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Index

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Invited Speakers

Thursday 4th April 2019

S 01

Clinical services for Neuromuscular Disease patients

Prof Sir Doug Turnbull
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Neuromuscular diseases collectively are common but many individual disorders are rare. Whilst there are generic measures that support many patients, individualised care is crucial. Developing a model enabling patients with rare forms of neuromuscular disease to be seen by a team that is highly skilled in managing their condition is important. Within the NHS, the development of the NHS Highly Specialised Services has been crucial to the implementation of this model of care. It allows patients with specific neuromuscular diseases to be seen (and investigated) in centres of excellence, allowing improvements in care and support. There are many other advantages of this model of care including the developing expertise of the clinical team caring for the patients, the production of clinical guidelines and the collection of clinical cohorts so important for clinical trials. This innovative model of care, combined with a universal health system, means that the UK has arguably the best standards of care for all patients with neuromuscular disease.

S 02

From Gene Discovery to Therapy

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The current generation of clinician scientists has benefitted enormously from the technological advances in molecular genetics that have expedited the identification of novel disease genes in ways that until a few years ago were unimaginable. This has also led not only to the exponential identification of novel disease genes but also novel molecular targets for therapeutic intervention.

Regarding the first phase of the genomic studies, the UK has benefitted from the network of clinical centres involved in rare neuromuscular diseases and funded via the Highly Specialised Services, and from the links with other European centres and with other large networks in US and Australia. The large collection of well characterized patients and the introduction for the first time of deep phenotyping using not only detailed muscle pathology but also muscle MRI, allowed the UK Centres to identify ~ 20 years ago some of the most common genes involved in congenital and in limb girdle muscular dystrophies, such as dysferlin and FKRP.

In the last few years the genomic revolution has provided a completely different dimension to the gene identification, allowing the characterization of ultrarare conditions which identification would have been impossible until just a few years ago.

The improved understanding of mechanism of disease has also led to the development of novel therapeutic interventions. For example, for a common condition such as Duchenne muscular dystrophy, the understanding of the rules that regulate individual exon splicing, and the ability to modulate splicing using antisense oligonucleotides, has led to the first genetic therapeutic option for the affected individuals. More recently, genomic studies using large population of DMD patients is providing for the first time both the molecular explanation for difference in clinical course between different patients. These studies are providing insight on potential novel therapeutic targets; and will also help to understand the extent of any therapeutic intervention for patients

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S 03

The FOR DMD trial: Individualized vs standardized treatment

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Over 16 years ago Professor Katie Bushby noted the "chaos" (at least 29 regimens) in corticosteroid (CS) treatment of Duchenne Muscular Dystrophy (DMD). She also set out to establish consensus guidelines for DMD care. Working with multinational clinicians and patient advocacy groups, she enlisted the Muscle Study Group (MSG) to collaborate with TREAT-NMD to address the wide variation in the use of CS by studying the long-term risks/benefits of the 3 most commonly prescribed regimens, daily prednisone, intermittent prednisone , and daily deflazacort, in a randomized, double-blind, comparative effectiveness trial. The resulting FOR DMD clinical trial enrolled 196 CS-naïve boys (age 4-7) from sites in 5 countries and involved nearly 200 investigators/staff. In 2020, after participants have completed 3-5 years of follow-up, the final results of the study will be analyzed. The deliverables expected of the study include: (1) identifying the superior CS regimen; (2) establishing the outcomes of systematically-applied consensus guidelines on side effects (metabolic, ocular, bone) and on DMD complications; and (3) exploring the relationship of genotype to complications of DMD and to benefits/side effects of CS treatment.

Knowing the superior regimen for long-term CS treatment in DMD remains of great importance, since novel treatments will likely require concomitant CS. Importantly, in advance of the final results we already know that 3-5 years of all 3 CS regimens have been well tolerated. This study is a tribute to Katie Bushby's vision and persistence and her skill in collaborating with the MSG and TREAT-NMD.

Acknowledgements: The FOR DMD investigators and steering committee; TREAT-NMD; the MSG. Funding by NINDS grant: U01 NS061799; the Parent Project for Muscular Dystrophy; the Muscular Dystrophy Association; Italian Telethon; Association Française contre les Myopathies-AFM: and by Marathon, PTC, Sarepta, Santhera Pharmaceuticals.

S 04

Vision DMD: developing an alternative to steroids

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Corticosteroids are routinely prescribed as part of the care recommendations in Duchenne muscular dystrophy (DMD). They are effective but the burden of side effects impacts clinical outcomes and detracts from patient quality of life. Developing safe and better tolerated treatments is an important goal for the management of DMD and other chronic inflammatory diseases. Vamorolone (VBP15), is a first-in-class ligand of the glucocorticoid receptor that shows the anti-inflammatory efficacy of corticosteroids (inhibition of innate immunity via activator protein 1 and nuclear factor kB) while reducing or eliminating many adverse effects of corticosteroids (transcriptional transactivation of genes due to binding the glucocorticoid response element).

Vamorolone demonstrated both efficacy and a reduction in adverse effects in DMD patients compared to traditional corticosteroids in a first-in-patient 24-week, open-label study (VBP15-003) following an acute treatment trial (VBP15-002), demonstrating that oral administration of vamorolone at dose levels of 0.25, 0.75, 2.0 and 6.0 mg/kg/day

was safe and well-tolerated over a 24-week treatment period.

A randomised, double blind, parallel group, placebo and active controlled study in 120 ambulant DMD boys ages 4 to <7 years is currently recruiting and aims to confirm the safety, efficacy, pharmacodynamics and population pharmacokinetics of vamorolone at daily doses of 2.0 mg/kg and 6.0 mg/kg versus prednisone 0.75 mg/kg/day and placebo over a 24-week treatment period and to evaluate persistence of effect over a total treatment period of 48 weeks. The study is being conducted in 34 sites across 11 countries.

Vamorolone has the potential to replace chronic corticosteroid treatment for many disorders where adverse effects detract from the quality of life of patients. Vamorolone studies in younger and older DMD patients are planned as well as studies in other neuromuscular conditions.

Vamorolone has been developed by ReveraGen Biopharma under a venture philanthropy model, with funding from international non-profit foundations, and the US and EU governments.

S 05

EURO-NMD, a reference network for neuromuscular diseases

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Rare diseases (RD) are defined in the European Union as life-threatening or chronically debilitating conditions that affect less than 5 per 10 000 people. It is well established that the small number of patients and the geographic dispersion is an obstacle to the diagnosis, access to care, research and improvement of medical expertise. The European Union has tried, over the years, to combat the lack of specific health policies for rare diseases in the different Member States, through the establishment of an overall strategy for Member States throughout the European Union.

The process behind the establishment of the European Reference Networks (ERNs) was a long one that started around 2004 with the creation of the Rare Diseases Task Force later replaced by the EUCERD Joint Action (EJA), led by Kate Bushby. These specialised working groups were responsible for the policy work and recommendations that were at the origin of the publication, in March 2014, of the Delegated and Implementing Acts by the European Commission (EC). These provide a framework for the creation of ERNs. ERNs are defined as networks of health care providers/centres providing highly specialised healthcare, with the purpose of improving access to diagnosis, treatment and care for patients across Europe. After the publication of the Delegated and Implementing Acts in March 2014, the EJA conducted a study to suggest a coherent grouping of the different RD in thematic areas. The suggested grouping intended to be a rational approach to RD ERN planning and to ensure coverage of all RD.

The work to establish EURO-NMD started in 2013 under Kate Bushby's lead. At that time we organised and ENMC workshop that has set the grounds for the neuromuscular ERN –" 200th ENMC International Workshop "European Reference Networks: Recommendations and Criteria in the Neuromuscular field", 18–20 October 2013". EURO-NMD builds and expands on the successful CARE-NMD and utilised the TREAT-NMD Alliance portfolio of resources.

EURO-NMD is a European Reference Network for the thematic grouping of rare neuromuscular diseases (NMDs), a broad group of related disorders that represent a major cause of mortality and lifelong disability in children and adults. NMDs collectively affect an estimated 500,000 EU citizens and result in significant costs for families and the healthcare system. EURO-NMD unites 61 of Europe's leading NMD clinical and research centres in 14 Member States and includes highly active patient organizations. More than 100,000 NMD patients are seen annually by the ERN.

The network aims are the harmonization and implementation of standards for clinical and diagnostic best practice; improving equity of care provision across Member States; decreasing time to diagnosis; increasing cost efficiency through better care pathways and access to specialist training and education. These aims will be addressed through the application of eHealth services, development and application of care guidelines, facilitation of translational and clinical research and development of educational programs.

S 06

The DMD-Hub, collaboration to address and increase trial capacity in the UK

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With Duchenne muscular dystrophy (DMD) clinical research at an unprecedented stage in terms of the number of possible therapeutic approaches coming to trials, the need to increase trial capacity for DMD trials in the UK and improve trial readiness was identified. Specifically, clinicians at established UK clinical trial centres involved in multiple DMD studies were reaching capacity, while centres that did have capacity lacked the expertise and needed support to run industry-sponsored clinical trials.

Kate Bushby was instrumental in leading a collaboration with the community to take decisive action. The 'Newcastle plan' workshop was organized to bring together 75 participants from the DMD patient and research community, as well as industry to address the issues. As a result, 8 DMD patient organizations agreed to fund 16 posts at a cost of £1.2 million to address the immediate capacity issue at experienced sites.

As part of a longer-term strategy the DMD-Hub was set up as a partnership between UK centres of excellence and Duchenne UK. With additional investment of £1.1 million from Duchenne UK, the DMD-Hub is facilitating the sharing of expertise and has successfully developed a network of trial-ready centres in the UK now able to take on interventional trials in DMD.

To date the DMD-Hub has funded 12 additional posts at 6 trial sites in Newcastle, Liverpool, Leeds, Birmingham, Bristol and Glasgow. In 2019 there are plans to support an additional 2 sites in London and Manchester. The Hub is committed to working with further sites (including Oswestry, Cambridge and adult sites) to facilitate them to take on upcoming industry- and academic-led trials. Ongoing training for other sites is expected to open up additional opportunities in subsequent years.

The DMD-Hub website (dmdhub.org) is a key resource for industry, clinicians and patients. It hosts an interactive map of the UK detailing clinical trial opportunities for patients, contains a repository of information and tools for sites and acts as a one-stop shop for industry / sponsors interested in conducting trials in the UK.

Partnerships with industry and sites are being enabled by the DMD-Hub to facilitate trial planning and innovative funding models are being implemented at DMD-Hub sites to ensure sustainability.

Future areas of interest for the DMD-Hub include addressing the issues related to gene therapy trials and the development of an Adult-Hub network to include the non-ambulant population.

The mission of the DMD Hub is to ensure all patients with DMD, including children and adults, have access to clinical research opportunities.

S 07

Promoting rare disease policies across Europe

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Leadership of the initial TREAT-NMD 'Network of Excellence' resulted in the increasing involvement of the Newcastle Muscle Team researchers in projects and advisory bodies dedicated to 'rare diseases' more broadly. Professor Kate Bushby, in particular, quickly became acknowledged as a leading expert in the field of rare disease policy. Rare diseases have, for many years, been recognised as a priority area for European cross-border collaboration: although the number of patients living with any single condition will -by definition- be small, the fact that there are over 7000 individual conditions classed as 'rare' equates to ca. 30 million patients across Europe. The rarity of these conditions poses particular challenges to patients and families, but also to health and social systems.

As vice-chair of the European Union Committee of Experts in Rare Diseases (2010-2013), and subsequent independent expert in the Commission Expert Group on Rare Diseases (2014-2016), Kate and her

team developed a unique reputation for Newcastle University as a seat of expertise not only in specific thematic areas such as neuromuscular diseases, but also in the formulation and implementation of policies supporting better diagnostics, treatment and care for all conditions classed as rare. By focusing on the commonalities, Kate and her team led the development of policies and recommendations across a broad range of topics, from national frameworks to rare disease registries and data capture, from cross-border genetic testing to network-building. The fruits of those policies are highly visible today, most notably in the creation of 24 European Reference Networks for rare diseases, and in the adoption of national plans and strategies by 25 European Member States: this impact is very evident in the UK, as well as in mainland Europe.

Kate's legacy in the rare disease field has continued, as her colleagues in the John Walton Muscular Dystrophy Research Centre continue to lead and shape international initiatives and projects with many ambitious goals: to identify future determinants of health and wellbeing for rare diseases; to reduce the diagnostic odyssey; to optimise the knowledge base and identify innovative solutions applied by one country which could be expanded to others; and generally to continue to strive towards collaborative policies with the power to alleviate the many difficulties and inequalities rarity creates.

S 08

What makes Genetics Human?

Professor Sir John Burn
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Times of rapid change can expose fault lines in society. Just as the mass of refugees from the Middle East elicited contrasting reactions which in turn stressed the existing partnerships in Europe, so the arrival of high throughput sequencing and point of care DNA testing will test the assumptions of Europe's nation states. Termination of pregnancy and population screening for genetic disorders test the diversity of opinion from the more individualistic and secular traditions of the North and West of Europe across to the countries of the East and South of Europe where traditional Catholic teaching holds sway. Memories of misuse and fears about impact on insurance deter many from whole genome sequencing and genetic databases while others, notably in the UK, press ahead with industrial scale sequencing in healthcare.

The emergence of non-invasive testing based on fetal DNA in maternal circulation will increase terminations of pregnancy for genetic disorders. Similarly, the availability of molecular targeted cancer treatments such as PARP inhibitors, PD1 blockers and BRAF specific therapy will prompt adoption of testing for germline defects in high penetrance "cancer genes" routine.

Point of care DNA testing for pharmacogenetic susceptibility, DNA/RNA tumour markers and the presence of pathogen sequences will democratise genomics. By testing cheaply and quickly for diagnostically relevant information at the clinical interface it becomes possible to use DNA technology to improve outcomes without eliciting negative reactions. Current restrictions on use of DNA technology in Europe will soon seem out of step with pressing clinical utility. A balance must be found which allows high throughput and point of care DNA tests to be ordered by a wide range of healthcare professionals and, in some cases, by members of the public while ensuring the hard won skills of professional geneticists remain available and their services financially viable.

Invited Speakers

Friday 5th April 2019

S 09

Genome Editing for Duchenne Muscular Dystrophy

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The advent of genome editing technologies, including the RNA-guided CRISPR/Cas9 system, has enabled the precise editing of endogenous human genes. We have applied these tools to the correction of mutations that cause genetic disease. For example, we engineered CRISPR/Cas9-based nucleases to correct the human dystrophin gene that is mutated in Duchenne muscular dystrophy patients. When we delivered these nucleases to cells from patients with this disease, the correct gene reading frame and expression of the functional dystrophin protein were restored in vitro and following cell transplantation into mouse models in vivo (Ousterout et al., Nature Communications 2015). When delivered directly to a mouse model of this disease, gene editing by the CRISPR/Cas9 system led to gene restoration and improvement of biochemical and mechanical muscle function (Nelson et al., Science 2016). In more recent studies we have shown that genome editing and dystrophin protein restoration is sustained in the mdx mouse model of DMD for one year after a single intravenous administration of AAV-CRISPR. We also confirmed immunogenic host response to Cas9 when administered via AAV vectors to adult mice, but show that the humoral and cellular immune response can be avoided by treating neonatal mice (Nelson et al., Nature Medicine 2019). Additionally, we have observed unintended genome and transcript alterations induced by AAV-CRISPR that should be considered for the development of AAV-CRISPR as a therapeutic approach. More recently, we have developed novel animal models of this disease for the preclinical development of therapies that will correct human disease-causing mutations. New constructs have been developed and validated for significant levels of gene correction and dystrophin restoration in this model. These studies demonstrate the potential for genome editing to be used to treat Duchenne muscular dystrophy and other neuromuscular disorders, and also highlight aspects of host response and alternative genome editing outcomes for further study.

S 10

Alternate gene therapy approaches to DMD

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Duchenne muscular dystrophy (DMD) occurs due to mutations in the *DMD* gene that result in an absence of the dystrophin protein, which plays a critical role in stabilization of the muscle sarcolemmal membrane via linkage to the transmembrane dystrophin-associated protein complex, and ultimate to the extracellular matrix. Gene therapy for DMD has largely concentrated on AAV-mediated delivery of microdystrophin gene constructs that have been engineered to fit within the approximately 5 kb size limit of AAV genomes, and typically encode critical dystrophin domains. Here we discuss two alternate gene therapy approaches. One approach utilizes AAV delivery of the *GALGT2* gene, encoding β-1,4-GalNAc transferase. Endogenous *GALGT2* is typically expressed in muscle only at the neuromuscular synapse and myotendonous junction, where utrophin replaces dystrophin in a utrophin-associated protein complex. Overexpression of *GALGT2* results in expression of utrophin throughout the muscle fiber, as well as upregulation of other proteins associated with muscle membrane stabilization, and a clinical trial of delivery of an rAAVrh74.MCK.GALGT2 vector via isolated limb infusion is underway. A second approach is directed

toward patients with the most common single exon duplication, using AAV delivery of non-coding U7 small nuclear RNAs (snRNAs) with sequences targeting exon 2 splice donor and acceptor sites that results in highly efficient exclusion of one or both copies of exon 2 from the mature mRNA transcript. In this case, either transcript is therapeutic, as exclusion of exon 2 results in translational initiation from a downstream internal ribosome entry site leading to a highly functional N-deleted protein isoform found in mildly affected Becker muscular dystrophy patients who have walked into their eight decade. Both approaches are complementary to microdystrophin therapies, to which they may also have theoretical advantages in subsets of patients.

S 11

AAV-mediated Gene Therapy in Neuromuscular Disease: Clinical Immunology Considerations

Byrne, Barry J
University of Florida, Powell Gene Therapy Center

Early onset neuromuscular disease (NMD) is often associated with severe or null mutations that lead to greater disease burden. The success of gene replacement strategies in this setting may be influenced by limited endogenous protein expression leading to anti-transgene immune response and loss of effective copy number in skeletal muscle due to somatic growth. In one example, Pompe disease is due to a deficiency or absence of the lysosomal enzyme acid alpha glucosidase (GAA), resulting in lysosomal glycogen accumulation that impacts striated muscle and the CNS, including defects of the neuromuscular junction (NMJ). Respiratory failure is the leading cause of morbidity and mortality in Pompe patients. AAV vectors expressing GAA have been evaluated in a phase I/II study in ventilator-dependent and independent pediatric Pompe patients. These studies are based on the finding that accumulation of glycogen in spinal motor neurons contributes to weakness and diaphragmatic dysfunction observed in Pompe disease. In a number of preclinical studies, we have found that restoration of GAA activity in muscle and neural tissue is able to reverse ventilatory insufficiency by reversing motor neuron dysfunction and restoring the integrity of the NMJ. The principle defect in the motor unit is related to deficiency of NMJ structure and function. New evidence also indicates the need for early intervention related to neural dysfunction since motor neurons show evidence of apoptosis in the murine model of Pompe. These deficits are present early in the mouse model and restoration of GAA activity in the muscle and neurons before 6 months of age leads to restoration of in situ force production. After 18 months of age, the loss in motor neurons leads to permanent deficits in force production of the tibialis anterior.

Clinical studies of AAV-mediated gene therapy have been pursued to address the fundamental aspects of gene therapy in a neuromuscular disease where patients are identified by severe early onset or newborn screening. Findings in non-clinical and clinical studies related to immune management in conjunction with AAV systemic delivery have paved the way for clinical studies in adults and younger subjects who are candidates for therapeutic AAV administration. The loss of neuromuscular junction formation is a major contributor to weakness and ventilatory failure and these deficits can be prevented by early administration of AAV-GAA. Studies which utilize next generation AAV vectors for systemic administration have led to efficient targeting of muscle and motor neurons for the early treatment of Pompe disease. Related studies in Duchenne muscular dystrophy also highlight the importance of identification and management of pre-existing anti-AAV antibodies which are able to reduce the efficacy of systemic AAV vectors. Practical considerations for the implementation of systemic AAV administration will be discussed for early onset neuromuscular disease.

S 12

Preventing transmission of pathogenic mitochondrial DNA mutations

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Mutations in mitochondrial DNA (mtDNA) are inherited exclusively from our mothers and can cause a broad range of debilitating and fatal diseases. Reproductive technologies designed to uncouple the inheritance of the mitochondrial genome from the nuclear DNA genome may enable affected women to have a genetically related child with a greatly reduced risk of mtDNA disease. Such technologies involve transplantation of the nuclear DNA from the egg of an affected woman to an enucleated egg from an unaffected donor. Nuclear genome transplantation can be performed either before or after fertilisation. The latter involves transfer of pronuclei, which separately contain the maternal and paternal genomes. In parallel with legal and regulatory reform to permit therapeutic application of so called "mitochondrial donation" techniques in the UK, we have conducted preclinical studies to test the safety and efficacy of pronuclear transplantation (PNT). Our findings indicate that PNT is compatible with development of embryos to the blastocyst stage, with no detectable effect on chromosome segregation and gene expression. Moreover, we find that the amount of mtDNA co-transferred with the pronuclei is minimal, accounting for 0-5% of the mtDNA contained in PNT blastocysts. Remarkably, this tiny amount mtDNA outcompetes the cytoplast donor mtDNA in some embryonic stem (ES) cell lines derived from PNT blastocysts. While the relevance of this to development in vivo remains to be established, the finding highlights the importance of ongoing research to minimise resurgence of pronuclear-associated mtDNA. In the meantime, the Human Fertilisation and Embryology Authority (HFEA) have approved the cautious use of PNT in the UK and our clinic has been granted the first licence for its therapeutic application.

S 13

Mitochondrial donation: predicting who might benefit?

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Primary mitochondrial (mt) DNA diseases affect up to 1in 5000 live births and can often have devastating clinical sequelae. There are currently no curative treatments with the focus of therapeutic strategies largely on palliation of symptoms. The emergence of assisted reproductive techniques, such as Mitochondrial Donation, offer the first real opportunity to prevent the transmission of some serious forms of mtDNA disease from mother to child. The Human Fertilization and Embryo Authority (HFEA), the statutory regulatory authority charged with regulating human embryo research in the UK, has devised, a detailed regulatory process for issuing individual licenses to couples considered suitable for Mitochondrial Donation. Clear licensing prerequisites include a risk of transmitting serious mtDNA disease and a predicted mtDNA mutant load in oocytes that would make Pre-implantation Genetic Diagnosis (PGD) unsuitable. In women of child bearing age with a confirmed diagnosis of mitochondrial disease due to pathogenic mtDNA mutations, reproductive counselling is considerably more complicated due to issues of mtDNA heteroplasmy, threshold effect and variable penetrance of specific mutations. Predicting mutant load in children is particularly complex as seriously affected children with high heteroplasmy levels can be born to asymptomatic mothers harbouring low levels of deleterious mtDNA mutations. Here I will discuss the complexities around such predictions and present new work on the derivation of a statistical model to forecast the proportion of children with mtDNA heteroplasmy levels expected below a cut-off value that aligns with current PGD practice guidelines for the most common heteroplasmic mtDNA pathogenic variant in adults. Our findings have not only considerable implications for women at risk of transmitting serious mtDNA mutations but are now being considered in the HFEA deliberations for the granting of treatment licenses for Mitochondrial Donation.

Posters and Platform Presentations

‡indicates a platform or flash presentation

Key:

Abstract Category	Abstract prefix
Muscular Dystrophies	D
Peripheral neuropathy	PN
Motor Nerve Disorders	MND
Neuromuscular Junction Disorders and Channelopathies	NMJ&C
Mitochondrial Disease	MD
Other Diseases and Diagnostics	OD

Dystrophy

D01

Selenoprotein related myopathy (SEPN1-RM): A comprehensive analysis of progression over time to guide clinicians and clinical trials

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Background: The term *SEPN1*-related myopathies (SEPN-RM) refers collectively to four autosomal-recessive disorders caused by mutations in the *SELENON* gene, with similar clinical features but with distinct muscle pathologies.

Aims and Methods: We present cross-sectional phenotypic data of a multi-centric large cohort of 61 patients with SEPN1-RM with pathogenic variant in *SELENON* gene. We also present longitudinal data on clinical course of 25 patients (21 families) aged 2.5-24 years over a mean follow-up of 6.3 years with

the aim to assess changes in motor abilities, respiratory function and scoliosis progression based on functional scores, lung function tests and Cobbs angle.

Results: In the cross-sectional data more than two-thirds (47/61; 77%) of patients presented at ≤ 2 years with hypotonia, poor head/neck control and development delay. 45/61 (74%) patients developed scoliosis at a mean age of 10.3 years with 18 patients undergoing scoliosis surgery (mean age 13.6 years). A total of 10/61 (16%) children lost ambulation within the first two decades of life. Nocturnal ventilator support was initiated in 3/4th (50/61; 82%) patients (with mean FVC 38% - n=20) at mean age of 13 years.

In the longitudinal data patient cohort, two-third of the children (n=16/25; 64%) had weight below the 2nd centile during the follow up period and 6/61 children (0.1%) needed nasogastric feeds and/or gastrostomy. Functional motor scores declined from mean total score of 33 to 27 with an estimated annual change in motor ability scores of -0.55; similar trends were also noted with timed tests indicative of a slow motor decline over time. 21/25 (84%) patients had spinal stiffness, preceding scoliosis by mean of 1.8 years. FVC trends showed a decline from a mean of 52% to 40% over a 4-year mean follow up. The estimated change in FVC % per year was -2.04 (SE 0.46; p value <0.001).

Conclusions: This large cohort of SEPN1-RM patients well describes the phenotype and details the clinical course of this rare condition, with emphasis on a paediatric population. Our data suggest that loss of independent ambulation occurs more frequently than previously reported; and the observed FMS and FVC trend data provide useful information on the slope of change over time. Our results emphasize the importance of a consistent multi-disciplinary assessment and management of these patients, to improve outcomes and inform clinical trials.

Abstract structure:

D02

An investigation of *DMD* genetic variation and conservation and its links to intellectual disability

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Background: *DMD* mutations in both males and females are linked to cognitive impairment of varying degrees. The degree of impairment does not correlate with the severity of muscle disease or with the "reading frame rule" and the pathogenic mechanism is poorly understood. This makes it impossible to reliably predict cognitive outcomes for specific mutations. Similarly, it is difficult to confidently ascribe pathogenicity to *DMD* variants identified in children undergoing genomic testing for intellectual disability, whether or not any muscle disease is present.

Aims: We sought to ascertain whether publicly available genomic datasets could be used to infer regions of functional and clinical importance within the *DMD* gene, with a particular focus on potential causes of intellectual disability.

Methods: Population variant data were obtained from the gnomAD database, while conservation scores were obtained via the UCSC table browser. Further variant information (including both copy number and single-nucleotide variants) and clinical phenotype data were obtained from the DECIPHER database.

Results: Preliminary analysis reveals regions with especially high degrees of conservation within the dystroglycan-binding domain of the dystrophin protein. These same regions also display especially low frequencies of population-level variation, further suggesting their functional importance. Analysis of clinical phenotype data is ongoing and these results will also be presented.

Conclusion: This study confirms the functional importance of the 3' region of the *DMD* gene and helps define specific regions that may be of pathogenic

relevance in *DMD*-related cognitive impairment. Furthermore, this data-driven approach to the definition of functionally important gene regions is applicable to any gene of interest and may provide a clinically useful tool in helping to resolve the pathogenicity of genomic variants.
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D03

An exploration of the relative progression of change in a range of upper limb (UL) parameters in boys with Duchenne muscular dystrophy (DMD)

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Background: The progression of DMD in the UL is not well documented in the literature, particularly in boys treated with glucocorticoids.

Aims: To explore the progression of change in isometric strength, joint range and function of the UL and investigate possible relationship between the above parameters.

Methods: 21 boys with mean age 10.4 (3.25) years (13 ambulant, 8 non-ambulant) with DMD treated with glucocorticoids were followed longitudinally for 4.5 years. UL myometry, goniometry and the Performance of the Upper Limb test (PUL) assessment data were extracted and analysed. The analysis included descriptive statistics, comparisons between ambulant and non-ambulant groups and correlation between the outcome measures.

Results: The PUL scores demonstrated an increasing trend in the boys up to 8 years old, followed by a plateau and then declined from approximately 11 years of age in a proximal to distal order. In our sample, the distal domain did not change significantly but the proximal and middle domain captured decline, particularly in the non-ambulant group. Goniometry scores demonstrated increasing presence and severity of contractures with age. In the ambulant group, approximately 28.3 % of the boys demonstrated shoulder contractures, 6.7% elbow contractures and 11.7% wrist contractures, while in the non-ambulant group, shoulder contractures were present in 62.1%, elbow contractures in 58.3% and wrist contractures in 33.3% of the boys. Myometry absolute values for shoulder flexors, elbow flexors, elbow extensors and wrist extensors showed no particular trend with increasing age. Myometry, goniometry and PUL were poorly correlated.

Conclusions: In this cohort, PUL scores declined in a proximal to distal direction. Joint contractures increased in a proximal to distal order. Myometry did not correlate with disease progression. Neither myometry nor goniometry were predictive factors for UL function in boys with DMD. Further research to evaluate the use of myometry in DMD may be beneficial as this is a commonly used outcome measure.
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‡D04

The common *COL6A1* deep intronic c.930+189C>T mutation in Ullrich Congenital Muscular Dystrophy is efficiently corrected by exon-skipping AONs treatment

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Background: Collagen VI-related congenital muscular dystrophies (COL6-CMD) are the second most common congenital muscular dystrophy variants and currently there is no cure available. COL6-CMD are caused by recessive or dominant mutations in one of the three genes encoding for the α-chains of collagen type VI (*COL6A1*, *COL6A2* and *COL6A3*). Depending on the type of mutations, COL6-CMD ranges from the severe Ullrich Congenital Muscular Dystrophy (UCMD) to intermediate phenotypes and the milder Bethlem Myopathy (BM). The commonest recurrent mutation is a deep intronic *COL6A1* mutation that induce the incorporation of a novel pseudo-exon in the transcript, causing a dominant gain of toxic function.

Aims: The aim of this study was to identify lead AON sequences designed to target a *COL6A1* deep intronic mutation and abolishing the inclusion of the pseudo-exon in the transcript, hence generating a normal transcript.

Materials and Methods: Skin fibroblasts cultured from 4 UCMD patients carrying *COL6A1* deep intronic c.930+189C>T mutation were used as cellular models. 15 AONs were designed and their efficiency was evaluated at both RNA and protein levels using quantitative real-time PCR, immunofluorescence and flow cytometry.

Results: Two lead candidates have been identified with promising results in skipping the pseudo-exon. These AONs specifically suppress the aberrant splicing and the incorporation in the mature transcript of the pseudo-exon, without altering the *COL6A1* wild-type expression at RNA level. Moreover, these two AONs lead to the restoration of collagen VI protein production in the extra cellular matrix (ECM) from all the 4 patients derived fibroblasts. The restoration of the protein production in the ECM was evaluated with both immunofluorescence and flow cytometry analysis. We are currently analysing the ultra microstructure of collagen VI protein in ECM with electron microscopy, to confirm normal collagen VI structure restoration after AONs treatment.

Conclusion: Our findings provide a proof of concept for AON exon-skipping as a therapeutic approach for one of the most common mutation in COL6-CMD.

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D05

Translational consequences of neurodegeneration in dystrophic nerves of *mdx* mice, as a model for Duchenne Muscular Dystrophy

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Background: In the childhood disease Duchenne Muscular Dystrophy (DMD) and the dystrophic *mdx* mouse model, intrinsic repeated bouts of skeletal muscle necrosis result in denervated neuromuscular junctions (NMJs). We hypothesise that this ongoing NMJ denervation results in premature progressive degenerative changes in the nerves innervating the dystrophic muscles contributing to their loss of function.

This dystrophic research builds upon our studies of normal age-related loss of skeletal muscle mass (sarcopenia) in healthy mice, associated with denervation of NMJs and myofibres. Our time course study of sciatic nerves from ageing C57Bl/6J mice (immunoblotting) showed increased levels of various neuronal proteins by 18 months (v SMI-32, ChAT) and many others by 22 months (Tau5, p62), indicative of neurodegeneration (2017. PMID: 27030741). Immunostaining of lumbar spinal cords from old mice shows loss of proprioceptive muscle afferents, with consequences for sensorimotor control of muscle function (2018. PMID: 30084046). These ongoing ageing studies provide the techniques for the studies in dystrophic rodents.

Aim: To test the hypothesis that premature progressive (irreversible) neuronal changes occur in the *mdx* mouse model of DMD.

Methods: Protein levels in sciatic nerves were quantified using Western immunoblotting.

Results: Our analyses of sciatic nerves of *mdx* compared with normal C57Bl/10Scsn mice by immunoblotting (n=8-10/group), show significantly increased levels (P≤0.05) of Tau5 and S100 by 13 months (9 months earlier than in normal mice), confirming our hypothesis; with markedly increased protein levels (e,g. Tau5) by 18 months (n=4-7/group.

Conclusions: Progressive neurodegenerative changes have potentially major adverse consequence for contraction and long-term function of dystrophic muscles. For growing DMD boys where relentless myofibre and NMJ damage occurs over many years, it is essential for clinical translation to know the extent to which neuronal/spinal cord function has been irreversibly altered at a particular stage of the disease, to optimise efficacy of clinical therapies that aim to stabilise or improve function of the DMD muscles.

For pre-clinical trials using dystrophic rodents, neuronal degeneration may prove a valuable readout to monitor longer-term benefits of therapies designed to prevent/reduce myonecrosis.

D06

Neuromuscular disorders in Qatar: Genetic Distribution and Proteomics

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Background: NMDs comprise an extensive clinically and genetically heterogeneous group of uncommon inherited disorders.

Aims: The objectives of our study are to find out about the genetic distribution of NMDs in Qatar, the frequency of the subtypes as well as the pattern of altered circulating plasma proteins in a sample of NMDs patients.

Subjects and methods: The first phase of the project has recruited 58 NMDs families having a variety of congenital myopathies/dystrophies and limb girdle muscle dystrophies. Whole genomic sequencing and large scale Somologic proteomic screening were applied.

Results: Congenital mersoine deficient muscle dystrophy (MDC1A) was the predominating type encountered in our NMDs cohort. All MDC1A patients were of the non-ambulatory type, the best achieved motor skill, in a proportion of patients, was the unsupported sitting. A founder mutation was identified in a group of Qatari patients. Variability in clinical phenotype's severity in terms of respiratory and feeding compromises, osteopenic changes, and brain images were clearly obvious among MDC1A patients with *LAMA2* founder mutation as well as other mutations. An interesting novel large N-terminal deletion identified in our study showed the retained transcription and translation of LAMA2 in affected cases. This finding points out the activation of an alternative LAMA2 promoter.

Sarcoglyconopathy and Fukutin Receptor related muscle dystrophies were of the recurrent dystrophies encountered in our cohort. Other rare subtypes were identified. WGS variants analysis revealed exciting clue to the atypical brain structural abnormalities identified in one of our patients. The proteomic screening approach revealed a set of 100 significantly differentially altered markers in patients versus controls. A number of naturally non-enriched muscle proteins has been identified specifically altered in patients indicating potential roles in promoting secondary changes of muscle damage process.

Conclusion: The findings of this study are the first to address the genetics of NMDs in Qatar. WGS identified nation specific founder mutations both in LAMA2 and sarcoglycan alpha and spotted a genetic background clarifying some of the atypical MDC1A-clinical characteristics. The "Omics", Whole Genome and Proteomic screening provide high capacities of better understanding of the pathophysiology of muscle damage in dystrophies.

D07

Global Phase 3 PolarisDMD Trial for Edasalonexent, an Oral NF-kB Inhibitor that Has Shown Consistent Positive Effects on Muscle Function in Boys with DMD

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Background: Edasalonexent (CAT-1004) inhibits NF-kB, a key link between loss of dystrophin and disease pathology, and which plays a fundamental role in the initiation and progression of skeletal and cardiac muscle disease in DMD.

Aims: To assess efficacy and safety of edasalonexent in the Phase 3 PolarisDMD trial.

Methods: PolarisDMD is enrolling boys with DMD from 4 to 7 years of age (before 8th birthday) with any mutation who are able to stand from supine in ≤10 sec. Key exclusion criteria include glucocorticoid use within 6 months, or recent use of investigational therapies. Edasalonexent/placebo will be given three times/day for 1 year, and ~125 boys will be enrolled in a 2:1 ratio, active:placebo, followed by an open-label-extension (OLE) study in which all boys receive edasalonexent. The North Star Ambulatory Assessment is the primary endpoint with timed function tests as secondary endpoints. Additional assessments include ambulatory heart rate monitoring, growth assessments and bone density.

Results: Edasalonexent has previously been studied in boys with DMD of the same age in the Phase 2 MoveDMD trial and OLE, which showed benefits on muscle function with clinically meaningful slowing of disease progression through more than a year of treatment compared to the off-treatment control period. Edasalonexent had a durable and positive impact on muscle enzyme tests including CK and other muscle biomarkers that were significantly improved. Lower leg muscle MRI T2, which correlates with muscle function and loss of functional abilities, was significantly improved compared to off-treatment progression, consistent with slowing of disease progression. Edasalonexent was well tolerated without evidence of steroid side effects. Height and weight increased age-appropriately, and the elevations in heart rate typically observed in DMD declined significantly toward age-normative values, suggestive of benefit.

Conclusions: Edasalonexent has demonstrated safety and efficacy in the MoveDMD trial, and the global Phase 3 PolarisDMD trial is currently enrolling. Edasalonexent is a potential oral foundational therapy for all affected by DMD.

‡D08

Non-invasive assessment of fibrosis in mouse skeletal and cardiac muscle by contrast enhanced magnetic resonance imaging using EP3533

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Background Increased levels of fibrosis in skeletal and cardiac muscle have been associated with a worse prognosis in Duchenne muscular dystrophy (DMD). Anti-fibrotic medications are being evaluated for amelioration of DMD. To date, MRI has not been used successfully to image fibrosis within skeletal muscle as an outcome measure.

Aims and Methods: EP3533 is a novel gadolinium-based contrast agent using a complex peptide with an affinity to collagen 1. Compared to standard Gd (DTPA), the relaxivity of EP3533 is five times higher per Gd atom. We acquired MRI scans from age-matched 40 week old *mdx* and BL10 mice, using EP3533 to detect and quantify fibrosis within skeletal and cardiac muscle.

Results: Significant differences in post-contrast R1 were demonstrated between *mdx* and BL10 mice using EP3533 (cardiac p=0.02, GCN p=0.04, TA p=0.04). All muscle groups had increased signal to noise ratio (SNR) at 5 minutes, which may be due to interference from Gd enhancement in blood vessels. Levels of SNR in the *mdx* mouse reached a plateau at 60-90 minutes, and did not return to pre-contrast levels after the scanning period (120 minutes). The BL10 mouse showed an initial peak at 5 minutes, and then plateaued at a much lower level from approximately 10 minutes onwards. Differences between *mdx* and BL10 mouse muscles in signal were demonstrated most clearly in the tibialis anterior (TA) muscles. Post-MRI the TA and gastrocnemius (GCN) muscles and the heart were removed for fibrosis quantification via Masson's trichome staining and the hydroxyproline assay. R1 change at 70 minutes in all muscles at follow up scan correlated significantly with Masson's trichrome. R1 change at follow up correlated in three out of four muscles with hydroxyproline. The strongest correlations to *ex vivo* measures of fibrosis were in the GCN muscle

Conclusion: The results of this study suggest that EP3533 can be used to quantify fibrosis within skeletal and cardiac muscles of *mdx* mice, showing a high degree of correlation to established histological and biochemical methods of fibrosis quantification. The results do provide support for the potential use of EP3533 as a non-invasive way to quantify fibrosis *in vivo*.

D09

DMD HUB: Expanding and facilitating clinical trials for Duchenne Muscular Dystrophy in the UK

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The DMD Hub was established as a unique partnership between Duchenne UK, the John Walton Muscular Dystrophy Research Centre in Newcastle and the Dubowitz Neuromuscular Unit at Great Ormond Street Hospital in London, to address trial capacity issues for Duchenne muscular dystrophy (DMD) trials in the UK.

Together we are able to address the issues and share expertise to establish a network of trial-ready centres in the UK, able to take on interventional trials in DMD. During the first 2 years we have supported the development of 6 trial sites and are working to facilitate additional sites to take on upcoming industry and academic-led studies.

The DMD-Hub website (DMDHub.org) is a key resource for industry, clinicians and patients. It hosts the clinical trial finder, which is an independent resource, regularly updated by a variety of reliable sources to provide timely information on existing and pending clinical trials for DMD in the UK. Links to centres and staff performing the trials are provided to facilitate clinical trial recruitment. The development of a Standard Operating Procedure for clinical trial recruitment will be linked to the clinical trial finder to facilitate fair and equitable access to clinical trials across the UK.

The clinical trial finder currently lists 26 trials in 12 UK sites. The JWMDRC-Newcastle and GOSH-London remain the sites with the highest number of trials, however it is clear that the DMD-Hub has successfully facilitated additional sites to take on trials.

The DMD-Hub toolbox, also available on the DMD-Hub website, is an ever evolving repository for training material and other resources to respond to the needs of sites and industry in the setting up and delivery of interventional clinical trials in DMD.

The DMD-Hub has expanded its focus to better include the DMD adult population. A meeting was convened with key stakeholders to prepare for the unique challenges of conducting clinical trials in this group. The DMD-Hub is committed to working on the recommendations identified during this meeting and supporting the development of the infrastructures needed.

D10

A novel in-situ hybridisation (ISH) assay mapping the in-frame pseudoexon 11 (pE11) expression in cultured dermal fibroblasts (CDF) and muscle in patients with severe collagen VI disease due to a deep intronic mutation in *COL6A1*

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Background: A recurrent, *de-novo* deep intronic mutation (intron 11) of *COL6A1* (c.930 + 189C>T) leading to a dominantly acting in-frame pseudoexon insertion, originally identified by a combination of muscle RNA and whole genome sequencing, has been recently identified in a larger international cohort to cause severe collagen VI-related disease (COL6-RD). cDNA analysis has shown lower expression of pE11-containing transcripts in muscle and CDF compared to wild type transcripts, despite the clinical severity and markedly reduced/mislocalised collagen VI, indicating a strong dominant-negative effect.

Aim: To develop an ISH assay for spatial and quantitative mapping of pE11, wild-type (WT) and total COL6A1 transcripts and study effects of the mutation on collagen VI assembly in muscle and CDF.

Methods: We developed customised highly specific short-length exon junction chromogenically tagged mRNA probes targeting E9E10 (total transcripts), E11pE11 (pseudoexon-containing transcripts) and E11E12 (WT transcripts) for brightfield detection (BaseScope, ACD bio-techne). A panel of recently authenticated antibodies against chain-specific (collagen VI A1, A2, A3) and multimeric collagen VI was selected for immunoanalysis in muscle and CDF. Muscle and CDF from four patients carrying the intronic mutation and unaffected controls (CTRL) were recruited for the study.

Results: Our current work is focused on optimising ISH assay conditions using housekeeping probes, and optimising collagen VI multimeric and chain-specific antibodies in control muscle and CDF. In CDF from all 4 patients, we have observed uniform, low-level cytoplasmic expression of mutant pE11-containing transcripts and a much higher expression of WT transcripts comparable to CTRL.

Conclusion: Work on ISH in muscle and immunoanalysis in muscle and CDF is in progress. We will correlate results with existing muscle pathology, quantitative collagen VI flow-cytometry and *COL6A1* RT-qPCR transcription data. Quantitative ISH for mutation mapping combined with high-resolution microscopy could be potentially useful tools for studying effects of dominant negative mutations on collagen VI assembly in COL6-RD, and the effect of therapies aimed at removing the retained intron from the mature transcript.

D11

Optimisation of a high–throughput digital script for multiplexed immunofluorescent analysis of the sarcolemmal dystrophin and associated protein complex (DPC) and myofibre regeneration in entire transverse sections of muscle biopsies in Duchenne muscular dystrophy (DMD)

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Background: The primary molecular endpoint for a number of gene-based therapeutic clinical trials in DMD is restoration or increased production of sarcolemmal dystrophin from the baseline, assessed by measuring the amount of dystrophin using Western Blotting (WB) and immunohistochemistry (IHC). With the ever-increasing numbers of DMD clinical trials there is an urgent need to develop robust, reliable and objective dystrophin quantification methodologies by IHC that can overcome limitations of existing methods.

Aims: To develop a fully automated digital high-throughput method for multiplexed immunofluorescent analysis of dystrophin, associated DPC proteins (alpha-sarcoglycan and beta-dystroglycan) and regenerating fibres (fetal/developmental myosin-positive) in entire transverse sections of muscle biopsies with rigorous optimisation of two key variables - exposure settings and background signal correction.

Methods: Multiplex immunostaining of fresh frozen sections and subsequent whole section fluorescent slide scanning (AxioScan ZEISS) enabled the assessment and quantification by image analysis (Definiens software platform) of entire tissue sections from DMD, Becker muscular dystrophy (BMD) and control (CTRL) muscle biopsies. Optimal exposure settings were determined using DMD, BMD and CRTL sections. Arbitrary thresholding was minimised using a novel dynamic thresholding strategy of correcting the background signal per myofibre.

Results: The script generated automated data for intensity, coverage and colocalisation for dystrophin and DPC at the single-fibre as well as whole section level, number of dystrophin-positive fibres in the entire section and number of regenerating fibres in correlation to the mean fluorescent intensities of dystrophin, DPC and colocalised dystrophin-DPC in the entire section from DMD, BMD and CTRL biopsies.

Conclusion: We present a novel digital dystrophin quantification script capable of multiparametric analysis of dystrophin in relation to key DPC proteins and myofibre regeneration allowing unbiased analysis of the amount of functional dystrophin in DMD/BMD samples. Rigorous optimisation strategies are implemented to demonstrate 'clinical trial readiness' and regulatory compliance. Comparative analysis of previously published digital methodology with our new script is ongoing.

D12

Preliminary results from the "Observational study of clinical outcomes for testosterone treatment of pubertal delay in Duchenne Muscular Dystrophy (DMD) (NCT02571205)"

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Background: Delayed puberty is almost universal in glucocorticoid-treated patients with DMD. We have demonstrated variations in the way this is managed and there is a pressing need to establish the impact of exogenous testosterone therapy on well-being, pubertal status and longer-term gonadal function as well as the musculoskeletal system in this group of adolescents.

Aims: This single centre study followed the progress of 15 adolescents with DMD and delayed puberty who were treated with incremental intramuscular testosterone injections using the testosterone ester 'Sustanon' every 4 weeks for 2 years.

Methods: Data on a range of endpoints including pubertal status and gonadal function was collected at 6 monthly intervals for 2 years. Patients were also assessed 3 months after the final testosterone injection.

Results: All 15 patients completed 27 months of follow-up. All were pre-pubertal at baseline with a testicular volume (TV) less than 4mls and baseline testosterone level <2nmol/l. 27 months later (3 months after the last testosterone injection) the mean testicular volume was 3.3 mls (range 1.5-6mls), Tanner stage G4P4 (range G4P4-G5P5) with a mean testosterone of 10.4 nmol (1.9-21.8). Mean FSH was 9.1 IU/L (range 1.8-38.3) and mean LH 8.6 IU/L(range <0.5-25.1). Qualitative interviews and treatment satisfaction questionnaire responses were favourable and no serious AEs were attributed to testosterone therapy. 2 AEs were felt to be associated with testosterone (acne and injection site reaction). 2 patients chose to stop testosterone injections at 18 months because of concerns about potential effects on mobility. Both continued to progress through puberty spontaneously.

Conclusion: Testosterone is not part of the standard of care in DMD but the described regimen was an effective and well-tolerated treatment for pubertal delay. Further follow-up is required but preliminary results suggest that this regimen promotes endogenous testosterone production.

D13

The utility of *mdx:cmah* as a model for assessing skeletal development in Duchenne muscular dystrophy (DMD). A comparison of the *mdx* and the *mdx:cmah* models

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Background and aims: Investigation of compounds to treat the defect in growth and skeletal development in DMD necessitates an appropriate pre-clinical model. The *mdx* mouse is commonly used but its phenotype is mild and few medications that have shown benefit in *mdx* have also shown efficacy in clinical trials. The *mdx*:*cmah* carries a human-like mutation in *Cmah* gene and has a more severe muscle phenotype[1], but its growth and bone have not been investigated. We aimed to investigate its use as a model for assessing skeletal development in DMD.

Methods: 6 male *mdx*, *mdx:cmah* and wild-type (WT) mice were sacrificed at 3, 5 and 7 weeks of age. Bone mass and architecture were assessed by micro-CT and breaking strength determined by 3-point bending. Growth was assessed by anthropometric measures and calcein labelling at the chondro-osseous junction. Bone transcriptome was assessed using the Qiagen osteogenesis pathway 84-gene array on humeral RNA.

Results: *Mdx*:*cmah* mice were smaller than WT at 3, but heavier at 7 weeks (p=0.02). 5-week-old *mdx:cmah* mice showed an increased growth rate (p=0.007). Gene profiling identified a 3-fold upregulation in *Mmp-10* and *Bmpr1b* expression in both *mdx* and *mdx:cmah* bone v WT and increased expression of many growth factors in *mdx:cmah*, including *Igf-1 (*30-fold v WT). *Igf1R* (10-fold) and *Vegf*a *(*32-fold). Micro-CT indicated that cortical bone area and fraction were lower in 3-week-old *mdx:cmah mice*

($p<0.05$) but cortical bone fraction was greater in both *mdx* and *mdx:cmah* v WT at 7-weeks ($p<0.05$). Tissue mineral density was higher in *mdx:cmah* than WT at 3 ($p<0.05$) and 7 ($p<0.01$) weeks.

Conclusion: The *mdx:cmah* mice shows clear evidence of catch-up growth that is also associated with an increase in bone development. This pattern does not mimic the typical DMD growth trajectory. Whilst its utility for studying growth and skeletal development may be limited, further studies of this model can shed light on the phenomenon of catch-up growth.

Ref: Chandrasekharan et al. Sci Transl Med (2010)

D14

Atomic force microscopy based assessment of peptide-conjugated phosphorodiamidate morpholino oligomer treatment of Duchenne muscular dystrophy

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Background: Antisense oligonucleotide (ASO) mediated splice modification is currently the most promising therapeutic intervention for Duchenne muscular dystrophy. ASO can be used for targeted exon exclusion resulting in the correction of aberrant reading frames and the production of an internally deleted, yet largely functional, dystrophin protein. Crucial modification resulting in a significant increase in bioavailability of the drug is conjugation of ASO to a cell-penetrating peptide. The peptide Pip9b2 was conjugated to a phosphorodiamidate morpholino oligomer (PMO). Atomic force microscopy (AFM) is a widely applied tool for biomechanical studies of pathologically altered samples. In AFM, mechanical properties of cells or tissues are quantified through the relative Young's modulus.

Aims: To estimate effectiveness of treatment of mdx mice using two different doses of Pip9b2-PMO. To utilize AFM for assessment of mechanical properties of sarcolemma following the treatment.

Methods: Male mdx mice received 2 systematic intravenous injections of 7.5 mg/kg or 15 mg/kg Pip9b2-PMO at 1-week interval. Two weeks after administration, Pip9b2-PMO-treated mice and their age- and sex-matched controls (C57BL/10, mdx) were sacrificed.

Results: Exon-skipping efficiencies in m. tibialis anterior were determined to be 50.4% and 82.1% resulting in restoration of dystrophin protein to 49.3% and 53.3% following 7.5 mg/kg and 15 mg/kg correspondently. The efficiencies of 37.5% and 55.4% in m. soleus were considerably lower than that in m. tibialis anterior in mdx given 7.5 mg/kg or 15 mg/kg correspondently. Treated mdx showed uniform dystrophin distribution in the sarcolemma. Elastic properties of individual muscle fibers were probed by AFM. Myofibers of C57BL/10 yielded Young's modulus values of 4.2 kPa on average, whereas those of mdx were 2.3 kPa indicating lower resistance of dystrophic muscles to deformation. Following the treatment of mdx where 50% of dystrophin restoration was reached, the Young's modulus didn't differ from that in C57BL/10, suggesting recovery of mechanical properties of myofibers. The Young's modulus of treated groups was considerably higher than that in untreated mdx. It was not found any difference in elasticity between groups treated by 7.5 mg/kg and 15 mg/kg, dystrophin protein content was equal between those groups.

Conclusion: Thus, Pip9b2-PMO treatment provides effective dystrophin restoration in hindlimb muscles of mdx reversing myofibers stiffness.

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D15

Genome editing to correct duplications in the dystrophin gene

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Background: Duchenne Muscular Dystrophy is a severe neurodegenerative disorder caused by deletions, duplications or point mutations in the DMD gene, which encodes dystrophin. In absence of dystrophin, muscle fibers degenerate and patients become wheelchair bound by their early teens. Cardiac and respiratory muscles are also affected, causing premature death by the third decade of life. Several approaches are currently being tested in clinical trials to treat DMD, but none of them are suitable to treat patients carrying duplications in dystrophin gene, which account for 10-15% of DMD cases.

Aims: In the present work we developed a genome editing approach that exploits CRISPR/Cas9 to correct duplications in the DMD gene by using a single guide RNA.

Material and Methods: We compared nuclear electroporation and integrating lentiviruses as tools to deliver CRISPR/Cas9 in patient-derived myoblasts carrying in-frame duplications. The efficiency of genomic editing was assessed as the ratio between untargeted and targeted amplicons in the T7 assay. Finally, Reverse Transcriptase-PCR and Western Blot were used to assess dystrophin restoration at transcriptional and protein levels.

Results: We have identified a nuclease able to efficiently target DMD intron 9, which would be suitable for gene editing in several patients harbouring DMD duplications. The estimated genomic targeting efficiency in Cas9-expressing cells obtained from DMD patients was around 28%. RT-PCR of treated versus untreated cells confirmed the restoration to wild-type dystrophin transcripts and finally Western Blot confirmed both dystrophin correction and a decrease of the mutated protein in edited cells.

Conclusion: CRISPR/Cas9 editing tool has proven to be suitable to remove large genomic duplications. Cells derived from DMD patients carrying duplications in dystrophin gene have been treated and corrected by using a single CRISPR/Cas9 gene therapy construct and, at later stages, corrected myoblasts will be assessed for their potential to functionally restore dystrophin production following transplantation in Duchenne Muscular Dystrophy animal models.

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D16

Engineering cell fate and artificial chromosomes in human iPS cells for next-generation gene and cell therapy of Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is due to mutations of the dystrophin gene and primarily affects skeletal muscles. Our study looks at different strategies to circumvent substantial obstacles in the development of therapies for this incurable disease. The limited availability of large number of cells and the large size of the dystrophin gene (2.4Mb) could be tackled by combining human artificial chromosome (HAC)-based gene correction and iPS cell-mediated production of transplantable myogenic cells. However, another significant hurdle is posed by cell delivery, as skeletal muscle is the most abundant human tissue and to date there are no systemically deliverable human iPS cell derived myogenic progenitors generated without integrating transgenes. This challenge could be addressed by modulating molecules involved in the developmental specification of pericytes, able to migrate through the vascular endothelium upon intra-vascular delivery, thus circumventing the requirement for multiple intra-muscular injections.

Aims: This study aims at generating safe, systemically-deliverable DMD iPS cell-derived myogenic cells genetically correctable with HACs containing the entire human dystrophin genetic locus.

Methods: DMD iPS cells have been generated with non-integrating strategies and then differentiated into expandable myogenic cells using both the myogenesis regulator MyoD (mRNA) and a trangene-free, small molecule-based protocol. Molecules involved in the developmental specification of perivascular cells able to migrate through the vascular endothelium upon systemic delivery were tested to modulate cell migration.

Results: We generated three DMD iPS cell lines, one of them already genetically corrected with HAC. We then derived inducible myogenic cells similar to skeletal muscle pericyte-derived mesoangioblasts. Preliminary results show that genomic integration-free MyoD mRNA drives a good myogenic differentiation. We additionally show that iPS cell-derived myoblasts acquire enhanced migration ability in vitro upon activation of signalling pathways involved in the developmental specification of pericytes. Notably, RNA sequencing data elucidate the gene expression shift underlying this acquired function, opening the way to the identification of druggable targets.

Conclusion: The generation of human iPS cell-derived myogenic cells able to migrate through an endothelial wall lays the basis for further pre-clinical studies exploring a new, safer genomic integration-free approach for ex vivo gene therapy of DMD.

D17

Novel high-throughput digital analysis to quantify the amount of functional sarcolemmal dystrophin and myofibre regeneration in Duchenne Muscular Dystrophy (DMD) clinical trial samples (exon 53 skipping with Golodirsen)

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Background: Exon skipping therapies in Duchenne muscular dystrophy (DMD) aim to modulate the pre-mRNA splicing of the DMD transcript using morpholino (PMO) antisense oligonucleotides (AONs) to restore the *DMD* open reading frame, leading to the production of a partially functional protein. However, the functionality of such restored dystrophin has been questioned.

Aims: To determine if restored dystrophin following treatment with Golodirsen (exon 53 skipping PMO) results in enhanced levels of dystrophin-associated proteins (DPC) at dystrophin positive regions of the sarcolemma in post-treatment muscle biopsies as a surrogate marker of functional dystrophin. Additionally, to assess if there is a reduction in the number of regenerating myofibres associated with restored dystrophin expression.

Methods: We developed a highly automated, high-throughput method for multiplexed immunofluorescent analysis of dystrophin and DPC in entire transverse sections of muscle biopsies. Using an optimised image analysis script developed in Definiens software applied to digitally scanned whole sections, we are able to generate data that allows colocalised quantification of dystrophin and DPC (alpha-sarcoglycan, beta-dystroglycan) at the sarcolemma and quantify the number of regenerating fibres (fetal/developmental myosin cocktail).

Results: Exposure settings following rigorous optimisation experiments yielded normalised dystrophin

and DPC intensity values in control sections without significant variation across different batches. Digital analysis performed on two samples revealed significantly higher mean alpha-sarcoglycan intensity value in dystrophin-positive sarcolemma compared with dystrophin-negative sarcolemma. We were also able to quantify the number of the regenerating fibres in these samples.

Conclusion: Our high-throughput digital analysis of samples blinded to treatment state demonstrated that the levels of DPC proteins are higher in dystrophin-positive compared to dystrophin-negative fibres, suggesting the dystrophin produced is able to drive assembly of a functional dystrophin-associated protein complex. The algorithm also allowed us to quantify the number of regenerating fibres in the entire muscle biopsy sections. Analysis of dystrophin-DPC-myofibre regeneration data on the entire study cohort is in progress and results will be correlated with treatment status following unblinding. We suggest that quantitative dystrophin-DPC colocalisation data and potentially the percentage of regenerative fibres could be used as a surrogate marker of the functionality of restored dystrophin in DMD.

D18

Long-term effects of cardio-active medications on left ventricular function in patients with Duchenne muscular dystrophy-related cardiomyopathy

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Background: Almost all patients with DMD develop progressive left ventricular dysfunction. Despite this, there has been little research into the response of this specific form of cardiomyopathy to standard 'heart failure' medications, deployed uniformly.

Aims: To describe the course of ventricular function before and after the introduction of a combination of ACE-inhibitor and beta-blocker in a large cohort of patients with DMD-related cardiomyopathy.

Methods: This was a retrospective analysis of serial left ventricular fractional shortening measures obtained in the four years before and four years after initiation of combination therapy with an ACE-inhibitor and beta-blocker at one hospital in the period January '95 to January 2015. Therapy was initiated consistently at the first outpatient attendance at which left ventricular FS < 28% ('possible cardiomyopathy') or FS < 25% or regional wall motion abnormality ('definite cardiomyopathy') was detected. Doses were up-titrated over subsequent visits, as tolerated. Eplerenone was not added during this time period.

Results: Results of 821 echocardiograms (> 2 scans / patient), performed on 126 DMD patients, aged 16.2 ± 4.7 at time of initial and 21 ± 7.3 years at their last analysed echocardiogram were reviewed. Mean age of first cardiomyopathy detection was 17.3 ± 4 years - but significantly older in those on steroid therapy (18.1 ± 3.4 versus 15.5 ± 4.1 years; $p = 0.003$). Group mean FS fell by 0.83% per year before therapy, but this reduced to 0.38% per year during four years on treatment. 'Poor-attenders' had lower FS% before dysfunction was detected. Ventricular function - already lost before treatment, was not regained thereafter but fell at the same rate as in those treated earlier. Neither age of detection of cardiomyopathy nor FS% at therapy initiation affected the beneficial response.

Conclusion: Combination drug therapy stabilises ventricular function but cannot prevent its slower decline over time. Cardiac therapies should be initiated no later than on first detecting ventricular impairment.

D19

Global FKRP Registry

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Background: The Global FKRP Registry is an international registry for individuals with conditions caused by mutation of the *Fukutin-Related Protein* (FKRP) gene: limb girdle muscular dystrophy 2I (LGMD2I, newly renamed LGMDR9) and the congenital muscular dystrophies MDC1C, Muscle-Eye-Brain Disease and Walker-Warburg Syndrome. The registry seeks to further understanding of the natural history and prevalence of FKRP-related muscular dystrophies (MD).

Aims: The purpose of the registry is to aid the rapid identification of eligible patients for clinical studies. It disseminates FKRP-relevant information to participants; provides a source of information to academics, industry and healthcare professionals; and supports the FKRP community.

Methods: Registration is patient-initiated through a secure online portal. Participants give their informed consent and are invited to complete a questionnaire about their condition. Data is reported by both patients and their healthcare professionals and includes: age of onset, presenting symptoms, family history, motor function and muscle strength, respiratory and cardiac function, medication, in addition to information on patient quality of life and pain.

Results: Currently, 643 patients (54% female, 46% male) are registered with the Global FKRP Registry, with an age range of 1 to 79 years. Registrations are from 38 countries, with greatest numbers from USA (28%), Germany (21%) and UK (12%). Diagnoses are reported as LGMD2I (90%), MDC1C (2%), other FKRP-related MD (3%), unspecified (5%). Seventy-one percent of patients are reported as being ambulant, 24% as non-ambulant and 5% as unspecified. The mutations reported within the registry are: 65% homozygous for the common mutation (c.826C>A), 29% heterozygous for the common mutation, 5% heterozygous with two unique mutations and 1% homozygous with a unique mutation which is not the common mutation.

Conclusion: The Global FKRP Registry is a valuable tool for the collection of patient data which informs academics, healthcare professionals and industry. For example, registry data may improve our understanding of phenotype-genotype correlation and inform improvements in standards of care. The registry represents a trial-ready cohort of individuals and supports the FKRP patient community.

D20

A complete cross-sectional and longitudinal study on plasma derived miRNAs revealed novel dysregulated signatures in DMD patients

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Background: Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disorder affecting 1 in 5000 newborn males, mainly caused by out-of-frame deletions or, more rarely, duplications, nonsense or other small mutations affecting *DMD* gene and therefore dystrophin protein production.

MicroRNAs are short (~20-23 nucleotides) noncoding RNAs that regulate gene expression; their dysregulation in serum and urine has been associated with many paediatric neuromuscular conditions including DMD.

MiRNAs are present in biofluids as free circulating molecules or included in exosomes small microvescicles (40-100 nm) secreted by different cell types.

Aims: To investigate the potential of plasma derived exosomal miRNAs as a novel non-invasive biomarker in DMD and find novel free circulating miRNAs cross-sectionally and longitudinally dysregulated, we performed miRNA profiling and validation analysis in DMD patients and healthy controls.

Moreover, we assessed if there was any association between miRNA levels in plasma and corticosteroid treatment.

Materials & Methods: The patients included in this study are part of a cohort of DMD boys in a multicentre 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 Serum/Plasma Focus microRNA PCR SYBR green-based panels (Exiqon), while validations were carried out by a qPCR TaqMan small RNA Assay (Life Technology).

Results: We detected novel miRNAs (both free-circulating and exosomal) dysregulated in plasma from DMD patients and validated those with the strongest abnormal expression.

We also detected a set of longitudinally dysregulated miRNAs between different timepoints (ΔT1-T2=24 months) in DMD patients.

Conclusion: Our findings indicate that a set of free-circulating miRNA is longitudinally dysregulated in plasma from DMD patients. Moreover we detected novel dysregulated miRNAs in plasma from DMD patients.

D21

Activity of Stereopure Antisense Oligonucleotides in Cellular Free-Uptake Models Predicts Exon Skipping and Dystrophin Protein Restoration in *mdx23* Mice

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Background: Wave Life Sciences has established a targeted approach to designing stereopure nucleic acid compounds based on sequence composition, chemical design, and backbone stereochemistry. Wave is using this approach to develop stereopure exon skipping antisense oligonucleotides (ASOs) as potential disease-modifying therapies for Duchenne muscular dystrophy (DMD).

Aims: To determine the effects of investigational stereopure ASOs targeting exon 23 *dystrophin* (*DMD*) pre-mRNA on exon skipping efficiency and dystrophin protein restoration in the *mdx23* mouse model of DMD and to determine the relationship between *in vitro* and *in vivo* effects.

Methods: *mdx23* mice received single or 4 weekly intravenous (IV) doses of ASO. Exon skipping efficiency was determined by TaqMan® assay, dystrophin protein restoration by western blot, and dystrophin localization by immunofluorescence microscopy. Serum enzyme markers of muscle and liver function were assessed. Cultured DMD patient-derived myoblasts or murine *mdx23*-derived myoblasts were incubated with ASOs under free-uptake conditions.

Results: Stereopure oligonucleotides targeting exon 23 induced dose-dependent exon 23 skipping *in vitro* in *mdx23*-derived myoblasts. *In vivo*, after a single IV dose, stereopure ASOs induced up to 16% exon skipping in target muscle tissues (gastrocnemius, diaphragm, quadriceps, heart) and restored dystrophin protein in gastrocnemius in *mdx23* mice for ≥28 days postdose. Four weekly IV doses of stereopure ASOs induced dose-dependent exon skipping of up to 20% in target muscle tissues of *mdx23* mice, yielding dystrophin protein restoration in target tissues, with 93% restoration observed in the gastrocnemius. Serum creatine kinase, aspartate aminotransferase, and alanine aminotransferase levels were reduced by 87%, 55%, and 79%, respectively, after 4 weekly doses. The dose relationship of exon skipping induced by stereopure ASOs was similar *in vitro* and *in vivo*. Transcript levels observed with free uptake of oligonucleotides in cultured myoblasts were predictive of transcript and dystrophin protein levels in the *mdx23* mouse.

Conclusion: Stereopure exon 23 skipping ASOs produced efficient exon skipping and dystrophin protein restoration in *mdx23* mice. Consistent results observed between *in vitro* and *in vivo* models support our approach of selecting clinical candidates targeting exon 51 and exon 53 using a free-uptake myoblast cell model.

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D22

Vision-DMD: an update on the drug development program of the orphan drug Vamorolone (VBP15) in Duchenne Muscular Dystrophy (DMD)

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VISION-DMD is the drug development program of orphan drug Vamorolone (VBP15) in Duchenne Muscular Dystrophy (DMD)

Vamorolone is a first-in-class dissociative steroidal drug developed by ReveraGen Biopharma under a venture philanthropy model, funded from international non-profit foundations, US and EU governments.

Vamorolone is administered as liquid solution taken daily.

The Phase IIa open-label, multiple ascending dose (0.25, 0.75, 2.0, and 6.0 mg/kg/day) studies (VBP15-002 and VBP15-003) recruited 48 steroid naïve DMD boys, age 4 to<7 years. Following a 2-week treatment period and 2-week washout period (VBP15-002), participants continued to receive vamorolone for 24 weeks (VBP15-003). A 2-year long-term extension study of vamorolone was offered to participants completing the phase IIa study.

Results of the phase IIa studies show vamorolone was well tolerated during 24-weeks treatment at tested doses. Pharmacodynamic biomarkers confirmed Vamorolone retains anti-inflammatory activities, but shows decrease in frequencies and severity of corticosteroid side effects. Bone loss was not seen with any dose of vamorolone, evidenced by serum osteocalcin. Adrenal suppression and insulin resistance were less impacted in vamorolone-treated DMD patients, relative to published glucocorticoid studies.

Additionally, dose-responsive improvement of muscle function was observed for 2.0 and 6.0mg/kg/day dose groups compared to natural history controls.

A phase IIb randomised, double blind, parallel group, placebo and active controlled study with double blind extension in 120 DMD boys has been initiated in 30 sites in 9 countries. 9 patients have been enrolled and 10 sites of 30 are recruiting. The study aims to evaluate safety, efficacy, pharmadynamics and population pharmacokinetic of vamorolone at daily doses of 2.0mg/kg and 6.0mg/kg versus prednisone 0.75mg/kg/day and placebo over 24-week treatment period and evaluates persistence of effect over a Treatment Period of 48 weeks in ambulant DMD boys aged 4 to<7 years.

Studies conducted demonstrated the efficacy and reduction in adverse effects in DMD patients using vamorolone compared to traditional glucocorticoids, supporting further testing of vamorolone as a safer alternative to long term glucocorticoids for DMD.

Studies in older and younger DMD patients are in development.

Vamorolone potentially could replace chronic glucocorticoids in disorders where side effects detract from patient quality of life.

D23

Respiratory Function Decline in Eteplirsen-treated Patients Diverges From Natural History Comparators Over Time

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Background: Patients with Duchenne muscular dystrophy (DMD) experience progressive degeneration of skeletal muscles, including those involved in respiration, and respiratory decline is linked to mortality. The onset of respiratory decline precedes increasing levels of clinical management as the percent predicated forced vital capacity (FVC%p) falls below established thresholds. The goal of treatment is to delay the time to reach these thresholds.

AIM: Motor function in eteplirsen-treated patients in Studies 201 and 202 diverges from natural history after 1 year of treatment. This analysis was conducted to determine if respiratory function follows a similar pattern.

Methods: We evaluated eteplirsen treatment effect in Studies 201 and 202 on FVC%p over time compared with well-matched natural history controls from the Cooperative International Neuromuscular Research Group Duchenne Natural History Study (CINRG DNHS) to determine if respiratory decline exhibits a pattern similar to what has been described for motor function. In Studies 201 and 202, pulmonary function tests were performed in all patients (N=12) every 24 weeks over the course of 216 weeks of eteplirsen therapy. FVC%p was plotted over time for both studies. This was compared with FVC%p of baseline age and FVC%p-matched CINRG DNHS patients over the course of 4 years to determine if eteplirsen-treated patients diverged from the CINRG DNHS patients. Two cohorts of CINRG DNHS patients were used as comparators: the All CINRG cohort (n=75), and the Genotyped CINRG cohort (n=67).

Results: The eteplirsen-treated patients in Studies 201 and 202 had similar baseline levels and similar decline compared with the All CINRG and Genotyped CINRG cohorts for the first year. A divergence appeared after Year 2 that continued to widen over the 4-year period. At Year 4, a difference of approximately 10% in FVC%p favored eteplirsen treatment in comparison to both CINRG DNHS cohorts.

Conclusion: The temporal pattern of divergence of respiratory function in the eteplirsen-treated patients from the natural history comparators is similar to the pattern of divergence seen for motor function.

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D24

Eteplirsen Is Well Tolerated in Men with Mild or Moderate Renal Impairment

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Introduction: Duchenne muscular dystrophy (DMD) is a rare, X-linked, fatal, neuromuscular disease caused by *DMD* gene mutations that disrupt the dystrophin messenger ribonucleic acid (mRNA) reading frame and prevent the production of functional dystrophin protein. Eteplirsen is a phosphorodiamidate morpholino oligomer (PMO) approved by the US Food and Drug Administration for treatment of DMD patients with mutations amenable to exon 51 skipping. Eteplirsen excludes exon 51 to restore the dystrophin mRNA reading frame and to enable translation of internally shortened dystrophin protein. PMOs have uncharged backbones and bind in a sequence-specific manner to RNA targets through Watson-Crick base pairing. PMOs represent a unique chemistry, structurally and biologically distinct from other synthetic antisense ribonucleic acid therapeutics, such as phosphorothioates (PSOs). Toxicities observed with PSOs have not been observed in nonclinical or clinical studies of eteplirsen. In DMD patients studied, approximately 64% of the total systemic clearance of eteplirsen 30 mg/kg (approved dose) is via renal excretion.

Aim: To determine the pharmacokinetics (PK), safety, and tolerability of eteplirsen in men with mild or moderate renal impairment.

Methods: This single-dose, parallel-group study enrolled male volunteers with mild (estimated glomerular filtration rate [eGFR] ≥60 to <90 mL/min; n=8) or moderate (eGFR ≥30 to <60 mL/min; n=8) renal impairment. Participants received intravenous (IV) eteplirsen 30 mg/kg, and postdose PK, safety, and tolerability results were evaluated and compared with demographically matched men with normal renal function (n=9).

Results: All enrolled participants completed the study. Total plasma clearance decreased by 27.5% (mild group) and 60.6% (moderate group), with proportional reductions in renal clearance (22.7% and 56.6%, respectively) and higher overall exposure compared with the normal group. The single IV dose appeared to be well tolerated by all groups. Three participants, one in each group, reported 6 treatment-related adverse events (AEs). These were dizziness (normal group); pyrexia (mild group); and pollakiuria, micturition urgency, and 2 events of incontinence (moderate group). All AEs were mild to moderate, considered nonserious, and resolved.

Conclusions: Eteplirsen was well tolerated across renally impaired and normal renal function groups. Exposure to eteplirsen increased as a function of decrease in renal clearance.

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D25

Arm cycling in Facioscapulohumeral Dystrophy (FSHD) patients: results of pilot study

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Background: FSHD sufferers live a long life with disability. Symptoms may develop in early childhood and weakness usually noticeable in the teenage years with 95% of affected individuals manifesting disease by age 20 years. The disorder impacts on the upper extremity and torso, impacting negatively on the muscle mass, shoulder mobility and functional tasks. Consequently chronic weakness of the shoulder and upper limbs negatively impacts independence of sufferers, prospects of employment and staying at work. At present there is no known cure and knowledge regarding the mechanisms underpinning FSHD is not sufficient to halt the progression of the disease via pharmacological interventions or gene therapy. Surgical interventions are used to improve scapular stabilisation but long term effect on disease progression is limited.

Aim: This study aims to test our hypothesis that shoulder muscle weakness and reduced range of

movements at upper limb joints would not affect arm cycling ability of FSHD patients.

Methods: patients were recruited from our clinical group and FSHD registry. They were assessed for muscle strength, contractures of upper limb, Oxford shoulder score. Subjects performed 2 minute of upper limb cycling x 5 at self-selected cadence (total 10 minutes work), with 30 seconds of rest between arm cycling exercise intervals. Rate of perceived exertion (RPE), cadence and video analysis was performed throughout the exercise period.

Results: 15 patients participated in the study; age 18-60 years, 9 males and 6 females. Patients had varying degrees of shoulder involvement with Oxford Shoulder Scores from 4-48. Participants gradually increased the cadence, maintained or increased resistance. All participants were able to complete the exercise programme. RPE was increased marginally by the 5th cycle. No significant problems were reported after the study. Participants were positive about this as a home exercise programme.

Conclusion: Static arm cycling offers a potential tool for upper limb rehabilitation for patients with FSHD in home setting but its effectiveness needs to be proven.

D26

Additional benefits of Ataluren: 2 case reports

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Background: Dystrophinopathy is a rare, severe muscle disorder, with nonsense mutations found in 13% of cases. Ataluren was developed to enable ribosomal read through of premature stop codons in nonsense mutation genetic disorders. Effectiveness of this drug has been assessed by National Institute of Clinical Excellence in the UK and the drug is available by managed access agreement (MAA) for patients after 5 years of age.

Methodology: We describe two patients, 7 and 21 years, on Ataluren. One patient had access to treatment by MAA and another older patient had treatment through Continued access programme from PTC pharmaceuticals. North star and physiotherapy assessments pre and post treatment were compared.

Results: Patient 1 has significant learning difficulties and attends special school. He has point mutation in exon 65 (c.9461T>A; p.Leu3154X). On muscle biopsy dystrophin was absent with labelling of rod domains and only a rare revertant-like fibres are seen with a C terminal antibody. Pre-treatment timed rise from floor was 34.8 seconds, 10 meters run was 11.2 seconds and North Star Ambulatory assessment was 7/34. These responded within 5 months of treatment on Ataluren to timed rise from floor to 11 seconds, 10m run to 8 seconds and North Star Ambulatory assessment to 15/34. According to assessors this difference was related to improvement in understanding of instructions.

Patient 2 has been on Ataluren from the age of 10 years, when he was enrolled in the first clinical trials, and subsequently he has continued on Ataluren post trials (2016). He has a point mutation (exon 59). He has no learning difficulties and became non-ambulant at 14 years. He currently is not ventilated with a FVC at 1.62; his cardiac status; LVEF 46%, FS 26%. His last assessment shows preservation in upper limb strength (PUL 2.0 21/42) and he currently self-propels on level ground.

Conclusion and significance: Effectiveness of Ataluren is proved for motor abilities in patients with DMD in research trials mainly concentrating on motor outcomes of ambulatory children. These two cases support its benefit outside what has been researched so far.

D27

Long-term efficacy of ataluren for the treatment of nonsense mutation Duchenne muscular dystrophy: observational data from the STRIDE Registry

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Background: Duchenne muscular dystrophy (DMD) is a fatal, X-linked disease, characterized by progressive muscle weakness. Approximately 10–15% of cases of DMD are caused by a nonsense mutation (nmDMD) in the dystrophin gene, which leads to translation of truncated, non-functional dystrophin. Ataluren is the first approved therapy to target the underlying cause of nmDMD, enabling formation of full-length, functional dystrophin.

Aim: In this study, long-term effectiveness of ataluren (40 mg/kg/day) in routine clinical practice was evaluated in patients with nonsense mutation Duchenne muscular dystrophy (nmDMD) enrolled in the international STRIDE Registry (N=213).

Methods: We compared data from registry participants (with ≥48 weeks between first and last assessment) with data from participants in Study 020 (NCT01826487) receiving ataluren (N=115) or placebo (N=115) for 48 weeks. Data were extracted from the registry on 9 July 2018.

Results: Registry participants had a mean (standard deviation, SD) age of 9.8(3.7) years at first assessment compared with 8.9(1.8) and 9.0(1.7) years in the ataluren and placebo arms of Study 020, respectively. Mean exposure to ataluren ranged from 71.8 to 121.6 patient-years depending on the different outcome measures used. Registry participants experienced smaller 48-week mean (SD) functional declines than patients receiving ataluren or placebo in Study 020, respectively, in 6-minute walk distance (n=66, 35.0[69.7], vs n=109, 42.2[84.9], and n=109, 57.6[98.8] meters), and time to walk/run 10 meters (n=61, 1.6[3.2] vs n=109, 2.3[5.2], and n=110, 3.5[6.4] seconds), stand from supine (n=55, 2.9[5.0] vs n=101, 3.8[6.1], and n=96, 3.9[6.9] seconds), climb 4 stairs (n=47, 1.2[2.2] vs n=105, 2.7[5.3], and n=103, 4.5[7.3] seconds) and descend 4 stairs (n=40, 0.5[1.5] vs n=106, 2.2[5.3], and n=100, 3.9[7.9] seconds). Safety outcomes were consistent with the known safety profile of ataluren.

Conclusions: Although the registry cohort consisted of older patients compared with those in Study 020, these patients showed smaller functional declines, suggesting that ataluren may slow disease progression in patients with nmDMD in routine clinical practice.

D28

Effect of Ataluren on Age at Loss of Ambulation in nonsense mutation Duchenne Muscular Dystrophy: Observational Data from the STRIDE Registry

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Background: Duchenne muscular dystrophy (DMD) is a fatal, X-linked disease, characterized by progressive muscle weakness. Loss of ambulation is a major milestone in disease progression. Approximately 10–15% of cases of DMD are caused by a nonsense mutation (nmDMD) in the dystrophin gene, which leads to translation of truncated, non-functional dystrophin. Ataluren is the first approved therapy to target the underlying cause of nmDMD,

enabling formation of full-length, functional dystrophin.

Aim: In this study, age at loss of ambulation was evaluated in patients with nmDMD taking ataluren for at least 12 months while enrolled in the international, multi-center STRIDE (Strategic Targeting of Registries and International Datasets of Excellence) Registry (N=207).

Methods: In this study, age at loss of ambulation was evaluated in patients with nmDMD taking ataluren for at least 12 months while enrolled in the international, multi-center STRIDE (Strategic Targeting of Registries and International Datasets of Excellence) Registry (N=207). Data were extracted from the registry on 9 July 2018. Kaplan–Meier analyses were used to investigate age at loss of ambulation.

Results: Mean (SD) age of registry participants starting ataluren treatment was 9.8 (3.7) and 89.2% were being treated with corticosteroids in addition to ataluren. At the date of data extraction, registry participants had a mean (SD) age of 11.6 (3.6) years. Mean (SD) exposure to ataluren within the registry was 372.6 (211.6) patient-years and 44.6% of patients had been on ataluren for more than 720 days. Mean (standard error) age at loss of ambulation in registry participants was 15.5 (0.3) years, and 50% of patients were still ambulatory at the age of 16.5 years. Safety outcomes were consistent with the known safety profile of ataluren.

Conclusions: Ataluren may delay loss of ambulation in patients with nmDMD.

D29

STRIDE: A Patient Registry Study Examining the Use of Ataluren (Translarna™) in Patients with Nonsense Mutation Muscular Dystrophy (nmDMD)

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Background: Ataluren (Translarna™) is the first-approved treatment for nonsense mutation Duchenne muscular dystrophy (nmDMD) in the European Union, in ambulatory patients aged 5 years and older.

Aim: The STRIDE Registry is an ongoing, multicenter, observational study aimed at building a patient data repository to provide real world experience regarding the treatment patterns for ataluren in routine clinical practice.

Methods: An enrollment of approximately 200 patients was targeted, based in part on the size of the nmDMD population in the EU. Patients are being followed for at least 5 years from the date of a patient's enrollment, or until withdrawal of patient consent, or death, whichever occurs first. The study is being conducted in the countries in which the drug is commercially available.

Results: Baseline data (as of 31.01.2018) from a cohort of 154 patients (98.1% males, n=151) with

nmDMD from sites across 12 counties were analyzed in terms of demography and clinical manifestations of nmDMD. In this cohort, the median age was 10.2 (5.0, 45.4) years; nmDMD was most frequently diagnosed between the ages of 5-10 years (n=74, 48.1%), and the median age at genetic confirmation was 5.5 years (0.02, 39.1). The median age of symptomatic detection was 2.5 years (0, 30) with a mean time (SD) between first clinical/biochemical symptoms and nmDMD diagnosis confirmation (i.e., identification of dystrophin gene mutation) is 3.0 years (2.5). The exclusion of female carriers from the analysis had minimal impact on median age (10.1 years; min, max: 5, 22.8) and median age at genetic confirmation (5.4 years; min, max: 0.9, 15.6). Additional descriptive statistics from demographic data are forthcoming.

Conclusions: These data suggest a need for increased awareness of nmDMD symptoms in order to reduce delays in diagnosis; the later median age at diagnosis observed here relative to recent results from the global DMD population (3.4 - 4.3 yrs) suggests that some countries may still face challenges with genetic testing for nmDMD.

‡D30

Caveolar dysfunction as a new player in cardiac disease in Duchenne Muscular Dystrophy

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Background: Cardiac failure is a major contributor to mortality in Duchenne muscular dystrophy (DMD). Current cardiac management relies on cardio-protective drugs that delay cardiac failure, but do not prevent it. In cardiac and skeletal muscles, dystrophin is an essential scaffold for a large glycoprotein complex with structural and signalling roles. Our previous research shows that dystrophin assembles different glycoprotein complexes in heart and skeletal muscle, suggesting distinct muscle-specific functions. Consistent with this, recent studies suggest that the C-terminus of dystrophin and a portion of its central rod domain are uniquely important for cardiac function. ΔR4-R23/ΔCT micro-dystrophin is a leading gene therapy construct that lacks the two putative cardio-protective domains and only partially protects from cardiac disease in the mdx mouse model of DMD. The molecular basis for the incomplete cardiac rescue provided by ΔR4-R23/ΔCT micro-dystrophin is currently unknown. This knowledge is important to guide the optimisation of micro-dystrophin gene therapy constructs to fully prevent development of cardiac disease.

Aim: To compare the protein complexes assembled by dystrophin and ΔR4-R23/ΔCT micro-dystrophin in the heart in order to identify differences in composition that may underlie the incomplete cardiac rescue offered by ΔR4-R23/ΔCT micro-dystrophin.

Materials: Wild-type, *mdx* and *mdx* mice that express ΔR4-R23/ΔCT micro-dystrophin were used in these studies. Proteomic analysis was performed on isolated dystrophin and ΔR4-R23/ΔCT micro-dystrophin protein complexes to compare their protein composition. Western blot analyses were performed to validate proteomic results and evaluate protein expression levels in total protein extracts. Heart tissue sections were immunolabelled to compare protein localisation. Electron microscopy experiments were performed to visualise ultrastructural differences between genotypes.

Results: We report here a new cardiac-specific interaction between dystrophin and the cavin protein complex that is not rescued by ΔR4-R23/ΔCT micro-dystrophin. Cavins play important roles in regulating the shape, function and dynamics of caveolae (specialised membrane domains implicated in cardiac contraction and disease). We discovered that the localisation of cavin-1, cavin-2 and cavin-4 is disrupted in the mdx heart and is not rescued by ΔR4-R23/ΔCT micro-dystrophin. A screen of different micro- and mini-dystrophins for their ability to interact with cavin proteins is ongoing.

Conclusion: Our results identify caveolar dysfunction as a new potential player in cardiac disease in DMD. These findings offer new insights into the cardiac interactions of dystrophin and are highly relevant to the optimisation of gene therapy approaches with enhanced protection from cardiac disease.

D31

Functional-capacity Outcome Measures for Myotonic Dystrophy (OMMYD)

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Background: Myotonic Dystrophy type 1 (DM1) is a multisystem disease with high heterogeneity representing an obstacle when defining outcome measures that can be reliable yet still valid for different phenotypes. The Outcome Measures for Myotonic Dystrophy (OMMYD) Consortium has proposed a set of Functional-capacity Outcome Measures highlighted for consideration in clinical trials for DM1.

Aims: For this study, a cohort of 213 patients enrolled in the natural history study of PhenoDM1 (Myotonic Dystrophy Type 1 Deep Phenotyping to Improve Delivery of Personalized Medicine and Assist in the Planning, Design and Recruitment of Clinical Trials) was analysed. We aimed to assess: [1] intra-session (i.e. trial to trial) reliability; and, [2] construct validity (i.e. associations with disease parameters) of the OMMYD-defined functional-capacity outcome measures.

Methods: The study visit assessments included: [1] Standard medical history and demographical data; [2] Strength assessments; [3] OMMYD-defined functional-capacity outcome measures (six-minute walk test, timed 10 m walk test, timed 10 m walk/run test, 30 seconds sit and stand test, and 9-hole peg test); and [4] disease-specific patient-reported outcomes that assess functional performance (including DM1-ActivC and MDHI subscales).

Results: By comparing selected OMMYD functional-capacity outcome measures to clinical manifestations of the disease and to the reported burden of illness, we assessed their sensitiveness to discriminate different disease phenotypes. Their association to muscle strength and with participants' perceived functional-performance provides an insight of these outcomes construct validity. Differences between sexes were attributed to height differences. All tests showed a significant difference between scores from the first trial to the second trial. Thirty seconds sit and stand test showed to discriminate for disease severity and test repetition assess different disease parameters apart from muscle strength or capability to walk.

Conclusion: This study established the feasibility and reliability of these tests in large-scale studies. This data will serve as reference values from a representative sample of DM1 adults for future clinical trials. These OMMYD tests can be used as a battery of outcomes or independently as some have shown overlapping on psychometric features.

‡D32

A Human iPS Cell-Derived Artificial Skeletal Muscle for DMD Therapy Development

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Background: Duchenne muscular dystrophy (DMD) is a genetic disorder characterised by progressive muscle wasting and severe weakness. It occurs in 1 in 5000 boys and is caused by the disruption of dystrophin, a protein that maintains the integrity of muscle cells and protects the cell membrane from rupturing during contraction and relaxation cycles. *Dystrophin* is the largest human gene, having different isoforms, and patients have various mutations leading to expression of dysfunctional protein forms. Exon-skipping (using anti-sense oligonucleotides), gene editing (using CRISPR/Cas9) and read-through technologies are promising therapies that produce a truncated yet functional protein. However, the development and optimisation of these therapies and determining their relative efficiencies, is limited by the lack of an overarching (temporally, economically and ethically viable) platform.

Aims and methods: We utilise CRISPR/Cas9 gene editing technology to develop dystrophin-detectable human induced pluripotent stem cells (iPSCs), by inserting a versatile multiple-reporter cassette, that can track different dystrophin forms' expression levels temporally and spatially, in real time and in fixed cells. Following that, iPSCs will be used in tissue engineering an in vitro human skeletal mini-muscle "organoid", maintaining the dystrophin-tracking properties.

Finally, once established, the developed modelling parameters and outcome measures will be used to assess the efficacy of different therapies on patient-derived DMD dystrophin-tracking artificial muscles to determine their ability to restore dystrophin function and ameliorate the dystrophic phenotype in each line.

Results and conclusion: The designed cassette has been developed and tested by insertion into skeletal muscle progenitor cells that have been differentiated and one of the cassette functions has been confirmed. Multiple lines are currently being developed and potential outcome measures are being researched.

This platform could accelerate research for therapies, offer more tailored and potentially personalised treatment, reduce need for muscle biopsies and provide a reliable method to quantify dystrophin. This reporting system can also be applicable to other neuromuscular conditions.

D33

Comparison of home-based versus hospital-based spirometry measurements in Duchenne muscular dystrophy

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Background: Respiratory function decline in Duchenne muscular dystrophy (DMD) is caused by progressive weakening of respiratory muscles. Standard of care guidelines recommend routine respiratory function monitoring to guide patient management. We compared respiratory function measurements obtained using a hand-held device allowing frequent home-based measurements to hospital-based spirometry measures in patients with DMD taking part in the DELOS trial.

Methods: Respiratory function data were collected from 64 DMD patients aged 10-18 years. All patients were required to have established respiratory function decline (peak expiratory flow <80%p) at baseline. Patients were treated with idebenone (900 mg/day) or placebo. Spirometry was conducted during hospital visits at baseline and 3 month intervals over 52 weeks. Patients also measured peak expiratory flow (PEF) weekly at home using the ASMA-1 device (Vitalograph). Data were analyzed using a mixed model for repeated measures method.

Results: Patients enrolled in the DELOS study were in an advanced stage of disease, with a mean age of 14.3 years. A majority (92%) were non-ambulatory and showed signs of significant upper limb weakness at baseline (59% had a Brooke's score of 5 or

above). The changes in PEF measured at home (expressed as percent of predicted, PEF%p) compared well with those from hospital-based measurements, showing a 5.6% ($p = 0.002$) treatment difference in favor of idebenone across all weekly visits, compared to a 6.27% difference at last visit using standard hospital spirometry ($p = 0.031$). Similarly to the hospital based data, the weekly ASMA-1 data indicated a stabilization of PEF%p in the idebenone group versus a consistent decline in the placebo group.

Conclusion: Home-based recording of respiratory function measures is reliable in pediatric and adolescent patients with DMD, and may prove useful when used in combination with hospital-based monitoring. These data further support the efficacy of idebenone in slowing respiratory function decline in DMD.

D34

Assessing idebenone's impact on respiratory function in Duchenne muscular dystrophy: Meta-analysis of two clinical trials

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Background: Patients with Duchenne muscular dystrophy (DMD) experience respiratory function decline that accelerates after they become non-ambulatory, leading to significant disease burden and, in many cases, death. The ability of idebenone to slow respiratory function decline in patients with DMD has been investigated in two randomized, placebo-controlled trials (the phase 2 DELPHI trial and phase 3 DELOS trial). DELPHI included patients (aged 8 to 16 years) irrespective of baseline respiratory function status, while DELOS required patients (aged 10 to 18 years) to be in the respiratory decline phase. DELPHI allowed the randomization of both glucocorticoid users and non-users, whilst DELOS included only glucocorticoid non-users.

Methods: Source data from both trials were pooled and analyzed for treatment effects on peak expiratory flow and forced vital capacity, expressed as a percentage of predicted (PEF%p and FVC%p). Change in PEF%p and FVC%p from baseline to weeks 26 and 52 (end of study) were analyzed using a mixed model for repeated measures, with study as a random effect.

Results: In total, 76 patients (DELPHI: 12; DELOS: 64) were in the respiratory function decline phase (PEF%p < 80%), of which 72 were not using glucocorticoids. Across all patients, the difference between the idebenone and placebo groups in PEF%p from baseline to week 52 was 8.0%p ($p = 0.003$). When omitting glucocorticoid users, this difference was 7.7%p ($p = 0.005$). For FVC%p, the treatment difference was 3.6%p ($p = 0.046$) in all patients, with a comparable difference of 3.5%p ($p = 0.051$) when omitting glucocorticoid users.

Conclusion: Results from a meta-analysis with data from two randomized, placebo-controlled trials (DELPHI and DELOS) demonstrate that idebenone slows respiratory function decline in patients with DMD. Additionally, the magnitude of treatment difference observed was unaffected by concomitant glucocorticoid use.

D35

Regulation of the dystrophin-associated glycoprotein complex composition by the metabolic properties of muscle fibres

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Background: Skeletal muscle is a highly compliant tissue that undergoes both qualitative and quantitative remodeling in response to mechanical, electrical and chemical stimuli. The dystrophin-glycoprotein complex (DGC) links the muscle cytoskeleton to the extracellular matrix and is responsible for force transduction and for protecting the muscle fibre from contraction induced damage (Kjaer, 2004). Mutations

in any of the proteins constituting the DGC are associated with muscular dystrophies and congenital myopathies (Wicklund and Kissel, 2014). Expression of DGC components have been shown to be altered in many myopathies. In contrast we have very little evidence of whether adaptive changes in muscle impact on DGC expression.

Aims: To investigate the relationship between muscle fibre phenotype and the DGC.

Methods: Immunohistochemistry, Western blot, Quantitative PCR, Semi-quantitative measurement of Collagen and Dystrophin Glycoprotein Complex (DGC) protein expression by immunofluorescence, Sarcolemma thickness measurement, and Imaging and analysis

Results: We show that the DGC composition is influenced by the phenotype of the muscle fibre.

Conclusion: Our work shows that the metabolic property of a muscle fibre is a key determinant in regulating the expression of DGC proteins at the sarcolemma.

D36

Patient Reported Outcome Measures in Dysferlinopathy – How suitable are they?

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Background: The COS is an international natural history study of patients with dysferlinopathy. Dysferlinopathy presents with heterogeneity in muscle weakness, including both distal and proximal phenotypes which poses significant challenges for developing appropriate clinical trial outcome measures as well as significant functional and quality of life challenges for patients. 204 patients, at 15 sites in 8 countries were evaluated using four Patient Reported Outcome Measures (PROMs)– Individualised Quality of Life Measure for Neuromuscular Disease, ACTIVLIM, International Physical Activity Questionnaire and for non-ambulant patients the Egan Klassifikation Scale (EK)

Aims: To review the suitability of these PROMs in this cohort over a period of three years. To summarise change scores over this time for the four PROMs.

Methods: Patients completed the InQOL, IPAQ, ACTIVLIM at annual visits over three years. The

EK was completed only if the patient was non-ambulant. INQOL is a quality of life measure that aims to capture the impact of key muscle disease symptoms (weakness, fatigue, pain, and locking), the impact on particular areas of life including activities, independence, relationships, and body image. Participants respond primarily using a 7-point Likert scale and also with some yes/no responses. The IPAQ assesses physical activity using a set of seven questions and hours of activity at different levels of intensity. The ACTIVLIM is a Rasch built measure of activity limitation and using a three-point grading system of impossible, difficult and easy and the EK was originally designed for Duchenne muscular dystrophy but assessing key "own functioning" in non-ambulant patients.

Data were analysed using descriptive statistics for each visit and over the period of the study and for rating scales (ACTIVLIM, EK, INQOL) modern psychometric analysis using Rasch analysis (RUMM2030 software) was also conducted to assess item fit, person fit, targeting, dependency and reliability

Results: Not all of the measures appeared to be suitable measurement tools in this disease specific cohort, this was particularly true for the INQOL. The ACTIVLIM appeared more robust. Change scores were noted for several of the measures over time.

	IPAQ	EK	ACTIVLIM	INQOL
Baseline	204	48	200	204
12 months	189	50	189	192
24 months	189	60	185	184
36 months	166	55	159	113

Discussion: Further work is required to adapt the current PROM's or alternative measures need to be selected.

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D37

A comparison of the utility between three muscle strength assessment methods in Dysferlinopathy

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Background: The COS is an international natural history study of patients with dysferlinopathy. Dysferlinopathy presents with heterogeneity in muscle weakness, including both distal and proximal phenotypes which poses significant challenges for developing appropriate clinical trial outcome measures. 203 patients, at 15 sites in 8 countries were evaluated using three methods to measure strength on a variety of upper and lower limb muscle groups

Aims: This study compared results between 3 methods of muscle strength testing used in the Jain Clinical Outcome Study (COS) for Dysferlinopathy performed at baseline and year 1 visits.

Methods: Manual muscle testing (MMT) uses the Medical Research Council scoring system to subjectively grade the amount of resistance the subject can withstand as the evaluator pushes against the extremity. Hand held dynamometry (HHD) uses a force transducer in a small device held against the extremity by the clinical evaluator. Quantitative muscle testing (QMT) uses a force transducer secured to a wall mounted fixed frame. All sites

completed MMT and HHD, while only 7 of the 15 sites had the QMT equipment available. Subjects also completed functional tests including as appropriate for their ambulatory status including adapted North Star Ambulatory Assessment for Dysferlinopathy, 10 metre walk test (10m) and 6 minute walk test (6MWT).

Results: The 3 strength testing methods were highly & significantly correlated with each other (r = 0.8, P<0.001). All 3 strength testing methods were highly & significantly correlated with functional tasks (r = 0.7 – 0.8, P<0.001). Age was significantly correlated with performance of all strength & functional tasks.HHD was the only strength testing method that demonstrated significant change in summed strength over one year. There was a trend toward decreased strength in Year 1 measured with QMA, but this was not significant. HHD was the only modality capturing decline consistently across muscle groups – thus demonstrating significant change across 1 year in total strength

Conclusion: Strength measurements are highly correlated with performance of functional tasks in Dysferlinopathy. Hand held dynamometry is sufficient for testing strength in key muscle groups & is sensitive to disease progression in 1 year

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D38

Clinical Outcome Study for Dysferlinopathy: Three years of natural history data for clinical trial readiness

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Background: The Jain Clinical Outcome Study (COS) is an international study evaluating patients with genetically confirmed dysferlinopathy. 203 patients were recruited across 15 sites and in 8 countries. Dysferlinopathy clinically presents with a spectrum of muscle weakness including both distal and proximal phenotypes which presents significant challenges for developing appropriate clinical trial outcome measures.

Aims: COS aims to develop understanding of disease progression and identify the most relevant outcome measures for this cohort to facilitate trial readiness in dysferlinopathy. Here we report three years of longitudinal functional data.

Methods: Patients attended six visits over three years. Physiotherapy medical and MRI assessments were conducted. Physiotherapy assessments included manual muscle testing (MMT) hand held dynamometry (HHD) and functional scales (North Star Assessment for Dysferlinopathy and Performance of Upper limb), as well as timed tests (rise from floor, 10 metre walk / run, four stair climb and descend; Timed Up and Go, TUG; Six Minute Walk Distance, 6MWD) and respiratory function testing (forced vital capacity and mouth inspiratory pressure).

Results: Significant change across all three years in respiratory function, muscle strength testing, timed function tests, 6MWT and ACTIVLIM. All timed tests except rise from floor showed significant and consistent decline across all three years. NSAD, 10m and TUG demonstrated the most significant

change. These results will be examined for differences between gender and ethnicity.

Conclusion: Progression in dysferlinopathy can be demonstrated using functional outcomes and dynamometry as measured by Physiotherapists over three years. Results support future study design and help power future clinical trials.
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D39

A Clinical Outcome Study for Dysferlinopathy: biobanking samples collected through a collaborative international multisite study

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Background: The Jain Clinical Outcome Study (COS) is an international study evaluating patients with genetically confirmed dysferlinopathy. 203 patients were recruited across 15 sites and in 8 countries. This three year study was extended for a two year time period until all sites completed year 3 visits. All sites completed year 3 visist, while some also completed year 4 and year 5 visits.

Further to the Clinical Outcome Study data collection allowing the characterization of the disease (results reported elsewhere), patients also had the option to donate biobank samples to the MRC Biobank for Rare and Neuromuscular Diseases curated at Newcastle.

Aims: COS aims to develop understanding of disease progression and support future scientific research

Methods: Standardised Protocols for collection, processing and shipment were provided to each site. Blood for DNA and RNA was collected at one time point only. Plasma and Serum were collected annually. Patients could opt to provide a skin sample to generate fibroblast cell lines. Urine was introduced from year 4 at Newcastle only. Samples have been shipped to the Newcastle MRC Centre Biobank for Rare and Neuromuscular diseases.

Results: 12 sites of 15 sites participated in the biobanking element.

While 97% patients opted to donate blood samples, only 43% patients opted to donate skin samples for fibroblasts.

Samples currently at Newcastle are as follows: Blood for RNA n142; DNA n155; Baseline Plasma n147; Year 1 Plasma n143; Year 2 Plasma n134; Year 3 Plasma n70 (pending final shipments); Baseline Serum n147; Year 1 Serum n143; Year 2 Serum n135; Year 3 Serum n70 (pending final shipments); Limited year 4 and year 5 samples are also available.

Conclusion: This is a valuable resource for scientists worldwide to develop the body of knowledge regarding Dysferlinopathies necessary to support design of future therapeutic targets and clinical trials.

The COS Study has been extended for two further years. COS2 will also collect urine across all participating sites.
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D40

Clinical Outcome Study in Dysferlinopathy: Medical comorbidities and polytherapy in a large population of dysferlinopathy patients

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Background: The Jain Clinical Outcome Study (COS) is the largest international natural history study In patients with genetically confirmed dysferlinopathy.

203 patients (aged 12-88) have been recruited across 15 sites in 8 countries. All patients were molecularly diagnosed and followed over 3 years.

Aims: To describe the comorbidities in a large cohort of COS patients at baseline and during follow-up (3 years).

To analyse the drugs and supplements taken before the screening and during the natural history study.

Methods: Medical history and comorbidities were collected by specific questionnaires administered by trained nurses or medical doctors. Medication used were categorised using the British National Formulary.

Results: The most common comorbidities at baseline were: cardiovascular (33 patients-16%-; 27 of which are hypertension), endocrine (21 patients-10%-; 10 of which are hypothyroidism) and respiratory (16 patients-7%-; 15 if which are asthma). 5 patients were diagnosed from autoimmune diseases. During follow-up, hypertension was diagnosed in 6 patients. 141 patients (69%) were taking drugs or supplements at the beginning of COS (range: 0-10 medications). 65 patients (32%) were on daily vitamins or nutritional supplements, 27 (13%) on anti-hypertensive, 25 on NSAIDs (12%), 16 (8%) on opioids and 16 (8%) on antidepressants. During follow-up, anti-hypertensive, antidepressants, opioids and anti-epileptic prescribed more commonly than other medications. Of note, before participation in COS and due to misdiagnosis with inflammatory myopathies, 56 patients (28%) had previously been treated with steroids, 16 (8%) with ivIg and 12 (6%) with immune suppressants.

Conclusion: Hypertension, asthma and hypothyroidism are the most common comorbidities in COS. Besides vitamins and supplements, anti-hypertensive, NSAIDs, opioids and antidepressants are the most prescribed drugs. 28% of the patients had previously taken steroids due to misdiagnosis.

We believe this information should be taken into account when designing interventional clinical trials. This Study has been supported by the Jain Foundation
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D41

The UK Myotonic Dystrophy Patient Registry: A Key Tool in the Facilitation of Clinical Research

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Background: The UK Myotonic Dystrophy Patient Registry (www.dm-registry.org/uk) is a patient self-enrolling online database collecting clinical and genetic information about both myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (DM2). The registry was established in May 2012 with support from Muscular Dystrophy UK (MDUK) and the Myotonic Dystrophy Support Group (MDSG), assisted by the TREAT-NMD Alliance and is coordinated Newcastle University.

Aims: The registry's primary aim is to facilitate and accelerate clinical research in Myotonic Dystrophy type 1 (DM1) and type 2 (DM2). The registry also aims to act as the most comprehensive distributor of information relating to upcoming academic and non-clinical studies in Myotonic Dystrophy.

Methods: The registry is used to capture longitudinal, self-reported data through an online portal available to patients and clinicians.

Results: Between May 2012 and December 2018, 749 patients have registered. In those who have a clinical diagnosis, 96% have DM1 and 4% have DM2. There is an even distribution of patients registered from both genders and 62% of patients were between 31 and 60 years old. Patients most frequently reported their current best motor function as either ambulatory unassisted (61%) or ambulatory assisted (30%). The most commonly reported symptoms were fatigue (74%) and myotonia (70%), with 62% of patients reporting both.

Conclusion: The registry has been a successful tool in assisting in the recruitment to a number of clinical studies since launching in 2012. It is an example of a novel, online-based, cost-effective, and patient-driven registry. 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.
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D42

The UK FSHD Patient Registry: A Key Tool in the Facilitation of Clinical Research

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Background: The UK FSHD Patient Registry (https://www.fshd-registry.org/uk/) is a patient driven, clinician verified tool designed to support clinical research. The registry shares a common dataset with many registries worldwide and is able to collect symptomatic information longitudinally. This online patient driven registry aims to facilitate and accelerate planning and recruitment of clinical trials. Patient reported outcomes are entered into a secure online portal that are then combined with clinician verified genetic details. Core clinical and genetic information has been collected through the registry with additional data about pain (MPQ-SF) and quality of life (INQoL).

Aims: The registry's primary aim is to facilitate and accelerate clinical research in to Facioscapulohumeral Muscular Dystrophy (FSHD). The registry also aims to act as the most comprehensive distributor of

information relating to upcoming academic and non-clinical studies in FSHD.

Method: The registry is used to capture longitudinal, self-reported data through an online portal available to patients and clinicians.

Results: Between May 2012 and December 2018, 916 patients have registered, 87% with a clinical diagnosis of FSHD1. There is an almost even distribution of patients registered from both genders and 59% of patients were between 31 and 60 years old. Muscle weakness was widely reported with periscapular shoulder weakness occurring most frequently (87%) followed by weakness of the hip girdle (67%) and facial muscles (67%) then foot dorsiflexor (64%). Onset of periscapular shoulder weakness was reported to occur before the age of 30 in 59% of cases, earlier than weakness in other areas. The use of a wheelchair was reported to begin most often after the age of 30 (76%).

Conclusion: A broad spectrum of patients have registered providing a new insight into the FSHD population in the UK. The Registry aims to help in the planning and recruitment of research. Sharing a common dataset with a growing number of FSHD registries around the world will allow the registry to achieve this locally and internationally. The registry is well placed to inform future clinical research and help develop of standards of care.

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D43

The Global Registry for COL6-related dystrophies

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Background: Mutations in the Collagen 6 genes (COL6A1, COL6A2 and COL6A3) cause a clinically and genetically heterogeneous group of rare diseases (Bethlem Myopathy, Ullrich Congenital muscular dystrophy (UCMD) and intermediate phenotypes) which are collectively known as the COL6-related dystrophies (COL6-RDs) . The newly launched Global Registry for COL6-related dystrophies (www.collagen6.org), funded by the Collagen VI Alliance through MDUK, allows secure capture and storage of data from individuals affected with a COL6-RD and from the medical professionals in charge of their care.

Aims: The primary objectives of the registry are to:
-Contribute to trial readiness of COL6-related dystrophies (RDs), allowing identification of genetically well characterised cohorts for participation in research
studies and clinical trials;
- Add to the understanding of disease natural history and prevalence;
- Assist doctors and other health professionals by providing them with up-to-date information on managing COL6-RDs, to help them deliver better standards of care for their patients;
- Stimulate industry interest in the COL6-RDs by demonstrating the availability of clinician validated data to support future trials and post marketing surveillance;
- Provide individuals with COL6-RD with up to date summaries of relevant current research and news via the website and newsletters.

Methods: Affected individuals can access information sheets about the registry, provide consent to being part of the registry and submit their data online. As part of the consent process, affected individuals consent to their clinicians entering data on their behalf and medical professionals are provided with a user account to allow them to enter data online. Requests for anonymous data from the registry are approved by the registry steering committee (containing professional and patient representation), who have overall oversight of the registry governance.

Results: Here we describe the development of the registry, plans for its future development and an analysis of the data contained within the registry thus far.

Conclusion: The Global Registry for COL6-related dystrophies is a new resource for the COL6-RD community which has potential to stimulate, accelerate and support clinical and basic research in this group of diseases.

Peripheral Neuropathy

PN01

Preliminary falls and functional balance data from the BALTiC study: A feasibility analysis of home based BALance Training in people with Charcot-Marie-Tooth disease

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Background: Charcot-Marie-Tooth disease (CMT) is the most common inherited neuromuscular disorder. Features include a slowly progressive, length dependent sensorimotor neuropathy. People with CMT report a high incidence of falls, though few groups to date have looked at exercise based interventions to improve balance in this population.
Aims: This study explored the feasibility and effect of delivering a home based training programme using multi-sensory rehabilitation and proximal strength training.

Methods: In this randomised controlled feasibility trial, 14 participants (m=21.4%) with CMT1A were randomised to either a 12 week home based exercise programme of multi-sensory balance exercises and proximal strengthening with a falls management session or a falls management session only. Participants self-recorded falls and activity for a planned 20 weeks. During the intervention, balance and strengthening exercises were prescribed in the home environment. Outcome measures included balance performance in static and dynamic posturography, functional balance tests, lower limb strength tests plus subjective measures of balance confidence and function. Brief qualitative interviews were also undertaken to capture participants' experience of taking part.

Results: No significant differences in disease severity existed between the two groups. Training was well tolerated with high participation levels. The intervention group had greater delays with completing the study in the aimed for 20 weeks because of work commitments of a small number (Intervention mean=32wks, SD±10.6 Control mean=24wks SD±2.6). Total reported falls were not significantly different (Intervention mean= 7 SD±5.2; Control mean= 4 SD±4; P=0.32).

Functional measures of balance showed larger improvement effect sizes, favouring the intervention group (Berg Balance Score, Hedge's G = 1.14; BESTest, Hedge's G 1.04, 10MTW, Hedge's G = 0.97, Functional Gait Assessment = 0.87). Centre of pressure variables showed moderate improvements in visual dependency (COP velocity with feet apart eyes closed, Hedge's G =0.48). Balance confidence showed small effect changes.

Conclusions: This RCT was safe and feasible. Functional measures show promise in this small and varied group with possible improvements in visual dependency. Now that feasibility has been demonstrated and, further exploration of efficacy will need to be undertaken on a larger sample.

PN02

Cross sectional study on CK levels in CMT and related disorders

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Background: Creatine kinase (CK) exists as different isoenzymes and is influenced by a number of factors including gender, race, muscle bulk and physical activity. An elevated CK may indicate acute muscle injury such as acute myositis or rhabdomyolysis or chronic muscle damage as is the case with chronic denervation in CMT. Aside from chronic denervation which occurs early in motor-predominant subtypes of CMT and later in other forms such as HSN, other factors including the duration of the disease and medications may influence CK levels.

Aim: To assess the cross-sectional correlation of CK with the CMTES, ulnar CMAP and molecular diagnosis in CMT and related disorders.

Methods: We retrieved CK values at specific time-points for 161 patients and correlated them with the corresponding CMTES and ulnar CMAP scores. Furthermore, we correlated the biochemical data of CK corrected for CMTES across all molecular diagnoses.

Results: We evaluated the CK in 63 females and 98 male patients. 73% (118) of evaluated patients had various subtypes of CMT (CMT1, CMT2, CMTi) 15% HMN (24), 8% HSN (13) and 4% HNPP (6). In our cohort the CK range was 39-2205. 48% (77) of patients had an elevated CK level and the majority were male. Abnormal CK levels were found in 40% of CMT1 patients, 55% of CMT2, 40% of CMTi, 67% of HMN and 38% of HSN. There was no difference in the average level of CK between the different groups of CMT subtypes. Patients with HMN, were the most likely to have elevated rather than normal CK levels and there was a trend of near-normal CK levels with higher CMTES. Impairment as measured by CMT Examination Score (CMTES) was on average 10.81 (± 5.23) (range 0– 21). Average ulnar CMAP scores at the corresponding time point was 5.01 (range between 0-9.6).

Conclusion: Based on this small cohort size, there was no statistically significant correlation between the CK level and CMTES or ulnar CMAPs. A CMT cohort with larger numbers would be able to assess more accurately assess if there is any correlation between CK and disease severity during the early stages of various CMT subtypes.

PN03

A novel homozygous mutation extending the PMP22 protein by 9 amino acid associated with an isolated severe sensory ataxia

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Background: Peripheral myelin protein 22 (PMP22) related neuropathies account for over 50% of inherited peripheral neuropathies, the so-called Charcot-Marie-Tooth. An increase or decrease in PMP22 gene copy number results in distinct phenotypes, ranging from CMT1A (Duplication), hereditary neuropathy with liability to pressure palsies (HNPP; single deletion) and CMT1E (Point mutations). Of note, the underlying pathological mechanisms are still poorly understood and homozygous mutations of PMP22 are very rare.

Aims: To present a patient with a new homozygous mutation in the PMP22 gene and a peculiar phenotype.

Patient: We have studied an 8-year-old girl who presented before the age of 1 year with severe locomotor delay, hypotonia and absent deep tendon reflexes. She never acquired independent ambulation and requires support also for standing in view of her severe sensory ataxia. Strikingly, her muscle power and muscle bulk are within normal limits in all segments and small sensory fibers are clinically spared.

Results: Nerve conduction studies showed an extremely severe sensorimotor demyelinating neuropathy (Conduction velocities<5m/s). Genetic analysis revealed a homozygous sequence change in the PMP22 gene causing the loss of termination codon (c.483A>G; p.*161Trpext*10). This variant is predicted to extend the protein by an addition of 9 amino acids and could potentially lead to loss-of-PMP22 function. Neurophysiological studies of the 2 heterozygous parents identified clear features supporting a diagnosis of HNPP in them.

Conclusion: PMP22-deficient human models are as rare as important to decipher the physiological function of the PMP22 protein *in vivo*. The predominance of large fiber sensory involvement in this and other rare similar cases suggests a pivotal role played by PMP22 in the embryogenesis of dorsal root ganglia in humans.

PN04

Plasma Neurofilament Light Chain Concentration compared with I-RODS in Stable Patients with Inflammatory Neuropathies on regular Intravenous Immunoglobulin

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Background: Blood neurofilament light chain (NfL) concentration using ultrasensitive single-molecule array (Simoa) assay has been explored in numerous neurological conditions. In acquired neuropathies, the stability of plasma NfL concentration in patients on regular intravenous immunoglobulin (IVIg) measured using ultrasensitive single-molecule array (Simoa) assay is unknown.

Aim: To perform a pilot, longitudinal study that assesses the reliability and validity of a Simoa assay measuring plasma NfL concentration in stable patients with inflammatory neuropathies (CIDP and MMN) on IVIg and assess for potential correlation with the inflammatory Rasch-built overall disability scale (I-RODS).

Methods: Blood samples and I-RODS were collected over two-time points in 20 clinically stable patients, on a regular IVIg regimen. Plasma NfL concentration was measured using an in-house developed Simoa assay.

Results: There were 14 patients with CIDP and 6 with MMN. 35% of patients were females, mean (SD) age of 56.6 (9.48) years receiving an average dose of 2.42 (2.25)g/kg/month. There was no significant difference in NfL concentrations or I-RODS scores between the two time-points (median= 11.38pg/mL vs. 9.97pg/mL, T= 47, p= 0.46) and (median= 63 vs. 63, T= 7.5, p= 1). NfL levels did not significantly differ between diagnoses (p= 0.91), or gender (p= 0.51). NfL results did not correlate with change in I-RODS (τ= 0.098, p= 0.76).

Conclusion: These results suggest that the Simoa assay is stable in a range of IVIg doses and reinforce I-RODS as a subjective marker of clinical stability. The lack of correlation between NfL results and I-RODS needs to be further evaluated in larger and more diverse groups. These results suggest that adequately treated inflammatory neuropathy does not have a significant, measurable signal of axonal damage, compared to normal control levels from other studies.

PN05

Intravenous Immunoglobulin Treatment and Risk of thromboembolic events

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Background: The potential risk of arterial thromboembolic events (aTEE) with the use of IVIg has been investigated in numerous groups of patients with conflicting results. Our unit has previously conducted a retrospective cohort study of 111 inflammatory neuropathy patients receiving regular IVIg and 333 neurology outpatients over a 30-month period that showed a higher incidence of arterial events in patients exposed to IVIg.

Aim: In neurology outpatients, evaluate individual risk factors (including IVIg exposure as a novel risk factor) and create a predictive model for risk of arterial TEE at two and a half years.

Methods: We performed multivariate logistic regression to assess the effect of individual risk factors. Kaplan-Meier analysis was used to estimate the arterial TEE cumulative incidence for patients for significant variables in combination and independently.

Results: Data on 445 neurology outpatients including 112 neuromuscular patients receiving

IVIg during this period was retrospectively collected between January 2014 and July 2016. These groups were matched on all cardiovascular risk factors except for smoking and diabetes. History of any IVIg exposure, hypertension and diabetes mellitus were significant contributors to aTEE outcomes, (odds ratio 3.3, 3.8 and 3.6, respectively, $p<0.05$). At two and a half years, Kaplan-Meier analysis showed cumulative probability of arterial TEE of 50% if patients had all three risk factors, compared to patients who had none of the risk factors; 15% if patients had both IVIg and hypertension, and 53% if patients had both IVIg and diabetes, compared to patients who had neither of these risk factors.

Conclusions: This review quantifies the contribution of IVIg and traditional cardiovascular risk factors in aTEE in neurology outpatients. It also provides a predictive model for future aTEE that will needs validation. Having a predictive model may be the first step to identifying ways to mitigate this risk given two of the three risk factors are modifiable.

‡PN06

Applications of unbiased datasets and machine learning for discovery of novel dHMN-causative variants

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Background: Despite advances in genomic technology, for many distal hereditary motor neuropathy (dHMN) patients, a single causative gene cannot be identified. The predictive capacity of combined annotation dataset for predicting relevant variants has been shown before using the Mitominer tool created within the MRC Mitochondrial Biology Unit. This is hindered, however, by the lack of a complete and robust proteome of the peripheral nerve or even the axon.

Aims: We utilize publicly available unbiased experimental datasets to highlight genesets enriched for axonal function and peripheral nerve biology, alongside individual variant characteristics to determine the qualities of causative genes and develop a machine learning tool which can assess the likelihood of any single variant causing dHMN.

Methods: Experimental data relating to genes either causative or not causative for dHMN, identified using ClinVar and ExAC, allowed us to identify patterns of data associated with genes causing axonal neuropathy. This data was then used to train a random forest machine learning predictive model, optimized for maximal separating causative variant from solved exome data.

Results: We find that there are several characteristics which distinguished neuropathy-causing genes, but that only certain experimental datasets were useful. We do find however that other public data not specifically related to motor neuropathies is also useful in the identification of dHMN genes. Machine learning methods effectively stratified the relevance of variants within patient exome data and were able to highlight variants of interest with unsolved cases.

Conclusions: We developed an approach to the challenge of recognising novel variants causing dHMN by identifying the characteristics of the genes currently associated with the condition. Furthermore, we have developed a machine learning model using these to distinguish between a set of potentially causative variants. Additional non-biased datasets relating to the peripheral motor neuron can be added to the model we have developed and will improve its accuracy, and the generation of these should be encouraged in future. Our bioinformatic tool applies a non-biased approach to the identification of novel dHMN variants appropriate for the next generation sequencing era.

PN07

Genetic investigation of inherited neuropathy in families from Middle East using next generation sequencing

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Background: Inherited peripheral neuropathies are a group of clinically and genetically heterogeneous disorders, that encompass Charcot-Marie-Tooth disease (CMT), distal hereditary motor neuropathy (dHMN), hereditary sensory neuropathy (HSN), hereditary sensory and autonomic neuropathy (HSAN) and hereditary neuropathy with a liability to pressure palsy (HNPP). Moreover, inherited peripheral neuropathies can be part of a more complex neurological syndrome. The advent of next generation sequencing has allowed the discovery of new disease-causing genes, however, 50% of patients with CMT2 remain without a diagnosis and reaching one remains a challenge due to the heterogeneity of the condition.

Aim: To explore the genetic background of undiagnosed inherited neuropathies in families from the Middle East using next generation sequencing.

Patients and methods: We recruited 11 families within the SYNaPS study, mostly consanguineous, with a clinical diagnosis of an inherited peripheral neuropathy. These families mostly originate from the Greater Middle East. Whole exome sequencing was performed. Variants were filtered using our bioinformatics system to only include rare homozygous and compound heterozygous variant with MAF<0.01 in publically available databases. Segregation analysis and confirmation by Sanger sequencing of candidate mutations is currently undertaken. In selective cases with novel phenotypic features, further functional studies will be performed.

Results: Neuropathy was the predominant feature in 6 families (100% consaguoinous) and 5 families presented with a neuropathy as a feature of a wider with multisystem condition. Likely pathogenic mutations were identified in 7 families (63.6%), with 4 variants being novel. All diagnosed cases were autosomal recessive (AR) type and variants were found in the following genes: *SH3TC2, MTMR2, NEFL, GAN, LAMA2, POLG,* and *WNK1*.

Conclusion: A genetic diagnosis has been obtained in most of families with an autosomal recessive inheritance pattern. Phenotype and genotype correlation was undertaken and unusual phenotypic features in some conditions have been identified.

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‡PN08

Frequent central nervous system, pachymeningeal and plexus MRI changes in POEMS syndrome

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Background: Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes (POEMS) syndrome is a rare multisystem disease associated with a plasma-cell dyscrasia. Although pachymeningeal involvement has rarely been described, the extent of central nervous system (CNS) involvement has not yet been extensively investigated.

Aim: To identify the characteristics and frequency of CNS involvement in POEMS syndrome.

Methods: We retrospectively evaluated CNS MRI findings from Europe's largest single-centre cohort of POEMS syndrome patients. Of seventy-seven patients who fit diagnostic criteria for POEMS syndrome, 41 had MRI brain and 29 had MRI spine examinations. A control group of 33 patients with chronic inflammatory demyelinating polyneuropathy (CIDP) was used as a comparator as this is the major differential diagnosis. Of these CIDP patients, 12 underwent both MRI brain and spine, 7 had MRI brain only and 14 had MRI spine.

Results: In 41 POEMS patients with MRI brain studies, we identified frequent smooth, diffuse meningeal thickening of the cerebral convexities and falx (n=29, 71%), of which 4 had meningeal collections. Seventeen (41%) had vascular abnormalities including white matter disease, of which 4 had established infarcts. Of 29 patients with MRI spine, 17 (59%) had thickening of the brachial and lumbosacral plexus. Conversely in 19 CIDP patients with MRI brain, none had meningeal thickening ($p < 0.0001$), however 8 (42%) had vascular abnormalities ($p = 0.85$). Of 26 patients with MRI spine, 9 (35%) had brachial or lumbosacral plexus thickening ($p = 0.06$).

Conclusions: In contrast to CIDP, POEMS patients frequently have pachymeningeal thickening. Vascular abnormalities and plexus thickening were common in both POEMS and CIDP. We propose that pachymeningitis should be incorporated into the diagnostic criteria for POEMS syndrome, which serves to distinguish features characteristic to POEMS syndrome which are not seen in alternative conditions within the differential diagnosis.

PN09

Optimising Response Assessment Following High-Dose Chemotherapy and Autologous Transplant for POEMS Syndrome

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Background: High dose melphalan autologous stem cell transplantation (ASCT) has emerged as an effective therapy for patients with systemic POEMS syndrome (**p**olyneuropathy, **o**rganomegaly, **e**ndocrinopathy, **m**onoclonal-protein and **s**kin changes).

Aim: To assess outcome measures following ASCT in POEMS syndrome and identify potential prognostication markers for disease progression.

Methods: We reviewed 42 patients who underwent melphalan ASCT over the last 18 years to measure outcomes following ASCT and optimise treatment response. Overall survival was 92.9%, one year survival was 94.6%, and progression free survival rate 80.1% (mean follow up 62.6 months, range 4-226 months). Three patients (16.7%) had engraftment syndrome, with no significant difference observed according to the mobilisation agent used. Average serum vascular endothelial growth factor levels improved from 4,959pg/ml (95% CI 3,895-6,190) to 489.5pg/ml (95%CI 416-1,132) at six months and 330pg/ml (95% CI 313-869) at one year. Significant neurological improvement was seen in all but two patients after ASCT, with median pre-transplant Overall Neuropathy Limitation Scale score of 6 improving to a post-transplant score of 2 ($p<0.01$). Seven percent of patients were bed bound before ASCT, but none were on most recent follow up. Nerve conduction studies demonstrated continued improvement even three years after ASCT. Formal haematological response was evaluable in 33/42 patients. No patient who achieved complete haematological response has relapsed by most recent follow up, whereas of those with less than complete response had a 31.6% relapse rate ($p=0.027$).

Conclusion: Long-term outcome is favourable in patients treated with ASCT for POEMS syndrome. VEGF, considered to be a surrogate biomarker for disease activity significantly improves following treatment, and patients who achieve complete haematological response have significantly better progression free survival than those who don't. Neurological improvement following ASCT is often ongoing for several years.

PN10

The Clinical Spectrum of MORC2 associated CMT2

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Background: The MORC2 gene encodes a member of the Microrchidia protein superfamily. It is a DNA-dependent ATPase that relaxes chromatin to facilitate DNA repair. Mutations in MORC2 associated with axonal Charcot Marie Tooth Disease were first described by Sevilla and Colleagues in 2016 and designated as CMT2Z.

Aims: We describe the clinical and neurophysiological features in three families identified though whole exome or mini exome sequencing at our centre who have MORC2 associated CMT2. We present a detailed discussion on the phenotypic spectrum described in the literature.

Patients: A now 36 year old man first walked at 18 months, and could only just run at age 15, presented at age 25 with evolving painful distal tingling and hand cramping and "locking." Detailed phenotyping and neurophysiological evaluation revealed axonal sensory and motor neuropathy with retinal dystrophy, pyramidal and subtle cerebellar signs, and higher mental function impairment (IQ 80). Following extensive evaluation including metabolic evaluation, targeted genetic testing and CMT2/ intermediate panels, whole exome sequencing identified a p.R252W mutation in MORC2. His child carries the same mutation, evaluation at age 5 demonstrated delayed gross motor and language skills, behavioural lability, and sensorimotor axonal neuropathy. Subsequently, a 27 year old female was found to have the same mutation, describing childhood onset of intellectual disability and delayed motor milestones, hearing impairment, intoed walking and upper limb involvement in her teens. She required a wheelchair at age 22. A further patient, now age 29 had delayed motor milestones, early foot deformity and scoliosis and patchy upper limb symptoms, has required a wheelchair when outdoors since age 27. She was identified to carry the E236G mutation. This patient's child had abnormal gait and falls when first walking at 15 months and on clinical examination had brisk lower limb reflexes with ankle dorsiflexion weakness with marked asymmetrical varus deformity. At age 4 there is marked speech and language delay and segregation of the mutation has been confirmed.

Conclusion: Detailed phenotyping seeking associated features such as hearing impairment, retinal dystrophy, ataxia, pyramidal features and neurodevelopmental impairment is of vital importance in the evaluation of peripheral neuropathy and pursuit of the diagnosis.

‡PN11

Pentanucleotide repeat expansion causes CANVAS and Late-Onset Sensory Ataxia

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Background: Late-onset ataxia is a common reason for neurological consultation, but its cause often remains idiopathic. Cerebellar dysfunction, but also proprioceptive or vestibular impairment, can lead to ataxia. When in combination, this more severe type of ataxia is termed cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS). Both sporadic and familial cases of CANVAS have been reported, suggesting the possibility of a recessive transmission of the disease

Aim: to identify the genetic cause of CANVAS and late-onset sensory ataxia

Methods: non-parametric linkage analysis and genome sequencing, functional studies

Results: we identified a recessive pentanucleotide repeat expansion as the cause of CANVAS and a common cause of late-onset sensory ataxia. The recessive repeat expansion showed full segregation in 23 cases from 11 families. Additionally, 33 (22%) out of 150 sporadic cases with late-onset ataxia carried the recessive repeat expansion. The percentage raised to 62% in patients with sensory neuronopathy and cerebellar involvement and 92% in full-blown CANVAS disease. The expansion resides in the polyA tail of an Alu element and differs in terms of both size and nucleotide sequence from the reference allele. Notably, the pentanucleotide repeat expansion does not affect expression of the repeat hosting gene at mRNA and protein levels in patient fibroblasts, lymphoblasts, muscle and brain tissue suggesting that there is no overt loss of function.

Conclusions: these data, together with the observation of an allelic carrier frequency of the expanded repeat of 0.7% in the European population, suggests that this biallelic pentanucleotide repeat expansion represents a frequent cause of late-onset ataxia, with clinical similarities and disease frequency to that of Friedreich's ataxia.

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Motor Nerve Disorders

‡MND01

miRNAs in the CSF of SMA patients as prognostic biomarkers of the potential response to Spinraza treatment

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Background: Spinal Muscular Atrophy is the most common genetic cause of infant mortality, resulting from homozygous deletion in the *Survival Motor Neuron gene 1* (*SMN1*). Spinraza (Nusinersen) is an antisense-oligonucleotide (AON) drug, which is the first FDA approved treatment for SMA. Although the majority of patients show a significant clinical improvement in response to Spinraza, some patients show minimal or no improvement.

Aims: To determine if micro-RNAs (miRNAs) in the cerebrospinal fluid (CSF) can be developed as prognostic biomarkers to predict the clinical outcomes of type I SMA patients in response to Spinraza treatment.

Methods: For discovery profiling, miRNA RT panels containing 752 miRs were performed on miRNAs extracted from the CSF samples of type I SMA patients at pre- (n=10) and 2 months (n=10) post- Spinraza treatment. 13 significantly differentially expressed miRNAs were identified ($p<0.05$) for further validation. Candidate miRNAs were validated using customized quantitative real-time PCR panels in CSF samples from 8 additional type I SMA patients, at pre-, 2 months post- and 6 months post- Spinraza treatment. The levels of miRNAs at pre-treatment were used in a stepwise multiple linear regression, with the percentage

change in CHOP-INTEND motor scores at 10 months post-treatment, a time point when a clear clinical response is expected, set as the dependent variable.

Results: The levels of 3 candidate miRNAs (CM) prior to Spinraza treatment were significant predictors (CM1: ß=-0.694, $p< 1x10^{-3}$; CM2: ß=0.518, p=0.002, CM3: ß=-0.273; p=0.013) for improvement of the motor ability of the patients, measured as the percentage change in CHOP-INTEND motor scores at 10 months post-treatment to pre-treatment.

Conclusions: These data show that CSF miRNAs could be employed as prognostic biomarkers to predict SMA patients' responses to Spinraza treatment. Further research is under way to increase the robustness of this prognostic panel by increasing sample size, identifying further prognostic miRNAs, and include longitudinal data at different time-points during treatment.

MND02

Determination of tissue-water T_2 of fat infiltrated upper and lower limb skeletal muscle with MRI in amyotrophic lateral sclerosis, Kennedy's disease and Duchenne muscular dystrophy

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Background & Aims: The aim of this work was to develop novel optimised quantitative magnetic resonance imaging (MRI) biomarkers to detect and monitor neuromuscular diseases. The primary objective was to estimate skeletal muscle-water spin-spin relaxation time (T_{2m}), a challenging endeavour in fat infiltrated muscles common in patients with these conditions.

Methods: A multi-component slice-profile-compensated extended phase graph (sEPG) model for multi-echo Carr-Purcell-Meiboom-Gill (CPMG) spin-echo sequence signals was implemented, with the fat signal modelled as two sEPG components with fixed parameters, and the remaining unknown parameters (B_1 field factor, T_{2m}, fat fraction (ff_a), global amplitude and Rician noise standard deviation) determined by maximum likelihood estimation. Through software simulations the performance of the method was compared with conventional least-squares parameter estimation, and the commonly assumed multi-exponential signal model. The algorithm was used to analyse clinical muscle study data including patient groups with amyotrophic lateral sclerosis (ALS), Kennedy's disease (KD) and Duchenne muscular dystrophy (DMD) – and their longitudinal follow up scans – and matched healthy controls. The processing pipeline to generate parameter estimate maps incorporated quality control according to quality of fit criteria and physical meaningfulness.

Results: Stable fitting was generally obtained, with little T_{2m} variation between muscles in healthy controls. In ALS and KD median T_{2m} were significantly elevated in varied patterns, but showed a trend to decrease in DMD, where other T_{2m} distribution histogram metrics such as the skewness and full width at quarter maximum differed significantly from those observed in healthy volunteers. Maps of estimated ff_a were qualitatively consistent with independently acquired Dixon fat fraction maps. Comparison of T_{2m} maps with short TI inversion recovery (STIR) MRI signal intensity distributions suggested that conventional STIR contrast may depend on factors additional to T_{2m}.

Conclusion: The data quality available from conventional clinical scanner CPMG protocols places limitations on the achievable accuracy and precision of multi-component transverse-relaxation decay parameter estimates. Nevertheless, the developed

methods provide reproducible measures which change with disease, and which may distinguish different pathological processes.

MND03

Mortality in patients with spinal muscular atrophy over the last 10 years in the Northeast of the UK

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Background: Infantile onset SMA is the most common genetic cause of death in infants (Kolb et al Ann Neurol 2017). Since the introduction of Standards of Care (Wang et al Consensus Statement for Standard of Care in Spinal Muscular Atrophy 2007), a more proactive approach to care for SMA resulted in improvements in the natural history of the disease**.** However, for SMA I infants the approach has been in most cases palliative until more recently with the introduction of novel treatments like antisense nucleotide and gene therapy.

Aims: In view of this dramatic change in the care of SMA patients, from a palliative approach to causal therapies, an audit was conducted to identify the mortality of SMA patients in the Northeast population over the last 10 years.

Results: In our cohort of neuromuscular patients, 77 were diagnosed with SMA 0, I, II or III. Currently 53 SMA patients are followed up in our service; of these 4 SMA I children are receiving intrathecal injection of Nursinersen. Twenty-four SMA patients died of the disease. Of this latter group, 2 patients had SMA 0 and died within their first month of life. Seventeen suffered from SMA I, 2 patients from SMA II and 3 from SMA III respectively. Four patients died while receiving Nursinersen treatment, at the age of 6, 7, 20 and 26 months after their second or third intrathecal injection. The causes of death were respiratory arrest, hypoxic ischaemic brain injury secondary to out of hospital cardio-respiratory arrest and pneumonia. Two of those patients were followed up out of area after having initiated Nursinersen at our site. Prior to the introduction of Nursinersen, all 13 patients with SMA I died at the mean age of 8 months. The age of the deceased patients with SMA II varied greatly between 3 years and 56 years. As for SMAIII, patients died aged on an average of 61 years.

Conclusions: Over the past 10 years 25% SMA 0-I patients died at an average age of 8.4 months (age range 0 – 20), 6% SMA II - III patients died at mean age of 47 years (age range 3- 75), cardiorespiratory failure being the most common cause of death in all cases.

MND04

Development of a Rasch optimised functional outcome measure for people with Spinal and Bulbar Muscular Atrophy

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Background: Spinal and Bulbar Muscular Atrophy (SBMA) or Kennedy's disease, is a rare neurodegenerative disease characterised by progressive weakness in the limb and bulbar muscles due to loss of lower motor neurons. In SBMA there are currently several functional rating scales in use. The revised amyotrophic lateral sclerosis functional rating scale (ALSFRS-R), the spinal and bulbar muscular atrophy functional rating scale (SBMAFRS) and the adult myopathy assessment tool (AMAT). The SBMAFRS was developed by clinicians from the ALSFRS for people with SBMA.

Aims: The aim was to combine items from three commonly used functional scales and optimise it specific to people with SBMA.

Methods: Three functional rating scales commonly used for people with SBMA were individually analysed. Item performance was evaluated using Rasch analysis. Individually, the items of the scales did not

cover the spread of the ability of individual persons. The individual items for the three scales were combined and repeated items were deleted. Additional items were discarded that did not fit the model or were not deemed clinically relevant to the clinical team. Items were rescored where there were disordered thresholds, and evaluation of item dependency and uni-dimensionality took place.

Results: 140 individual observations were entered into the analysis. The resulting scale consisted of 23 items and 17 categories were rescored. There was a good overall fit to the Rasch model (X^2 = 53.18, p=0.22). Reliability indices were 0.76 and item fit residuals were 0.02. Person location mean was high compared to the item location mean. Dependency was observed between stepping and speech; sit to stand, stairs and rise from supine. The clinical team justified retaining the items as they are functionally distinct. The scale was not uni-dimensional on testing (15.7% significant t-tests), but there was no clear delineation in items between the positively loaded and negatively loaded items.

Conclusion: We have combined items from three functional scales to create a tailored tool for SBMA. There are some issues of dimensionality and dependency, but the performance of the scale has been optimised to create a more clinically relevant tool specific to people with SBMA, with improved item performance.

MND05

Is Protein Intake Associated With Fat Free Mass In Type I Spinal Muscular Atrophy?

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Background: Spinal Muscular Atrophy Type I (SMA1) is a severe autosomal recessive neuromuscular disease characterized by degeneration of alpha motor neurons in the spinal cord with subsequent muscular atrophy and increased fat free mass (FFM). Amount of energy and dietary protein intake play a key role in maintenance of FFM, but optimal protein needs in SMA still remain unclear.

Aim: we investigated the association between FFM and daily food protein intake (PI) in a SMA1 sample with an optimal energy intake according to sex, age and energy metabolism.

Methods: 14 children with SMA1 (57%M; mean age:6 months, range 4-13) were recruited among a large sample involved in an ongoing longitudinal study, only if they presented an energy intake +/- 20% of energy expenditure assessed by indirect calorimetry. WHO growth charts were used as reference values for BMI. FFM was investigated by DXA and FFM/length ratio (g/cm) was calculated. Mean energy and nutrients' intakes by 3-days food dietary records were compared with age-based dietary recommendations. Descriptive results are expressed as median values with interquartile ranges (IQR).

Results: BMI Z-Score was -2.5 (IQR=-4.1—-1.7). FFM was 63% (IQR=62—69%) and FFM/length ratio was 63g/cm (IQR=59—66g/cm). 100% of patients had an energy intake in line with measured energy expenditure; only 43% satisfied the carbohydrates' recommendations, while 71% consumed more fat than recommendations. Proteins/kg BW were 1.9g/kg (IQR=1.4—2.1g/kg): +46% of recommendations (IQR=5—+83%). PI/kg BW (r=0.293, p=0.308) and adequacy index (r=0.486, p=0.078) were not correlated with FFM and FFM/length ratio.

Conclusion: SMA1 children with adequate daily food intake consumed more lipids and proteins and less carbohydrates than what recommended. Higher protein intake was not related to FFM and FFM/length rato; amount of PI in SMA1 children does not seem to contribute to the improvement of FFM. Further studies are needed to optimize protein requirements in SMA1 children.

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MND06

Respiratory trajectories in SMA type 2 and non-ambulant type 3 paediatric patients within the SMAREACH UK network

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Background: The availability of new therapeutic options for SMA2 and 3 has led to modified clinical phenotypes. However, the natural history of respiratory involvement in these conditions has not been fully outlined so far. We hypothesized that the progression of respiratory and motor function would decline differently among SMA2 and SMA3 non-ambulant patients.

Aims: Main aim is to identify the yearly decline of respiratory function and its correlation with motor function.

Patients and Methods: Retrospective analysis of SMA2 and non-ambulant SMA3 paediatric patients (age<18years) consented for SMAREACH UK natural history study. Nine-year data (Jun2010-Sep2018) were collected from study forms. Patients with at least 2 years follow-up data were included. We excluded patients in any interventional clinical trial. Anthropometrics, clinical data (establishment of Non-Invasive ventilation (NIV), cough assist) were collected. Motor function scores such as Hammersmith functional motor scale (HFMS) and Revised performance of upper limb (RULM) were collected at each visit. Forced vital capacity absolute, (FVC) and % predicted (FVC %pred.) were collected from spirometry.

Results: Out of 314 SMA2 and 3 patients, 153 met the inclusion criteria and had a full dataset available. 118/153 were SMA2, 16/153 were non-ambulant SMA3, 19/153 unknown. Median age at first visit was 5.6 (IQR 3.4-9.8) and 11.6 (IQR 4.9-15.8) years for SMA2 and 3 respectively. 44/134 (33%) had started NIV, main reason being recurrent low tract respiratory infections followed by hypoventilation detected at sleep study (12 vs 9). 39/134 (29%) had started cough assist. FVC %pred. yearly rate of decline was 3.2% in SMA2 patients and 4.0% in combined SMA2 and 3. In SMA2 patients we observed a steeper reduction of FVC %pred. up to 10 years of age, followed by a slower yet regular decline. Conversely, in SMA3 patients FVC %pred. started declining after the age of 10. In SMA2 HFMS at baseline correlated with FVC absolute (r=0.8, p=0.01) while both HFMS (r=0.40 p=0.22) and RULM (r=0.4, p=0.0.14) correlated with FVC% pred.

Conclusion. In SMA2 and 3, the respiratory function expressed as lung volumes, declines progressively from an early age and is correlated with motor function.

MND07

Feeding difficulties in children and adolescents with SMA type II

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Background: Spinal muscular atrophy(SMA) is a progressive motor neuron disorder caused by

deletion of the *SMN1* gene. The most prominent feature is muscle weakness of axial and limb muscles. Scoliosis and respiratory problems are common comorbidities in SMA types I and II as a reflection of paraspinal and respiratory muscle weakness. Weakness of bulbar muscles and muscles of the gastrointestinal tract have been described to a lesser extent, mostly in severe SMA (type I). Disease course of feeding difficulties in intermediate SMA (type II) are relatively unknown. The implementation of disease-modifying therapies will change motor function and survival in SMA, but effects on non-motor co-morbidities are unknown. The natural course of feeding difficulties needs to be explored in order to have a reference for treatment effects on these symptoms.

Aims: Identify feeding difficulties and their disease course in treatment-naïve SMA type II patients.

Patients and methods: We included all SMA type II patients from the GOSH SMA database. Data was retrieved through retrospective chart review. Growth curves including BMI were analysed adjusted for age and gender.

Results: We included 47 patients with a mean age of 10years (range 2-19yrs). Seven (72%) had a relatively mild SMA type II phenotype and achieved the ability to stand or walk with support in addition to sitting. Mean follow up time was 6years(range 1-15yrs). 31 of 47 Patients (66%) were underweight. Reported feeding difficulties included unsafe swallowing, (severe) weight loss or both. Feeding difficulties were present in 48% of patients. Fourteen patients(30%) needed gastrostomy because of severe weight loss(40%), swallowing difficulties(40%) or both(20%). Six out of 15 patients had recurrent pulmonary infections with improvement after gastrostomy suggestive of recurrent (silent) aspiration.

Conclusion: Feeding difficulties were present in 48% of patients with SMA type II, especially with more severe weakness. However, enteral feeding was needed in only 30% of patients and was indicated when severe weight loss or swallowing difficulties with or without (silent) aspiration were present.

MND08

Survival of motor neuron (SMN) protein levels before and after treatment with risdiplam (RG7916) in patients with Type 1 to 3 spinal muscular atrophy (SMA) compared with healthy subjects

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Background: Spinal muscular atrophy (SMA) is characterised by motor neuron loss and muscle atrophy, due to reduced levels of survival of motor neuron (SMN) protein from loss of function of the *SMN1* gene. While *SMN1* produces full-length SMN protein, a second gene, *SMN2*, produces only low levels of functional SMN protein. Risdiplam (RG7916; RO7034067) is an investigational, orally administered, centrally and peripherally distributed small molecule that modulates *SMN2* pre-mRNA splicing to increase SMN protein levels.

Aim: To report on the SMN protein levels measured in studies of risdiplam in patients with Type 1 SMA (FIREFISH - NCT02913482) and patients with Type 2 and 3 SMA (SUNFISH - NCT02908685; JEWELFISH - NCT03032172).

Methods: At abstract submission, SMN protein data are available from 63 patients with Type 2 and 3 SMA and 21 patients with Type 1 SMA, at baseline prior to treatment and after treatment with risdiplam. SMN protein levels in 49 healthy subjects have been collected in two other studies. This is the first detailed comparison of SMN protein levels across SMA types and healthy individuals, between copy numbers, across a wide age range (3.3 months to 52 years in patients with SMA), and in longitudinal data for patients receiving risdiplam versus patients on placebo. The same procedures and assays were used for SMN protein sample collection and analysis, enabling a robust comparison.

Results: In SUNFISH and JEWELFISH, SMN protein increased in a dose-dependent manner upon treatment with risdiplam, with a median 2.5-fold increase (range 1.5–3.5) at the highest dose. In FIREFISH, an individual SMN protein increase of up to 6.5-fold (range 1.6–6.5) was observed at the highest dose.

Conclusion: This increase in SMN protein is expected to lead to significant clinical benefit, based on the comparison of SMN protein levels in different SMA severity types and healthy subjects.

MND09

Developing a bulbar motor neuron protocol to study Spinal and Bulbar Muscular Atrophy

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Background: Spinal and Bulbar Muscular Atrophy (SBMA) is a rare progressive motor neuron disease caused by an X-linked, expanded polyglutamine repeat in the androgen receptor gene. It affects male patients who present with wasting and weakness of facial, bulbar and limb muscles in their fourth to sixth decade Spinal motor neurons (MNs) have been studied in mouse models of SBMA, pathological specimens and increasingly in MNs derived from patient induced pluripotent stem cells. However, there are currently no established protocols for studying bulbar MNs.

Aims: To develop a protocol to generate bulbar MNs from SBMA patient-derived iPSCs.

Methods: SBMA IPSCs (SB1, SB3, SB5 and SB6) were kindly provided from the lab of Dr Kurt Fischbeck at the National Institute for Neurological Disorders and Stroke (USA) through an MTA. Three lines of patient derived and three lines of control iPSCs were plated to 100% confluency and then differentiated into neuroepithelium using small molecules (dorsomorphin, SB431542 and CHIRR) in N2B27 media. At day 7 the cells were patterned using 5 developmentally rationalised experimental conditions. At day 14 MN precursors were treated with 0.1μ M purmorphamine for a further 4 days. The precursors were harvested at day 18.

Results: RNA was extracted from the cells and qPCR used to identify the experimental conditions which most closely demonstrated the HOX gene expression pattern found in bulbar MNs. The closest experimental conditions were fine-tuned to optimise the protocol. They will be retested for HOX gene expression pattern. These precursors will undergo terminal differentiation using Compound E. At day 7 of terminal differentiation the cells will be fixed and immunocytochemistry will look for evidence of Phox2B expression which defines bulbar MNs.

Conclusions: These bulbar MNs will be used to further explore the pathogenic mechanisms which underlie motor neuron diseases. Defining the patterning cues for bulbar v spinal MNs will lay the foundation for regionally specified astrocytes for further study.

MND10

UK Spinal Muscular Atrophy Registry

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Background: The UK SMA Registry collects clinical and genetic information from individuals with spinal muscular atrophy (SMA). Launched in 2008, the registry holds patient-entered data on over 500 SMA patients living within the UK and Ireland. SMA is a recessive, progressive neuromuscular disorder with a prevalence of approximately 1–2 per 100,000 persons.

Aims: The purpose of the registry is to aid the rapid identification of eligible patients for clinical studies. It disseminates SMA-relevant information to participants; provides a source of information to academics, industry and healthcare professionals; and supports the SMA community.

Methods: Registration is patient-initiated through a secure online portal. Participants give their informed consent and are invited to complete a questionnaire about their condition, including: genetic confirmation and SMN2 copy number; clinical diagnosis; SMA classification; current and best motor function; wheelchair use; scoliosis surgery; gastric/nasal feeding; ventilation status; family history.

Results: Currently, 512 participants are registered with the UK SMA Registry, with an age range of six months to 84 years. The greatest number of participants report a diagnosis of SMA type 2 (40%), followed by individuals with SMA type 3 (31%) and with SMA type 1 (12%) (17% unspecified). Considering motor function, 31% percent of individuals are unable to support themselves in a seated position, 37% are able to sit unsupported and 23% are able to walk (9% unspecified). Sixty-three percent of participants either always or sometimes use a wheelchair and 13% never use a wheelchair (24% unspecified).The majority of registry participants do not use a ventilation aid. Occasional use of non-invasive ventilation is reported by 16% and permanent use, by 1% of participants. Use of invasive ventilation is lower, with reported occasional use by 1% and permanent use, by 1% of participants (12% unspecified for each).

Conclusion: The UK SMA Registry is a valuable tool for the collection of patient data which informs academics, healthcare professionals and industry. It represents a trial-ready cohort of individuals and supports the SMA patient community.

MND11

The impact of spinal surgery on respiratory and motor function and weight gain in patients with SMA II and non-ambulant III

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Background: Scoliosis is a significant orthopaedic complication in Spinal Muscular Atrophy (SMA). Bracing is palliative and surgical intervention frequently required. Studies in small cohorts have reported the impact of spinal surgery on respiratory function. No data are available on motor function and weight.

Aim: To better understand the impact of spinal surgery on respiratory and motor function and weight in patients with SMA II and non-ambulant III.

Methods: We retrospectively reviewed the notes of SMA patients who underwent spinal surgery at Great Ormond Street Hospital (last 10 years). Data were collected up to 5 years both before and after the procedure as Forced Vital Capacity (FVC) %, Hammersmith Functional Motor Scale (HFMS), Revised Upper Limb Module (RULM), and weight

trajectories (UK-WHO growth charts). Cobb angle values and post-operative pain were also documented.

Results: The notes of 33 patients (26 SMA II, 7 SMA III) were reviewed. 24 underwent spinal fusion, 4 traditional growing rods (GR, followed by final growth spinal fusion in 2), 3 magnetic GR. Mean age at surgery was 10.9 years (range 5.4 – 16.7), mean pre-operative Cobb angle 68 degrees (range 35 – 97). Within the first year 69% showed FVC % decline (mean -11.4, range -1 — -26), 31% showed FVC % improvement (mean +10.5, range +1 - +20). Motor scores showed progressive worsening especially in gross motor function with relative spared upper limbs function. 15 patients negatively deviated from previous growth curve in the first year after surgery; 5 presented significant weight loss (>5% of total weight) successfully treated with food supplements and/or gastrostomy.

Conclusions: Most patients presented respiratory function decline early after surgery. A drop in motor function was noticed which was not reported before. Significant weight loss was detected in a proportion of patients suggesting this aspect needs careful management. Further studies are planned to address post-surgical pain.

MND12

Challenges in the implementation of nusinersen treatment for SMA in the UK

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Spinal muscular atrophy (SMA) is a rare inherited neuromuscular condition and until recently no drug treatment was available. Nusinersen is an antisense oligonucleotide and is the first drug approved by FDA and EMA for all types of SMA.

Following the positive outcome of an interim analysis of the Phase 3 ENDEAR study in SMA type 1, Ionis Pharmaceuticals and Biogen announced the early termination of the ENDEAR study and that all patients on the study would be transitioned onto an Open Label Extension (OLE) study given the positive results. In addition, the company opened an Expanded Access Program (EAP) to offer access to the drug, free of charge, to all patients with SMA1.

This exciting news for the SMA community has, however, been accompanied by major frustrations due to delays in the implementation of both the OLE and EAP and more recently delays in the overall NICE approval process of nusinersen.

The main challenges have been around allocating NHS resources for the EAP as the cost of delivering the drug was not met by the company. Unfortunately, the whole process took more than six months to see the first child being treated in the UK as part of the EAP. Initially, access was allowed only to local patients registered at the sites authorised to deliver the EAP, raising concerns about rationale of inclusion criteria and equity to accessing treatment. Since then additional sites have been able to deliver this new treatment and all eligible SMA1 children in the UK had access to the treatment.

The EAP closed to all new SMA patients in November 2018 and whilst most EU countries are administering the treatment in clinical practice, in England we are still going through a NICE appraisal process which is causing delays in accessing the treatment and raises serious concerns around equity to accessing the treatment.

MND13

AVXS-101 Gene-Replacement Therapy (GRT) for Spinal Muscular Atrophy (SMA): From Bench to Bedside

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Background: Onasemnogene abeparvovec (AVXS-101) is an investigational, one-time GRT that treats the genetic root cause of SMA, a progressive

neurological disease. AVXS-101 delivers the survival motor neuron gene (*SMN*) via a self-complementary adeno-associated serotype 9 viral vector (scAAV9) that crosses the blood-brain barrier. AVXS-101 is designed for immediate and sustained expression of SMN protein in non-dividing neurons, allowing for rapid onset and durable therapeutic effect.

Aims: Report AVXS-101 GRT development for SMA.

Methods: SMA mice (*SMN2*$^{+/+}$,*SMNΔ7*$^{+/+}$,*Smn*$^{-/-}$) received intravenous scAAV9-*SMN* or scAAV9-*GFP* at P1; survival and motor function were assessed. Non-human primates (NHPs) received intravenous scAAV9-GFP; transduced cell types were assessed. In the phase 1 trial (NCT02122952), symptomatic SMA1 patients received a one-time AVXS-101 infusion at low (n=3) or proposed therapeutic dose (n=12). Safety (primary objective), event-free survival (no death/permanent ventilation), sitting unassisted (secondary objectives), CHOP-INTEND, and other milestones were assessed.

Results: In SMA mice, scAAV9-*SMN* improved survival (>200 versus 15 days in controls), and increased motor function (90% had righting ability at P13 versus ~20% in controls). In NHPs, scAAV9-*GFP* efficiently targeted motor neurons throughout the CNS. In the phase 1 trial, all patients survived, event free, at 24 months. In the therapeutic dose cohort, 11/12 patients reached CHOP-INTEND ≥40; 11 sat unassisted ≥5s, 10 for ≥10s, 9 for ≥30s. Two patients crawled, stood, and walked. In the long-term follow-up study (LTFU), 2 more patients sat ≥30s and 2 stood with support. No patient received nusinersen during the 24-month study period. Four patients had an asymptomatic transient rise in serum aminotransferase. The oldest patient is 59.2 months of age with 53.3 months of follow-up post–AVXS-101 therapy (as of September 27, 2018).

Conclusion: AVXS-101 demonstrated transformational event-free survival, motor function, and milestone improvements in symptomatic SMA1 infants. Long-term safety is being monitored for 15 years (LTFU). Phase 3 trials in SMA1 patients in the US and Europe are ongoing. Additional trials investigate AVXS-101 in presymptomatic SMA, and in older patients using intrathecal administration. This experience with SMA is hoped to inform other programs that build on the AveXis platform to bring much-needed gene therapy to patients with rare diseases.

‡MND14

FIREFISH Part 1: Early clinical results following an increase of survival of motor neuron protein (SMN) in infants with Type 1 spinal muscular atrophy (SMA) treated with risdiplam (RG7916)

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Background: Spinal muscular atrophy (SMA) is characterised by motor neuron loss and muscle atro-

phy, due to reduced levels of survival of motor neuron (SMN) protein from loss of function of the *SMN1* gene. While *SMN1* produces full-length SMN protein, a second gene, *SMN2*, produces low levels of functional SMN protein. Risdiplam (RG7916; RO7034067) is an investigational, orally administered, centrally and peripherally distributed small molecule that modulates *SMN2* pre-mRNA splicing to increase SMN protein levels.

Aim: To report on the FIREFISH Part 1 dose-finding study (NCT02913482), an ongoing, multicentre, open-label, two-part, seamless study of risdiplam in infants aged 1–7 months with Type 1 SMA and two *SMN2* gene copies.

Methods: Part 1 is exploratory and principally assesses the safety, tolerability, pharmacokinetics and pharmacodynamics of different risdiplam dose levels (enrolment complete). Confirmatory Part 2 (n=40) assesses safety and efficacy of risdiplam, with a primary endpoint of the proportion of infants sitting without support for 5 seconds after 12 months.

Results: Part 1 interim analysis presents a dose-dependent increase in SMN protein levels in whole blood, with an up to 6.5-fold increase vs. baseline after 4 weeks of treatment at the highest dose of risdiplam (2.0–6.5-fold). To date (data-cut 07/09/18), no safety-related stopping rules have been met, and none of the following events have been reported: loss of ability to swallow, tracheostomy, or permanent ventilation. Part 1 motor milestone, safety, and survival data for infants that have been treated for a minimum of 6 months will be presented.

Conclusion: The up to 6.5-fold increase in SMN protein observed in Part 1 is expected to lead to clinical efficacy based on the differences in SMN protein levels between SMA severity types (e.g., Type 2 vs. Type 1 with differences of ~2-fold). Part 2 of the FIREFISH study is ongoing.

‡MND15

MRI detection of human motor unit fasciculation in Amyotrophic Lateral Sclerosis

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Background: Patients with Amyotrophic Lateral Sclerosis (ALS) typically wait 12 months between symptom onset and receiving a definitive diagnosis. This delay prevents the early instigation of life-prolonging therapies, and hampers timely recruitment into clinical trials. There is therefore a strong need to develop improved diagnostic technologies for ALS. A hallmark of ALS is the presence of fasciculation; random and irregular muscle twitching produced by involuntary activation of individual skeletal motor units. The micro-movement of muscle fibres during fasciculation offers a target for new diagnostic approaches.
Aims: To evaluate the potential of a novel diffusion weighted MRI protocol to detect fasciculation in patients with ALS.

Methods: We have developed a novel diffusion weighted MRI protocol which is sensitive to micrometer-scale movement of skeletal muscle and which can detect activity of motor units [1] – referred to as Motor Unit MRI (MUMRI). The legs of 4 patients with confirmed ALS and 6 healthy controls were scanned using the MUMRI method, acquiring time series of scans in both legs at rest over multiple 3 minute epochs. The frequency and spatial distribution of spontaneous motor unit activity was assessed.

Results: Patients with ALS showed a significantly higher rate of spontaneous motor unit activation at rest than controls (mean 99.1 per minute, range 25.7-161 in patients versus 7.7 per minute, range 4.3-9.7 in controls, $p<0.05$, Students t-test). The percentage muscle cross sectional area in which fasciculation

was detected was significantly higher in patients than controls (15.9%, SD 2.8 vs 2.9%, SD 1.6).

Conclusion: Our results are the first demonstration of the ability of MR imaging to detect fasciculation in patients with ALS. This technique offers unprecedented insights into human skeletal muscle physiology and pathophysiology. MUMRI can potentially be extended to whole body assessment and could provide a rapid, pain free and sensitive means of diagnosing and monitoring patients with ALS as well as other neuromuscular disorders.

References: 1: Porcari et al, Proc ISMRM, p330, Paris 2018.

Neuromuscular Junction Disorders and Channelopathies

NMJ+C01

Investigating muscle phenotype change with age in monogenic disease may provide new insights into the normal ageing process

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Background: It is often reported that the severity and frequency of acute attacks of paralysis in people with both hyperkalaemic and hypokalaemic periodic paralysis starts to reduce around age forty. Forty is also the age around which optimal motor performance in master athletes starts to decline and above which pathological changes e.g. up to 5 COX negative fibres on muscle biopsy, are accepted as within normal limits for age.

Aims:

1. To determine if the reduction in periodic paralysis attack severity with age is conserved across species.
2. To investigate whether the reduction in periodic paralysis attack severity with age is a result of normal muscle ageing or ageing in the presence of single ion channel dysfunction.

Methods: To minimise genetic and environmental confounders and standardise the paralytic attack, male hyperkalaemic periodic paralysis (HyperPP) mutant mice were compared to their wild-type brothers. All animals were housed in the same environment. A paralytic attack was induced on soleus muscle *ex vivo* in a tissue chamber maintained at 30 degrees Celsius using a solution containing 10mM potassium and 1.3mM Calcium as previously described. The results from young adult (13 to 26 weeks, n=5 WT, n=5 Hyper PP), middle-aged (43 to 70 weeks, n=4 WT, n= 5 Hyper PP) and old (>95 weeks, n=5 WT, n=8 Hyper PP) mice were compared.

Results: Young and middle-aged soleus lost significantly more force than old soleus. This was true for both HyperPP (one-way ANOVA $p=0.007$) and wild-type (one-way ANOVA $p=0.009$) animals. This was not simply due to a reduction in absolute force as maintenance of force also occurred in old soleus that had greater or equal baseline tetanic force to young soleus. Tetanic force for 2 out of the 5 old wild-type soleus muscles was increased throughout the period of hyperkalaemia. This was never observed in young or middle-aged wild-type, or young, middle-aged or old HyperPP soleus.

Conclusions: A reduction in periodic paralysis attack severity with age appears to be conserved across species. Our data suggests this phenomenon is the result of 'normal' muscle ageing rather than the chronic consequence of single ion channel dysfunction.

NMJ+C02

Differences in Muscle Phenotype Severity between Humans and Mice with Monogenic Disorders may help Identify Novel Therapeutic Pathways

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Background: The four transgenic mouse models of periodic paralysis recapitulate many features of the human disease. They have similar pathology, the same gender difference in phenotype severity and muscle weakness is consistently induced on exposure to extremes of potassium. However, none of the mouse models have ever been observed to have a spontaneous attack of weakness and the potassium concentration needed to induce an attack is more extreme than that reported for humans.

Aims:

1. To identify differences in normal mouse and human muscle excitability and explore these differences using selective pharmacological blockade.
2. To test whether the identified differences protect against spontaneous attacks of weakness in mice with periodic paralysis.

Methods: Muscle velocity recovery cycles (MVRCs) can be used to indirectly measure muscle excitability *in vivo*. In human muscle there are two phases of increased conduction velocity. The first, known as early supernormality, has a correlate in nerve and reflects passive decay of charge that has accumulated in the sarcolemma. The second, known as late supernormality, is specific to muscle and reflects activity-induced potassium accumulation in the t-tubules. MVRCs have not previously been reported in mice. MVRCs were recorded under anaesthesia in Wild-type C57BlJ6 mouse Tibialis Anterior (TA), using QTRAC software, as described for humans. Intraperitoneal injection of 9-Anthracene-carboxylic-acid was used to block ClC-1 channels and micromolar ouabain to selectively block the t-tubule Na/K pump. MVRCs were recorded pre- and post-pharmacology in the same animal.

Results: In contrast to human MVRCs, mouse MVRCs had no late supernormality. Blockade of ClC-1 induced late supernormality in mouse TA ($p=0.002$, t-test with Welch correction). Blockade of the t-tubule Na/K pump induced late supernormality in 3 of 14 TAs exposed to ouabain compared to 0 of 66 not exposed to ouabain ($p= 0.007$, two-tailed Fisher exact test).

Conclusions: MVRCs can be recorded in mice. Our data suggest that, compared to humans, mice have more effective t-tubule potassium buffering and clearance. This is due, at least in part, to an increased ClC-1 conductance and t-tubule Na/K pump activity. The next step will be to determine if this protects against spontaneous attacks of weakness in mice with periodic paralysis.

NMJ+C03

Anderson Tawil Syndrome: expanding the phenotype and assessing cardiac risk

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Background: Anderson Tawil Syndrome (ATS) is a rare neuromuscular channelopathy traditionally characterised by periodic paralysis, cardiac arrhythmias and dysmorphic features. Mutations in the KCJN2 gene are associated with ATS. Accurate and early diagnosis is important in facilitating treatment of episodic paralysis and preventing potentially life-threatening cardiac events.

Aims: To fully characterise the phenotype in a carefully stratified cohort including cognitive deficits and cardiac risk.

Patients: At the Nationally Commissioned Highly Specialised Service for Channelopathies we have one of the largest cohorts of patients with ATS in the world. Patients with a genetically confirmed diagnosis were consented and enrolled into our Channelopathy cohort study. Clinical and family history, examination findings and investigations including

neurophysiology, psychometric, cardiac and radiological assessments were reviewed.

Results: 66 patients were identified with KCJN2 mutations. Comprehensive clinical information has so far been collected for 18. Cardiac symptoms were prominent. Two thirds (11) had daily or very frequent palpitations. 27% (5) reported shortness of breath. Serious cardiac complications occurred in 4 patients (ICD insertion, left ventricular dysfunction/ cardiomyopathy). 39% (7) reported pain which has previously not been appreciated in ATS. At least 44.4%(8) patients had fasciculations. 22%(4) patients had minimal decrement (<48%) on Long Exercise Tests (LET) despite having episodic weakness. Lower limb Muscle MRI was abnormal in 6 patients. Daytime somnolence was reported, prompting sleep studies. Neuropsychometric testing suggests slowed processing speed. While dysmorphic features exist, these can be subtle. Short stature is not ubiquitous, with a height range of 147-180.34cm. Heterogeneity within families was commonly seen.

Conclusions: The phenotypic spectrum of ATS is broader than currently appreciated including frequent pain, fasciculations and daytime somnolence suggestive of nocturnal hypoventilation. Fasciculations may suggest additional lower motor neurone pathology. A negative LET is common and should not deter from pursuing genetic testing. Cardiac symptoms are common and complications beyond ventricular ectopics require screening for. Data analysis is ongoing.

NMJ+C04

Applied Neuromuscular-Junction Facility to investigate structure and function of mammalian neuromuscular junction

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Background: The Neuromuscular junction (NMJ) is the specialized chemical synapse that mediates the transmission of action potentials in motor axons to skeletal muscle fibers. The NMJ is the best characterized cholinergic synapse and its study for many years has provided much of our general knowledge of synapse structure, function and development. While an increasing number of molecular defects are known to cause impaired neuromuscular transmission, in very few cases has the impact of these molecular defects on the structure and function of the NMJs been characterized in detail, much less understood. Partly as a result of this lack of knowledge, available treatments for diseases of the NMJ are limited in number and efficacy.

Aims: To address these concerns we aimed to establish the Applied NMJ Facility to study NMJ structure and function.

Methods: Here, we are initially using murine nerve-muscle preparations for electrical recording combined with high-resolution laser confocal microscopy (LSM880) allowing accurate investigation of NMJ functionality at a single synapse resolution. It is our intention to extend our structural and functional NMJ studies to human biopsy samples later on this year.

Result: Our facility can provide detailed information about synaptic transmission at the NMJ, using both extra- and intra-cellular electrical recording, as well as high resolution imaging of NMJ morphology, membrane recycling, mitochondrial membrane potential and Ca^{2+} dynamics.

Conclusion: This is a broadly applicable technique which can be adopted to investigate alterations of NMJ activity. Furthermore it will facilitate the development and assessment of new therapies in mouse models of neuromuscular diseases, including peripheral neuropathies, motor neuron disorders and myasthenic syndromes.

NMJ+C05

Exploring novel treatments for disorders that feature defects of the neuromuscular junction structure

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Congenital myasthenic syndromes (CMS) are a group of inherited disorders that affect the signal transmission at the neuromuscular junction (NMJ) and share the clinical feature of fatigable muscle weakness. In *DOK7*-CMS compromised neurotransmission is due to small and destabilised NMJs. DOK7 is a cytoplasmic-adaptor protein that amplifies the signalling from muscle-specific receptor tyrosine kinase (MuSK), that is responsible for the formation and stabilisation of the NMJ. It has recently been shown that large amounts of DOK7 protein in muscles generate enlarged NMJ in mice, which are very efficient in signal transmission and have no reported detrimental effects.

This study aims to identify small molecules that can specifically upregulate the amount of DOK7 in muscles.

First, the level of upregulation needed to generate the enlarged NMJ is undefined. We used a retroviral vector harbouring a human *DOK7* expression cassette to infect wild-type C2C12 and subsequently titrate levels of DOK7 needed to generate enlarged AChR clusters. A 4-5 fold increase in DOK7 protein levels proved to be enough to mimic the generation of enlarged synapses *in vitro*.

Subsequently, approximatively 5000 small molecules from a library of muscle-specific compounds were used in screening experiments. Initial screenings were performed in triplicates on a C2C12 reporter cell line designed to contain a 1-Kb sequence of the DOK7 promoter fused to the Luciferase-reporter. Confirmatory results were performed for potential candidate upregulating compounds on a similar EGFP reporter cell line. Five potential hits that show significant upregulation after being tested on both reporter cell lines, are being further investigated. They will provide the basis for a more detailed analysis of the effects of DOK7-upregulation in cell culture biological assays and ultimately for *in vivo* testing.

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‡NMJ+C06

Salbutamol modulates postsynaptic specialisation at the neuromuscular junction in a mouse model of ColQ myasthenic syndrome

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Background: The β-adrenergic agonists salbutamol and ephedrine have proven to be an effective therapy for human disorders of the neuromuscular junction (NMJ), in particular many subsets of congenital myasthenic syndromes (CMS). However, the mechanisms underlying this clinical benefit is unknown.

Aims: In order to explore the effect of salbutamol in CMS, we investigated the effect of salbutamol treatment on the NMJ in the ColQ deficient mouse, a model of end-plate acetylcholinesterase deficiency.

Methods: ColQ$^{-/-}$ mice received 7 weeks of daily salbutamol injection, and the effect on muscle strength and NMJ morphology was analysed.

Results: Salbutamol to a gradual improvement in muscle strength in ColQ$^{-/-}$ mice. In addition, the NMJs of salbutamol treated mice showed significant improvements in several postsynaptic morphological defects, including acetylcholine receptor density and area, but did not affect nerve terminal area or

axon diameter. Salbutamol had no measurable effect on muscle fibre size or fibre type proportion.

Conclusion: These results suggest that β-adrenergic agonists lead to clinical benefit in CMS by inducing long-term structural changes at the NMJ, and that these effects are primarily at the postsynaptic membrane.

‡NMJ+C07

Congenital myasthenic syndrome due to a mutation in a nuclear membrane protein

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Background: Next generation sequencing has led to the identification of an increasing number of unexpected gene mutations affecting synaptic transmission at the neuromuscular junction. Here we have identified a family with a novel congenital myasthenic syndrome (CMS) with mutations in a nuclear envelope protein. We describe both the characterisation of this disorder and our investigation of the underlying pathogenic mechanism.

Aim: To learn how a ubiquitously expressed nuclear envelope protein mutation can cause a disorder where the dominant symptoms are due to defective neuromuscular transmission.

Results: The index case and his brother have mild fatiguable muscle weakness in shoulder abductors, finger extensors and ankle dorsiflexors, and mild wasting of deltoid and medial gastrocnemius. They showed decrement of 40% and 26% on RNS at 3Hz respectively. Whole exome sequencing identified a homozygous single nucleotide deletion c.127delC; p.P43fs in *TOR1AIP1*. The gene encodes nuclear envelope protein LAP1 (lamin-associated protein 1), of which at least two isoforms (LAP1B and shorter LAP1C) exist. The variant, which is predicted to ablate the expression of LAP1B but not LAP1C, was shown by Sanger sequencing to segregate with disease. Mutations in *TOR1AIP1* are rare and an effect on neuromuscular transmission has not previously been reported. Trapezius biopsy showed mild variation in muscle fibre size, and no detectable expression of LAP1 in muscle nuclei. Electron microscopy revealed abnormal herniated nuclei. A mouse model for the disorder was obtained in which LAP1 is ablated in striated muscle. Mice were initially strong, with normal EMG and diaphragm spontaneous and evoked endplate potentials. They became weak from 6-7 weeks of age and by 12 weeks of age showed up to 15% decrement at 20Hz RNS. Diaphragm spontaneous and evoked endplate potentials became prolonged, and EDL and soleus endplates were very fragmented with some sprouting and denervation. Synaptic AChR levels were unchanged in the model mouse diaphragm compared with wild type (^{125}I-BuTx), but extra-synaptic AChRs increased 3-fold. MuSK expression was decreased. H&E staining showed a large variation in fibre size with central nuclei. Electron microscopy showed muscle fibre morphology ranging from normal to very disorganised and atrophied, and with abnormal endplates structure.

Conclusion: Our data is consistent with denervation and muscle degeneration and regeneration, with ongoing remodelling of the NMJs, and impaired neuromuscular transmission caused by defective synaptic structure.

Mitochondrial Disease

M01

The phenotypic and genotypic spectrum of *MT-ATP6*-related mitochondrial disease

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Background: Mutations in *MT-ATP6* are associated with maternally-inherited Leigh syndrome (LS) and neurogenic weakness, ataxia and retinitis pigmentosa (NARP). Early studies suggested a strong correlation between mutant heteroplasmy level and disease severity in two common pathogenic variants, m.8993T>C/G.

Aims: To determine the phenotypic and genotypic spectrum of *MT-ATP6*-related mitochondrial disease.

Methods: A retrospective, observational cohort study (January 2009 – October 2018) of individuals referred to the three national mitochondrial centres in Newcastle upon Tyne, London and Oxford that comprise the NHS Highly Specialised Service for Rare Mitochondrial Disorders of Adults and Children.

Results: We identified 88 clinically affected individuals (median current age 26.5 years, range 0.75–74 years, interquartile range: 33.3 years) and 37 asymptomatic family members from 60 pedigrees. Fifteen patients (17%) died, and four patients were lost to follow up during the study period. The common clinical features were cerebellar ataxia (83%), peripheral neuropathy (74%), and cognitive dysfunction (65%). Thirty-six per cent of patients manifested with Leigh syndrome (LS), and only 7 patients exhibited a pure NARP syndrome. The median age of onset was younger in patients with LS compared to those presenting with other clinical phenotypes (1.5 vs 15 years, $p<0.001$). Four adult patients developed unexpected, subacute brainstem dysfunction. Nine pathogenic variants were identified in our patient cohort, and five of them accounted for 91% of all cases. The maternal transmission of the pathogenic *MT-ATP6* variants was established in 68 patients (77%), and the mutation likely arose *de novo* in three patients (3%). Logistic regression modelling revealed a correlation between the risk of clinical manifestation and mutant heteroplasmy level in five common mutations; the chance of being clinically-affected increasing significantly when the mutant load exceeded 50%.

Conclusion: This is the most extensive observational study of *MT-ATP6*-related mitochondrial disease to date. Our data highlight that sequencing of the *MT-ATP6* gene should be included in the diagnostic workup of patients with both cerebellar ataxia and neuropathy. Clinicians should be aware that brainstem crisis can occur in adult patients without previously manifesting LS. Moreover, the expression threshold for the mutant mtDNA heteroplasmy associated with common, pathogenic *MT-ATP6* variants have important implications for genetic counselling and reproductive options.

M02

FBXL4 related mitochondrial disease is associated with pyruvate dehydrogenase deficiency

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Background: *FBXL4* related mitochondrial disease or FBXL4 deficiency is a severe autosomal recessive mitochondrial disorder, typically characterised by neonatal or infantile onset lactic acidosis, failure to thrive, muscular hypotonia, and variable additional features. FBXL4 deficiency was first described as a disorder of mitochondrial DNA (mtDNA) maintenance associated with mtDNA depletion and combined mitochondrial respiratory chain deficiency. FBXL4 protein is localised to mitochondria but its function remains unknown.

Aims: To identify patients with FBXL4 deficiency and assess the associated biochemical defects.

Patients and Results: *FBXL4* sequencing of 85 patients with suspected mtDNA depletion syndrome in Oxford did not identify any cases of FBXL4 deficiency. Analysis of other patients with suspected mitochondrial encephalomyopathy presenting neonatally or in infancy identified 5 unrelated patients homozygous or compound heterozygous for pathogenic / likely pathogenic variants in *FBXL4*. All 5 patients were found to have pyruvate dehydrogenase deficiency in fibroblasts (0.23-0.57 nmol/mg protein/min; normal range 0.6-0.9), whereas there was no evidence of mitochondrial respiratory chain deficiency in muscle (results available for 4/5 patients).

Conclusion: We propose that PDH deficiency is a common consequence of loss of functional FBXL4 protein and has a major contribution to the disease phenotype. If substantiated, this finding may have important treatment implications for patients with FBXL4 deficiency.

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M03

Exploring the Impact of mtDNA Mutations on the Metabolic and Epigenetic Profiles of hIPSC-Derived Myogenic Cells

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Background: A number of chromatin modifying enzymes are reliant on intermediate products of metabolism, underscoring a focal point that links a cell's metabolic state with its transcriptional profile. Metabolic impairments caused by mitochondrial DNA (mtDNA) mutations have the potential to impact the epigenetic landscape and might represent an unexplored pathomechanism contributing to the disease that warrants further investigation.

Aims: To develop a cell model for exploring novel pathomechanisms contributing to mitochondrial disease in skeletal muscle, including the impact mtDNA mutations have on enzymatic chromatin modifications.

Methods: In this study we utilised human induced pluripotent stem cell (hIPSC) technology to establish an *in vitro* model of mitochondrial disease. hIPSC clones have been generated from patients harbouring the heteroplasmic m.8344A>G mt-tRNA^{Lys} and m.3243A>G mt-tRNA$^{Leu(UUR)}$ mutations most commonly associated with myoclonic epilepsy with ragged red fibres (MERRF) and Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndromes respectively. By taking advantage of the random segregation of mutant mtDNA in patient fibroblast populations, we obtained hIPSC lines with high heteroplasmy for disease modelling alongside isogenic control lines with low/undetectable levels.

Patient-specific hIPSC lines were then differentiated into disease-relevant myogenic cell types by recapitulating developmental signalling gradients that occur during myogenesis. Differentiated cells express key myogenic regulatory factors (MyoD, Myogenin) and form multinucleated MyHC^{+}/Titin^{+} myotubes whilst retaining PAX7^{+} satellite-like cells.

Results: The m.3243A>G mutation appears to have a detrimental effect on overall myogenicity. Myotubes with high m.3243A>G load also show impairments in mitochondrial function and fail to initiate expression of certain MyHC isoforms which might indicate an impairment in myotube maturation. ChIP-Seq libraries are currently being subjected for genome-wide sequencing in order to assess locus-specific changes in metabolically sensitive epigenetic marks that might underlie observed impairments. This *in vitro* model provides new insight into pathomechanisms of mitochondrial disease in skeletal muscle. Epigenetic modifications and/or expression of specific loci that are affected by mtDNA mutations are being tested as new targets for novel therapeutic interventions. Ben O'Callaghan is supported by a PhD studentship from the MRC Centre for Neuromuscular Diseases.

‡M04

Mutations in *POLRMT* impair mitochondrial transcription and are associated with a spectrum of mitochondrial disease clinical presentations

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Background: The vast majority of mitochondrial disorders result from mutations in components of the nuclear-encoded mitochondrial DNA (mtDNA) maintenance machinery and oxidative phosphorylation (OXPHOS) subunits. The role of the mtDNA transcription machinery in mitochondrial disease, however, remains relatively unknown. The mitochondrial RNA polymerase (POLRMT) is the sole RNA polymerase in mitochondria and is responsible for the transcription of the mitochondrial genome.

Aim: To characterise the clinical and molecular nature of novel *POLRMT* variants that underlie the mitochondrial disease-associated phenotype present in five unrelated individuals.

Patients and Methods: Using whole-exome sequencing, we identified novel recessive and dominant *POLRMT* variants in five individuals presenting with a variety of clinical problems, ranging from global developmental delay, hypotonia and growth defects in childhood to late onset progressive external ophthalmoplegia (PEO). Where investigated, these defects were accompanied by either a mosaic cytochrome *c* oxidase deficiency in skeletal muscle and/or multiple respiratory chain enzyme deficiencies. Mitochondrial mRNA and OXPHOS protein levels were assessed in mutant *POLRMT* fibroblasts (3 of 5 patients). In addition, recombinant mutant POLRMT proteins were generated in order to determine the effect of *POLRMT* variants on mitochondrial transcriptional activity *in vitro*.

Results: Functional characterisation of patient fibroblasts revealed a defect in mitochondrial mRNA synthesis, although no mtDNA deletions or copy number abnormalities were identified. Mild decreases in the levels of both OXPHOS subunits and fully-assembled complexes were observed *in vivo,* whilst functional *in vitro* characterisation of the investigated recombinant *POLRMT* variants revealed that patient mutations exhibited variable, but deleterious effects on mitochondrial transcription.

Conclusion: Our results demonstrate for the first time, that pathogenic variants in the *POLRMT* gene can cause a spectrum of clinical phenotypes ranging from childhood-onset developmental delay to late-onset PEO and emphasise the importance of defective mitochondrial transcription as a disease mechanism.

M05

Mild disease spectrum and trajectory in *MTFMT*-related Leigh syndrome

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Background: Mitochondrial methionyl-tRNA formyltransferase (MTFMT) is required for the initiation of translation and elongation of mitochondrial protein synthesis. Pathogenic variants in *MTFMT* have been associated with Leigh syndrome (LS) and mitochondrial multiple respiratory chain deficiencies.
Aims: To elucidate the spectrum of clinical, neuroradiological and molecular genetic findings of patients with bi-allelic pathogenic *MTFMT* variants**.**

Methods: Retrospective cohort study combining new cases and previously published cases.

Results: In this multi-centre study, we identified eight new patients with pathogenic *MTFMT* variants and 30 other previously reported cases. The median age of presentation was 14 months (range: birth to 17 years, interquartile range (IQR) 4.5 years), with developmental delay (59%) and motor symptoms (47%) being the most common presenting clinical features. Raised serum lactate was evident in 83% of patients (range 2.7 – 14.3 mmol/L, normal < 2.2), and a structural cardiac abnormality was observed in 12 patients. MRI head findings included symmetrical basal ganglia changes (62%), periventricular and subcortical white matter abnormalities (55%), and brainstem lesions (48%). Six patients (18%) had neither basal ganglia nor brainstem signal abnormalities. Twenty-five of 34 patients (74%) were alive at the time of their last clinical follow up (median 6.8 years, range: 14 months-31 years, IQR: 14.5 years); 29% of patients survived into adulthood (≥18 years). Isolated complex I and combined respiratory chain deficiencies were identified in 31% and 59% of the cases, respectively. Reduction of mitochondrial complex I and complex IV subunits was identified in all patient fibroblasts analysed (n=13). Sixteen pathogenic *MTFMT* variants were identified, of which c.626C>T represents a founder mutation in the European population.

Conclusion: Patients that harbour pathogenic variants in *MTFMT* have a milder clinical phenotype and disease progression compared to those patients with LS caused by other nuclear-driven mitochondrial defects. Evaluation of patient fibroblasts may preclude the need for a muscle biopsy to prove causality of novel variants.

M06

Molecular mechanisms of mitochondrial disease: pathological and genetic studies in Mendelian disorders of mtDNA maintenance

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Background: Mitochondrial DNA (mtDNA) deletions are an important pathological mechanism in adults with mtDNA maintenance disorders. These mtDNA deletions clonally expand within post-mitotic cells (neurons and muscle) causing mitochondrial respiratory chain deficiency.

Aim: To attempt to correlate mitochondrial genetics and respiratory chain deficiency at a single cell level and assess whether deleted mtDNA species have a replicative advantage.

Methods: We used immunofluorescence to quantify mitochondrial respiratory chain deficiency in muscle biopsies from patients with mtDNA maintenance disorders (n=16). Using the same tissue section from a subset of patients (n=6), we performed laser microdissection and single cell genetic analysis to investigate the relationship between mtDNA genetics and respiratory chain deficiency. For a further 3 patients then used single molecule PCR in combination with real time PCR to determine if a replicative advantage explains the accumulation of mtDNA deletions.

Results: Quadruple immunofluorescence demonstrated no obvious differences between patients with dominant or recessive mutations, with the majority of patients showing some fibres with isolated CI deficiency and some fibres with both CI and CIV deficiency. Genetic analysis demonstrated major arc deletions to be more common and showed a clear correlation between deletion level and respiratory chain deficiency. We find that 62.8% of respiratory chain deficient muscle fibres contained a single deletion, 34.6% two deletions and 2.6% three deletions. In cells with multiple mtDNA deletions we find that 56.7%the smallest mtDNA deletion (Largest mtDNA molecule) clonally expands to the highest level.

Conclusion: There is no genotype based pattern of respiratory chain deficiency. We demonstrate a clear correlation between the level of mtDNA deletion and extent of respiratory chain deficiency within a single cell. Single molecule PCR in combination with real time PCR demonstrates that there appears to be no replicative advantage for smaller mtDNA molecules.

M07

Understanding multi-dimensional respiratory chain deficiency phenotypes in single skeletal muscle fibres

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Background: Mitochondrial diseases are heterogeneous diseases that can arise due to mutations in either the nuclear or mitochondrial genomes. These conditions are further complicated by the polypoid nature of mitochondrial DNA (mtDNA), which means that mtDNA mutations can be heteroplasmic and lead to a mosaic pattern of deficiency within tissues such as skeletal muscle. Limitations in techniques to date have allowed either highly multiplexed analysis of protein expression or spatially resolved analysis of the expression of a small number of proteins.
Aim: To optimise and use imaging mass cytometry for the assessment of mitochondrial proteins in skeletal muscle of patients with mitochondrial disease.

Methods: We used imaging mass cytometry to investigate changes in respiratory chain complexes I-V (CI-V) and mitochondrial mass markers. Skeletal muscle was assessed from patients with nuclear encoded CI mutations (n=2), single, large-scale mtDNA deletions (n=2), the m.3243A>G mtDNA point mutation (n=2) and three other tRNA variants (n=3).

Results: Imaging mass cytometry demonstrated isolated and severe CI deficiency in 100% of muscle fibres in patients with nuclear encoded CI mutations. In comparison mosaic patterns of CI, CIII and CIV respiratory chain deficiency was observed for patients with mtDNA variants. Most interestingly, in tRNA patients we find that fibres with CI deficiency have a compensatory increase in CII and CV.

Conclusion: Imaging mass cytometry allows correlation of a large number of mitochondrial proteins and thus in-depth characterisation of respiratory chain deficiency. Furthermore, it will allow us to correlate respiratory chain deficiency with a range of cell signalling and other mitochondrial markers.

M08

A novel multiplex chromogenic immunoassay for evaluating mitochondrial respiratory chain complex I and complex IV defects in diagnostic muscle biopsies

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Background: The investigation of clinically suspected mitochondrial disease (mtD) includes performing a skeletal muscle biopsy for biochemical/histochemical assessment of mitochondrial respiratory chain (RC) defects. COX-SDH histochemistry detects RC-complex IV (CIV) defects, but RC-complex I (CI) defects cannot be detected histochemically. CI/CIV defects are common in mtD. Immunohistochemical evaluation of RC-complex defects relies on reduced amount of the assembled complex associated with catalytic deficiency, detectable with RC subunit-specific monoclonal antibodies.

Aims: Our aim was to design a dual chromogenic immunoassay (DCI) for evaluating CI/CIV defects in diagnostic muscle biopsies to complement the existing histochemical assays.

Methods or Patients or Materials: In the DCI optimised protocol, primary antibodies (Abcam), TOMM20 (mitochondrial mass), NDUFB8 (CI) and MTCO1 (CIV) were co-incubated (TOMM20+CI and TOMM20+CIV), and then TOMM20 developed to yellow and the other marker to teal (Discovery/Ventana Systems) with co-localising antibodies visualising as green. The DCI and COXSDH assays were performed in serial frozen sections. 23 biopsies were assessed: 15 with genetically confirmed mtD (mtDNA rearrangements/point mutations/depletion), 4 with high clinical/histological suspicion of mtD, and 4 unaffected controls. % COX and CI/CIV-deficient fibres were counted in two random fascicles, with high concordance amongst % COX-negative and CI/CIV-deficient fibres.

Results: Control sections stained as a mosaic dark green (type I fibres) and light green (type II fibres) pattern. Completely CI/CIV-deficient fibres stained yellow, and partly CI/CIV-deficient fibres stained yellow-green, and were easily detectable due to good visual colour contrast. The DCI detected more CI-deficient fibres in 7/19 cases and more CIV-deficient fibres in 5/19 cases compared to COX-negative fibres (average 6%). Most COX-negative fibres had dual CI+CIV defects with DCI. Segmental and partial CI/CIV defects were detectable. Equivocal COX-SDH stained fibres were often strongly CI/CIV-immunodeficient.

Conclusion: In conclusion, our multiplex DCI reliably detects CI/CIV defects comparable in sensitivity to the COX-SDH histochemical assay, is easy to evaluate due to a good visual contrast between CI/CIV positive and negative fibres and can be easily co-opted to routine diagnostic work. Studies are underway to develop a quadruple chromogenic immunoassay for digital evaluation of CI/CIV defects.

M09

A novel *MT-TG* mutation associated with adult-onset multisystem mitochondrial disease

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Background: Collectively, mitochondrial disorders are among the most common inherited neurological disorders. The prevalence of adult mitochondrial disease caused by mitochondrial DNA (mtDNA) mutations is around 1:5,000.

Aims: To describe the clinicopathological and biochemical profile of an adult harbouring a novel mutation in *MT-TG*.

Patient: A 39 year old female presented with sensorineural hearing loss since her late teens, visual impairment with bilateral cataracts, retinal dystrophy and subsequent bilateral retinal detachments in her 20s, hypothyroidism in her 30s, secondary amenorrhoea, and short stature on examination. There was no family history of neurological or neuromuscular disease. Blood lactate was elevated and histochemical staining revealed frequent ragged red and cytochrome *c* oxidase (COX) deficient fibres in muscle tissue. Spectrophotometric analysis of mitochondrial respiratory chain activity revealed reduced activity of complexes I and IV prompting mitochondrial DNA genetic analysis.

Results: Sequencing of the entire mitochondrial genome revealed the novel missense mutation m.10038G>A in *MT-TG*, encoding tRNA glycine, at a heteroplasmy level of 92% in muscle, 40% in urine and 15% in blood. Single fibre segregation studies confirmed a higher mutation load in COX deficient fibres (n=27, 95.3%) as compared with COX positive fibres (n=26, 78.9%; p=0.0005).

Conclusion: We describe a novel pathogenic mutation in *MT-TG*. The m.10038G nucleotide is highly conserved and the m.10038G>A mutation was present at variable heteroplasmic levels in different tissues. Histochemical and biochemical evidence of impaired mitochondrial protein synthesis was indicated in muscle tissue, and single fibre studies confirmed the biochemical defect segregated with high mutant levels. As such, it is highly likely the m.10038G>A is pathogenic. The majority of mtDNA mutations occur in the 22 mtDNA-encoded tRNA genes. However, despite over 270 reported tRNA mutations, only five reside within *MT-TG*. The cause of the variability in prevalence of mutations among tRNA gene remains unknown.

M10

The role of mtDNA heteroplasmy, copy number, age and nuclear factors in the clinical heterogeneity associated with the m.3243A>G mutation

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Background: Mitochondrial disease associated with m.3243A>G, the most common pathogenic mitochondrial DNA (mtDNA) mutation, is clinically heterogeneous. People who harbour m.3243A>G can present with a range of clinical features that progress at variable rates, making an accurate prognosis difficult.

Aim: To describe and understand the cause of m.3243A>G-associated heterogeneity.

Patients and Methods: We examined the phenotypic profile of 238 m.3243A>G carriers from the Mitochondrial Disease Patient Cohort UK using the Newcastle Mitochondrial Disease Adult Scale (NMDAS) and evaluated which commonly assayed tissue (blood, urine, skeletal muscle) represents the m.3243A>G mutation load and mtDNA copy number most associated with disease burden. We then modelled the role of heteroplasmy level, age and additive nuclear genetic factors in the development of specific phenotypes within 46 pedigrees from the cohort.

Results: Blood heteroplasmy declines by ~2.3%/year and males have ~20% higher m.3243A>G mutation load in urine; we present formulas to adjust for these effects. Age and m.3243A>G heteroplasmy level in all three tissues are associated with dis-

ease burden (R^2 range=0.18-0.27, P<0.001), with blood heteroplasmy (corrected and uncorrected) being most strongly associated. A greater proportion of the variation in disease burden is explained if mtDNA copy number in skeletal muscle is included (R^2=0.40, P<0.001). Common phenotypic features include hearing impairment, psychiatric involvement and ataxia, however, age and heteroplasmy levels are poor predictors of severity for individual phenotypes (pseudo-R^2≤0.17). We found high to moderate heritability estimates for psychiatric involvement, cognition, ataxia, migraine and hearing impairment (h^2 range=0.40-0.76, P<0.05).

Conclusion: Our results indicate that m.3243A>G heteroplasmy, skeletal muscle mtDNA copy number and age explain some of the variation in m.3243A>G-related disease burden, however, nuclear genetic factors also influence clinical outcomes. This study paves the way for future work identifying these nuclear modifiers.

M11

Engaging with patients with mitochondrial disease to make them better decision makers

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Background: The Wellcome Centre for Mitochondrial Research, Newcastle supports world class research into mitochondrial disease. Our Centre also strives to be world class at involving patients and the public with our research, thereby allow us to develop an effective research strategy for the benefits of patients.

Aim: Our existing and future aims are: To engage with patients throughout the UK about our research, helping them to become better decision makers; to build a community of empowered expert patients and carers who are involved in developing and implementing a research and engagement strategy for our mutual benefit and to build capacity and engagement expertise within our staff to create a listening culture in our scientific community that is responsive to patient needs.

Methods: We have incorporated patients' and carers' views in the strategic direction of the Centre; developed our engagement conferences and patient/carer focus groups; delivered an international multistage design event with the purpose of involving people living with mitochondrial disease in the design and development of ideas that could transform their lives.

Results: Members of the Wellcome Centre for Mitochondrial Research continue to be involved with annual patient engagement conferences, regular focus groups discussing policy and research issues and the design of a patient/public focused website. We have devised and delivered a very successful not-for-profit venture, the Myto project, where researchers from Newcastle University's Open Lab teamed up with the Wellcome Centre for Mitochondrial Research and TU Eindhoven in order to hold three design events across the UK, Netherlands and Italy. These design events sought to involve people living with and caring for mitochondrial disease in the creation and development of digital tools to enrich their lives and spread important messages about mitochondrial disease.

Conclusion: Through engagement we continue to stimulate national and international debate on issues affecting our patients, and create strategic partnerships with patient and public groups. This allows us to ensure research advances make a meaningful difference to the lives of patients with mitochondrial disease.

M12

Clinical research activity within the Wellcome Centre for Mitochondrial Research

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Background: The Wellcome Centre for Mitochondrial Research (WCMR) aims to transform the lives

of patients with mitochondrial disease. Utilising the expertise of clinical and laboratory team members, we work closely with patients to improve their lives and the lives of future generations affected by mitochondrial disease.

Aims: We seek to generate substantial improvements in the health and wellbeing of patients with mitochondrial disease. Our goal is to offer every patient the opportunity to take part in studies and clinical trials that may ultimately lead to new therapeutic strategies.

Methods: Within the Centre, close proximity between the clinical and laboratory team provides an ideal environment for maximising translational research opportunities.

Results: Currently we have one commercial phase 1 and one commercial phase 2/3 CTIMP in set-up and have two further phase 1 CTIMPs in feasibility. We are currently delivering a phase 3 combined CTIMP/device trial, working with a commercial Sponsor. We are also in the process of setting up an MRC funded drug repurposing study which will be the largest study ever undertaken in participants with mitochondrial myopathy and which was designed in collaboration with patients. In addition to CTIMP studies, we are delivering, preparing to deliver or have recently completed, 10 observational and interventional studies. These include follow-up of children born via Mitochondrial Donation, 'deep phenotyping' studies and a number of studies aiming to identify optimal outcome measures for future studies. We also continue to collect data and tissue for the Wellcome Centre for Mitochondrial Research Patient Cohort and Newcastle Mitochondrial Research Biobank which provide an invaluable resource for internal and external researchers.

Conclusion: We work closely with research sponsors, including The Newcastle upon Tyne Hospitals NHS Foundation Trust, with our academic and commercial collaborators and also with other support organisations (e.g. UKCRC Clinical Trials Units) to obtain the necessary regulatory approvals and ensure that our research is conducted to the highest possible standards.

‡M13

Preventing the transmission of mitochondrial DNA disease utilising preimplantation genetic diagnosis

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Background: Pathogenic mitochondrial (mt) DNA variants are responsible for a broad spectrum of chronic, multisystem presentations that can be present at birth or develop later in life. mtDNA disease is progressive, causing debilitating physical, developmental, and cognitive disabilities and with no effective cure. Given mtDNA is strictly maternally-inherited, the ability to prevent the transmission of mtDNA disease is possible using preimplantation genetic diagnosis (PGD) and more recently, mitochondrial donation.
Aims:

1. To determine success rates for mtDNA PGD
2. To ascertain the segregation patterns of individual pathogenic mtDNA variants and how representative a single cell biopsy (8 cell stage) is of the entire embryo
3. To generate prediction models for the likelihood of PGD success for important pathogenic mtDNA variants

Patients: Data were collected from 80 PGD cycles, representing 43 patients and 12 pathogenic mtDNA variants, generated by three international, licenced centres offering mtDNA PGD.

Results: The live birth success rate for mtDNA PGD was 15.2% per PGD cycle. The majority of pathogenic variants displayed a wide range of heteroplasmy within individual embryos. However, the m.8993T>G and m.9176T>C pathogenic variants resulted in a binomial distribution of heteroplasmy levels within individual embryos. A high mutation load of the pathogenic m.3243A>G and m.8344A>G variants in patients showed significant association with a high level of heteroplasmy of the pathogenic variant in the patient's embryos. Subsequent laboratory investigations of disaggregated blastocyst cells, following unsuccessful PGD, confirmed that blastomere biopsy at the eight cell stage is representative of the total mutation load in the embryo. Prediction models were generated and demonstrate that for the majority of pathogenic mtDNA variants, as the maternal heteroplasmy level increases the success rate for PGD decreases, due to a lack of embryos with a low mutation load.

Conclusion: Our data show that mtDNA PGD can successfully prevent the transmission of mtDNA disease, however for many pathogenic mtDNA variants, a high level of maternal heteroplasmy confers a low likelihood of achieving embryos with a suitably low mutation load to warrant implantation. Therefore for some patients who harbour high levels of a heteroplasmic pathogenic mtDNA variant, Mitochondrial Donation may represent a more suitable alternative to PGD to prevent the transmission of mtDNA disease.

‡M14

Small molecules counter-select deleterious mitochondrial DNA variants by inhibiting their replication in human cells

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Background: Pathological variants of human mitochondrial DNA (mtDNA) were first reported over 30 years ago; yet treatments are still lacking for this devastating group of disorders. Deleterious mutations often affect some, but not all, of the many copies of the mtDNAs -a state known as heteroplasmy- and, typically, disease manifests only if the proportion of the mutant molecules exceeds a threshold. Thus, even a modest increase in the proportion of wild-type mtDNA should be curative. The nucleus ultimately determines the heteroplasmy level of mtDNA variants, but the molecular mechanisms underpinning the selection for or against deleterious mtDNAs are still poorly understood. **Aims:** To determine the cellular processes affecting the selection of mtDNA variants, and to use this information to identify small molecules that favour the propagation of wild-type mtDNA.

Methods: We used both unbiased and targeted approaches to study human cells carrying the most common pathological mtDNA, m.3243G. Then, having identified specific features of cells that are capable of selecting wild-type over mutant mtDNA, we screened for compounds that target the relevant pathways and processes to determine whether any decreased the level of mutant mtDNA.

Results: Our studies of cells that do, or do not, select wild-type mtDNA have led to the discovery of two small molecules that induce the selection of wild-type mtDNA in somatic cells, and restore the mitochondrial respiratory capacity without inducing cell death. One of these compounds has been administered to human subjects previously and so can potentially be rapidly translated to the clinic. Analysis of the pathways involved in the process has enabled us to begin dissecting the underlying mechanisms of actions of the small molecules, which involve the inhibition of the replication of the DNA molecules as function of the mitochondrial fitness.

Conclusion: There is an urgent unmet clinical need for therapies to treat patients with mtDNA-related disorders. The identification of small molecules that favour the selection of wild-type mtDNA represents an important advance. Moreover, since the compounds rectify the genetic defect itself by decreasing the number of copies of the mutated gene, they, can potentially, not only arrest disease progression, but also reverse it. An experimental medicine study will evaluate the safety and efficacy of one of the compounds in patients with m.3243G mtDNA.

M15

A homozygous two exon deletion in *UQCRH*: matching mouse and human phenotypes

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Background: The ubiquinol:cytochrome *c* oxidoreductase hinge protein (UQCRH) is a very small subunit of complex III (CIII) of the oxidative phosphorylation (OXPHOS) system. UQCRH connects the subunits cytochrome *c* with the cytochrome *c*1. Thus, it plays an important role in the assembly of CIII. Mitochondrial disease presentations linked to *UQCRH* are not yet described, although somatic *UQCRH* variants have been linked to different types of cancer.

Aims: To confirm the pathogenicity of a homozygous deletion of exons 2 and 3 in *UQCRH* identified by whole exome sequencing and autozygosity mapping and to compare the human phenotype to the phenotype of a mouse model with the equivalent deletion of *Uqcrh*.

Patients and Methods: We report two male first cousins from a consanguineous family with recurrent episodes of severe ketoacidosis, excess blood ammonia and hypoglycaemia and signs of encephalopathy. Brain MRI's showed no abnormality and between episodes the health of the two patients was

entirely normal. A mouse model was created by deletion of exons 2 and 3 of *Uqcrh* and screening of the mouse phenotypes was performed.

Results: In patient fibroblasts, steady-state levels of CIII subunit UQCRC2 were decreased and Blue-Native PAGE assessment showed a slightly smaller assembly of CIII compared to controls. Patient fibroblasts also demonstrated low CIII enzymatic activity and decreased maximal respiration by Seahorse analysis. The murine presentation was more severe, with progressive functional impairment and premature early adult death. Enzymatic activity and protein expression of OXPHOS complexes were investigated in mouse tissue revealing significant decreases in CIII activity in heart, brain and liver. mRNA expression analysis in mouse liver embryonic fibroblasts (MEFs) revealed decreased expression of the *Uqcrh* transcript, more severe in the homozygous animals compared to heterozygotes. Complexome profiling of heart samples from wild-type and homozygous mice revealed decreased CIII expression and altered assembly profiles in homozygotes, in agreement with the human patient data.

Conclusion: We describe the first patients with bi-allelic mutations in *UQCRH*, with an identical mutation in the mouse mimicking several phenotypic aspects. Notably, bi-allelic variants in *UQCRC2* and *UQCRB*, two other subunits of complex III, result in a similar episodic clinical presentation.

M16

Bladder dysfunction in patients with mitochondrial disease

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Background: Mitochondrial diseases often present with a spectrum of clinical features with multi-organ involvement. Since mitochondrial diseases are often characterised by a loss of smooth muscle function, it is plausible to hypothesise that mitochondrial dysfunction may contribute to bladder dysfunction.

Aim: To determine the presence of bladder dysfunction in patients with mitochondrial disease.

Methods: In this observational cohort study of patients with genetically confirmed mitochondrial disease, we assessed bladder function using three tools: a validated questionnaire measuring severity scores; bladder diary measuring nocturnal polyuria index and bladder scan measuring bladder voiding efficiency.

Results: Between 31st October 2017 and 30th June 2018, 65 consecutive patients aged 18years or over, with a genetic diagnosis of mitochondrial disease without cognitive impairment were recruited. Twenty percent of patients manifested with bladder dysfunction as defined by one of: ICIQ-LUTS* Questionnaire severity score of >60%; NPI* >35% or BVE *<70%, necessitating referral for further urological investigations. ICIQ-LUTS questionnaire severity scores demonstrated no patients reported severe LUTS, based upon on 60% severity threshold. Bladder diary revealed 40% of the cohort reported nocturia (≥2x/night) whilst 13.2% were noted to have nocturnal polyuria. Finally, 18% of the cohort were found to have high post-void residual volumes (bladder voiding efficiency < 70%).

Conclusion: Our findings suggest bladder dysfunction is under recognised in this patient group and support the need for clinical urological assessment. A combination screening approach is best suited, as seen in other chronic disease settings. Further work is required to devise a composite score to evaluate LUTS and extend this study to a larger patient group.
* *ICIQ-LUTS = International Consultation on Incontinence Modular Questionnaire – Lower Urinary Tract Symptoms*
**NPI =Nocturnal Polyuria Index*
**BE = Bladder Voiding Efficiency*

M17

Guidelines for physiotherapy in mitochondrial disease

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Background: The physical manifestations of Mitochondrial disease are extremely varied. Muscle symptoms spanning from fatigue, muscle pain and weakness and neurological symptoms can be as varied as ataxia or dystonia. Research investigating the efficacy of physiotherapy interventions in people with Mitochondrial disease is lacking. However, many symptoms and impairments seen in people with Mitochondrial disease are also seen in other disease populations. Therefore this guidance includes references from research evidence for other neurological and neuromuscular populations alongside research including people with mitochondrial disease.

Aim: To provide indications for referral to physiotherapy; to highlight the importance of physiotherapy for people with mitochondrial disease and provide examples of the relevant outcome measures for use within the clinical setting.

Methods: This expert consensus opinion was written and developed by physiotherapists based within the specialist mitochondrial centres in Newcastle, London and Oxford. The guidance was also reviewed by members of the multi-disciplinary team based within these centres, colleagues from acute and community services and patient groups. Where possible this guidance is supported by existing evidence-based knowledge.

Results: This guidance is intended to be of interest to the following people or organisations; healthcare professionals; people with Mitochondrial disease and their carers; patient support groups; commissioning organisations; service providers and researchers.

Conclusion: In view of the diverse presentations of mitochondrial disease, we recommend that all patients diagnosed with a mitochondrial disorder should be able to access a specialist physiotherapist. This will enable patients to receive advice about their condition and be referred for on-going physiotherapy in their locality as required. Local physiotherapy services are encouraged to obtain support in the management of this rare condition from the physiotherapists that work within the three mitochondrial disease centres for more specialised advice.

M18

PROSPER 2B: Prospective Observational Study of PatiEnts with mitochondrial depletion syndrome, RRM2B

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Background: Mitochondrial diseases are an important group of inherited neuro-metabolic disorders that invariably exhibit multi-organ involvement, are relentlessly progressive, and result in high disease burden and premature death. To date, over 70 patients have been reported in the literature manifesting with a spectrum of *RRM2B*-related mitochondrial disease. While pharmacological agents are emerging for this mitochondrial disease little is known about its natural history.

Aim: To deeply phenotype patients harbouring recessive mutations in *RRM2B* over 12 months to enable the development of disease-specific, patient-centric outcome measures for future studies.

Methods: Patients (pediatric and adults) with a genetically proven diagnosis of recessive RRM2B-related mitochondrial disease enrolled in the Medical Research Council Mitochondrial Disease Patient Cohort Study in Newcastle, Oxford and London will be invited to participate.
Participants will then be asked to attend Newcastle to complete the following clinical assessments at study start and end:

(a) Demographics and anthropometrics. Physical examination and Vital signs.
(b) Disease burden (NMDAS).
(c) Diurnal variation in skeletal muscle and liver glycogen by in vivo 13C and 1H magnetic resonance spectroscopy.
(d) Assessment of bulbar function.
(e) Pulmonary Function
(f) Muscle Strength and Motor Function
(g) Functional capacity
(h) Physical activity monitoring
(i) Bowel Dysfunction
(j) Symptom Severity

Interim visits will also be performed at a venue convenient to the participant. These visits will include assessments that are deemed feasible.

Results: The study is scheduled to start February 2019. The results from the study will analysed to assess to the feasibility and validity of assessments for use in future clinical trials.

Conclusion: This study will establish relevant clinical biomarkers for future clinical trials in RRM2B. The interrogation of feasible, valid and sensitive battery of clinical markers is imperative when new pharmacological treatments are in the pipeline.

M19

High-throughput screening to discover mitochondrial therapeutics

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Background: Mitochondrial dysfunction is a key pathogenesis in numerous neuromuscular disorders. There are currently no effective curative treatments available to patients with mitochondrial dysfunction.

Aim: To develop a range of high-throughput assays to discover new treatments for mitochondrial dysfunction

Methods: We have designed and adapted several assays to enable the application of high-throughput fluorescent imaging techniques to screen thousands of small molecules and natural products. Due to the multi-faceted nature of mitochondria our assays are able to improvements in various aspects of mitochondrial function including mitochondrial biogenesis, mitochondrial turnover, mitochondrial REDOX status and oxidative phosphorylation rate.

Results: We validated our assays using previously described modifiers of mitochondrial function. Here we show that the effect of these control compounds is reproducible even in 384 well format. Once verified, we used a selection of the assays to screen compound libraries and show that not only can we detect compounds which improve mitochondrial function, but that they are more efficacious than previously reported compounds.

Conclusion: Development of high-throughput assays is key to discovering new treatments for mitochondrial dysfunction. The time invested to develop these assays is already paying dividends with the discovery of several promising new potential mitochondrial therapeutics.

M20

Cardiac manifestations of mitochondrial disease in children and adolescents

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Background: Mitochondrial disease in children is an immensely-diverse group of disorders caused by nuclear or mitochondrial DNA defects. Prevalence of cardiac involvement in childhood mitochondrial disease is not well-characterised, thus resulting in uncertainties about cardiac health surveillance.

Aims: To determine the spectrum of cardiac abnormalities in genetically-confirmed childhood mitochondrial diseases.

Methods: Children and adolescents under 18 years were identified from the UK mitochondrial Disease Patient Cohort (REC 13/NE0326) and were followed up regularly in Newcastle, UK. Medical records, electro-cardiographies (ECG), echocardiograms and genetic reports from 2009 to 2018 were reviewed.

Results: Fifty two children with pathogenic mitochondrial-DNA (mt-DNA) and fifty three children with nuclear-DNA (n-DNA) mutations were identified. The most common mt-DNA mutation was the m.3243A>G followed by m.11778G>A, m.8993T>C, m.8993T>G and m.9176T>C. The most common n-DNA defect was the *POLG* gene mutation followed by the Complex I subunit assembly genes, the *SUCLA2* and the *SURF1* genes. Out of the 96 clinically-affected children and adolescents, the three most prevalent phenotypes were ataxia, weakness and developmental delay.

Overall, cardiac abnormalities were identified in 9 patients (9.4%): mt-DNA mutation(n=7) and n-DNA defect(n=2). In the mt-DNA group, hypertrophic cardiomyopathy was identified in four patients (m.3243A>G, m.8993T>C, m.8993T>G and m.4300A>G). One patient who harboured the m.3243A>G mutation had coarctation with multiple ventricular septal defects while the other patient with the same mutation had dysplastic aortic valve with stenosis and regurgitation. Asymptomatic right bundle branch block was identified in a 17-year-old who harboured the m.13513G>A mutation.

In the n-DNA group, only two patients had hypertrophic cardiomyopathy (recessive mutations in *AGK* and *NDUFS1*). The patient who harboured the *AGK* mutations developed hypertrophic cardiomyopathy with biventricular systolic and diastolic impairment. His two elder siblings (adults) had severe cardiomyopathy requiring heart transplant at age 13 and 14 years, respectively. The cardiac manifestations were significantly more frequent in mt-DNA defects (15.9% vs 3.8%, p=0.047).

Conclusion: Cardiac manifestations featured more frequently in those with mt-DNA mutations than those with n-DNA defects. In the n-DNA defects, cardiac disease with mitochondrial disease is genotype-specific. Therefore, cardiac surveillance should be targeted to mitochondrial DNA mutations and high-risk nuclear genotypes.
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M21

Truncating MT-ATP6 mutations: expanding the molecular and phenotypic spectrum of mitochondrial ATP synthase disorders

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Background: Mitochondrial DNA (mtDNA)-related ATP synthase disorders cause maternally-inherited Leigh syndrome and neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP). Most MT-ATP6/8 mutations are missense with relatively uniform levels across tissues, and high mutant loads frequently correlate with severe clinical phenotypes.

Aims: To describe the clinical phenotypes and functional consequences of truncating *MT-ATP6* mutations.

Methods: We report three unrelated probands with mitochondrial encephalomyopathy harbouring truncating *MT-ATP6* mutations. Structural and functional consequences of the *MT-ATP6* variants were investigated with Blue-Native Gel Electrophoresis (BNGE), Microscale oxygraphy, reactive oxygen species (ROS) measurement in patients' tissues, and with transmitochondrial cybrid cell lines.

Results: Patient 1 presented with adult-onset ataxia, chronic kidney disease and diabetes, while Patient 2 had myoclonic epilepsy and ataxia; both harboured the novel m.8782G>A; p.(Gly86*) mutation. Patient 3 exhibited cognitive decline, with posterior white matter abnormalities on brain MRI, and severely impaired renal function requiring transplantation. The m.8618dup; p.(Thr33Hisfs*32) mutation, previously associated with NARP, was identified. All three probands demonstrated a broad range of heteroplasmy across different tissue types. Blue-native gel electrophoresis of cultured fibroblasts and skeletal muscle tissue confirmed multiple bands, suggestive of impaired complex V assembly. Microscale oxygraphy showed reduced basal respiration and ATP synthesis, and increased reactive oxygen species generation. Transmitochondrial cybrid cell lines studies confirmed the deleterious effects of the novel m.8782G>A; p.(Gly86*) mutation.

Conclusion: We expand the clinical and molecular spectrum of *MT-ATP6*-related mitochondrial disorders to include leukodystrophy, renal disease and myoclonic epilepsy with ataxia. Truncating *MT-ATP6* mutations may exhibit highly variable mutant levels across different tissue types, an important consideration during genetic counselling.

M22

Targeting metabolic-epigenetic axis to promote oxidative phosphorylation activity in the presence of mitochondrial dysfunction

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Background: Mitochondrial impairment, which results in a metabolic imbalance, determines changes in activities of metabolites-dependent enzymes. Chromatin modifying enzymes reliant on metabolites link metabolism with gene transcription and overall cell function. Therefore, we aim to shift this program towards the oxidative respiration. The rationale of this therapeutic strategy is to overcome the oxidative metabolism deficit threshold at which disease manifests.

Aims: This project aims to delineate compounds, which boost oxidative mitochondrial function. This strategy has a potential to target a broad spectrum of diseases, where mitochondrial function is compromised including neuromuscular and neurodegenerative disorders.

Methods: We take advantage of the established in vitro human model system where human induced pluripotent stem cells derived (iPSCs) derived-myogenic progenitors are induced towards the mature myotubes characterized by expression of oxidative and glycolytic Myosin Heavy Chains (MYH) isoforms. Those two distinct fibre types correlate with oxidative and glycolytic metabolism, respectively. To this end, iPSCs-derived myogenic progenitors carrying mitochondrial mutations vs. isogenic control lines are differentiated in the presence of small compounds. Small compounds compatible with this assay are available commercially. Treatments, which enhance or cause a shift from glycolytic to oxidative fibre types are selected for further analysis. Fibres isoforms are

detected based on immunofluorescent staining and quantified as an output read-out.

Results: We have performed the proof of concept experiment, which demonstrates an absence of MYH isoforms in the presence of the mitochondrial mutation (m.3243A>G), indicating lack of fibres maturation. The same mutant line subjected to the treatment with one of the tested compounds - inhibitor of epigenetic reader targeted against bromodomain (I-BET762) induces Fast MYH. Therefore, treatment with epigenetic modifiers can prove to be of therapeutic benefit applicable to disorders where mitochondrial dysfunction plays a major role.

M23

Neuromuscular disorders and PREFER project: understanding patient preferences and their importance in the drug development process

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Background: Patient preferences are defined by the Food and Drug Administration (FDA) as "The relative desirability or acceptability to patients of specified alternatives or choices among outcomes or other attributes that differ among alternative health interventions". Over the last decades there has been an increasing recognition of the importance of patient preference information in the decision making process along the drug development. Rare diseases in particular have been highlighted as sensitive groups that could beneficiate from patient preferences. However, there is not yet consensus on how and when better to incorporate patient preferences into decision making to better inform concerning stakeholders. In 2016, the European public-private partnership PREFER ('Patient Preferences in Benefit and Risk Assessments during the Drug Life Cycle') launched, a five year project funded by the Innovative Medicines Initiative-2 Joint agreement. The PREFER project aims to assess when and how patient preferences should be incorporated into the drug development process. More specifically, provide a set of systematic recommendations to assess, engage and include patient preferences during the development, approval, and post-approval of new therapies.

Methods: As part of the PREFER project's work-package 3, a case study in neuromuscular disorders will be completed that will inform the PREFER aim. This case study will assess patient and caregiver unmet health priorities and risk tolerance for neuromuscular treatments by systematic qualitative and quantitative approaches. An initial series of ten face-to-face interviews and four focus groups will be completed to elicit a set of attributes that will later be included on a large-scale survey expected to reach up to 700 participants (patients and caregivers) with myotonic dystrophy type 1 and mitochondrial disorders.

Results: In parallel to this conference, focus groups with patients and caregivers will be ongoing as part of the case study.

Conclusion: This study will set precedents that not only will inform the PREFER project aims but also stakeholders interested in these diseases about current unmet health needs that patients and caregivers established as priority and the risks they are willing to undertake to treat them.

Other diseases and diagnostics

OD01

The development of an immunoblotting external quality assurance scheme

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Background: The Muscle Immunoanalysis Unit (MIU) is an ISO 15189 accredited laboratory which

offers a highly specialised national referral service for the diagnosis of Limb Girdle Muscular Dystrophy (LGMD) - progressive muscle diseases that produce weakness in a limb-girdle distribution. Analysis is undertaken on frozen muscle biopsies, utilizing immunohistochemistry and immunoblotting techniques to study protein expression and focus genetic analysis. The unique immunoblotting process used in MIU was developed by the department and is not offered by any other UK laboratory. As a result, there is currently no external quality assurance (EQA) scheme available for this technique.

Aims: To develop an international scheme to assess the quality of immunoblots by suitably accredited laboratories.

Methods: The scheme was trialled with Hospital Sant Pau (Barcelona) and MIU as participants. The referring centre emailed an electronic image of the blot to the assessing centre, which reviewed the image and completed a standardized scoresheet. The referring centre checked the responses against the original report to determine consistency.

Results: Results from the initial trial assessment highlighted advantages and limitations of the methodology. Further discussion and modifications of the scoresheet are in progress to establish guidelines for an unambiguous standardized assessment.

Conclusion: We propose to develop a format and procedure for an external quality assessment scheme for immunoblotting. This is supportive of requirements necessary for ISO 15189 accreditation, in which medical laboratories seek confirmation of confidence in the results of diagnostic testing through participation in suitable comparisons. Further data is required and the expansion of the scheme to involve additional participants will be explored.

OD02

Diagnostic outcomes of the Highly Specialised Services for Rare Neuromuscular Disorders -Congenital Muscular Dystrophies and Myopathies (HSS CMD/CMY)

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Background: Congenital muscular dystrophies (CMD) and congenital myopathies (CM) are rare neuromuscular conditions with major clinical, pathological and genetic heterogeneity. Diagnostic gold standard is identification of pathogenic variant/s in a disease causing gene. Diagnostic abilities are hindered by the clinical and genetic complexity of CMD and CM, size and polymorphic nature of many disease genes, such as *RYR1*, *TTN* and *NEB* genes, and the high number of private mutations identified in patients.

Aims: To describe diagnostic outcomes of the HSS for CMD and CM in the UK.

Methods: Review of the clinical, pathology and genetic diagnostic outcomes in HSS CMD/CMY in the 2016-2017 financial year. All patients referred to the service underwent gatekeeping and specialised advisory service, with multidisciplinary review of pathology and genetic results. Sequencing of full coding and short flanking intronic sequences and duplication/deletion analysis of 82 CMD and CM genes was performed by next generation sequencing.

Results: A total of 516 patients were referred to the service. A definite genetic diagnosis was achieved in 160 patients (31%). Ninety of these patients (17%) had parallel referrals to the clinical/pathological/genetic services. Clinical assessment was offered to 201 patients and majority of these had muscle biopsy and muscle MRI review as well, if available. A definite genetic diagnosis was achieved in 117/201 patients seen in our clinic (58%). Fifty patients had a diagnosis of CM (25%) and 13 of CMD (5%). A muscle/skin biopsy review was completed in 128/516 patients and 95/128 (74%) obtained a definite pathological diagnosis. A diagnosis of CMD or CM was excluded on clinical and/or pathological grounds in 86/516 patients.
Conclusion: This review highlights the importance of specialist validation of genetic analysis results by expert clinical and pathological review. The higher diagnostic yield in patients with clinical assessments at GOSH reflects the benefits of the combined muscle pathology and muscle MRI imaging review, systematically applied to all patients seen in clinic. This outcome review also highlights a high number of patients in whom a diagnosis of CMD/CM was ruled out, helping to redirect diagnostic investigations.

OD03

Novel recessive *MYO18* gene pathogenic variants in a patient with congenital myopathy and mini core pathology

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Background Recessive variants in *MYO18B*, encoding an unconventional myosin protein, have been described in three unrelated patients with congenital myopathy, with classic nemaline rods or dense bodies reminiscent of nemaline rods in two patients. Cardiomyopathy, short stature, Klipper-Feil anomaly and dysmorphisms were variably present.

Aims: To describe a novel patient with congenital myopathy and *MYO18B* variants.

Patient: The patient, of Philippine origin, presented with severe motor delay, achieving independent ambulation at age 5 years. Now aged 17 years he has facial weakness with ptosis, axial weakness with progressive thoracic scoliosis and proximal more than distal limb weakness, affecting lower more than upper limbs. He remains ambulant but cannot rise independently from the floor. Generalised hypermobility with ankle contractures is present. Respiratory function is normal, but he has mild feeding difficulties. CK was normal at age 12 years. Lower limb muscle MRI at age 15 years showed asymmetric fatty infiltration and bulk reduction of glutei, hip extensors, flexors and adductors, soleus and gastrocnemii, with asymmetric involvement of semitendinosus and tibialis anterior muscles. Muscle biopsy showed myopathic size variation, unevenness of oxidative staining with few distinct mini-cores, slow fibre uniformity and a distinct population of very small fetal myosin-positive fibres uniformly dispersed across fascicles. No nemaline rods were observed on light and electron microscopy.

Results: Next generation sequencing of 55 genes responsible for congenital myopathies identified two novel heterozygous *MYO18B* gene variants. The c.4131delC p.(Ala1378fs) results in a premature termination of the MYO18B protein and is predicted to be pathogenic. The second variant, c.39+5G>T, has not been previously reported and bioinformatics splicing predictions were not informative. The variants were confirmed *in trans*. To assess the significance of the c.39+5G>T variant, transcript analysis was performed on muscle tissue with RT-PCR of mRNA and amplification of cDNA. The assay showed an abnormal fragment length, indicating and effect of the variant on mRNA splicing.

Conclusions: We have identified a novel patient with recessive *MYO18B* gene variants. This report further expands the current knowledge on this rare form of myopathy, suggesting that mini-cores without nemaline rods can also be part of the associated pathological spectrum.

OD04

A novel patient with multiple pterygium syndrome and nemaline myopathy due to recessive *TPM2* gene variants

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Background: Pathogenic variants in *TPM2* have been associated with a variable clinical spectrum, including congenital myopathies and distal arthrogryposis, all but one with dominant inheritance. A homozygous pathogenic variant in *TPM2* has been reported in a single family with nemaline myopathy (NM) and Escobar variant multiple Pterygium syndrome (EVMPS).

Aims: We report the second patient with recessively inherited *TPM2*-related EVMPS and NM in a patient from a consanguineous family.

Patient: Reduced fetal movement and scoliosis were antenatally detected. He was hypotonic at birth, with kyphoscoliosis, multiple upper and lower limb contractures, pterygia at shoulders and knees and mild ptosis. He required nasogastric tube feeding and gastrostomy from 10 months of age. Creatine kinase was normal. He sat at 18 months and was able to pull up on furniture and do a few steps at age 3.5 years. Intelligence and speech development was normal. He had non progressive thoracolumbar kyphoscoliosis with lower thoracic and lumbar spine fusion (T4-S1). There was no respiratory compromise.

Results: Muscle biopsy from right vastus lateralis muscle showed myopathic size variation and type I fibre predominance. Few cores/mini cores and sparse, small nemaline rods were seen only at the ultrastructural level. A novel homozygous intronic sequence variant, c.564-2A>C was found in the *TPM2* gene, predicted to abolish the consensus acceptor splice site for exon 6b. Unaffected parents were heterozygous for the variant and expected to produce 50% of TPM2.2 which appears to avoid muscle weakness.

Conclusions: We have identified a novel homozygous intronic *TPM2* gene variant affecting exon 6b splicing in a patient with EVMPS and NM. Interestingly, the only other homozygous TPM2 variant was also found in exon 6b and expressed only in two of four TPM2 isoforms (TPM2.2 and TPM2.3). This report confirms role of TPM2 as cause of EVMPS and the importance of TPM2 expression in early period of prenatal life.

OD05

GNE Myopathy Patient Registry

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Background: The GNE Registry is an international, patient reported, disease specific database which provides the neuromuscular community with a valuable, flexible and sustainable resource of disease specific information. The registry can be used to support planning and recruitment for clinical trials, and to capture real-world patient data (including data that may contribute to post marketing surveillance and standards of care). The GNE Registry is managed by the John Walton Muscular Dystrophy Research Centre (JWMDRC, Newcastle University, UK) and supervised by the Steering Committee. The GNE myopathy registry helps to facilitate translational research. The registry is available in 7 languages, collecting data since March 2014 with over 350 participants from over 30 countries worldwide (www. http://www.treat-nmd.eu/gne/patient-registries/international-registry).

Aims: To contribute to the disease understanding by collecting reliable information; facilitating clinical trial readiness and translational research by helping the recruitment of patients in the clinical trials; helping to overcome the scarcity of resources and the geographic isolation of patients; informing patients and the GNE myopathy community of latest developments in scientific research and disease management, via newsletters.

Methods: Patient reported data gathered through disease specific questionnaires (GNEM-FAS, quality of life (SF12) and other non-validated questionnaires.

Results: The registry has allowed a genotype-phenotype study to be conducted (Reference). Ten newsletters have been produced in 7 languages and distributed to the registry participants and to the neuromuscular community via TREAT-NMD network. The registry helped to facilitate recruitment for patient advocacy meetings, clinical trials (NCT02736188, NCT02377921) and natural history study (NCT01784679). The registry has also participated in EURORDIS and MDUK research, namely The Voice of rare disease patients and GNE patient's day.

Conclusions: The International HIBM Patient Registry is a valuable tool for this ultra-rare disease community

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OD06

Self-management in neuromuscular diseases: a qualitative exploration of the patient perspective

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Background: The concept of self-management has seen a growth in interest from health policy-makers over the past two decades and is frequently regarded as a way of reducing the burden of long-term illness and demand on health services, whilst simultaneously improving outcomes for individual patients. It has consequently become a key focus in many areas of healthcare but has had little exploration in people living with neuromuscular diseases (NMDs). This study seeks to use qualitative research methods to explore the concept of self-management from the perspective of patients with NMDs, to inform future interventions and research in the area.

Methods: This study will use semi-structured interviews with patients at one regional specialist centre for neuromuscular diseases. Recruitment will continue until data saturation, indicated by no new themes arising from the data. Semi-structured interviews will explore 1) how people manage living well with their condition; 2) successful strategies; challenges; 3) unmet support needs; 4) areas of importance for living well with NMDs; 5) resources used and role of staff in self-management support. Interviews will be audio recorded and transcribed. Sampling will be purposive to ensure a breadth of people with a range of diagnoses and ages. Three main groups will be targeted with 5-10 participants in each group: 1) Early disease onset with rapid progression; 2) early disease onset with slow progression over the lifespan; 3) late disease onset with slow progression. Thematic analysis will be used to code data and identify the key domains and themes. An exploratory comparison of themes will be undertaken between the three identified groups and those with and without affected family members. Preliminary themes will be sent to participants for

respondent validation to gain feedback and refine interpretation of data.

Conclusion: This study is the first to explore self-management from the perspective of NMD patients using qualitative methods. This study seeks to use qualitative research methods to explore the concept of self-management from the perspective of patients with NMDs, to inform future interventions and research in the area.

OD07

The clinical and genetic spectrum of a UK cohort of paediatric and adult patients with *MYH7 gene related* skeletal myopathies

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Background: Pathogenic variants in the MYH7 gene, encoding for the slow beta cardiac myosin heavy chain, are responsible for a wide spectrum of mostly dominantly inherited myopathies affecting cardiac and skeletal muscles, including hypertrophic or dilated cardiomyopathy, Laing type distal myopathy, congenital fibre type disproportion, scapuloperoneal myopathy and myosin storage myopathy MYH7 gene variants have so far been reported only in two UK families with skeletal myopathy.

Aims: To describe clinical and genetic spectrum of a UK cohort of patients with *MYH7* gene related congenital myopathy.

Methods: Patients were recruited at the Dubowitz Neuromuscular Centre and National Neurology and Neurosurgery Hospital in London. A retrospective review of medical notes of patients with genetically confirmed *MYH7* gene related myopathy was done, including demographics, family pedigrees, clinical features, neurophysiology, skeletal muscle MRI, and the muscle biopsies.

Results: We identified 8 novel patients from 5 families with *MYH7* gene pathogenic variants**.** Four were females and four males, with age ranging between 2 and 44 years**.** Onset ranged between 1 and 7 years. Patients presented variable patterns and severity of muscle weakness and wasting or muscle hypertrophy, with wide intra an inter-familial variability. Cardiomyopathy was diagnosed in 2 patients and one commenced targeted cardiac treatment. One patient had reduced respiratory function, with force vital capacity being 29% of the predicted value at age 18 years. Muscle biopsies were available in 2/8 cases. Both showed myopathic changes with minicores, and one case showed marked fast fibre predominance. There was no evidence of myosin storage. Lower limb muscle MRI was performed in two patients and showed hypertrophy of gastrocnemii and in one patient diffuse involvement of the muscles with streaky fatty infiltration. We identified 5 *MHY7* dominant variants (one missense, and four in-frame single amino acid or single exon deletions).

Conclusions: This work further expands current genotypic and phenotypic knowledge on *MYH7* gene related myopathies and aids diagnosis and clinical management of patients with this rare disease.

OD08

High-Fidelity Disease Modelling of Skeletal Muscle Laminopathies Using Human Ips Cells and Artifical Skeletal Muscles

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Background: Laminopathies are severe heterogeneous genetic diseases caused by mutations in A-type lamins, which are encoded by the *LMNA* gene. These proteins together with Lamin B1 and B2 form the nuclear lamina: a mesh-like structure located underneath the nuclear membrane which helps maintaining nuclear shape and regulating gene expression. Laminopathies affect multiple cell types and can be tissue-specific or systemic, with some subtypes affecting striated muscle, peripheral nerve and adipose tissue, while others cause multisystem disease with accelerated aging. Although different mechanisms have been proposed, the precise pathophysiology of laminopathies remains unknown; additionally, their rarity and lack of easily accessible cell types for ex vivo studies negatively impact on therapy development.

Aims & Methods: To bypass these hurdles, here we used induced pluripotent stem (iPS) cells from patients with skeletal muscle laminopathies such as *LMNA*-related congenital muscular dystrophy and limb-girdle muscular dystrophy 1B, to model disease-associated phenotypes *in vitro*. iPS cells from three skeletal muscle laminopathy patients were differentiated into skeletal myogenic cells and myotubes.

Results: Characteristic cellular phenotypes were observed in all *LMNA*-mutant iPS lines, including nuclear shape abnormalities and mislocalisation of proteins of the nuclear lamina. Notably, complex modelling in three-dimensional (3D) artificial muscle constructs resulted in recapitulation of nuclear abnormalities with higher fidelity than standard bi-dimensional (i.e. monolayer) cultures and identified nuclear length as a robust and objective outcome measure. Finally, we will present and discuss current efforts and future applications of this novel 3D organoid-like platform for therapy development and drug screening for skeletal muscle laminopathies.

Conclusion: These results demonstrate that patient-specific iPS cells can model phenotypic readouts of skeletal muscle laminopathies with high fidelity upon 3D differentiation *in vitro*, laying the foundation for future therapy screening platforms for skeletal muscle laminopathies and other severe muscle disorders.

‡OD09

Natural history of sporadic inclusion body myositis: a longitudinal observational study investigating outcome measures for clinical trials

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Background: Sporadic inclusion body myositis (IBM) is the most common age-related acquired myopathy. Distinct early clinical features include selective weakness of quadriceps and forearm muscles. Pathological features of the disease include both inflammatory and degenerative features, which, together with the nature of progression and unresponsiveness to immunosuppressants suggest a possible neurodegenerative mechanism.

Aims: Our aim was to longitudinally evaluate clinical, quantitative (muscle strength) and functional characteristics of the disease in order to develop reliable statistical models of progression over time that can be used in clinical trials.

Methods: In a prospective cohort study, we recruited 205 patients with a diagnosis of IBM made by a neuromuscular expert and fulfilling any of the following research diagnostic criteria: 1) Griggs' criteria, 2) MRC (Hilton-Jones) criteria, or 3) ENMC 2011 criteria. Patients were enrolled across three centres (London, Oxford and Newcastle). Patients underwent bilateral manual muscle testing (MMT)

on 23 muscle groups using the expanded (0-5) MRC scale, quantitative muscle testing (QMT) using the HUMAC Norm CSMi dynamometer and grip testing using the handheld dynamometer (where available) at each follow up visit. Disability scoring was performed at each follow up visit using the IBM Functional Rating Scale (IBMFRS). The time at which a patient first required a mobility aid was recorded. Linear multilevel models were fitted to investigate the progression of MRC scores, IBMFRS scores and quantitative myometry scores over time. Cox proportional hazards regression was used to model time to use of a mobility aid in patients with IBM.

Results: In the preliminary analyses we have been able to generate reliable multilevel statistical models of disease progression using those muscle groups that are worst affected by the disease over time. We have also demonstrated the progression of IBMFRS over time confirming its utility in monitoring disease progression. We studied predictors of time to use of a mobility aid. Final results will be presented at the meeting.

Conclusion: This study will prove to be the largest prospective observational study in patients with IBM to date and will reveal useful outcome measures that can be used to trial therapeutics in clinical trials.

OD10

Novel loss-of-function mutation in *ACBD5* found in family with ataxia

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Hereditary autosomal recessive cerebellar ataxias are a highly heterogeneous group of disorders that affect the cerebellum and connected regions of the nervous system causing a range of symptoms including uncoordinated voluntary movements, problems with eye movements and dysarthria. *ACBD5* encodes for the peroxisomal membrane protein Acyl-coA binding domain 5 (ACBD5) which is involved with peroxisomal metabolism and selective autophagy. A homozygous deleterious indel mutation has been previously reported in an ACBD5-deficient patient with ataxic symptoms, with impaired β-oxidation of very-long-chain fatty acids (VLCFA) (Ferdinandusse et al., 2017).

Aims: To investigate the autosomal recessive mutation of *ABCD5* found in a family with ataxia by exploring its pathogenic mechanism and functional effects.

Methods: Blood and skin biopsies taken from consented family members (two affected brothers, one unaffected sister and both parents). DNA was extracted from blood and the skin biopsies were used to grow primary fibroblast lines for all family members. Western blots using protein from primary fibroblast lines, anti-ACBD5 antibody, cDNA sequencing and metabolic screening were used to confirm type of mutation, pattern of inheritance and effect on protein ACBD5.

Results: Western blots, immunofluorescence staining, and cDNA sequencing show a loss of function mutation in *ACBD5*, with nonsense mediated decay (NMD). Metabolic screening revealed an increase in very long chain fatty acids in the affected patients.

Conclusion: The molecular techniques used show a loss of function mutation that shows NMD. Metabolic screening shows an increase in very long chain fatty acids which hints at a possible peroxisomal mechanism. Future work will centre around further exploration of a possible mechanism, focusing on peroxisomal and mitochondrial metabolism and function, using an additional ACBD5-deficient line and ABCD1-deficient cell lines that cause adrenoleukodystrophy (ALD) that may have a similar mechanism.

References: ACBD5 deficiency causes a defect in peroxisomal very long-chain fatty acid metabolism. Ferdinandusse S et al., 2017. J Med Genet 54:330-337

OD11

The TREAT-NMD Advisory Committee for Therapeutics (TACT): Facilitating Drug Development in Neuromuscular Rare Diseases

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Background: Established in 2009 as part of TREAT-NMD, TACT is an expert multidisciplinary body that provides the neuromuscular community with independent, confidential and objective guidance on advancing new therapies (whether novel or repurposed) for rare neuromuscular diseases.

Aims: The goal of each TACT review is to position the potential therapy along a realistic and well-informed pathway to or through clinical trials, and eventual registration, by evaluating supporting preclinical data and other critical drug development considerations necessary for objective decision-making. The output is detailed advice leading to the improved design and conduct of studies that generate more meaningful data and have the increased potential to be funded longer term.

Methods: Applications are reviewed by a bespoke panel of world-leading experts drawn from the TACT committee of around 65 members in response to the specific needs of a particular application. All reviews are conducted under strict confidentiality agreements and conflict declarations. An online and thorough review process is followed by a face-to-face meeting between the panel and the applicant. A confidential and independent report detailing the panel's advice is provided to the applicant within 4-6 weeks of the meeting. TACT conducts two review cycles each year, considering up to four applications each time.

Results: To date TACT has held 18 review meetings, 10 in Europe and 8 in the North America, and has reviewed a total of 53 applications from both academic investigators and industry. TACT has worked closely with existing infrastructures established by the National Institute of Health (NIH) and the European Union, and major patient organisations in multiple regions around the world to ensure the process assists the neuromuscular community as a whole.

Conclusion: Feedback shows that TACT has generated recommendations that have greatly helped investigators, including industry, in evaluating their potential compounds. It has encouraged applicants to consider their development programs in a methodical fashion with clear go/no-go decisions and with optimal use of funding and resources. Examples of the impact of TACT review advice are given.

‡OD12

Frequency of Coronary Artery Disease in People with McArdle Disease

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Background: McArdle disease (glycogen storage disease type V; GSDV) is a rare metabolic disorder characterised by the inability to use glycogen as an energy source in skeletal muscles. Consequently, affected people experience pain during physical activity and exercise. Complications include muscle contractures, muscle breakdown (rhabdomyolysis), compartment syndrome and kidney failure. Exercise-related symptoms and anxiety about causing muscle damage can be a major barrier to exercise and many patients lead sedentary lifestyle. Sedentariness may increase the prevalence of coronary heart diseases (CAD) in this patient population. Proving this theory may help improving patients' care by supporting exercise training in this patient population and early investigation for CAD.

Aims: Review the medical notes of patients with McArdle disease to analyse the occurrence of CAD, risk factors and treatments provided in this population.

Methods: A retrospective, single-site study was performed in the UK. Medical notes from patients seen between April 2017 and April 2018 (period of

one year) were reviewed. Collected data included genetic diagnosis, age at McArdle disease diagnosis, current age, gender, presence/absence of CAD, age at CAD diagnosis, symptoms of cardiac disease, cardiac disease treatment type, level of physical activity / sedentariness, other risk factors for cardiac disease (smoking, hypercholesterolemia, hypertension, family history of cardiac disease, etc.), body mass index and past medical history.

Results: Preliminary data from 73 patients will be presented.

Conclusion: Preliminary data on CAD frequency in a sample of patients with McArdle disease will be discussed.

OD13

Comorbidity and mortality in patients with sporadic Inclusion Body Myositis (sIBM)

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Background: Sporadic Inclusion Body Myositis (sIBM) is an age-related acquired myopathy with male predominance. The disease results in progressive limb weakness and loss of function leading to severe disability and having a major impact on quality of life. In a recent meta-analysis, high variability in sIBM prevalence estimates and quality of the studies was found. The meta-prevalence using the four articles of highest quality was 45.6/million.The natural history of sIBM remains largely unknown and available data on mortality and comorbidity in these patients is extremely limited. Survival rates in patients with sIBM seem to be comparable to the general population.

Aims: To evaluate the cause of death and contributing factors in sIBM patients seen at the UCL Queen Square Centre for Neuromuscular Diseases.

Methods: Data from patients with sIBM fulfilling 2011 ENMC criteria were reviewed. Causes of death were obtained from the death certificates, which were retrieved from the General Register Office. Collection and review of clinical data of the living and deceased patients was done. Underlying comorbidities were extracted.

Results: Seventeen deaths were recorded. Mean age of death was 75.6±7.7 years. Mean age of death in the UK is 79 years for males and 83 years for females (Office for National Statistics). The most frequent primary cause of death was respiratory (60%). The most common associated comorbidities were cardiovascular (21%) and rheumatological (19%).

Conclusions: These preliminary data suggest that there might be a possible impact of sIBM on lifespan. Aspiration pneumonia was the leading cause of death in our cohort. Regular monitoring including SALT evaluation and advice should be undertaken.

OD14

A versatile, modular digital script for automated high-throughput multiparametric myofibre analysis in brightfield and epifluorescent paradigms

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Background: Digital scripts are vital for unbiased, high-throughput multiparametric analysis of muscle landscapes in frozen/fixed histology sections with useful diagnostic and research applications. By extrapolating far greater quantities and parameters of pathological data compared to traditional manual assessment digital scripts open up novel opportunities for deep subset analyses and pathway mapping in tissue sections.

Aims: To develop a versatile, modular digital script that can be tailored to desired study specifications applicable to a variety of neuromuscular conditions.

Methods: Entire sections of skeletal muscle stained either fluorescently or chromogenically were scanned using a digital slide scanner. The images were exported to the Definiens Tissue Studio and Developer software platform that was used to develop subsequent image analysis modules.

Results: Initially the script was developed to map oxidative changes in the mixed fibre-type gastrocnemius muscle, with COX-SDH staining, in a long-term rodent model of critical illness and recovery. Staining intensity translated to digital heatmaps as surrogate indicators of mitochondrial biogenesis. Reliable fibre separation was then achieved with subsequent introduction of a ubiquitously expressed membrane marker stain (spectrin/laminin) to create a 'mask' for defining the sarcolemma of each myofibre. Brightfield analysis of muscle fibre diameter in 'histologically normal' paediatric muscle biopsies provided good correlation between whole section counts and manually selected regions of interest, generating age-stratified data on muscle fibre size. Current modules measure numerous indices related to the level of expression of markers, and the coverage of markers above a given threshold in myofibre subpopulations using multiplexed immunofluorescent staining. We have developed an optimised module for multiparametric quantification of dystrophin and associated proteins in biopsies from Duchenne and Becker muscular dystrophy patients. Separate modules are under development for quantitative immunoanalysis of complex I/IV defects in patients with primary mitochondrial disease and dot-quantitation for RNA in-situ hybridisation assays.

Conclusion: Our unique modular approach allows for continuous machine learning, increasing the script's capacity to generate a variety of high-throughput qualitative and quantitative datasets tailored to a wide range of neuromuscular applications.

OD15

Vacuolar myopathy with valosin containing protein (VCP)-positive intranuclear and cytoplasmic inclusions: report of two cases with early and late childhood-onset disease

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Background: Paediatric-onset protein aggregation and vacuolar myopathies (PAVM) are rare familial or sporadic disorders with marked clinical, pathological and genetic heterogeneity. Extensive pathological work-up in muscle biopsies may be required to narrow the hunt for the putative disease-causing gene/protein. A multisystem proteinopathy (MSP) causing several phenotypes in adults including rimmed vacuolar myopathy, motor neurone disease, frontotemporal dementia and Paget disease of bone, unified by pathological aggregation of ubiquitin and TDP-43 has been linked to mutations in *VCP*, *HNRNPA2B1*, *HNRNPA1* and *SQSTM1*. Intranuclear and cytoplasmic VCP+ inclusions are present in

most cases of *VCP*-MSP. VCP+ aggregates are hitherto not reported in other PAVM except sporadic inclusion body myositis and some extra-skeletal neurodegenerative disorders

Aims: Here we describe two clinically diverse early and late-childhood onset cases with unifying muscle pathology of a vacuolar myopathy with VCP-positive intranuclear and cytoplasmic inclusions resembling the classic *VCP*-MSP.

Patients: PI, a 21-year-old male presented at age 16 with aching pain in his left forearm and progressive difficulty straightening the left hand fingers. Remaining neurological assessment was normal. PII, a 22-year old female presented at age 9 with toe walking and delayed motor milestones. She developed rapidly progressive weakness and lost ambulation at age 14. She is currently fully wheelchair dependent, and uses long-term non-invasive ventilation. There is no family history of neuromuscular disease in both patients.

Results: CK was normal, EMG myopathic in left forearm finger flexors and intrinsic hand muscles, and muscle MRI showed oedema and fatty infiltration affecting left forearm and finger flexors in PI. Muscle biopsies from both patients following extensive histochemical, immunohistochemical and ultrastructural work-up showed identical pathology resembling that of classic *VCP*-inclusion body myopathy with chronic myopathic/dystrophic changes, rimmed vacuoles, sarcoplasmic and intranuclear protein aggregates/inclusions (VCP/ubiquitin/TDP43+) and tubulofilamentous inclusions. Extensive molecular genetic studies to date are negative including whole exome sequencing in PII.

Conclusion: Further molecular genetic diversity may underpin the unifying VCP+ inclusion body pathology in these atypical paediatric presentations. Recruitment to other next-generation-sequencing platforms is under consideration.

OD16

Clinical research activity in the John Walton Muscular Dystrophy Research Centre

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The John Walton Muscular Dystrophy Research Centre (JWMDRC) team encompasses a number of specialists performing world-class translational research to bring diagnosis, care and therapy to people with neuromuscular diseases. These diseases range in scope from Duchenne Muscular Dystrophy and Spinal Muscular Atrophy, to Limb Girdle Muscular Dystrophies and Myotubular and Centronuclear Myopathy. The team aims to use the knowledge obtained from translational research to offer patients with genetic and acquired neuromuscular diseases the opportunity to take part in studies and clinical trials, which may lead to new treatments and improve the health outcomes and quality of life for all patients and their families. Multidisciplinary in nature, the JWMDRC team works together to collaborate on many different clinical trials and studies in both adult and paediatric populations. Current and upcoming projects include interventional clinical trials, translational research, natural history studies, patient registries and BioBanks, plus exciting advancements in gene therapy. The team is active in the conception and design of local, national and international commercial and academic studies. The JWMDRC coordination team is responsible for obtaining MHRA approval, Health Research Authority (HRA) and Research Ethics Committee approval, local confirmation of capacity and capability from Research and Development, National Institute for Health Research support and adoption and study management throughout the whole process. Every member of the team is instrumental in conducting

research in line with Good Clinical Practice (GCP) that is facilitated by the coordination team to produce the highest professional level of neuromuscular clinical research in Newcastle and the North East.

OD17

Immune-array genotyping association analysis in a large cohort of sporadic inclusion body myositis and controls

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Background: Sporadic inclusion body myositis (sIBM) is characterized by a combination of inflammatory and degenerative changes affecting muscles. While the primary cause is unknown. Genetic factors contributing to the aetiology of sIBM. Our last whole exome sequencing association analysis in sIBM and controls identified 15 common SNPs within Major histocompatibility complex (MHC) region. This finding is inconsistent with the previous reports that MHC associated with the risk of sIBM. However, because this region is in high degree of linkage disequilibrium (LD) existing among alleles, it is very difficult to know the causal allele among these in LD.

Aim: My aim from this work is to explore the sIBM cases-controls genotyping array association by investigating the potential association with HLA-related genes.

Methods: A total of 434 Caucasian patients with IBM were recruited from many countries through International IBM Consortium Genetic Study (IIBMCGS). These cohort will be genotyped by immunochip array a custom-designed, high-density genotyping chip that covers genes known to be associated with a variety of autoimmune diseases.

Expected result: We expected to identify the most significant risk allele within HLA genes. This include the alleles identified in the recent association study that showed a strong association with HLA-DRB1*03:01, HLA-DRB1*01:01 and HLA-DRB1*13:01. Promising association might be identified with large sample size. We are aiming to reach 550 sIBM cases.

OD18

Overnight Pulsoximetry for respiratory progression screening in a Neuromuscular Service

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Background: Progressive respiratory involvement it's been described in some patients with neuromuscular disorders (NMD) being the result of progression of weakness in the inspiratory muscles in combination to alteration of the mechanical properties of the lungs and chest wall (1). In patients with NMD, clinical assessment of respiratory function and performance of pulmonary function tests are of

great value to screen for nocturnal respiratory disorders, weakness of the diaphragm, hypercapnic respiratory insufficiency and potentially cough deficiency(2). The screen the rate of progression and the need of potential referral to specialized respiratory services for additional interventions becomes critical in over time.

Aims: To explore the use of Overnight Pulsoximetry (OP) as an initial screen to evaluate respiratory progression in neuromuscular patients.

Methods or Patients or Materials: A retrospective audit of OP tests was performed from January 2017 to July 2018. Reason for referral and outcome of the OP were reviewed alongside diagnosis, age, FVC and FVC% and presence of signs of nocturnal hypoventilation.

Results: A total of OP 83 tests were audited (23% from pediatric patients). There was a range of diagnosis. Duchenne Muscular Dystrophy (14%) and Fascioscapulohumeral Muscular Dystrophy (13%) were the most common ones.
Main reason (75%) for referral was the presence of hypoventilation signs. Low values of FVC%, drop in FVC from sitting to lying or incapacity to perform FVC test in clinic were the other ones. The OP outcome for the majority of patients (42%) indicated absence of objective changes in overnight traces. Only 29% were referred to respiratory specialist services (13% to Sleep Services under the suspicion of Obstructive Sleep Apnea). The remaining demonstrated either a combination of normal SPO2 mean with an altered Sleep Apnea Index (SAI), moderately altered SPO2 mean but stable in comparison to previous studies or moderately altered SPO2 mean but with no symptoms. The presence of artefact traces resulting in the need of repeating the study was found in 16%.

Conclusion: OP appears to be a valuable screening assessment for those patients who report signs of hypoventilation with an unclear respiratory involvement signs. Further exploration of confounding factors such as fatigue, musculoskeletal pain, is strongly encouraged to discriminate from respiratory progression.

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OD19

Exercise training outcome measures in Neuromuscular Diseases: a systematic review

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Background: Efficacy of exercise interventions in patients with myopathy has long been a contentious topic, heightened by the variability of outcome measures across studies. We systematically reviewed outcome measures utilised to assess exercise training in patients with muscle diseases and performed a meta-analysis for outcome measures that were most frequently employed.

Aim: Evaluate the relevance and usefulness of the most frequently utilised outcomes to evaluate the benefit of exercise training in patients with muscle diseases.

Methods: A systematic review and meta-analysis were performed according to PRISMA. We searched MEDLINE, Embase, Scopus, WoS, SPORTDiscus, CDSR, CENTRAL, and DARE from inception to April 2018. Articles were eligible according to predefined criteria. The most frequently utilised outcome measures were evaluated by a random-effects meta-analyses pre, post and training, non-training.

Results: Our search identified 33,727 records; 130 articles fulfilled inclusion ctiteria, comprising 1,808 patients with muscle diseases. We observed a significant increase in maximal aerobic capacity and peak power pre *versus* post exercise training (P<0.0001) and exercise training *versus* control (P<0.05). Compared to post-training, 6 Minute Walk Test (P<0.05), timed sit to stand tests (P<0.05), rise from supine (P<0.005), SF-36 (P<0005), fatigue severity scale (P<0·0001), central nuclei (P<0.05), type I/type II muscle fibre area (P<0.005), and citrate synthase (P<0·0001) were effected. We noted no effect on the Timed up and Go, Quality Of Life domains, capillary density, fibre type composition, strength and creatine kinase compared with non-exercise controls and/or post-exercise.

Conclusion: An array of outcome measures currently exist to assess exercise efficacy and safety in patients with myopathy. Greater consensus of the most appropriate outcome measures to be used in clinical research and clinical practice is required. A comprehensive review of outcome measures utilised will facilitate academics, clinicians, and pharmaceutical companies in trial design. These findings have major implications in the context of clinical trial readiness.

OD20

Physical activity interventions and therapeutic exercise in rare neurological disorders: a protocol for a scoping review

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Background: Rare neurological conditions have a significant cost burden on health and social care services. Neurological impairments result in increased dependence and sedentary lifestyle. Although enhancing physical activity is considered imperative in these conditions, the effectiveness of interventions on physical activity and therapeutic exercise across rare neurological disorders remains unclear. The aim of this protocol is: to synthesise the body of evidence on the effectiveness of physical activity interventions and therapeutic exercise for people with rare neurological diseases.

Methods: a scoping review will be undertaken across a broad range of rare conditions, such as the ataxias (e.g. Friederich's ataxia), hereditary spastic paraparesis, Huntington's disease, neuromuscular diseases (e.g. polyneuropathies, myasthenia and muscular dystrophies), motor neurone disease, atypical parkinsonisms. A two-phase search strategy will be conducted: a preliminary search will be conducted using keywords such as physical therapy, physiotherapy or exercise. Relevant keywords of systematic reviews will be extracted to form a second list of terms that will be used to run the final searches through the following databases: Pubmed, Embase and CINAHL. Only full-text systematic reviews in English will we included with at least one outcome measure on physical activity (e.g. walking, endurance or balance). Only studies concerning adults over the age of 18 will be considered. Pharmacological studies will be excluded. Data extraction: titles and abstracts will be screened for eligibility by one reviewer against inclusion/exclusion criteria. The full-text of papers that are potentially suitable will be retrieved and checked by a second reviewer, who will also independently review a random sample of 10% of the total papers for inclusion or exclusion. Any discrepancies will be discussed and resolved by a third party. Data will be extracted from each eligible review including, where possible: research designs, population sample sizes, details of interventions and any control, outcome measures, study results and effect sizes. A narrative synthesis of findings will be undertaken.

Conclusion: this synthesis should help to identify the available evidence on physical activity interventions and therapeutic exercise and areas of unmet needs in individuals with rare neurological disorders.

‡OD21

Clinical presentation of patients with *TANGO2* mutations

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Background: Autosomal recessive mutations of the Transport And Golgi Organization protein 2 (TANGO2) have recently been identified as the cause of a rare metabolic disorder with a distinct clinical and biochemical phenotype of recurrent metabolic crises, hypoglycemia, lactic acidosis, rhabdomyolysis, arrhythmias and encephalopathy with cognitive decline.

Aims: To further elucidate the clinical presentation of patients with *TANGO2* mutations.

Patient and methods: We report nine patients from seven independent families carrying *TANGO2* mutations with a complex presentation including developmental delay, recurrent metabolic crises, muscle weakness, rhabdomyolysis, cardiac arrhythmias, encephalopathy and refractory seizures with onset in early childhood. We studied muscle histology, biochemical measurement of respiratory chain enzyme activities in skeletal muscle and immunoblot analysis of fibroblasts and muscle.

Results: All nine patients carried autosomal recessive pathogenic *TANGO2* mutations. One patient was homozygous for the reported deletion of exons 3-9, another carried the same deletion at hemizygosity with a 22q11.21 microdeletion inherited *in trans*. The other patients carried four novel homozygous mutations (c.262C>T/p.Arg88*; c.220A>C/p.Thr74Pro; c.380+1G>A exon 5 essential splice-site variant), and a further novel heterozygous mutation (c.11-13delTCT/p.Phe5del) which was hemizygous in cDNA, indicating the loss of the other allele. These variants are very rare and predicted to be pathogenic. Immunoblot analysis in fibroblasts (3 patients) and muscle (1 patient) detected a decrease of TANGO2 protein levels.

Conclusions: These nine new cases expand the clinical presentation, and further elucidate the molecular mechanism contributing to the phenotype, of patients with *TANGO2* mutations.

OD22

MDUK Oxford Neuromuscular Centre - Changing the landscape for clinical trials by developing a major centre in the UK

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Neuromuscular diseases (NMD) are an important group of inherited and acquired disabling conditions caused by impairment of peripheral nerve and / or skeletal muscle function, and leading to premature death or major chronic disability, often exacerbated by cardio-respiratory involvement. Over the last decade there has been dramatic progress in the development and testing of experimental therapies for NMD, exemplified by the unprecedented number of possible therapies for Duchenne muscular dystrophy (DMD) coming to trial, the extensive patient registries and work on outcome measures and regulatory interactions led by TREAT-NMD and other groups. However, to date, involvement of clinical trial sites in the UK outside the main centres at Newcastle and London has been limited, especially in industry funded studies. There are thus two current strategic imperatives: first, to invest in and accelerate the preclinical discovery, development and translation of improved, second generation experimental therapies for DMD and other NMDs; and second, to dramatically increase UK clinical trial capacity.

As such, the MDUK Oxford Neuromuscular Centre, directed by Professor Matthew Wood, was founded in 2019 by Muscular Dystrophy UK and the University of Oxford to focus on the urgent mission of accelerating the discovery, development and deployment of new medicines to combat devastating neuromuscular diseases. The Centre builds on the already excellent research, training and patient care in Oxford to drive the development of novel experimental therapies more rapidly and increase national clinical trial capacity in neuromuscular diseases.

The core strategic and scientific objects of the Centre are to:

- Develop enhanced clinical infrastructure and clinical trial capacity in adult and paediatric neuromuscular disease;
- Deliver new experimental medicines to impact neuromuscular disease;
- Advance neuromuscular gene discovery to identify new therapeutic targets and biomarkers;
- Develop new and improved stem cell and animal models for neuromuscular disease;
- Build capacity through recruitment and a PhD training programme;
- Build partnerships with industry to advance the core translational objectives.

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Author Index

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