIV compliance in particular in DMD with autism/LD, as low weight and untreated respiratory failure can hastily precipitate into premature deaths. Starting the process of familiarization with NIV in the paediatric setting and early RIG/PEG insertion may help to reduce the number of deaths at young ages. Multi-centre, prospective surveillance of mortality in DMD are needed to further clarify risk factors for early mortality and improve care and life expectancy.

D41 Downregulation of miR-29 and miR-23 in urine of Duchenne muscular dystrophy patients

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Background: Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disorder affecting 1 in 5000 newborn males. It is more commonly caused by out-of-frame deletions or, more rarely, duplications, nonsense or other small mutations affecting DMD gene and consequently dystrophin protein production. Affected boys develop progressive muscle weakness, leading to loss of ambulation, if untreated by age 9. Glucocorticoid therapy has proven to preserve some degree of muscle function and delay loss of ambulation. MicroRNAs are short (~20–23 nucleotides) non-coding RNAs that regulate gene expression; their dysregulation in serum has been associated with many paediatric neuromuscular conditions including DMD. MiRNAs are present in urine where they are included in small microvesicles called exosomes (40–100 nm), which are secreted by different cell types.

Aims: To investigate the potential of urinary miRNAs as a novel non-invasive biomarker in DMD, we profiled their pool isolated from urinary exosomes of ambulant and non-ambulant DMD patients and age matched controls. From these, 6 candidate miRNA were selected for further validation, based on their reported involvement in skeletal muscle related diseases, including DMD and Facioscapulohumeral muscular dystrophy (FSHD). Finally, we assessed if there was any association between miRNA levels in urine and corticosteroid treatment.
Materials and methods: The patients included in this cross-sectional study are part of a cohort of DMD boys in a multicenter natural history study registered in clinicaltrials.gov (NCT02780492). Samples from patients recruited in London, Paris, Newcastle and Leiden were analysed. qPCR microRNA profiling was performed using a miRCURY LNA™ Pick-&-Mix microRNA PCR SYBR green-based panels (Exiqon), while validations were carried out by a qPCR TaqMan small RNA Assay (Life Technology).

Results: We detected 53 miRNAs expressed in urine from DMD patients and validated those with the strongest abnormal expression (n=6) in a larger cohort of patients. We found significantly lower expression of miR-29c-3p and miR-23b-3p in ambulant and non-ambulant DMD patients respectively, compared to controls. In addition, miR-29 levels were differentially affected by different steroid regimens.

Conclusion: Our findings indicate that exosomal urinary miR-23 and miR-29 are promising novel non-invasive biomarkers for DMD.

‡D42 Myostatin is a reliable biomarker for monitoring drug response in DMD

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Muscular dystrophies are characterized by weakness and wasting of skeletal muscle tissues. Myostatin being a negative regulator of muscle mass, its down-regulation has been seen as a promising tool to counterbalance the muscle wasting observed in neuromuscular patients but most showed limited efficacy. We have recently demonstrated that circulating myostatin levels are dramatically reduced in patients affected by a muscle wasting or atrophying disease (Mariot, Nat Comms 2017). We also provided in vivo evidence in the Mtm1 KO mouse model, which shows highly atrophic muscles, that a down-regulated myostatin pathway can be reactivated by correcting MTM1 rescue. These results suggest that myostatin could be a reliable biomarker for treatment efficacy in neuromuscular diseases.

In this project, we evaluated this possibility in the GRMD canine model for DMD. Indeed, we have recently demonstrated that systemic or locoregional administration of an AAV vector coding a canine myostatin reduces the physiological decline in muscle strength of treated limbs in the treated animals in a dose dependent manner (Le Guiner, Nat Comms 2017). We observed that measures of circulating myostatin levels were sufficient to distinguish control, untreated and locoregionally or systemically treated GRMD dogs, thus demonstrating that circulating myostatin is a reliable biomarker for monitoring the effect of myostatin gene therapy in DMD.

‡D43 Profile of circadianly regulated metabolic genes in dystrophic heart

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Duchenne muscular dystrophy (DMD) a monogenic disorder caused by the lack of the integral structural protein, dystrophin, which results in severe muscle wasting and cardiomyopathy in affected boys. Indeed, cardiorespiratory complications are the predominant cause for mortality in DMD patients. We have recently shown that circadian rhythm is disrupted in dystrophic mice as a direct result of the lack of dystrophin protein. It is well reported that disruption of circadian rhythmicity leads to perturbed metabolism and an array of disorders including obesity, diabetes and cardiovascular disease. Disturbed cardiac metabolism in DMD patients and dystrophic mice is also well described, and thus it would be interesting to learn whether pertinent metabolic genes which are known to be circadianally regulated, are disrupted in dystrophic mice. Here we show for the first time, significant changes in the differential expression patterns of multiple genes involved in free fatty acid and glucose metabolism, in 2 mouse models of DMD compared to control mice. These findings provide the foundation for further research to better understand the metabolic/circadian milieu and its effect on dystrophic heart, so that we may devise strategies to augment cardiac metabolism, in an effort to halt the deterioration in cardiac phenotype.

‡D44 Extracellular myomiR abundance is not clearly correlated with skeletal muscle dystrophin expression in mdx mice with skewed X-chromosome inactivation

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Background: microRNAs (miRNAs) are short, ~22nt, non-coding RNA species involved in post-transcriptional gene regulation. A subset of miRNAs, the myomiRs, have been found to be important for the proliferation and differentiation of myoblasts. We recently discovered that extracellular myomiRs are minimally-invasive indicators of muscle turnover and are highly elevated in the serum of Duchenne Muscular Dystrophy (DMD) patients and dystrophic animal models, making them attractive biomarkers.

Aims: Here, we sought to understand the relationship between levels of dystrophin protein in skeletal muscle and the corresponding serum abundance of the myomiRs and other putative miRNA biomarkers.

Methods: To this end, male mdx mice were crossed with female XistΔhs model. Overall, both mdx and mdx-XistΔhs samples exhibited much higher serum miRNA expression levels compared to controls. Unexpectedly, there was no clear correlation between dystrophin expression in skeletal muscle and myomiR restoration towards wild-type levels, as has been widely reported after exon skipping therapy to restore dystrophin expression. Conversely, several miRNAs (miR-193b-3p, miR-370-3p and miR-483-3p) did show a gradation of serum abundance levels with differential muscle dystrophin expression, though these miRNAs were less sensitive and less specific biomarkers relative to the myomiRs.

Results: We first compared mdx and C57 serum samples and analysed expression patterns of 63 miRNAs that may play a role in the disease. This led to identification of 12 lead candidate miRNA biomarkers, including the myomiRs, that were further investigated in the mdx-XistΔhs model. Overall, both mdx and mdx-XistΔhs samples exhibited much higher serum miRNA expression levels compared to controls. Unexpectedly, there was no clear correlation between dystrophin expression in skeletal muscle and myomiR restoration towards wild-type levels, as has been widely reported after exon skipping therapy to restore dystrophin expression. Conversely, several miRNAs (miR-193b-3p, miR-370-3p and miR-483-3p) did show a gradation of serum abundance levels with differential muscle dystrophin expression, though these miRNAs were less sensitive and less specific biomarkers relative to the myomiRs.

Conclusion: These results underscore that the relationship between dystrophin levels and normalisation of extracellular miRNA abundance is more complex than initially thought and warrants detailed further investigation.
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