Abstracts of the
17th UK Neuromuscular Translational Research Conference

17th and 18th April 2024

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Invited Speakers

Wednesday 17th April 2024

S01

Safety of Gene Therapies

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The last few years have seen a dramatic increase in clinical trials using AAVs to deliver therapeutic transgenes to individuals affected by a variety of neuromuscular disorders. One AAV has received global approval for infants and young children with SMA; FDA has recently conditionally approved another AAV for a subset of DMD boys. Multiple ongoing clinical trials will reach planned endpoint in 2024. While there are differences in the extent of clinical response, often dependent on the stage of disease at the time of the intervention, the success of AAV gene therapy is incontrovertible with thousands of patients having experienced benefits. Adverse events have however been observed in many trials and in the real-world settings, using different AAV serotypes and different transgenes, suggesting that at least some of the observed events are class dependent. In my presentation I will discuss the different types of adverse events encountered after systemically administered AAV vectors in completed and ongoing clinical trials. Review of public information and published manuscripts reporting adverse events for AAV gene therapies in neuromuscular disorders. Multiple studies have reported activation of the innate and adaptive immune responses after exposure to AAV, as well as humoral and cellular responses to preexisting host immunity. Anti transgene reaction in CRIM negative patients have also been described. In rare cases, the severity of these responses led to the demise of patients affected by DMD (cardiac, acute respiratory distress syndrome), SMA (thrombotic microangiopathy, liver failure) and myotubular myopathy (liver failure). Adverse events, including severe ones, following AAV gene therapies are relatively common. I will discuss the importance of excluding comorbidities that might exacerbate specific adverse reaction, and the importance of carefully monitoring patients after receiving AAV, as most of the adverse events can be managed adequately with the appropriate intervention. While there is an ongoing effort to reduce viral load and improve relevant tissue targeting with next generation AAVs, we need to improve our skills in the optimal multidisciplinary monitoring and management of patients receiving these innovative therapies.

S02

Accelerating new therapies for rare neuromuscular diseases

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Major advances in genomics have been paralleled by unprecedented technological developments underpinning a new age of gene-based therapeutics, with the potential for the very first time of addressing the genetic causes of thousands of rare diseases, including neuromuscular diseases. The classical example is Duchenne muscular dystrophy (DMD), where modulation of pre-mRNA splicing or replacement of the DMD gene can restore a viable reading frame and the expression of functional protein. While significant progress has been made and first-generation therapies have been successfully developed, a major challenge is overcoming low efficacy and poor delivery to affected tissues. We have developed a range of peptide- and extracellular vesicle-based platform technologies to overcome this challenge. For example, peptide-oligonucleotide compounds provide greatly improved muscle and heart delivery and are currently in clinical development for DMD and myotonic dystrophy. Future challenges include the large number of rare neuromuscular diseases (most of which lack effective therapies), the presence of

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ultra-rare variants that lack the commercial incentives for industry investment, and the need to identify patients pre-symptomatically to achieve the greatest therapeutic benefit. The Oxford-Harrington Rare Disease Centre (OHC) is a partnership between the University of Oxford and the Harrington Discovery Institute in Cleveland with a mission focussed on development of new therapies for rare diseases. The vision for how the OHC is addressing these challenges will be discussed.

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S03

Treatments for ATTR amyloidosis

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Hereditary transthyretin (ATTR) amyloidosis is, in the absence of disease–modifying therapy, a progressive and fatal condition with a high disease burden for patients and their carers. Over the last decade there have been remarkable treatment advances in ATTR amyloidosis. Current therapies revolve around attempting to slow or halt ongoing amyloid formation and consist of small molecule drugs (tafamidis and acoramidis) to stabilise the TTR tetramer and prevent its dissociation into amyloidogenic monomers, and drugs which reduce circulating TTR concentration by targeting hepatic production known as a 'gene silencers' (comprising the RNA interference therapeutics patisiran and vutrisiran and the antisense oligonucleotide therapies inotersen and eplontersen). The TTR stabilizers have shown unequivocal benefit in the increasingly diagnosed ATTR amyloid cardiomyopathy (ATTR-CM) and tafamidis is approved for this indication as well as being approved in Europe for hereditary ATTR amyloid polyneuropathy (ATTRv-PN). The gene silencers have been shown to benefit the polyneuropathy in patients with ATTRv-PN and are approved and funded for this indication in the NHS; they are currently being tested in ATTR-CM in phase 3 clinical trials with promising early results. CRISPR/Cas9-based in vivo gene editing with NTLA-2001, administered by a single intravenous infusion, has been shown in a first-in-human study among patients with ATTR amyloidosis to be safe in the short to medium term and to achieve remarkable knockdown of serum TTR, expected to translate into improved clinical outcomes. Lastly, novel therapies which are specifically designed to accelerate natural clearance of ATTR amyloid are being evaluated in a number of ongoing clinical trials. The new therapeutic landscape has already revolutionised outcomes for patients with ATTR amyloidosis and, coupled with the increase in early diagnosis, promises to further improve quality of life and longevity among patients with this hitherto underdiagnosed and untreatable condition.

S04

DMD / DM1 Therapy Advances - Next Generation RNA Therapies Update

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RNA therapeutics for neuromuscular diseases have made impressive progress over the past few years with the approval of several first-generation drugs. Manipulation of mutant RNA can be achieved using synthetic antisense oligonucleotides, which are short, synthetic, single-stranded DNA analogs, either by modulation of splicing (to induce splice switching) or by inactivation. Some clinical failures have also revealed some challenges associated with their uptake and intracellular kinetics and highlighted the need to develop alternative chemistries, conjugates, or delivery systems to improve targeted delivery to muscle. Multiple next-generation drugs are now in clinical development, including some novel chemistries, but also compounds conjugated to peptide or antibodies. This talk will summarize the

latest advances in RNA therapies for Duchenne muscular dystrophy (DMD) and Myotonic type 1 (DM1).

S05

Can all persons with neuromuscular diseases benefit from exercise training?

John Vissing, MD, PhD
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It is evident from a large body of evidence gathered in the last decades that aerobic conditioning is safe and improves muscle function in patients affected by a variety of myopathies. Much less is known about training effects in neurogenic conditions, whether fuel supplements can enhance exercise performance, if very weak patients also can benefit from training and what intensity should be the target for training. Recent studies in patients with motor neuron diseases (MNDs) indicate that longer duration aerobic training is not as beneficial as in myopathies, because patients fatigue. The fatigue is likely neural in origin, because fewer and larger motor units have to fire more frequently in MNDs. New training paradigms, such as short-term high-intensity training, have shown better effect and compliance in patients with MNDs. Another unanswered question is whether very weak patients can benefit from training, and if so, how can we train them? A general notion has been that very weak patient, i.e. less than 10% of normal strength, cannot be trained because the muscle has reached an end-stage with no possibility to improve. Recent studies, however, suggest that this notion is wrong, and that the trouble has been that no suitable training tools have been developed for very weak patients. Assisted cycle training and antigravity walking/running on a treadmill offer new avenues for training in these patient groups. Fuel supplements are known to enhance exercise performance in a number of metabolic myopathies. But the question is whether patients with non-metabolic neuromuscular diseases can benefit from dietary supplements before or during exercise? The talk will discuss experiments that address these questions, and suggest avenues for new developments in the field.

Thursday 18 April 2024

S06

Role of Physiotherapy Rehabilitation in the Landscape of Genetic Therapies

Dr Gita Ramdharry
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The weight of evidence is gaining strength on the benefits of exercise and rehabilitation for people living with neuromuscular diseases, yet long term engagement in physical activity remains a challenge for many. This talk will address two questions:

(1) What is the role of exercise and rehabilitation interventions with the introduction of disease modifying drugs? Will they become obsolete, or could they actually inflate treatment effects with multi-modal drug/rehabilitation delivery?
(2) The majority of exercise trials to date focus on physiological or impairment level change but are not translating to long term engagement. Should we now be moving on from simply training muscles, to changing behaviour?

S07

Innate immune system induction in mitochondrial disease

Professor Yanick J Crow
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Mitochondria represent a potential source of immunogenic nucleic acid - where each cell contains multiple mitochondria, and diverse nucleic acid forms are generated during the transcription and replication of mitochondrial DNA (mtDNA). Loss of function of PNPT1, a component of the mitochondrial degradosome, was the first described Mendelian mitochondrial cytopathy associated with enhanced

type I interferon signalling, showing notable phenotypic overlap with the paradigm type I interferonopathy Aicardi-Goutières syndrome. More recently, pathogenic mutations in ubiquitously expressed ATAD3A were shown to cause an upregulation of type I interferon signalling through the dsDNA sensor cGAS, resulting from a leakage of mtDNA into the cytoplasm. These observations, implicating both mitochondrial RNA and DNA as inducers of interferon signalling, raise the possibility that innate immune system engagement may contribute to the phenotype of (other) monogenic mitochondrial-related disorders. Furthermore, type I interferon induction by mitochondrial-derived nucleic acid has also been implicated in a number of common neurodegenerative diseases.

S08

Immunological Disease Mechanisms and New Treatments in Inflammatory Myopathies

Professor Pedro M Machado
UCL Queen Square Institute of Neurology

Idiopathic inflammatory myopathies (IIMs) are a group of rare autoimmune diseases with a broad spectrum of clinical manifestations, most frequently sharing the feature of immune-mediated muscle injury. Patients can be categorised as having juvenile myositis (JM), dermatomyositis (DM), immune-mediated necrotising myopathy (IMNM), anti-synthetase syndrome (ASS), inclusion body myositis (IBM), and controversial nomenclature subsets such as polymyositis (PM) and overlap myositis. These distinctions are made based on clinical, serological and pathological findings. Interferon (IFN) pathways have been identified as major contributors to the pathogenesis of some subtypes of IIMs. DM is associated with a prominent type 1 IFN signature, whereas small studies have described a type 2 IFN signature in patients with ASS and those with IBM, although additional larger studies are needed to further characterise these associations. In contrast to DM, ASS, and IBM, the muscle in patients with IMNM shows low levels of IFN pathway activation. IBM is unique among IIMs owing to its molecular signature involving highly differentiated cytotoxic T cells that evade immune regulation. Emerging therapies in IIM currently in clinical trial development include neonatal Fc receptor (FcRn) inhibition (Efgartigimod, Nipocalimab), dual toll-like receptor (TLR) 7/8 inhibition (Enpatoran), complement inhibition (Ravulizumab), anti-CTLA-4 therapy (Abatacept), targeting type 1 IFN (via TYK2–JAK inhibition: Brepocitinib; JAK–STAT inhibition: Baricitinib, Tofacitinib; ILT7 inhibition: Daxdilimab; or anti-INF beta therapy: PF-06823859), and CAR-T cell therapy (targeting CD19 or B cell maturation antigen). In IBM, ABC008/Ulviprubart (a humanised monoclonal antibody against KLRG1, which selectively depletes highly differentiated T cells) and Rapamycin (Sirolimus, an inhibitor of the mammalian target of rapamycin (mTOR)), are being tested. The landscape of idiopathic inflammatory myopathies is evolving rapidly, driven by an enhanced understanding of underlying disease pathogenesis. The development of innovative therapeutic trials, coupled with the development of standardised outcome measures, holds promise for expanding our therapeutic strategies.

S09

Novel Treatments for Immune Mediated Neuropathies including CIDP, Nodopathies and GBS

Professor Michael PT Lunn
Centre for Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery, Queen Square and Department of Neuromuscular Disease, Institute of Neurology, UCL

The inflammatory polyradiculoneuropathies that are GBS and CIDP are a diverging group of diseases, where increasing understanding of pathogenesis, pharmaceutical developments and ‘borrowing’ drugs from diseases with analogous mechanisms has led to progress in treatment. This lecture will aim to give an overview of recent developments in mechanistic understanding and the therapeutic opportunities this presents. Molecular mechanisms of axonal and Schwann cell damage in Guillain-Barré syndrome have been finessed to such an extent that a number of potential therapeutic targets can be explored. A single dose of IVIG remains basic GBS treatment with second doses shown to be harmful. Plasma exchange remains a reasonable alternative where available. Complement inhibitors are a focus of interest, but further refinement of targets and de-

livery with proof of efficacy is required to move to use. Inhibiting calpain to protect cells MAC-targeted could be a focus of attention if non-toxic clinical agents can be found. CIDP has diverged into typical CIDP and autoimmune nodopathy, enshrined in the 2021 CIDP Guidelines. Many autoimmune nodopathies have IgG4 predominant pathomechanisms; IVIG is not efficacious and complement inhibition is unlikely to work. However, targeting the B-cell compartment (CD20 positive or negative), gives rational options for treatment in this generally more aggressive group of diseases. Beyond corticosteroids, IVIG and plasma exchange for CIDP other treatments shows promise but await the results of controlled trials. Mycophenolate is effective in service-evaluation models compared to other oral immunosuppressants. Rituximab works in 40-70% of patients with non-nodopathy CIDP. Sphingosine-1-phosphate agonists were disappointingly negative in a single RCT, possibly for design reasons. FcRN and complement inhibitors are very likely to become part of CIDP treatment algorithms in the near future.

S10

Autoimmune Myasthenic Syndrome - The Recent Dramatic Progress in Treatment of Autoimmune Myasthenia Gravis

Dr Jennifer Spillane
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Myasthenia Gravis (MG) is an autoimmune disease of the neuromuscular junction that causes fluctuating fatigable neuromuscular weakness. Approximately 80% of cases are caused by antibodies against the acetylcholine receptor (AchR) at the neuromuscular junction and a variable proportion of the remainder have antibodies against MuSK or LRP4, which are important post-synaptic clustering proteins The spectrum of severity ranges from mild intermittent ptosis to respiratory failure requiring ventilatory support. Ocular symptoms such as ptosis and diplopia are the presenting features in 80% of patients but most patients go to develop generalised disease. There are various treatment options for generalised MG. Pyridostigmine is the first line treatment but provides symptomatic relief only. There is randomised evidence for thymectomy in seropositive generalised disease. Acute severe exacerbations are treated with Intravenous immunoglobulin or plasma exchange. The mainstay of treatment, however, rests on immunosuppression using corticosteroids and non-specific agents such as azathioprine, mycophenolate mofetil and methotrexate Although a large proportion of patients with MG achieve disease control with these measures, others have refractory disease with ongoing symptoms, frequent exacerbations and dependence on rescue therapies. Many patients are exposed to long term high dose steroids. In recent years, increased understanding of the pathogenesis of MG has led to the development of newer agents with a more specific mechanism of action, improved efficacy and a rapid onset of action. Anti-complement agents and neonatal Fc receptor blockers target pathogenic mechanisms that are directly involved in MG, have shown their efficacy in phase 3 randomised controlled trials and are set to become part of the treatment paradigm in generalised MG. Anti B Cell therapies are increasingly used in MuSK MG. There are various other targets and treatment options under investigation including anti cytokine treatment, other B Cell targeting strategies, autologous stem cell transplant and CAR-T therapy. These advances have the potential to revolutionise the management of generalised MG but questions remain as to where these newer treatment options will fit into the treatment pathway.

S11

Improved diagnosis of patients through systematic variant resolution

Professor Conrad (Chris) Weihl
Head of Neuromuscular Section, Department of Neurology, Washington University in St Louis

Whole exome sequencing approaches have revealed the enormous variability in coding and non-coding regions of disease causing genes. In particular, rare or singelton variants in disease genes are often defined as variants of unknown significance (VUSs) when interpretated by genetic testing labs. This often leaves both patients and clinicians without a clear path forward. Resolving VUSs is essential for patient diagnosis, enrollment in clinical studies, defining the prevalence of disease subtypes and ultimately the implementation of disease modifying

therapies. Our group has been developing several approaches to streamline and improve variant resolution LGMD disease genes via a multi-tiered approach. These approaches include the development of both phenotypic and biomarker guidance in variant interpretation. The development of high throughput deep mutational scans and functional assays to categorize both pathogenic and benign variants in LGMD disease genes. And; finally, validation of pathogenic variants using alpha fold prediction, deep learning approaches and artificial intelligence. Improving patient diagnosis through the validation of genetic variants will increase the pool of patient available for clinical studies and amenable to future therapies.

S12

Gene Modifiers in Mitochondrial Diseases

Dr Sarah Pickett
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Inherited mitochondrial DNA mutations lead to varied clinical outcomes; individuals with identical pathogenic variants can exhibit vastly different presentations. This is exemplified by the most common pathogenic heteroplasmic variant, m.3243A>G, which causes severe neurological disease (MELAS, characterised by stroke-like episodes) but also causes a wide variety of phenotypes, including deafness and diabetes. Phenotypic clustering in families and moderate to high heritability estimates for individual phenotypes suggest a significant role for nuclear background in shaping phenotype. Using tools from complex disease genetics, such as genetic linkage, association analysis and polygenic risk scores, we aim to identify and characterise the nuclear factors that drive m.3243A>G-related phenotypes. We have found that the genetic architecture of nuclear factors influencing m.3243A>G-disease differs between phenotypes. Cardinal neurological features of MELAS likely stem from a few influential nuclear genes, while other presentations are likely polygenic and complex. Our studies highlight the value of international collaboration and large patient cohorts in understanding rare disease pathophysiology.

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Posters, Flash and Platform Presentations

‡ indicates a platform or flash presentation

Key:

Abstract Category	**Abstract prefix**
Dystrophy Pre-Clinical	D
Dystrophy Clinical	DC
Peripheral Neuropathy	PN
Motor Nerve Disorders	MND
Neuromuscular Junction Disorders and Channelopathies	NMJ&C
Mitochondrial Disease	MD
Other Diseases	OD
Diagnostics and cross-cutting therapies	DCC

Dystrophy Pre-clinical

D01

Localisation of various dystrophin isoforms in the mouse brain

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Background: Duchenne muscular dystrophy (DMD) is a severe neuromuscular disease caused by mutations in the dystrophin gene, resulting in muscle degeneration and a shortened life expectancy. DMD patients also have a higher prevalence of intellectual disability, anxiety, attention deficit hyperactivity disorder, and autism spectrum disorder than the general population. The presence of at least seven alternative promoters, two polyA addition sites and multiple alternative splicing results in several dystrophin isoforms with different expression patterns and putative roles. Mutations affecting only the full-length isoforms (Dp427) have been linked to emotional behavioural problems, while those also affecting shorter isoforms are also associated with intellectual disability in DMD patients. DMD mouse models that carry a mutation affecting Dp427 dystrophin isoforms display an enhanced fear response, and increased anxiety- and depressive-like behaviours. We hypothesised that dystrophin isoforms have differential expression in different brain areas and that their deficiency results in the different comorbidities present in patients.

Aims: The aim of this study was to investigate the different dystrophin isoforms localisation at both RNA and protein levels in mouse brain.

Methods/Materials: In this study, C57BL/10 mice (wild-type), *mdx5cv* mice lacking Dp427 and *mdx52* mice lacking both Dp427 and Dp140 were used. Capillary western blot was used to identify the protein levels of 427 kDa, 140 kDa and 71 kDa, qPCR analysis for RNA expression levels and BaseScope for RNA localisation, in different brain regions including cortex, hippocampus, cerebellum, midbrain and olfactory bulbs.

Results: Our results revealed that Dp427c expression is high in the cortex, while Dp427p1 and Dp427p2 are highly expressed in the cerebellum as demonstrated in capillary western blot analysis. *Mdx52* and *mdx5cv* mice had very low Dp427 transcript levels, due to the effect of the frameshifting mutation on mRNA stability, while neither of these *mdx* mouse models expressed Dp427 protein. Additionally, we confirmed that Dp71 is the most expressed isoform in the brain.

Conclusion: These newfound data add knowledge on the molecular networks associated with emotional and cognitive comorbidities in DMD.

D02

A new generation of arylhydrocarbon receptor (AhR) antagonists as utrophin modulators for the treatment of Duchenne muscular dystrophy

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Background: Utrophin upregulation has been suggested as a means to compensate for the missing dystrophin in Duchenne muscular dystrophy (DMD) with the advantage of being independent of patient genotype. The first in class utrophin modulator ezutromid was developed from a phenotypic screen through to a Phase 2 clinical trial. Promising efficacy and evidence of target engagement were observed in DMD patients after 24 weeks of treatment, however trial endpoints were not met after 48 weeks. Further investigations identified ezutromid had sub-optimal solubility and metabolic stability, in combination with induction of CYP1A which appears to be responsible for the lack of sustained clinical efficacy. We demonstrated that ezutromid binds to the arylhydrocarbon receptor (AhR) with high affinity, and that antagonism of AhR by ezutromid leads to utrophin upregulation, confirming AhR as a viable target for utrophin functional replacement therapies. [1]

Aims: To identify a small molecule drug which increases utrophin levels in muscle as an effective treatment of DMD.

Methods/Materials: Compound design is based on the following assays: Luciferase reporter assay performed on H2K-*mdx* utrnA-luc cells; analysis of CYP1A1 expression levels by RT-qPCR in HepG2, AML12 and H2K-*mdx* cell lines; western blot analysis of UTRN and AhR in iDMD and H2K-*mdx* cell lines and ADME data.

Results: Following euzutromid, a second generation of active compounds (phosphinates) was designed, with improved stability and solubility.[2] However, they exhibited hepatotoxicity and could not be progressed *in vivo*.[3] A third generation of compounds was designed to improve their stability further and maintain their activity. This led to a novel series of stable compounds showing promising results in luciferase and protein. *In vivo* efficacy results did not correlate to *in vitro* findings. Further investigation of the AhR signalling pathway identified CYP1A1 as a downstream protein of interest, as inhibition of CYP1A1 correlates to AhR antagonism.[4] Therefore, we chose measurement of CYP1A1 expression in HepG2 cell line as our primary assay. Any identified active compounds were also tested in AML12 and H2K-*mdx* cell lines to confirm preliminary results. Any validated compounds underwent pharmacokinetics and measurement of utrophin protein levels. This optimised assay workflow has allowed us to identify a fourth generation of novel compounds showing high potential to become *in vivo* candidates.

Conclusion: This work has allowed the identification of novel lead molecules as AhR antagonists with better efficacy and improved properties compared to ezutromid. Those novel compounds are now being optimised to get an *in vivo* candidate.

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D03

Necroptotic cell death mediates both cardiomyopathy and myopathy in preclinical animal models of Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is a progressive muscle degenerative disorder, culminating in a complete loss of ambulation, hypertrophic cardiomyopathy and cardiorespiratory failure. Necroptosis is a genetically programmed form of necrosis dependent upon the RIPK1/3 and MLKL axis. It is involved in several inflammatory and neurodegenerative conditions. Pharmacologic inhibitors of necroptosis are currently being developed and tested in clinics for non-muscle-related disorders.

Aim: To determine the role of necroptosis in all degenerating tissues in DMD: ambulatory, respiratory muscles, and heart.

Methods/Materials: Evidence of the activation of the necroptotic axis was examined in dystrophic tissues from Golden Retriever muscular dystrophy (GRMD) dogs and R-DMDdel52 rats, a novel rat model for DMD that closely recapitulates the human disease. A functional assessment of the involvement of necroptosis in dystrophic animals was performed on mdx mice that were genetically depleted for RIPK3. Dystrophic mice aged from 12 to 18 months were analysed by histology and molecular biology to compare the phenotype of muscles from mdxRipk3$^{+/+}$ and mdxRipk3$^{-/-}$ mice. Heart function was also examined by echocardiography in 40-week-old mice.

Results: in *sartorius* and *biceps femoris* muscles from GRMD dogs, RIPK3 expression positively correlated to myonecrosis levels. RIPK3 was also found elevated in the diaphragm. In the slow-progressing heart phenotype of GRMD dogs, the phosphorylated form of RIPK1 at the Serine 161 site was strongly increased in cardiomyocytes. In DMDdel52 rats, cardiomyopathy is more severe than in GRMD dogs. A similar p-RIPK1 upregulation to GRMD dogs was found in DMD rat hearts and associated with a marked overexpression of Ripk1 and Ripk3, indicating ongoing necroptosis. Compared to mdxRipk3$^{+/+}$ littermates, mdxRipk3$^{-/-}$ mice displayed decreased compensatory hypertrophy of the heart. They showed an increase in the relative wall thickness and a reduction in the left ventricle mass.

Conclusion: Our data highlight molecular and histological evidence that the necroptotic pathway is

activated in degenerative tissues from dystrophic animal models, including the diaphragm and the heart. We also provide the genetic proof of concept that selective inhibition of necroptosis in dystrophic condition improves both histological features of muscles and cardiac function, suggesting that prevention of necroptosis is susceptible to providing multiorgan beneficial effects for DMD.

D04

Role of the ECM in the progression of muscular dystrophies

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Background: Muscular dystrophies are a group of disorders characterized by chronic muscle damage, inflammation, fibrosis and, ultimately, loss of function resulting in disability and reduced life expectancy. The extracellular matrix (ECM) facilitates transfer of mechanical force, repair of damaged muscle and is also important in signalling. In patients with muscular dystrophies, ECM is accumulated and represents the main component of fibrotic tissue. ECM is now recognised as an active party in the process of muscle degeneration and not only a passive consequence of the disease. However, neither the composition of the ECM nor its role influencing muscle cells behaviour has been characterized fully.

Aims: Characterize the composition of ECM in the skeletal muscles of patients with muscular dystrophies and its ability to influence the behaviour of muscle cells.

Methods/Materials: Muscle samples from 4 month old sarcoglycan null mice and age-matched controls were decellularized using a combination of 1%SDS and 1% Triton-X and washed with water to isolate the ECM. Mass spectrometry was performed on dystrophic and healthy ECM to identify molecules that were upregulated in the dystrophic matrix. Decellularized ECM (dECM) was used to create a coating for cell culture dishes, on which myoblasts and FAP cells were grown. Proliferation was measured using CyQuant assay. We are now assessing the fibrotic and adipogenic differentiation capacity of the cells using in-cell-western for collagen-I and perilipin respectively.

Results: 196 Matrisome proteins were found to be differentially expressed between disease and control. There was no significant difference between the proliferative potential and the differentiation in cells that were grown on an ECM coating or a gelatine control coating.

Conclusion: Decellularization is a promising method for the isolation and study of ECM proteins from whole muscle samples. There are many differentially expressed ECM proteins when comparing diseased and healthy muscle. ECM coating is a viable method for culturing cells and has potential to be used to investigate the bidirectional relationships between ECM and cells.

D05

A multiplex 3D human iPS cell-derived muscle platform for evaluation of neuromuscular gene therapies

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Background: The structure and function of skeletal muscles are disrupted in various neuromuscular and musculoskeletal disorders, including muscular dystrophies wherein adeno-associated virus (AAV)-

mediated gene therapy has emerged as a promising treatment, but their clinical success is hindered by modest efficacy and adverse reactions. These limitations can be addressed by employing robust, humanized models that can predict cell- and tissue-specificity, toxicity, and efficacy during the early stages of AAV gene therapy development.

Aims: To develop an invitro human platform based on 3D-engineered skeletal muscles for evaluating gene therapy vectors with high efficacy, minimal toxicity, and selective tissue targeting.

Methods/Materials: Human iPS cell (hiPSC)- derived 3D organoids were used access AAV serotypes expressing GFP transgene. Live-microscopy, immunocytochemistry and qPCR were used to access the expression of GFP transgene and lineage markers.

Results: To evaluate the compatibility of 3D hiPSC-based platform for invitro AAV transduction, we assessed transduction efficiency and expression patterns of natural AAV serotypes. Morphological and molecular analyses of GFP transgene revealed a dose- dependent increase in transgene expression in transduced muscles, accompanied by deep tissue penetration into the 3D tissues. Further, we observed that a 14day time course is sufficient to distinguish different serotypes in terms of peak expression. Next, we leveraged the multi-cellular and multi-lineage nature of our 3D platform to investigate the tropism of natural and recombinant AAVs (rAAVs) in bilineage 3D muscles containing isogenic myofibers and motoneurons. We observed preferential tropism of neuronal-specific rAAVs in motoneurons over myofibres, validating the fidelity of our platform compared to conventional assays involving preclinical models. Additionally, we confirmed that neuronal-specific rAAVs outperformed conventional AAV serotypes in transducing motoneurons in 3D muscles. Finally, we presented our ongoing efforts to expand beyond multilineage, establishing a multiorgan (oid) platform to conduct similar assays in tissues known to be off-targeted by AAVs, along with strategies to introduce therapeutically relevant transgenes for evaluation in this innovative, quasi vivo platform.

Conclusion: We present a hiPSCs-derived 3D skeletal muscle-based platform to assess and develop next-generation AAV vectors and therapeutic molecules.

D06

Generation of a *de novo* intronic junction in the *DMD* gene through CRISPR/Cas genome editing as a potential therapy for a high proportion of Duchenne muscular dystrophy patients

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Background: Duchenne Muscular Dystrophy (DMD) is caused by mutations across the DMD gene. The subsequent absence of dystrophin protein compromises muscle stability and contractility and gives rise to progressive muscle wasting. Different gene therapies are being investigated, such as AAV micro-dystrophin delivery, premature termination codon read-through, exon-skipping and utrophin upregulation. Nevertheless, these therapies require repeated administration, could carry an adverse immunological risk and some are restricted by mutation specificity. Such problems may be circumvented with genome editing.

Aims: We aim to create a *de novo* junction between introns 18 and 55 (deleting exons 19-55), using CRISPR/Cas, to express an internally truncated functional dystrophin from the endogenous *DMD* locus. The gRNAs are designed to target introns and produce a ~800 kbp deletion. It is estimated that this strategy would eliminate ~81% of total DMD mutations.

Methods/Materials: To produce the deletion of exons 19-55, gRNAs for *Staphylococcus aureus (Sa)* Cas9 were designed and screened individually *invitro* for cleavage efficacy using TIDE analysis. The optimal gRNAs for each intronic site in murine *Dmd* were multiplexed into an *Sa*Cas9 construct. Top multiplexed gRNAs were tested in Neuro2A and C2C12 cells to confirm edited deletion by PCR and Sanger Sequencing. Gene editing constructs were packaged into AAV9 vectors, locally injected into 2-months old *mdx* mice and functional efficacy assessed by muscle electrophysiology. As a positive control, a cDNA construct expressing D19-55 dystrophin was generated and tested in *mdx* mice for protein expression.

Results: The positive control plasmid significantly increased dystrophin positive fibres *in-vivo* by plasmid intramuscular injection, indicating that D19-55 dystrophin is expressible and has the potential for beneficial effects when expressed in sufficient levels. Deletion of exons 19-55 was confirmed at DNA level *in-vitro*. Multiplex construct and co-delivery of gRNAs packaged into AAV9 were assessed *in-vivo* but no beneficial effects on muscle physiology were seen likely due to inability to detect a deletion in treated muscle samples using the techniques employed.

Conclusion: This study shows the development of a universal genome editing strategy from theory to *in-vivo* testing, exploring limitations of achieving a large deletion *in-vivo* and highlighting potential functionality of a new truncated dystrophin.

D07

Characterising the expression and localisation of dystrophin mRNA during skeletal muscle regeneration

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Background: Dystrophin (dp427) is essential for muscle membrane integrity: without dystrophin, myofibres are susceptible to contraction-induced damage, as seen in Duchenne muscular dystrophy (DMD). The dystrophin gene is enormous (2.3 megabases): production of a single 14kb dp427 mRNA requires ~16 hours of polymerase activity. Dystrophin transcription is also complex: dp427 5' sequence is always present in excess of 3', i.e. even in healthy muscle most dystrophin mRNA is nascent, not mature. Transcription is initiated concertedly, only for transcripts to be subsequently degraded shortly after completion (i.e. supply outstrips demand).

Aims: We investigated the hypothesis that this inefficient system allows post-transcriptional control of dp427 mRNA (via degradation, rather than initiation), allowing faster responses to sudden increases in demand, such as during muscle repair. Skeletal muscle is highly regenerative: following injury, quiescent resident satellite cells activate, proliferate, differentiate into myoblasts, and then fuse to form new myofibres, allowing muscle to recover from even substantial myofibre loss. Myoblasts do not express dp427, however: transcription commences only post-mitotically, after cell fusion. Myotubes must therefore produce new dystrophin rapidly, to protect the sarcolemma throughout growth to mature myofibres: a marked increase in demand.

Methods/Materials: We investigated dystrophin expression in BaCl-injured mouse muscle throughout the repair process (N=5, 2-28 days), using a combination of qRT-PCR and single-transcript multiplex fluorescence *in-situ* hybridisation to reveal timing, stability and localisation of individual dp427 transcripts.

Results: Nascent dp427 expression within regenerating myofibre nuclei is comparable to uninjured muscle, suggesting basal transcriptional initiation rates are already close to maximal. These immature fibres are, however, host to markedly greater numbers of sarcoplasmic mature dp427 mRNAs (with concomitant increase in 3':5' ratio): expression within regenerating myofibres is consistent with reduced degradation of mature mRNAs, to meet demands of increased translation.

Conclusion: Transcription from long genes is slow and unresponsive: overproduction, with control by post-transcriptional degradation, circumvents this, allowing muscle cells to address and meet increases in demand rapidly. This has therapeutic implications for exon-skipping, as demand in dystrophic muscle is very high: this mRNA stabilisation model implies that corrected transcripts will be preferentially preserved, and thus of unexpectedly high translational value.

D08

The role of collagen VI in satellite cell function

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Background: Collagen VI-related congenital muscular dystrophies (ranging from severe Ullrich to mild Bethlem) are caused by mutations of one of the three genes (*COL6A1*, *COL6A2* and *COL6A3*) encoding α-chains of collagen type VI. Collagen VI is one of the major components of the skeletal muscle extracellular matrix (ECM) and a key element in the skeletal muscle stem (satellite) cell niche. It connects the ECM to the basal lamina of muscle fibers. Collagen VI is produced mainly by fibroblasts, but there is evidence that satellite cells also contribute to its production. It has been shown that satellite cell self-renewal and functionality are reduced in a mouse model of collagen VI deficiency. However, it is not clear if this was caused by lack of collagen VI in the satellite cell niche, in the satellite cells themselves, or both.

Aims: Aims of our study include determining if satellite cells from Col6a2(-/-) mice are dysfunctional *in vitro* inside and/or outside of their niche, whether they are functional and contribute effectively to skeletal muscle regeneration after their transplantation and if they are able to generate functional satellite cells *in vivo*.

Methods/Materials: In the study we cultured isolated satellite cells and single myofibers from the Col6a2(-/-) and control wild-type mice. Moreover, *mdx* nude mice were used for transplantation of Col6a2 (-/-) and Col6a2 (+/+) satellite cells.

Results: Our data reveal an important role of collagen VI in regulating satellite cell function. Satellite cells in COL6 knockout mice are dysfunctional *in vivo* and *in vitro*. In addition, satellite cells with collagen 6 deficiency are defective in their contribution to skeletal muscle regeneration *in vivo*.

Conclusion: We show that satellite cells are directly affected by the lack of collagen VI and that this ECM component regulates satellite cell function both *in vitro* and *in vivo*. This suggests that effective therapeutic approaches for collagen VI-related muscular dystrophies needs to target not only fibroblasts, but also satellite cells.

D09

Anti-fibrotic drugs for Duchenne Muscular Dystrophy (DMD)

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Background: Duchenne muscular dystrophy (DMD) is a genetic condition caused by mutations in the dystrophin gene. This leads to progressive muscle fibre degeneration and weakness. Fibro-adipogenic progenitor cells (FAPs) are tissue-resident mesenchymal cells that can differentiate into adipocytes or fibroblasts and have a prominent role in muscle regeneration. Fibrotic and adipogenic differentiation lead to interstitial fibrosis and adipogenic

accumulation, which contributes to the characteristic muscle weakness of DMD. Although the signalling pathways activated by FAPs in dystrophic muscle have not been fully studied, our lab have demonstrated that tyrosine kinase inhibitors (TKIs) and ROCK inhibitors have anti-fibrotic activity against FAPs. Therefore, we aim to disrupt the differentiation process and target such mechanisms using these inhibitors.

Aims: The aim of this project is to analyse if a series of therapeutic compounds can safely and effectively reduce fibrotic and adipogenic differentiation of human control and DMD FAPs in vitro. The second aim is to investigate if there is a reduction of fibro-fatty tissue expansion in the DBA/2J-mdx murine model of DMD. Our final aim is to translate this research into treatments for patients with DMD at various stages of disease progression.

Methods: 339 TKIs were screened by evaluating collagen-I expression of immortalised human FAPs with In-Cell Western assays. The drugs that reduced collagen-I expression by at least 30% were tested in FAPs from control individuals and patients with DMD. Next, we investigated the effect of drugs on cytotoxicity, mitochondrial membrane permeability, apoptosis, proliferation and migration.

Results: 10 drugs were identified to reduce collagen-I expression of FAPs cultured in fibrogenic medium by at least 30%. Across all 10 drug candidates, there was no significant effect on cytotoxicity or mitochondrial membrane permeability and no apoptosis was induced. There was a trend towards a decreased proliferation rate compared to the positive control with all drugs apart from one compound, although this is not statistically significant. There was also a trend towards decreased migration of FAP cells.

Conclusion: In vitro studies allowed the selection of 4 promising candidates that will now progress to in vivo investigation to confirm if they reduce fibrosis and muscle degeneration in DMD.

D10‡

Targeting adipogenic differentiation of fibro-adipogenic precursors in Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is a genetic disease characterized by the loss of muscle fibres and its replacement by fatty-fibrotic tissue. The primary cells responsible for the fibrotic and fat tissue expansion are the fibro-adipogenic precursor cells (FAPs). These are mesenchymal stem cells that can differentiate into fibroblasts or adipocytes. There is limited knowledge on what molecular mechanisms are driving FAPs cell-fate. Recently, Wnt/Hedgehog/Notch pathway was highlighted as one of the pathways potentially involved in the differentiation of FAPs into adipocytes.

Aim: To study the effect of drugs targeting the Wnt/Hedgehog/Notch pathways in the differentiation of FAPs from patients with DMD and control individuals.

Methods: We screened 169 drugs targeting the Wnt/Hedgehog/Notch pathways using immortalized human FAPs cultured in adipogenic differentiation medium for 6 days. To evaluate adipogenic differentiation, Perilipin-1 expression was assessed using in-cell western assay. The drugs that reduced at least 30% perilipin expression in immortalised FAPs, were then tested in primary FAPs obtained from muscle biopsies of patients with DMD and control individuals (n=3-4). We evaluated the effect of drugs on perilipin-1 expression and FAPs relevant functions, such as cell viability and cell membrane permeability- using luminescent and colorimetric assays, and apoptosis, and proliferation- using fluorescent assays. Further, we tested the drugs effect on FAPs mitochondrial function using Seahorse XF cell Mito stress test.

Results: We successfully identified 14 drugs that reduced at least 30% perilipin expression on FAPs cultured in adipogenic medium. Three drugs (Bruceine D, RGB (Free base), GSK-3 inhibitor-1) reduced FAPs adipogenic differentiation without causing cytotoxicity and affecting FAPs proliferation. The drugs preserve the mitochondrial health of the FAPs and do not affect their bioenergetic profile.

Conclusion: We have identified three drugs that could potentially reduce adipogenic differentiation of FAPs while maintaining cell viability and mitochondrial function. Currently, I am dissecting the molecular pathways inhibited by these drugs *in vitro*. Overall, targeting the adipogenic differentiation of FAPs could potentially diminish fat infiltration and slow down muscle degeneration in DMD.

D11‡

Advanced *in vitro* modelling of *LMNA*-related congenital muscular dystrophies using patient-derived iPSCs for development of gene editing strategies

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Background: Laminopathies are severe diseases caused by mutations in the *LMNA* gene which encodes the proteins lamin A/C. These type V intermediate filaments assemble to form a proetinaceous meshwork structure called the nuclear lamina. This lies beneath the inner nuclear membrane where it maintains a repressive, heterochromatic environment. Laminopathies are categorised based on the tissues they impact, with striated mucle (skeletal and cardiac) being the most commonly affected tissues. *LMNA*-related congenital muscular dystrophy (L-CMD), in particular, is the most severe form of striated muscle laminopathy, causing premature death due to cardiorespiratory distress. Key pathological hallmarks of these diseases are nuclear shape abnormalities and lamin protein mislocalisations. However, research into disease mechanisms and therapies is limited due to a lack of scalable, humanised disease models and limited access to patient samples.

Aims: To address these hurdles, we aimed to model hallmark skeletal muscle laminopathy phenotypes such as nuclear shape abnormalities and lamin protein mislocalisations *in vitro*, in both 2D and a 3D artificial muscle platforms. Further, we aimed to use these models for the development of exon skipping strategies for amenable mutations using antisense oligonucleotides (AONs) or CRISPR-based permanent exon removal.

Methods/Materials: Three patient-derived *LMNA*-mutant iPSC lines were differentiated into myogenic cells *in vitro* and disease-associated differentiation, transcriptomic and nuclear phenotypes were assessed. Further, AON and dual gRNA-based CRISPR strategies were applied to cells and assessment of phenotypic amelioration was carried out.

Results: Results show defects in myogenesis are not an early pathological event in iPSC-derived L-CMD cells. Recapitulation of hallmark nuclear shape abnormalities and lamin mislocalisation phenotypes were observed in 2D artificial muscle models and nuclear elongation was exacerbated in the more mechanically challenging 3D artificial muscle platform. Analysis of nascent transcription demonstrated *LMNA*-mutant nuclei remain transcriptionally active, despite severely impacted nuclear integrity. The use of AON and CRISPR-based exon skipping approaches generated stable, shortened *LMNA* products. In particular, CRISPR-treated cells showed amelioration of nuclear shape and lamin A/C mislocalisation phenotypes.

Conclusion: iPSCs can effectively model L-CMD phenotypes and can be further utilised as a platform

for the devlopment of gene-therapy approaches. Specifically, we demonstrate the potential of an exon-skipping strategy for amenable *LMNA* mutantions.

D12‡

Therapeutic effect of AAV-mediated delivery of linker proteins in a mouse model for LAMA2-related congenital muscular dystrophy

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Background: LAMA2-related muscular dystrophy (LAMA2 MD or MDC1A) stands as the most prevalent form among congenital muscular dystrophies, arising from mutations in *LAMA2* - the gene encoding laminin-α2 (merosin). This protein serves as one of the constituent chains in the heterotrimeric extracellular matrix protein laminin-211 (α2β1γ1). The considerable size of laminin-α2's coding sequence (9.4kb) and the heterotrimeric structure of laminin-211 render AAV-mediated gene replacement impossible.

Aims: In this study, we describe the development and efficacy of a preclinical AAV-based gene therapy aimed at functionally substituting laminin-211 with two small proteins. These linker proteins, miniagrin (mag) and αLNNd, have been designed to fit within AAVs and serve the purpose of functional replacement by utilizing laminin-411 as a scaffold.

Methods/Materials: Intravenous co-injections of AAVs expressing mag and αLNNd were administered to LAMA2 MD mice. We assessed the ameliorative effects of this treatment on the disease phenotype—by measuring muscle weight, muscle function, and histology—as well as the peripheral neuropathy, employing both muscle-specific and ubiquitous promoters.

Results: The linker proteins were expressed at high levels in skeletal muscle and peripheral nerves and lead to a robust and highly significant amelioration of the muscular dystrophy phenotypes. This improvement manifested as increased body and muscle weights, enhanced grip strength, normalized mean myofiber size, and reduced fibrosis. Notably, the use of a ubiquitous promoter additionally mitigated nerve pathology, as evidenced by the alleviation of progressive hindlimb paralysis observed in LAMA2 MD mice.

Conclusion: Our findings indicate that systemic delivery of AAVs expressing these two linker proteins holds promise as a potential therapeutic strategy for LAMA2 MD patients. Given that the linker proteins are derived from proteins (agrin, nidogen-1, and laminin- 1) expressed in LAMA2 MD patients, this treatment is likely to be well-tolerated.

D13

Identification of reference microRNAs in skeletal muscle of the DE50-MD canine model of Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is a fatal muscle wasting disease caused by mutations in the dystrophin gene. Three microRNAs (miRs) known as "dystromiRs" (miR-1, miR-133a and miR-206) are dramatically elevated in serum of DMD patients and animal disease models including the DE50-MD canine model; however, affected muscle typically shows reduced or unchanged dystromiR expression compared to controls. To test whether dystromiRs or other miRs of interest are altered in muscle of DE50-MD animals, a panel of stable reference miRs is needed. Expression of the small nuclear RNA U6 is often used, however there is limited work investigating its stability across different tissues, species or disease states.

Aims: To establish a panel of reference miRs for quantification of miRs of interest in WT and DE50-MD dogs over a range of ages and muscle groups.

Methods/Materials: RNA was extracted from WT and DE50-MD dog (N=6) vastus lateralis muscle

samples collected longitudinally at 3, 6, 9, 12, 15 and 18 months of age, and from muscles collected post-mortem (N=3; cranial tibial, semimembranosus, lateral triceps and diaphragm). 87 RNAs were quantified in a subset of 6-month-old WT and DE50-MD muscles (N=4) using the QIAcuity miFinder panel. Reference gene algorithms geNorm, BestKeeper and Normfinder identified the most stable subset of 8 RNAs which were then quantified in all RNA samples, alongside snRNA U6. The most stable miRs of this subset, accounting for genotype, muscle and age, were used to normalise quantities of dystromiRs miR-1, miR-133a and miR-206.

Results: microRNAs miR-191, let-7b, miR-125a and miR-15a were the most stable miRs tested, while snRNA U6 performed poorly. DystromiR expression, normalised to the geometric mean of the panel of reference miRs, was lower for miR-1 and miR-133a in DE50-MD compared to WT muscles, while miR-206 levels did not significantly differ between genotypes.

Conclusion: A normalisation factor derived from miRs-191, let-7b, miR-125a and miR-15a appears suitable for normalising miR expression data from WT and DE50-MD muscle over a range of ages and muscle types.

D14

Transcript imbalance in Duchenne muscular dystrophy: implications for antisense oligonucleotide therapy

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Background: Duchenne muscular dystrophy (DMD) is a rare neuromuscular disease caused by mutations in the DMD gene that lead the reduction or absence of dystrophin protein. One of the most promising treatments for DMD are antisense oligonucleotides that modulate exon incorporation in DMD pre-mRNA, restabilising the frame altered by mutations. However, the intricate nature of the DMD transcription and especially its unique phenomenon of non-homogeneous expression thought its length, known as "transcript imbalance," may affect efficacy of RNA therapies. Despite its original description in 1995, transcript imbalance remains inadequately explored and overlooked in the era of treatments targeting the DMD pre-mRNA.

Aims: This study aims to provide a comprehensive evaluation of transcript imbalance across various DMD mutations type and genomic localizations. The goal is to characterise the correlation between different type and location of mutations with this phenomenon and contribute insights for the development of more effective DMD treatments.

Methods/Materials: RNA extracted from 15 DMD and 4 healthy control biopsies were investigated using FluiDMD cards—a microarray loaded with TaqMan systems covering all DMD exon-exon junctions throughout the transcript length.

Results: The study evaluated and compared the rate of DMD transcript imbalance among different DMD mutations, specifically nonsense, duplication, and deletions occurring in both the 5' and 3' regions of the DMD gene. Imbalance rates were also compared between DMD biopsies and myotubes and against healthy controls.

Conclusion: The correlation of imbalance rates with patients' mutation patterns revealed completely new and valuable insights able to improve our understanding of DMD mRNA dynamics in patients. Our observations might contribute to explain the difference in protein restoration observed in DMD patients undergoing antisense oligonucleotide therapy compared to the mdx23 mouse, typically used in the preclinical development.

D15

New potent carbohydrazide-pyrimidine based chemical probes allow identification of new molecular target for utrophin upregulation

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Background: Duchenne Muscular Dystrophy (DMD) is a severe genetic condition characterized by mutations on the DMD gene producing dystrophin, fundamental for preserving muscle integrity. Lack of dystrophin results in a degenerative muscle-wasting clinical picture currently without cure that demands innovative therapeutic strategies. Upregulation of utrophin, a paralogue of dystrophin, represents one of the most valuable approaches for DMD, regardless of mutation or disease progression. However, the molecular pathways leading to this mechanism have been poorly explored. We recently identified and optimized a carbohydrazide-pyrimidine small molecule (OX1914) that demonstrated a distinct mode of action from the first-in-class utrophin upregulator ezutromid, offering potential for identifying new druggable molecular targets for modulating utrophin.[1,2]

Aim: Utilizing cutting-edge affinity-based proteome profiling approach, our goal is to identify the targets of newly synthesized carbohydrazide-pyrimidine-based small molecules with high potency in upregulating utrophin. This target deconvolution aims at enhancing our understanding of molecular pathways leading to utrophin upregulation, paving the way for new therapeutic options.

Methods/Materials: Chemical probes incorporating a diazirine for covalent binding with target proteins and an alkyne for protein analysis were synthesized based on previous structure-activity relationship studies. Activity was confirmed in a cell-based gene reporter assay for Utrophin A and B promoters. For protein profiling, immortalized cell lines from mdx mice (LUmdx) and human patients (iDMD) were treated with chemical probes, and photo-crosslinking was initiated by UV irradiation. Cell were then harvested, lysed, and chemically linked to a fluorophore or biotin for in-gel fluorescence and protein enrichment. Processed samples were then alkylated, reduced, and trypsinized for LC/MS proteomics analysis.
Results: We successfully designed and synthesized a new set of photoactivable chemical probes that preserve nanomolar potency in promoting utrophin regulation. Using these probes we were able to identify new molecular targets, details of which will be disclosed during the conference.

Conclusion: Despite the lack of therapeutic agents for DMD, utrophin upregulation emerges as a valuable strategy. This work unveils new druggable targets for upregulating utrophin, offering a promising starting point for the development of small molecule-based therapies for DMD and addresses the crucial gap in understanding utrophin regulation mechanism.

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D16‡

Differential TfR1 expression patterns impact TfR1 targeting delivery strategies in neuromuscular disease models

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Background: The transferrin receptor, TfR1, has become an important target for enhancing the delivery of drugs across muscle membranes and the blood-brain barrier, offering a strategy for antisense oligonucleotide (ASO) delivery in treating a number of neuromuscular diseases, including myotonic dystrophy 1 (DM1) and spinal muscular atrophy (SMA). Despite this, little is known about TfR1 turnover and its expression patterns in different tissue types and different disease models.

Aims: To assess anti-TfR1 conjugated ASOs as a treatment strategy for neuromuscular diseases and provide information on TfR1 expression across murine and human neuromuscular disease models.

Methods: We treated DM1, HSA-LR mice and SMA mice with ASOs conjugated to an anti-TfR1 antibody and analysed efficacy in correcting splicing events and compound biodistribution via ELISA. Using qPCR, western blot and IHC, we measured and compared the expression of TfR1 mRNA, total protein and cell surface protein in 4 highly studied neuromuscular disease mouse models: MDX52, HSA-LR, LC15 and SMA, and their respective WT strains: FVBC and C57BL/10. Finally, we characterise TfR1 expression in differentiated DM1 human and primary mouse skeletal muscle myoblasts, employing ICC and FACS.

Results: We successfully delivered anti-TfR1-ASOs to brain and heart in both SMA and DM1 mouse models but only in SMA mice was the penetration and treatment of skeletal muscles observed. Analysis of anti-TfR1-ASO concentration in the skeletal muscles revealed a higher concentration in the SMA model. Exploration of TfR1 expression reveals differences in skeletal muscle and heart on comparison of DM1 mouse models to SMA and WT FVB/N mice. Furthermore, assessment of TfR1 expression in human skeletal muscle cells shows TfR1 downregulation in differentiated DM1 myoblasts.

Conclusions: We demonstrate that TfR1 expression is not uniform among disease and wild-type animal models, impacting the ability TfR1 targeting compounds to reach target tissues. We provide valuable information on receptor expression and availability at the cell surface in a number of neuromuscular disease models with implications for drug delivery systems seeking to exploit TfR1. Furthermore, variations in TfR1 expression patterns in DM1 and human cellular and murine models highlights species differences that must be considered upon therapeutic translation into humans.

D17‡

Interactome analysis of dystrophin isoforms in the mouse brain

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Background: Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder caused by mutations in the X-linked DMD gene, resulting in the disruption of functional dystrophin protein production. DMD patients exhibit progressive muscle weakness and are at a heightened risk of respiratory and cardiac failure. Notably, a significant proportion of DMD patients also experience intellectual disability and/or neurobehavioral complications, such as autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD) and anxiety, which have been linked to deficiency of different isoforms in the brain. The presence of at least seven alternative promoters, two polyA addition sites and multiple alternative splicing sites results in several dystrophin isoforms with different expression patterns and putative roles. The variability in DMD isoform deficiency, determined by the location of the gene mutation, is associated with varying degrees of brain comorbidities among affected individuals. DMD mouse models that carry a mutation affecting Dp427 dystrophin isoforms, display an enhanced fear response and increased anxiety- and depressive-like behaviours. We hypothesised that the various dystrophin brain isoforms interact with different protein complexes in the brain.

Aims: The aim of this study was to identify potential candidate dystrophin protein interactors in the mouse brain, to better understand DMD brain comorbidities.

Methods/Materials: In this study, *mdx5cv* mice lacking Dp427, *mdx52* mice lacking both Dp427 and Dp140 and DMD-null mice lacking Dp427, Dp140 and Dp71 were used. Different brain regions (cortex, hippocampus, cerebellum, midbrain and olfactory bulbs) were used for immunoprecipitation followed by mass spectrometry analysis to identify dystrophin's protein interactors in the brain. The proteins were further validated with immunohistochemistry and western blot analysis.

Results: Our results revealed that different proteins interact differently with Dp427, Dp140 and Dp71 in the different brain regions. Some of these proteins are related to ion channels, synaptic GABAergic transmission, neurodegeneration, and brain development.

Conclusion: This is the first study depicting the protein interactions of Dp427, Dp140 and Dp71 in the mouse brain, thus this knowledge will address the molecular networks associated with emotional and cognitive comorbidities in DMD.

D18

Optimizing the efficacy of antisense oligonucleotides for the treatment of Duchene Muscular Dystrophy

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Background: Duchene Muscular Dystrophy (DMD) is a severe X-linked neuromuscular disorder that causes progressive muscle weakness and premature death. DMD is caused by mutations on the dystrophin gene (*DMD*), causing a shift on the reading frame that results in an overall reduction or depletion of dystrophin. One promising approach for the treatment of this neuromuscular disorder is the use of exon skipping antisense oligonucleotides (ASOs) to restore the reading frame of the *DMD* gene leading to production of shorter but functional dystrophin protein. To date, four ASOs have been conditionally approved by the U.S. Food and Drug Administration (FDA). However, their treatment efficacy remains low and there is a current need of developing better ASOs for the treatment of DMD.

Aims: This study, which is part of the UKRI funded interdisciplinary consortium TransNAT, aims at developing ASOs of improved efficacy by testing different chemical modifications and conjugations to different moieties to optimize their cellular uptake and intracellular trafficking on different models of skeletal muscle.

Methods/Materials: The cellular trafficking of ASOs will be assessed by small nucleotide detection using miRNAscope combined with immunocytochemical assays to analyse their intracellular location, along with qPCR and western blot assays to evaluate the levels of exon skipping and protein restoration.

Results: Various muscle cell models derived from DMD patients will be employed: immortalized myoblasts and iPSCs will be differentiated into myotubes and treated with the different ASOs. After treatment, the cells will be fixed to evaluate the intracellular localization of ASOs. In parallel, mRNA and protein will be collected from the treated and untreated cells to evaluate the levels of skipped and unskipped *DMD* transcript and the levels of dystrophin protein restoration. The best candidates will be then administered to DMD animal models for further evaluation of their effectiveness *in vivo* and their biodistribution.

Conclusion: With this study, we expect to gain a better understanding on the role of different chemical modifications of ASOs on their endocytosis, intracellular trafficking, and treatment efficacy to, ultimately, design safer and more efficient ASOs-based therapies for DMD.

D19

Developing an effective RNA editing approach to target the nonsense mutation in the key functional region of DMD

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Background: Duchenne Muscular Dystrophy (DMD) is a rare genetic disorder characterised by progressive muscular weakness, due to mutations in the 79 exons of the DMD gene. Mutations after exon 60 of the DMD gene remain challenging by exon-skipping therapies since these exons are essential for dystrophin functionality. Alternative approaches that could correct these mutations without losing the functional motifs are therefore important. Base editing, which is capable of converting A-to-I or C-to-U, offers a promising strategy in correcting point mutations.

Aims: We aimed to develop an effective RNA editing approach targeting a nonsense mutation at exon 68 to restore the reading frame of the DMD gene.

Methods/Materials: A REPAIR (RNA editing for Programmable A to I Replacement) approach was adapted to correct the c.9851G>A nonsense mutation in the DMD transcripts. Five guide RNAs (sgRNA1-5) were cloned into a U6-mini-dCas13x-ADAR2 base editing construct(mxABE), respectively. In parallel, a 'traffic-light' reporter plasmid: mCherry-Ex68*-GFP(CEG), with the nonsense mutation (*) was constructed. The GFP expression would only be triggered upon an A to I conversion at the mutation site. The CEG and the sgRNA-mxABE plasmids were co-transfected into HEK293T cells, and the editing efficiency at the protein level was determined by the percentage of GFP+ cells within the mCherry+ population. At the mRNA level, the A-to-I conversion ratio was measured by Sanger sequencing via RT-PCR using primers spanning the mutation site.

Results: In transfected cells, we demonstrated an editing efficiency of the sgRNA-mxABEs ranging from 10-35% at both the RNA and protein level, with sgRNA5 being the most effective guide RNA. Furthermore, there was no evidence of any bystander or off-target editing in the flanking regions of the DMD mutation site.

Conclusions: We demonstrated successful editing of DMD nonsense mutation using mxABE construct to restore the reading frame, without introducing bystander effects. Next step will focus on investigating the editing efficiency and the restoration of the full-length dystrophin, via an AAV delivery approach, on DMD patient-derived myoprogenitor cells, a more clinically relevant cell model. This would pave the way for developing a novel personalized therapy to treat DMD patients with point mutations.

D20

Role of Stiffness in the Commitment of Fibro-adipogenic Progenitors in Duchenne muscular dystrophy

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Background: Duchenne Muscular Dystrophy (DMD) is an X-linked disorder produced by mutations in the Dystrophin gene. Clinical progression of DMD is characterized by loss of muscle fibres and accumulation of fibro-fatty tissue, impairing patient's muscle function. Dystrophin is a subsarcolemmal protein that protects muscle fibres from the processes of contraction and relaxation. Thus, lack of DMD induces muscle fibre damage. Fibro-adipogenic progenitors (FAPs) are key actors in the process of muscle degeneration as they differentiate

into fibroblast and adipocytes, contributing directly to the accumulation of fibrotic and fat tissue. Mechanical stress induced by increased stiffness in muscle niche can induce FAPs' differentiation. Until now, it is unknown if tissue stiffness influences FAP's differentiation in Duchenne.

Aims: to study the role of stiffness on driving FAPs' cell fate into fibroblast and adipocytes in Duchene Muscular Dystrophy *in vitro*.

Methods/Materials: We evaluated the cell behaviour of FAPs seeded on surfaces at different stiffness (0.2 up to 10^6 kPa) using Cytosoft® plates coated with ultra-pure collagen to ensure cell adherence. FAPs were isolated from biopsies from patients and control individuals (n=3-5, per group). To isolate FAPs we performed fluorescent-activated cell sorting (FACS) and cultured Platelet Derived Growth Factor Receptor Alpha (PDGFRα) + and CD56 - cells. Next, we evaluated the role of 8 and 10^6 kPa of stiffness when differentiating FAPs. The differentiation was done by treating the cells with TGF-β1 (3 days) or with Adipogenic media (10 days). As a read out of the differentiation process, we assessed the levels of extracellular matrix proteins (i.e Collagen I, Fibronectin, a-SMA) and adipogenic markers (i.e FABP4) by western blot.

Results: Our results show a decreased cell survival in soft surfaces (<8 kPa). This was even more evident in FAPs DMD. In addition, we found increased commitment of FAPs into adipocytes at higher levels of stiffness. The effect of the stiffness was less clear when evaluating differentiation of FAPs into fibroblasts.

Conclusion: Our study suggests, for first time, that higher stiffness prompts differentiation of human FAPs into adipocytes.

Dystrophy Clinical

DC01

Introducing pre assessment unit for DMD boys

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Background: Duchenne Muscular Dystrophy (DMD) patients undergo muscle biopsies as part of their clinical trials, requiring a general anaesthetic (GA). The patient is reviewed by the anaesthetist the morning of the procedure to confirm they are clinically well for the procedure. The anaesthetic will review their cardiac and respiratory function, due to the characteristics of the condition their function can have declined resulting in them not being fit for a general anaesthetic and as a result the patients being cancelled last minute.

Aims: To identify a pathway for DMD boys to be reviewed in advance to confirm they are fit for the GA, if intervention is needed this can be put in place and resulting in the patients not being cancelled on the day of the procedure.

Methods/Materials: A pre assessment unit had been established in our hospital and so the possibility of the patients being reviewed in this department in advance of the procedure was explored. The process was discussed with the pre assessment unit adn the wider research team. The costs and pathways were discussed and identified.

Results: All DMD research patients are seen in the pre assessment unit prior to having a GA, they are reviewed by the anaesthetist and if interventions are needed to ensure the patient is fit for the GA this is then discussed with the research team and implemented pre surgery. We add the additional costs to the study costings to ensure the sponsor reimburse the hospital and department for this process.

Conclusion: Patient cancellations has been reduced, all aspects are reviewed from heart scans, lung functions and current medication. If adjusts are required these changes are made to ensure it is safe for the patient to go under a GA. The process is more streamlined as the anaesthetic team have had the chance to review the patient in advance and they are aware of the patient before the day of the procedure.

DC02

Psychopharmacological treatments in children and adolescents living with Duchenne Muscular Dystrophy (DMD): insights from a single-centre cross-sectional study

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Background: Approximately half of DMD patients have cognitive and neurobehavioral symptoms, which can be related to mutation site. Intellectual disability affects 1 in 3 people with DMD. Other neurodevelopmental comorbidities frequently seen in DMD include attention deficit hyperactivity disorder and social communication difficulties/autism spectrum disorder. Furthermore, mood (anxiety, depression) and obsessive-compulsive symptoms, and behavioural problems have a higher prevalence in

DMD. The 2018 DMD care guidelines recommend mental health screening at neuromuscular clinic visits and the involvement of mental health clinicians for further assessment and management in case of positive findings. The effectiveness of psychosocial/psychotherapeutic approaches alone in the management of severe neurobehavioral symptoms can be limited, and psychopharmacological interventions may be required. However, to date there is little research in terms of real-world data and guidelines clinicians can refer to on this regard.

Aims: To investigate the use of psychopharmacological treatments in a paediatric cohort of DMD patients.

Methods/Materials: This is a single-centre cross-sectional study. We will review the clinical notes of DMD patients currently under the care of the Dubowitz Neuromuscular Centre, Great Ormond Street Hospital (GOSH) for Children, London. As the GOSH neuromuscular service does not routinely offer a child psychiatry service for these patients, the details of the psychopharmaceutics prescribed in external services – largely Child and Adolescent Mental Health Services (CAMHS) – will be analysed. The following data will be collected: number of DMD patients receiving psychopharmacological treatment(s), age range, DMD mutation subdivided by the expected effects on dystrophin isoform expression - Group 1 (Dp427 absent, Dp140/Dp71 present), Group 2 (Dp140 unknown), Group 3 (Dp427/Dp140 absent, Dp71 present), and Group 4 (Dp427/Dp140/Dp71 absent), drug(s) received, indication, dose, duration of treatment, perceived benefit (yes/no/unknown) and adverse events if reported by the patient/his caregiver(s).

Results: There are currently 250 DMD patients aged 1-18 years in our database, and results are being analysed.

Conclusion: Data from this study will contribute to raise awareness on this unmet aspect of the multidisciplinary care for DMD patients. Large prospective studies are required to test the safety and efficacy of currently available psychopharmacological treatments in children and adolescents living with DMD.

DC03

The John Walton Muscular Dystrophy Research Centre Patient Registries – Supporting neuromuscular research since 2008

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Background: The John Walton Muscular Dystrophy Research Centre coordinates 3 national and 3 international neuromuscular patient registries. With over 4,500 participants across all registries, the valuable data collected can be used to support research, clinical trial readiness, and patient care in a number of different ways.

Aims: Demonstrate the value of patient registries to the neuromuscular field by describing the key ways in which registries, their participants, and data, can be used, both nationally and globally.

Methods: Our patient registries have been used to aid recruitment to clinical trials and natural history studies, collect data for specific research projects, to provide real world data for post-marketing surveillance, and to disseminate disease-specific information to the patient and clinician community. In this poster, we provide case studies for each of these uses from across the registries. We also describe the process by which researchers and industry partners can request support from the registries or how to request data reports.

Results: Patient registries remain a valuable asset to the neuromuscular field, and further engagement from the research community continues to increase their value.

Conclusion: The John Walton Muscular Dystrophy Research Centre patient registries are an effective tool to support research in the neuromuscular field.

DC04

The BMD Hub: a network enabling trials, recruitment to studies, and care for Becker muscular dystrophy patients in the UK

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Background: Over the past few years, there has been an increased interest in Becker Muscular Dystrophy (BMD), with upcoming natural history and interventional clinical trials. However, these clinical trials are complex, and access is often limited to patients followed up in a small number of neuromuscular centres. The emerging clinical trials in BMD offer an opportunity to consider whether the DMD Hub network model (dmdhub.org) may be utilised to develop a network for BMD to support clinical trial delivery.
Aims: To enhance clinical trial access for patients with BMD in the UK, increase awareness and provide accurate and updated information about clinical research and upcoming clinical trials in BMD.
Methods: The BMD Hub will be established, working closely with clinical care sites, patient organisations, and industries, and will include

A) a network of health care professionals and coordinators with a specific interest in BMD.
B) a network of sites interested in and capable of delivering clinical trials in BMD.
C) a website as educational resource for health care professionals, patients, and families.
D) the Central Recruitment Database (CRD) for BMD utilising the DMD model (https://www.dmdhubrecruits.org) as a resource for patients with BMD.

Results: Learning from the experience in DMD, the BMD Hub will promote clinical trial readiness in BMD in the UK, by supporting sites in setting up and running clinical trials, facilitating a more equitable access to clinical research to patients and addressing specific industry needs. Establishing a network to support clinical research and addressing educational gaps will also support the implementation of care, offering an opportunity to develop standardised assessments and care guidelines for BMD. Finally, with the changing landscape of Duchenne Muscular Dystrophy (DMD) trials, now including the adult population, the BMD Hub could address the upcoming challenges for DMD, including recruiting from the older age group and transition to adult services.

Conclusion: The BMD Hub aims to foster collaboration, leading to the improvement of care and research in BMD. Expanding on its success with DMD, the CRD stands as a valuable tool, ensuring fair and equal access to clinical trials for BMD patients in the UK.

DC05‡

Differences in executive function skills used during planning and navigation observed in boys with DMD

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Background: Duchenne Muscular Dystrophy (DMD) is associated with neurobehavioural comorbidities, such as autism, anxiety, and impairments in cognitive function. Work with dystrophin-deficient mice has found evidence of behavioural differences and impairments on tests of cognitive and behavioural functioning, that are ameliorated upon dystrophin restoration. Clinical trials for genetic therapies restoring dystrophin and delaying the course of disease progression require robust outcome measures to assess improvements and maintenance of cognitive and behavioural functioning alongside physical ability.

Aims: The D-Brain study aims to identify areas of cognitive and behavioural difficulty in children with DMD, that can be used as outcome measures to assess the effectiveness of genetic therapies. We

present a preliminary subset of the D-Brain assessments, comparing aspects of executive function in boys with DMD compared to healthy controls.

Methods: Parent reports from Behaviour Rating Inventory of Executive Function, 2nd Edition (BRIEF-2) and observational data from Behavioural Assessment of the Dysexecutive Syndrome for Children (BADS-C) from 21 boys with DMD (Mage = 11.40, SD = 2.54) were compared to 17 age-matched controls (Mage = 12.85, SD = 2.58). Two subtests were included in the analysis: a cognitively demanding task with little structure requiring planning (Zoo Map 1) and a structured version of the task with predefined steps to follow (Zoo Map 2).

Results: Independent sample t-tests of BRIEF-2 showed that compared to controls, the DMD group experience more difficulties in planning and organising ($t(36) = 2.79$, $p = .009$), inhibitory control ($t(36) = 2.75$, $p = .010$), shifting attention and flexible problem solving ($t(36) = 4.96$, $p < .001$). Similarly, boys with DMD showed a marginally significant trend of impaired performance vs. healthy controls in Zoo Map 1 ($t(34) = -1.95$, $p = .059$), while there was no significant difference in performance in Zoo Map 2 ($t(29) = 0.41$, $p = .685$).

Conclusions: Our findings suggest that children with DMD may struggle with several aspects of executive functioning, particularly in the ability to plan and organise behaviour, and inhibition. This supports previous animal and human studies indicating executive functioning difficulties as a result of dystrophin deficiency in the brain and highlights executive functioning difficulties as a possible focus for dystrophin-restoration therapies and DMD-specific behaviour interventions.

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Peripheral Neuropathy

PN01

Sensory ataxic neuropathy in two families with heterozygous PNPT1 variants

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Background: Inherited neuropathies encompass a range of diseases from those in which the neuropathy is the sole or predominant feature of the disease to those in which the neuropathy occurs as part of a multisystem disease. Those with sensory ataxic neuropathy (SAN) exhibit loss of proprioception and vibration sense with preservation of muscle power. Biallelic variants reported in polyribonucleotide nucleotidyltransferase-1 (PNPT1) have been associated with variable phenotypes varying from syndromic hearing loss to multisystem Leigh disease[1]. Recently, heterozygous variants in PNPT1, with incomplete penetrance and variable expressivity, have been associated with cerebellar ataxia and prominent sensory neuropathy[2]. The clinical manifestation is diverse including gait disturbance, upper limb incoordination, nystagmus, dysarthria, scoliosis, and sensory neuropathy with decreased reflexes. Other clinical features include cognitive impairment and hearing loss have been described. Barbier et al, 2022, identified two novel heterozygous PNPT1 variants in two families with autosomal dominant sensory and cerebellar ataxia[3].

Aims: We report 2 families with heterozygous PNPT1 variants with SAN.

Methods/Materials: Whole genome sequencing was performed in 2 families with autosomal dominant SAN.

Results: Pathogenic heterozygous splice site (c.2014-3C>G) and nonsense (Arg715Ter) variants were detected, and confirmed on sanger sequencing, in family 1 and 2 respectively. All patients in both families presented with an isolated SAN clinically and neurophysiologically. In family 1, two patients developed additional cerebellar and autonomic signs respectively. On the other hand, family 2 only have SAN. Neurophysiology showed a sensory neuropathy in all patients and brain imaging was consistent with a cerebellar atrophy in 3 individuals.

Conclusion: We report two heterozygous PNPT1 variants (including 1 novel) in 2 families with a predominant SAN. This demonstrates the genetic and phenotypical heterogeneity of PNPT1 and identifies PNPT1 as a cause of isolated SAN.

PN02

Hereditary Sensory Neuropathy Serine Trial (SENSE) Protocol

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Background: Hereditary Sensory Neuropathy type 1 (HSN1) is a rare autosomal dominant neuropathy characterised by profound sensory and motor involvement. It arises from variants in the SPTLC1/SPTLC2 genes, inducing a gain of function mechanism in the serine palmitoyl transferase enzyme. This produces neurotoxic deoxysphingolipids, ultimately causing the neuropathy. While L-serine has shown promise as a potential treatment, a prior small study failed to meet the primary outcome measure. In response, we have developed a quantitative lower limb muscle MRI Fat Fraction protocol, designed as a highly responsive biomarker for HSN1.

Aims: Our primary objective is to evaluate the effectiveness of L-serine in stopping or slowing disease progression in HSN1 and, concurrently, to validate the lower limb MRI Muscle Fat Fraction protocol as the primary outcome measure in future inherited neuropathy trials.

Methods: This is a phase II, randomised, double-blind, placebo-controlled trial involving individuals over 18 years with genetically confirmed SPTLC1/2 diagnosis, capable of undergoing MRI without sedation and with Charcot Marie Tooth Examination Score (CMTES) ≤ 26. Exclusion criteria include factors such as recent foot surgery, diabetes, pregnancy/breastfeeding and current use of L-serine. Participants will take 400mg/kg/day of L-serine or dextrose powder (placebo) for 12-months. The primary outcome measure is the difference in lower limb muscle fat fraction over 12-months between L-serine treated and placebo-treated groups using MRI. Secondary/exploratory measures which will be performed at baseline and 12-months later include biomarkers (neurofilament light chain levels, plasma deoxysphingolipid levels), thigh intraepidermal nerve fibre density on skin biopsy, CMTNSv2, CMTNSv2-R, CMTESv2, CMTESv2-R and questionnaire-based assessments (CMT health index quality of life score, Neuropathic Pain Symptom Inventory, Neuropathic pain diagnostic questionnaire, Brief pain inventory and pain diary).

Results: Recruitment commenced in August 2023 with a target of 50 patients. Currently as of December 2023, 24 patients have completed screening, 15 have been randomised and recruited.

Conclusion: Results of the SENSE trial will be presented at a future Neuromuscular Translational Research meeting.

PN03

Establishment of a cellular model of SPTLC1-associated hereditary sensory neuropathy 1A to evaluate the efficiency of antisense oligonucleotides

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Background: Hereditary Sensory Neuropathy 1A (HSN1A) is an inherited progressive peripheral neuropathy. A missense dominant p.C133W mutation in the serine palmitoyl transferase long chain subunit 1 (*SPTLC1*) gene (the founder mutation in the UK) leads to the accumulation of toxic metabolites which impair the peripheral nerves. The use of Gapmer-antisense oligonucleotides (Gapmer-AONs) is a promising treatment for HSN1A by specifically silencing the mutant transcripts of *SPTLC1* to reduce the production of toxic metabolites. Our group has successfully identified several lead AONs that target the C133W mutation at the mRNA level. To further evaluate their efficiency at the protein level, a robust cellular model is needed.

Aims: We aim to establish stable reporter cell lines expressing either the mutant or wild-type human *SPTLC1* gene to evaluate the mutant-specific silencing efficiency of AONs at the protein level.

Methods/Materials: Mouse neuroblastoma cells, Neuro2A, were transduced by lentiviral vector containing mutant (C133W) or normal human *SPTLC1* fused with EGFP/mCherry transgene, and then characterised. Cell lines stably expressing mutant or normal SPTLC1-EGFP were transfected by human AONs at different concentrations to validate the protein level efficiency of the candidate AONs. The outcomes were the percentage of $EGFP^{+ve}$ cells as measured by Fluorescence-activated Cell Sorting (FACS) and SPTLC1 protein expression levels as measured by western blotting.

Results: The stable reporter cell lines expressing either the mutant (C133W) or normal SPTLC1-EGFP were successfully established using lentiviral transduction. After the treatment, all the selected candidate AONs showed effective suppression of the mutant SPTLC1 protein, without any effect on the normal SPTLC1 protein expression, consistent with the effect previously detected at the mRNA level.

Conclusion: The *SPTLC1* reporter cell lines provide a stable and sensitive cellular model for high-

throughput evaluation of AONs on mutant-specific protein knockdown efficiency. Data achieved from this model system also provides strong evidence that Gapmer-AON therapy is a promising strategy to treat diseases caused by dominant gain-of-function mutations, such as HSN1A. Future studies will focus on the functional restoration of the lead AONs, both in vitro and in vivo, subsequent to the reduction of the toxic metabolites after effective AON treatment.

PN04‡

Antisense oligonucleotide mediated mutant Sptlc1 silencing as a therapeutic strategy for hereditary sensory neuropathy type IA: A proof of concept study in a mouse model

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Background: Hereditary sensory neuropathy type IA (HSN1A) is a severe condition caused by dominant mutations in the serine palmitoyl transferase long chain subunit 1 (*SPTLC1*) gene, which encodes a subunit of serine palmitoyltransferase (SPT), a rate-limiting enzyme for sphingolipid metabolism. Mutated *SPT* causes accumulation of neurotoxic metabolites Deoxysphingoid Bases (DSBs), leading to axonal loss of the sensory nerves.

Aims: Our overarching aim is to develop a novel therapy for HSN1A by utilising Gapmer-antisense oligonucleotides (AONs) to selectively suppress mutant transcripts of the *SPTLC1* gene and reduce toxic metabolites. This study aims to provide proof-of-concept evidence for this strategy in a mouse model of HSN1A carrying the S331F mutation (c.992 C>T).

Methods/Materials: A panel of AONs targeting the mouse S331F mutation were designed with different chemical modifications and screened in dermal fibroblasts derived from Sptlc1^{S331F} mice. Lead AONs were identified by their selective silencing efficiency on mutant *Sptlc1* transcripts. The AONs were further validated *in vivo* in Sptlc1^{S331F} mice following subcutaneous injections, using different treatment regimes.

Results: AONs with locked nucleic acid (LNA) modification presented strong potency and selectivity in silencing the S331F transcripts in cultured fibroblasts. This was also evidenced when treating Sptlc1^{S331F} mice by a single subcutaneous injection, with the lead AON achieving approximately 95% and 65% silencing of the mutant transcripts in liver and dorsal root ganglia (DRG), respectively. After repeated injections, N-acetylgalactosamine-conjugated LNA-AON showed not only sustained silencing at the mRNA level, but also a significant reduction of blood DSBs at the functional level. Moreover, AON with 2-O-methoxyethyl (MOE) modification also achieved therapeutic effects at both mRNA and DSBs levels after repeated subcutaneous injections.

Conclusion: Our study provides strong proof-of-concept for an AON-mediated allele-specific silencing approach in treating gain-of-function disorders such as HSN1A. AONs with either LNA or MOE modification offer promising therapeutic benefits *in vivo* by targeting key organs affected in HSN1A and providing functional restoration. Based on this success, future efforts will focus on developing AON

therapy to target the human C133W founder mutation, by validating the lead AONs in a novel humanized SPTLC1^{hC133W} mouse model recently established in our lab.

PN05

Brainstem single-nucleus RNA sequencing in a highly prevalent length-dependent motor axonopathy of horses

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Background: Single-nucleus RNA sequencing (snRNAseq) provides further understanding of neuromuscular disorders by defining the transcriptomes of healthy and diseased cell sub-populations. However, studying axonopathies of long motor nerves is currently limited by the short axons of rodent models and sparsity of human tissues. The equine Recurrent Laryngeal nerve (RLn) (up to 2.5m) has its neuronal cell bodies in the Nucleus Ambiguous (nAmb). Recurrent Laryngeal Neuropathy (RLN) is a highly prevalent distal axonopathy of horses that has been proposed as an appropriate model for human length-dependent motor axonopathies. While snRNAseq has provided valuable exploration of human diseases, it has so far had minimal application in veterinary species.

Aims: Apply snRNAseq technologies to equine samples to define the cell populations of the equine brainstem's nAmb and identify a transcriptomic signature of RLN.

Methods: The left and right nAmb were located grossly using visual anatomical landmarks and removed from snap-frozen brainstems of 5 adult Warmblood geldings with varied RLN severity characterised by histological examination of the RLn. Nuclei were isolated using an optimised mouse brain protocol (10x Genomics) and captured using a 10x Chromium Next Gem Single Cell 3' kit. Using Cell Ranger and Bioconductor packages for analysis, 1000 cells were randomly selected from the data set for further pilot analysis. Clusters were identified using marker genes outlined from published studies in other species to define distinct cell populations within the equine brainstem's nAmb.

Results: 8 clusters were identified as "Oligodendrocyte 1", "Oligodendrocyte 2", "Oligodendrocyte precursor cells", "Astrocytes", "Microglia 1", "Microglia 2", "Endothelia", "Neuron 1", and "Neuron 2". Alongside neuronal markers, "Neuron 2" population also exhibited numerous cell stress factors, including upregulation of HSPB1, UBB, HSPA1A and SOD1, indicative of heat shock and oxidative stress pathways.

Discussion: This study shows that snRNAseq technologies can be adapted and utilised in horses and establishes a neurological cell population standard for use in future research. It indicates the presence of cell stress factors in neuronal cell populations from the nAmB. Future work will determine whether these are disease-associated and indicative of cellular responses in this prevalent distal motor axonopathy.

PN06

Whole exome sequencing in hereditary neuropathies: experience from an Indian centre

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Background: Diagnosis of inherited neuropathies has dramatically improved with the development of next generation sequencing (NGS). Nevertheless, the diagnostic rate remains very heterogeneous worldwide. To increase NGS global access, the MRC-funded International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) project was created.

Aim: Our aim was to analyse whole exome sequencing (WES) data of an Indian monocentric cohort.

Methods/Materials: Through the ICGNMD project, we included nine patients with a suspected hereditary neuropathy from one centre in South India. Clinical data was collected by the local neurology team. All patients underwent WES.

Results: Five patients out of nine were male (56%). The mean age at onset of the neuropathy was 11.6 years old (range 2 to 26). None of the patients had family history of peripheral nerve disease and five had consanguineous parents. A genetic diagnosis was confirmed in 3 patients (33%) with pathogenic variants in MFN2, SPTLC1 and SH3TC2. The causative heterozygous variants found in MFN2 and SPTLC1, respectively p.Thr105Met and p.Cys133Tyr, had previously been described in the literature. A novel nonsense homozygous mutation was discovered in the SH3TC2 gene of the third patient: p.Glu423Ter. The patient had a history of family consanguinity and presented with typical CMT4C associated distal weakness, scoliosis, pes cavus and hearing impairment. This variant was not reported in gnomAD4 and was classified as "very strongly pathogenic" by in-silico predictors. Loss of function variants are known to be pathogenic in this gene. In 4 patients (44%) we identified variants of uncertain significance in the following genes: KIF1A, IGHMBP2, SPG11 and GDAP1. More data is needed to investigate these candidates. In the last two patients of our cohort (22%), WES data analysis retrieved no results.

Conclusion: This single centre cohort study from India highlights the potential diagnostic yield of WES for hereditary neuropathies in under-investigated countries.

PN07‡

Optimising a muscle-specific AAV-BDNF gene therapy in mouse models of Charcot-Marie-Tooth Disease

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Background: Charcot-Marie-Tooth disease (CMT) is an inherited peripheral neuropathy characterised by slowly progressive, length-dependent motor and sensory nerve dysfunction, usually presenting in adolescence and causing life-long disability. CMT2D results from mutations in the *GARS1* gene encoding glycyl-tRNA synthetase (GlyRS), which cause conformational changes that mediate aberrant protein interactions. For example, we have shown that several GlyRS mutants mis-associate with the extra-cellular domain of the neurotrophin receptor, TrkB, contributing to neurotrophin signalling impairments and perturbation of *in vivo* axonal transport of signalling endosomes in CMT2D mice. Accordingly, we have shown that intramuscular injection of AAV-tMCK-proBDNF in pre-symptomatic *Gars*$^{C201R/+}$ mice reverses axonal transport deficits and additional features of the neuropathy after 1 month.

Aims: We are investigating the effect of longer-term treatment in the *Gars*$^{\Delta ETAQ/+}$ mouse model of CMT2D, which possesses a patient-specific mutation. This will determine whether longer exposure of muscles to enhanced BDNF levels restores axonal transport speeds and restricts the onset of symptoms.

Methods/Materials: Pre-symptomatic *Gars*$^{\Delta ETAQ/+}$ mice were treated with AAV8-tMCK-proBDNF via intramuscular injection into the lower hindlimbs and after 3 months, the following phenotypes were assessed: grip strength, muscle mass, plasma and muscle BDNF levels, neuromuscular junction innervation, and *in vivo* axonal transport of signal-

ling endosomes. Additionally, we have generated a highly muscle-tropic MyoAAV to determine the effects of systemic treatment.

Results: After 3 months of AAV8-tMCK-proBDNF treatment, we saw no phenotypic improvements in $Gars^{\Delta ETAQ/+}$ mice. Perhaps accounting for this, the levels of BDNF in both injected muscles and plasma were considerably reduced compared to the 1-month treatment. However, our new MyoAAV-BDNF capsid drives much higher BDNF levels within target muscles, even following systemic injection, offering an alternative approach to enhance intramuscular BDNF expression.

Conclusion: The therapeutic effect of AAV8-tMCK-proBDNF is lost with prolonged treatment duration. We hope to overcome this using a novel MyoAAV to determine whether enhanced and prolonged BDNF availability in muscle is a viable therapeutic strategy for CMT2D.

PN08

A UK wide research registry for inherited peripheral neuropathy

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Charcot-Marie-Tooth disease (CMT) is one of the commonest genetic neurological diseases affecting 1 in 2500 people). We have established a UK wide database for patients with CMT who are willing to be contacted for ethically approved research projects. Up until now there has been no such database for individuals with CMT in the UK representing a major barrier for recruiting to clinical trials for this rare group of diseases. The UK CMT database is hosted in the secure UCL 'safe data haven' and has undergone favourable ethical review (IRAS 319927). Any individual with CMT resident in the UK can register on the database via the following weblink https://redcap.idhs.ucl.ac.uk/surveys/?s=DN44N9HM84NH7XN7. This means that other than directing interested and eligible patients with CMT to this webpage, the clinician is not required to consent or enter data for the participant. A part time database manager will be employed to ensure the accuracy of the entered demographic and clinical data. Clinicians caring for database participants may therefore be contacted by the database manager to corroborate clinical details and in particular the type of genetic variant.

PN09‡

Investigating loss of Replication Factor Complex subunit 1 (RFC1) function in CANVAS patients and heterozygous AAGGG expansion carriers

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Background: CANVAS is a recessively inherited condition caused in most cases by biallelic AAGGG expansions in RFC1. Despite the recessive mode of inheritance, RFC1 transcript or protein expression appear unchanged. Yet, the identification of compound heterozygous null variants causing CANVAS suggests a role of RFC1 function in the disease pathogenesis.

Aims: To investigate the disease-causing mechanisms underlying CANVAS.

Methods: Here we show that pathogenic AAGGG expansions form stable nucleic acid structures compatible with G-quadruplexes in vitro and lead to transcription inhibition in vitro and in reporter assays in a repeat-length-dependent manner.

Results: We confirmed that RFC1 transcript and protein expression is preserved in bulk post-mortem cerebellar tissue and IPSC neurons. Also, long-read RNA sequencing did not show changes in RFC1 transcript processing or splicing. Nonetheless, patients derived lymphoblasts showed increased susceptibility to DNA damage, exhibiting reduced survival and earlier activation of apoptosis when treated with the DNA damaging agents cisplatin or oxaliplatin. Furthermore, we found that neuron-specific knock-down of gnf1 - the fruit fly RFC1 orthologue - led to decreased survival, progressive motor impairment and increased neuronal DNA damage in adult flies, and that these phenotypes were exacerbated by cisplatin treatment. Because of the known toxicity of platin on sensory neurons, and given the key role of RFC1 in DNA damage repair, we speculated that AAGGG expansions might increase the susceptibility to chemotherapy induced neuropathy in humans. Indeed, in a multicentre cohort of subjects who received oxaliplatin for an underlying neoplasm, heterozygous RFC1 expansion carriers showed an increased risk of developing a severe neuropathy compared to non-carriers (25/34, 73% vs 172/336, 52%, p=0.01).

Conclusion: Although the exact mechanisms causing the selective neuronal loss in CANVAS remain unknown, our in vitro, fruit fly, and human data suggest that RFC1 function is relevant to the disease pathogenesis, and that treatment with DNA damaging agents may unmask a hypomorphic effect of AAGGG expansions.

PN10

Foot Ulceration in Charcot-Marie-Tooth Disease and Related Disorders: Prevalence and Contributing Factors

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Background: Foot ulcers are a common issue among individuals diagnosed with Charcot-Marie-Tooth (CMT) disease and related disorders. These ulcers primarily stem from sensory loss and structural foot abnormalities. The interplay of peripheral neuropathy, muscular imbalances, and altered foot mechanics leads to pressure points, skin breakdown, and ultimately foot ulcers, increasing morbidity.

Aims: To investigate the occurrence of foot ulcers in patients with CMT and related disorders at our centre, ascertaining prevalence across various genetic subtypes and identifying associated risk factors.

Methods: We conducted a retrospective analysis of our clinical database and records of patients attending our inherited neuropathy clinics.

Results: Among 1982 patients with CMT and related disorders visiting our clinics, 101 individuals (5%) reported having foot ulcers. Of these cases, 70 (69%) were male and 32 (31%) were female, with an average age of 48 years (range 16-75). The average CMT Examination Score (CMTES) was 15.45 (± 5.49), (range 3 - 30). Among those reporting ulcers, 51% (52) were diagnosed with hereditary sensory neuropathy (HSN), with the majority having HSN1 (38/52) due to SPTLC1 and SPTLC2 variants (73%). Additionally, 48% (48) were diagnosed with CMT, and among these, 68% (33/48) had CMT1A due to the PMP22 duplication. 58% (59/101) of patients with ulcers exhibited foot deformities, with pes cavus being the most prevalent deformity (41/59). Decreased ability to feel was reported by 95% (96/101) of all patients.

Conclusion: Mitigating the risk of ulcers and associated complications in CMT requires preventive measures such as patient education, orthotic interventions, and footwear modifications. Regular foot care management through podiatry services is integral in the multidisciplinary approach to address CMT and related disorders.

PN11

Muscle-specific GDNF administration as a therapy for Charcot-Marie-Tooth subtype 2D

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Background: Charcot-Marie-Tooth disease (CMT) is a group of inherited peripheral neuropathies characterised by slowly progressing distal weakness, muscle wasting and sensory dysfunction. CMT subtype 2D (CMT2D) is caused by dominantly inherited mutations in *GARS1*, which encodes the aminoacyl-tRNA synthetase GlyRS, a housekeeping enzyme linking glycine to its cognate tRNA. These toxic gain-of-function mutations allow GlyRS to aberrantly interact with other proteins, such as neurotrophin/Trk receptors, hinting at these pathways as potential therapeutic targets. In agreement with this, we recently showed that enhancing brain-derived neurotrophic factor expression in muscles alleviates defects in axonal transport in mouse models of CMT2D.

Aims: The aim of this work is to assess whether boosting glial cell line-derived neurotrophic factor (GDNF), the most powerful motor neuron survival factor, in muscles of CMT2D mice, is a viable approach to restrict the appearance of neuropathic phenotypes.

Methods/Materials: $GARS^{\Delta ETAQ/+}$ mice harbouring a CMT2D patient mutation were injected with recombinant GDNF into the tibialis anterior muscle, and *in vivo* imaging was performed, to assess axonal transport of neurotrophin-containing signalling endosomes. The neuropathic mice also received intramuscular injections of adeno-associated viral vectors encoding GDNF under a muscle-specific promoter (AAV8-MHCK7-GDNF), as well as other recombinant growth factors from the GDNF family, prior to intravital imaging of axonal transport and muscle weight measurements.

Results: We show that recombinant GDNF injection into tibialis anterior muscle rescues the impairment in signalling endosome axonal transport *in vivo*. Similarly, increasing GDNF availability for 1 month via AAV8-MHCK7-GDNF delivery also shows preliminary positive effects on this phenotype. Amongst the additional GDNF-family neurotrophic factors, the most promising results so far were detected with intramuscular injection of recombinant Artemin.

Conclusion: Boosting intramuscular levels of GDNF, either acutely via recombinant protein injection or long-term via muscle-specific AAV delivery, improves impaired *in vivo* axonal transport and muscle weight in CMT2D mice.

Motor Nerve Disorders

MND01

Understanding the lived experiences and factors contributing to bladder and bowel dysfunction in Adults with Spinal Muscular Atrophy (SMA): Audit of Practice

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Objective: To explore the lived experiences of people experiencing bladder and bowel dysfunction by identifying the contributing factors and impact on their quality of life (QoL).

Background: SMA is a genetic disease that results in progressive muscle weakness and atrophy due to degeneration of the anterior horn cells in the spinal cord and lower brainstem motor nuclei caused by mutations in the Survival Motor Neuron 1 gene. Bladder symptoms are common in people with SMA for uncertain multifactorial reasons. Neural degeneration, behavioural factors, and disability are risk factors leading to symptomatic presentation.

Methods: A multi-disciplinary team designed the audit tool to assess urinary symptoms, medication, and management from retrospective notes of patients attending the SMA annual clinic at St Georges. Participants were given a combined questionnaire on the QoL and urinary symptoms profile.

Result: The audit included 28 patients with SMA Type 2 and 3 (mean age 38), 22 sitters, and 6 ambulant receiving gene-modifying therapy (Nusinersen 11 and Risdiplam 17). The audit identified 2 bladder augmentations, 3 urinary retentions, 3 urology referrals, and 3 hospitalised for constipation, renal stones, and urinary obstruction. The impact of QoL and lived experience will be discussed.

Conclusion: The study highlights that bladder symptoms have under-recognised comorbidity in adults with SMA. Due to its considerable impact on QoL and well-being, future research and care standards are required to address this unmet need.

MND02

Investigating the developmental expression of the SMN protein in mouse brain

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Background: The extended survival of treated children with early-onset Spinal Muscular Atrophy (SMA) is enabling us to appreciate novel neurodevelopmental phenotypes including delayed cognitive and communication development, and other neurobehavioural complications. These observations unveil that brain-related comorbidities can be present in a proportion of early-onset SMA patients and suggest a possible role of SMN in brain development. Previous animal studies have shown that early post-natal treatment with an *SMN2* splicing modifier small molecule restored axonal growth and maturation in the spinal cord, but in utero treatment was required to fully rescue axonal development and function.

Aims: To elucidate the effects of SMN deficiency and of early postnatal versus prenatal treatment on brain development, both at the structural and molecular levels.

Methods/Materials: The Taiwanese severe SMA mouse model (with two copies of the human *SMN2*) is used, where mice were treated at P0 daily, with SMN-C8, a small molecule *SMN2* splicing modifier. Brain tissue were collected at P3, P7, P14, P21 and P28 from wildtype (WT) and treated SMA1 mice, as well as untreated SMA1 mice at P3 and P7. Pregnant

mice, carrying SMA1 offspring, were also prenatally treated with SMN-C8 at E13 and tissue were collected at the same timepoints after birth. Tissue from untreated SMA type 3-like (mild phenotype) with four copies of human *SMN2* were also collected.

Results: The expression of *SMN* transcripts and protein levels in brain tissue was investigated. Preliminary data show that levels of full-length (FL) SMN and Δ7 transcripts and SMN protein in brain decrease with age in WT, SMA1 and SMA3 mice, consistently with data in humans. SMA1 mice treated with SMN-C8 have increased levels of SMN protein in cortex and cerebellum, compared to untreated SMA1 mice of the same age. Immunohistochemistry will also be used to study markers of neuronal growth cones and axonal regeneration or markers for cholinergic neurons.

Conclusion: This study highlights the developmental and spatial changes of the SMN protein in the mouse brain from prenatal to post-natal ages and the additional effects of prenatal versus early postnatal treatment on brain development.

MND03

Mortality in Spinal Muscular Atrophy in the era of disease-modifying therapies

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Background: With the availability of novel disease-modifying therapies, survival in Spinal Muscular Atrophy (SMA) has increased, but mortality is not rare in severely affected cases.

Aims: To characterise causes of mortality in children with SMA over the last five years since the introduction of disease-modifying therapies.

Methods: Retrospective case review of all patients with SMA attending our paediatric service, who died between 2019 and 2023. Data was collected from electronic medical records and SMA-REACH database.

Results: A total of 146 patients with SMA were seen in our institution in the last 5 years (1 SMA0; 61 SMA1; 56 SMA2; 28 SMA3). Eleven patients with SMA died in this time-period: 1 SMA0(9%), 7 SMA1(64%), 3 SMA2(27%) and 0 SMA3. Median age at death was 13 months (range 7weeks-21 years): the median age of death in SMA-1 cohort was 9 months (range 7weeks–8.5years) and in SMA-2 cohort was 19 years (range 17years–21years). Two patients received Nusinersen treatment, 8 patients were treatment naïve (6 were not eligible; 1 RIP prior to commencement; 1 parent declined). One with SMA-2 had previously been on a clinical trial (olesoxime). Four patients were wheelchair users (3 SMA2, 1 SMA1) and one child achieved independent sitting. Physiotherapy scores at last review were available in 7 cases: 5 had CHOP scores: median score 19/64(range 7-36/64); 2 had SMA scores: 5/69 and 7/40. Three patients were orally fed, 2 patients were fed via gastrostomy and 6 patients had nasogastric tube-feeding. Seven patients required non-invasive ventilation (ranging from 12 hours to 22 hours/day), 1/11 required ICU invasive ventilation and 3/11 did not require respiratory support. Cough assist device was in use in 4/11 cases. Five patients died in intensive care setting, 2 died at home/hospice and 1 patient died in their local hospital. Place of death was unknown in 3 cases. Cause of death was available in 8 cases. Respiratory illness was the precipitating cause in 3 cases, respiratory failure in 5 patients and one patient had cardio-respiratory arrest secondary to acute aspiration. A ceiling of care agreement was in place in 5/11 cases and resulted in withdrawal of care.

Conclusion: In contrast to the well-known natural history, only 7% of SMA patients attending our institution died. The majority of these patients had SMA-1, at severe end of the spectrum and were also treatment naïve. Respiratory related deaths occurred in 88% of known causes of death.

MND04

The role of S-Nitrosylation in the pathogenesis of SBMA

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Background: Spinal and bulbar muscular atrophy (SBMA) is an X-linked and late-onset progressive neuromuscular disease caused by a CAG repeat expansion in the *Androgen Receptor* (*AR*) gene caused by molecular mechanisms, which to this day remain elusive. S-nitrosylation (SNO) is a redox-triggered post-translational modification that governs protein functionality through the covalent reaction of nitric oxide (NO)-related compounds with a cysteine thiol group found on the target protein. In physiological conditions, SNO can serve as a significant regulator of signal transduction pathways, much like phosphorylation. However, ageing or exposure to environmental toxins can result in anomalous SNO reactions that impact protein misfolding, mitochondrial fragmentation, synaptic function, apoptosis, or autophagy. These alterations are significantly known to contribute to the mechanism of several neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. Although importantly, SNO is therapeutically targetable, how SNO contributes to SBMA pathogenesis is unknown.

Aim: The overarching objectives of this research programme are to investigate whether: i) SNO occurring on AR protein is altered in SBMA and ii) is a target for therapy in this condition.

Methods/Materials: We have employed the resin-assisted capture (SNO-RAC) assay for protein S-nitrosothiols, coupled with mass spectrometry in human-derived myoblasts from both unaffected and affected individuals. The effect of SNO on wild-type and polyQ mutant AR biology has been tested in the human-derived myoblasts using several molecular biology techniques, from transactivation assays to confocal microscopy using fluorescence recovery after photobleaching (FRAP).

Results: S-nitrosylation of specific cysteines within AR critically controls the ability of AR to promote the assembly and functionality of the transcriptional machinery (e.g. MED1, BRD4, RNA polymerase II) onto the chromatin and that this process is impaired in SBMA.

Conclusion: Our findings suggest that SNO plays a pivotal role in the pathogenesis of SBMA, affecting the assembly of the transcriptional machinery onto chromatin, which is disrupted in this disease. This discovery opens exciting avenues for therapeutic intervention in SBMA and underscores the importance of further research in unravelling the intricate connections between SNO and neuromuscular disorders

MND05

Characterising access to care management for adults living with SMA in the UK through Adult SMA REACH Data collection study

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Adult SMA REACH, a national clinical network and data collections study, aims to characterise adults living with Spinal Muscular Atrophy (SMA) in the UK, understand the impact of new treatments on the natural history of SMA and standardise care provision. SMA is a form of motor neuron disease caused by a mutation in the survival motor neuron 1 gene (SMN1) which results in a wide disease spectrum affecting infants and adults. The recent advances in drug development and European Medicines Agency approvals of Disease modifying therapies (DMT) highlight the need for standardised, longitudinal, real-world data collection across this emerging cohort and timely access to care provision. Using the Adult SMA REACH dataset we aim to establish how many

and which patients have access to specific aspects of care provision The dataset comprehends 370 patients across 19 sites in the United Kingdom, with over 1200 patient visits at the time of writing. Data from adult patients enrolled in the Adult SMA Reach project will be analysed. Data includes validated functional outcome measures, reported care management and access to care interventions. This includes physiotherapy provision, use of mobility aids, respiratory and cardiac care. We will categorise patients according to their SMA type, mobility status and treatment. Using this strategy, we will be able to describe access to care intervention and provision for adults living with SMA in the UK using the Adult SMA Reach data set. Cross-sectional analysis is ongoing and will report on access to care management and its impact on disease severity. Due to the current availability of Disease Modifying Treatments, and improved care standards, there is an opportunity to identify gaps in care provision and inequality in access to care and treatment. Ultimately this project will help in providing better care for adults with SMA in the UK.

MND06

Exploring the role of microRNA-125b-5p in regulating microglial mediated neuroinflammation in Spinal Muscular Atrophy

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Background: Spinal Muscular Atrophy (SMA) is a neuromuscular disorder associated with mutations in the survival motor neuron 1 (*SMN1*) gene. The intricate molecular mechanisms underlying motor neuron degeneration in SMA remain elusive, primarily due to the widespread expression of the SMN protein and its involvement in various cellular processes. Recent investigations have highlighted the significance of microRNAs in fine-tuning various molecular processes, including microglial-mediated neuroinflammation. Among these, microRNA-125b-5p stands out as a microglial-enriched microRNA known to target the TNFAIP3, a crucial regulator of the NFKß1 pathway.

Aims: Our study aims to explore the impact of microRNA-125b-5p on the TNFAIP3/NFKß1/TNF-neuroinflammation pathway in SMA.

Methods/Materials: We employed previously isolated spinal cord tissues from two distinct groups of mice: the severe SMA (*hSMN2*)2+/-; Smn-/- and heterozygous unaffected (*hSMN2*)2+/-; Smn+/- Taiwanese mice. New-born SMA mice at postnatal day 0 (PND 0) were treated with a single subcutaneous injection of a 25-mer morpholino antisense oligomer (PMO25) at 40μg/g targeting *hSMN2* exon-7 to promote exon inclusion. Spinal cord tissues were collected from SMA untreated, SMA treated with PMO25, and heterozygous littermate control mice, at PND 7. Using real-time quantitative PCR, we measured changes in expression of h*SMN2-FL*, *Tnfaip3*, and microRNA-125b-5p transcripts in different groups of mice.

Results: In the SMA mice, there was a notable increase in the expression levels of microRNA-125b-5p. Conversely, when these mice were treated with PMO25, there was a significant reduction in microRNA-125b-5p levels. This reduction in microRNA-125b-5p coincided with an increase in the expression of *SMN2-FL*, as observed at both the RNA and protein levels. Importantly, no discernible differences were detected in *Tnfaip3* transcript levels across the various groups of mice.

Conclusion: Elevated levels of microRNA-125b-5p in SMA mice highlight a significant connection between SMN and this microRNA. With this association established, our next objective is to confirm the post-transcriptional effects of microRNA-125b-5p by assessing TNFAIP3 protein levels. In addition, we are currently in the process of developing a mouse microglial cell line with reduced SMN expression. This model will enable us to directly link microRNA-125b-5p levels with changes in neuroinflammatory markers regulated by the NFKß1 pathway.

MND07

The integration of PROMs and clinician reported data: a holistic approach to characterise disease burden and treatment impact

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Background: UK SMA Patient Registry collects patient-reported outcome measures (PROMs) from individuals living with spinal muscular atrophy (SMA) in the United Kingdom and Ireland. In 2022, PROMs collection was introduced in the registry to supplement clinical and genetic data held therein. PROMs capture the perspectives of adults and caregivers of young people living with SMA about the impact of their condition and treatment, their quality of life and activities of daily living. Importance of the patient voice is increasingly recognised and valued. Currently, SMA therapies Nusinersen and Risdiplam are available in the UK via managed access agreements (MAAs). The collection of clinical and patient-reported data will inform review of treatment impact by UK regulatory authorities.

Aims: In collaboration with clinical networks Adult SMA REACH and SMA REACH UK, the registry aims to collect PROMs data of 100 Nusinersen and 100 Risdiplam patients. PROMs will be aligned with Adult SMA REACH and SMA REACH clinical data, anonymised, analysed and submitted to regulatory authorities for consideration as part of the treatment MAAs.

Methods: Registration is patient-initiated through an online portal. Patients are invited to complete questionnaires about their condition and PROMs: EQ-5D; Global Impression of Change; SMA Independence Scale; and a free-text box. Enabled through patient consent and data sharing agreements, patient-level PROMs data is shared with each patient's SMA REACH clinic and with the SMA REACH coordination teams. In clinic, the data informs patient care. At project coordination level, PROMs are aligned with clinical data collected by SMA REACH.

Results: The registry has 647 participants: 445 adults (16+years); 202 paediatric (<16years). PROMs have been completed by 212 adults and by the caregivers of 86 paediatric patients. The fraction of PROMs able to be aligned with Adult SMA REACH and SMA REACH clinical data will be presented.

Conclusions: The UK SMA Patient Registry represents a well-defined cohort of individuals with SMA and is a valuable tool for the collection of SMA real-world data reported by treated and treatment-naïve patients. PROMs collection by the patient registry supports UK SMA data collection and supplements Adult SMA REACH and SMA REACH clinical data, assisting in therapy evaluation by regulatory authorities.

MND08‡

Using innovative Data Modelling methods to improve data quality-learning from Adult SMA REACH a real-world data collection study

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Background: Adult SMA REACH is a Real World Data collection study for Adult Spinal Muscular Atrophy (SMA) patients across United Kingdom. The aim of Adult SMA REACH is to improve the knowledge about the impact of new treatments in the natural history of the diseases but also to support the approval of Nusinersen and Risdiplam in UK under the Manage Access Agreement Programme (MAA). The generation of Real-World data requires the standardization and systematic collection of data generated during routine clinical visits and the constant monitoring of data quality to ensure that the data reported to regulatory agencies (NICE and NHSE) meet the highest quality standards. Adult SMA REACH includes data from 19 different sites with 370 patients and 1200 follow-up visits. The manual monitoring of data for quality check was time consuming and this highlighted the need for innovative methods of data monitoring.

Aims: We aimed to increase data quality using innovative data modelling methodologies.

Methodology: Innovative data modelling techniques enable data representation as a structured collection of interconnected entities, enhanced with metadata. This format outperforms conventional tabular representations, facilitating more profound and intricate analysis.

Results: Using this methodology, we created an automated software for data validation, consistency checks, completeness analysis and treatment tracking. This enabled us to provide continuous, hands-free monitoring of evolving datasets. The software also allowed us to reveal systematic issues on some sites impacting data quality, allowing us to provide targeted and tailored assistance.

Conclusions: The application of new methodologies has allowed us to speed up the data monitoring process from a time-consuming process to a process carried out in seconds. We plan to expand our work by standardizing a data-agnostic data modelling methodology, allowing for a quick and flexible adaptation to different project scenarios within the John Walton Muscular Dystrophy Research Centre, Newcastle.

MND09

Investigating RNA editing dysregulation in spinal and bulbar muscular atrophy

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Background: RNA editing by deamination is a widespread and essential post-transcriptional modification, catalysed by the Adenosine Deaminase Acting on RNA (ADAR) enzymes. Upon binding to double-stranded RNAs, they convert adenosine into inosine, interpreted as guanosine by the translation machinery. The functional impact of RNA editing on cell biology ranges from changing amino acid sequences of proteins (recoding) to altering splicing patterns of pre-mRNA. Recent advancements in high-throughput sequencing techniques have shown that neurons are amongst the cells with the highest levels of RNA editing, with millions of A-to-I conversion events across the transcriptome. Aberrant RNA editing has been identified in various neurological disorders, for vastly unknown reasons and with unexplored consequences. Whether RNA editing is impaired in polyglutamine diseases is unknown.

Aims: The overarching aim of my DPhil project is to explore the role of RNA editing in the mechanisms of toxicity in polyglutamine diseases, using spinal and bulbar muscular atrophy (SBMA) as disease model. Specifically, I will test the working hypothesis that the transcriptomic dysregulation in polyglutamine disorders is disrupting the ADAR interactome, preventing efficient canonical editing.

Methods/Materials: Using deep transcriptomic data-sets from SBMA patient-derived iPS-motor neurons and diseased tissues, we profiled the RNA editing events in this disorder. Within the same data-sets, we are now looking at differentially expressed ADAR interactors. Moreover, we employed single-molecule tracking microscopy techniques to elucidate the dynamic behaviour of ADAR enzymes within neuronal cells in disease contexts.

Results: From the editome analysis, we observed reduced overall editing of the non-coding sequences, and differential editing of targets involved in neuronal survival in SBMA motor neurons. These results are coupled with evidence of altered enzyme dynamics and binding activity in presence of the polyQ androgen receptor.

Conclusion: With this work, we aim to unravel a previously unrecognised mechanism of pathogenesis in repeat expansion disorders, focusing on RNA editing impairment. Successful completion of this project will also help identify new therapeutic approaches, targeting at restoration of ADAR editing activity.

MND10

DNA damage is linked to TDP-43 pathology in ALS

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Background: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that precipitates motor neuron degeneration. A staggering 97% of ALS cases exhibit TDP-43 proteinopathy. The pathomechanisms underlying this disease have remained elusive due to the disease's heterogeneity and the inherent difficulties in accessing living human motor neurons.

Aims: This study aims to decipher the pathomechanisms of ALS by leveraging large biobanks of human induced pluripotent stem cell-derived motor neurons (iPSMNs) derived from individuals diagnosed with ALS. Previous iPSMNs studies in ALS have been constrained by small, underpowered cohorts. This study addresses this limitation by employing a larger and more diverse sample.

Methods: We have compiled a comprehensive compendium of 429 iPSMNs derived from 15 distinct datasets, complemented by 271 post-mortem spinal cord samples. These samples span 10 unique ALS mutations and sporadic ALS. We utilized reproducible bioinformatic workflows to analyze RNA-sequencing data from these samples.

Results: Our analysis unveiled a robust upregulation of p53 signalling across the ALS spectrum, evident in both iPSMNs and post-mortem spinal cord samples, and across genetic subtypes. Intriguingly, p53 activation was most pronounced with C9orf72 repeat expansions, but was notably weaker with SOD1 and FUS mutations. We also discovered that TDP-43 depletion potentiates p53 activation in both post-mortem neuronal nuclei and cell culture, thereby establishing a functional link between p53 activation and TDP-43 depletion. Additionally, ALS iPSMNs and post-mortem tissue exhibited an enrichment of splicing alterations, somatic mutations, and gene fusions.

Conclusions: These findings, derived from large-scale analyses, suggest that ALS motor neurons exhibit a heightened DNA damage response, potentially resulting from genomic instability, which may contribute to motor neuron death. This integrated transcriptomic study offers valuable insights into the pathomechanisms of ALS and unveils potential therapeutic strategies for tackling neurodegenerative diseases.

Neuromuscular Junction Disorders and Channelopathies

NMJ&C01‡

DOK7-AAV9 gene therapy in a novel mouse model for Congenital Myasthenic Syndrome caused by mutations in *CHRND*

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Congenital Myasthenic Syndromes (CMS) are genetic disorders of the neuromuscular junction (NMJ) characterised by fatigable muscle weakness. In CMS patients with mutations in the RAPSN gene, the mutations impair acetylcholine receptor (AChR) clustering at the motor endplate and cause reduced AChR surface expression. A CMS patient with clinical features of RAPSN CMS including muscle weakness and respiratory crises from birth was found to have compound heterozygous mutations in the AChR delta subunit. One of these was p.R396H in the cytoplasmic loop of the delta subunit. We have previously confirmed that AChR clustering in myotubes is impaired if AChR harbours this mutation. We further designed a dR399H C57BL/6 mouse model, provided by the MRC GEMM program and characterised the mouse model up to postnatal week 20 confirming it reflects many characteristics of congenital myasthenic syndrome. The muscle adapter protein Dok-7 is essential for amplifying and activating the receptor kinase MuSK which ultimately orchestrates the clustering and maintenance of AChR clusters. In this study we administered therapeutic dose of adeno-associated virus serotype 9 (AAV-9) vector encoding the human DOK7 gene to dR399H C57BL/6 mouse model at postnatal week 11 to mice that showed symptoms of fatigable muscle weakness tested by inverted screen test. This was compared to model mice treated with only 0.9% saline. Neuromuscular junction (NMJ) function was evaluated by weekly inverted screen tests, electromyography at age 6 and 20 weeks postnatal life, and ex-vivo electrophysiological recordings of hemidiaphragm-phrenic nerve preparations. NMJ morphology was assessed by fluorescent immunostaining followed by confocal microscopy at the end of the study. Administration of DOK7-AAV9 resulted in enlargement of NMJs, reversed the fatigable muscle weakness and improved decrement tested by repetitive nerve stimulation in approximately half of the mice. Positive ex-vivo electrophysiological features such as an increase in the amplitude of miniature endplate potentials and endplate potentials as well as increased quantal content was seen. These results suggest that DOK7-AAV9 gene therapy may be beneficial for a range of neuromuscular junction disorders that cause AChR clustering defects and the DOK7-AAV9 gene therapy may also be beneficial when administered later in life when symptoms are present.

NMJ&C02

Remote monitoring of cardiac risk in Andersen-Tawil Syndrome

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Background: Andersen-Tawil Syndrome (ATS) is a rare channelopathy typically characterised by periodic paralysis, cardiac arrhythmias and dysmorphic features caused by mutations in the KCNJ2 gene. There is a significant risk of cardiac morbidity and mortality making regular cardiac monitoring a crucial aspect of clinical assessment. Conventional methods are limited by short monitoring times, discomfort or invasive procedures and their complications. New technologies may overcome these limitations.

Aims: To determine if remote, hand-held, monitoring devices are useful for detecting cardiac arrhythmias and ECG changes in patients with ATS.

Methods: Suitable patients with ATS who attend the Skeletal Muscle Channelopathy clinic in our centre were given KardiaMobile devices (KMD), sample size was restricted to number of devices available.

During the 8-week study period, patients were asked to record an electrocardiogram (ECG) at home using the KMD while asymptomatic once per week and during any cardiac symptoms. Quantitative and qualitative analysis of all ECGs were completed. Where available, data was compared to already implanted cardiac devices.

Results: 11 patients completed the study, 6 had a severe cardiac phenotype. Average baseline QTc was prolonged. Subtle artefact in baseline ECGs hindered U wave identification. 18% of asymptomatic ECGs recorded arrythmias (ranging from PVCs to cycles of bigeminy or trigeminy). 7 symptomatic ECGs were returned with PVCs, bigeminy, trigeminy or irregular heart rate seen, however with some traces significantly obscured by artefact.

Conclusion: Remote, handheld cardiac monitoring devices may be useful in detecting prolonged QTc and asymptomatic arrythmias in patients with ATS. However, techniques/training to overcome artefactual symptomatic readings need to be considered and larger studies with direct comparison to conventional cardiac monitoring methods are needed.

NMJ&C03‡

A remarkable response to a new treatment in a patient with refractory myasthenia gravis: a case report with video recordings

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Background: Myasthenia gravis (MG) is an autoimmune disorder that can present at any age and cause a variety of symptoms. Patients with MG with acetylcholine receptor antibodies (AChR Ab) have their disease driven by IgG1 and IgG3 antibody subclasses. These cause their pathology in part by activation of the complement system. New therapies such as complement inhibitors target this pathway specifically and offer a new approach to treatment in patients who may not respond to conventional therapy. Here, we present a case of a young female with refractory myasthenia gravis who had an excellent respond to a daily subcutaneous form of C5-inhibitor.

Case Report: A 12 year-old female was diagnosed with generalised myasthenia gravis with acetylcholine receptor antibodies and no thymoma. She was treated aggressively with conventional therapies including prednisolone, mycophenolate mofetil, rituximab and ciclosporin but showed little response and remained profoundly disabled, dependent for all activities of daily living and unable to attend school or leave home. Her only partially effective treatment was plasma exchange weekly, which was required to prevent respiratory failure. In addition, she experienced numerous severe adverse events from her treatment which themselves required treatment. At the age of 21 she was enrolled into a clinical trial for the C5 complement inhibitor. Once unblinded and on the drug, she displayed a remarkable response with a marked improvement in her symptoms within 2 weeks. After 2 years on the C5 inhibitor, she is now asymptomatic, fully independent and active, and on no other treatment for her MG. She has suffered no adverse effects from this treatment.

Conclusion: This case demonstrates the importance of new therapies in MG, and their potential use in refractory cases or those where conventional therapies are not tolerated. Ensuring that these new therapies are available to patients can prevent long-standing disability, adverse events and suffering.

NMJ&C04

Widespread Immune Dysfunction in Refractory Myasthenia Gravis

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Background: Many patients with Myasthenia Gravis (MG) are refractory to standard immunosuppressive therapy. In addition to not fully understanding the mechanisms behind refractory disease, there is a lack of biomarkers to predict treatment outcome to help guide treatment decisions.

Aims: We aimed to examine the circulating profile of those with MG of differing severity and treatment requirements to look for markers associated with refractory disease.

Methods/Materials: Peripheral blood mononuclear cells and plasma were isolated from whole blood of 15 healthy individuals and 37 individuals with acetylcholine-receptor antibody positive MG or differing treatment requirements. Flow cytometry was used to determine cell frequencies and phenotypes. LEGENDPLEX assays were used to determine concentration of soluble molecules, and ELISA for the concentration of complement proteins.

Results: Refractory patients demonstrate expansion of memory B cells, mainly switched memory B cells expressing IgG, mirrored by a reduction in mature B cells. Memory B cells are higher in those with early-onset compared to late-onset MG. Preliminary stimulation studies have identified higher production of cytokines TNF-α, IL-6 and IL-10 from B cells in MG compared to control. Regulatory T cell (Treg) frequencies, along with soluble CD25 levels, are lower in refractory cases, and correlate negatively with disease severity and quality of life scores. Dendritic cell (DC) frequencies are also reduced in refractory cases, whereas monocytes are expanded. Circulating levels of complement proteins C3, C5 and clusterin are higher in refractory cases. Additionally, there is higher expression of complement receptors CD21 and CD35 on B cells, and CD55 (DAF), CD46 (MCP) and CD59 (protectin) on both CD4 and CD8 T cells in patients compared to controls, particularly in refractory cases.

Conclusion: A broad immune dysfunction in patients with refractory MG has been identified, more so in those with early-onset MG, which existing therapies are not designed to fully address. Memory B cell, Treg and DC frequencies, as well as complement proteins and receptors, appear to be markers of refractory disease. Further work is required to determine if these findings could predict refractory disease at baseline. Therapies targeting Treg expansion may be helpful in refractory MG.

NMJ&C05

A UK experience of symptomatic treatment of myotonia with Lamotrigine

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Background: Lamotrigine has recently been shown to be effective for symptomatic treatment for non-dystrophic myotonia; little data on real-world clinical experience exists.

Aims: To report our clinical experience of using Lamotrigine for treatment using the Myotonia-Behaviour-Score (MBS) in patients with myotonia.

Methods: We retrospectively evaluated the MBS from a UK single-centre of patients attending the Nationally Commissioned Highly Specialised Service for Channelopathies. The MBS was collected at pre treatment, six months follow up and after the highest dose increase was reached.

Results: Out of 26 patients on Lamotrigine, 12 were evaluated to date. Of those half (6) had *CLCN1* mutations and other half (6) *SCN4A* mutations, with mean (SD) age of 43.4 (15.6) years. Mean reduction in MBS of seven patients after six months of treatment was from 3.6 to 1.7, which was statistically significant (p=0.0176). There was no significant difference in MBS reduction in five patients with average treatment duration of 2.4 (± 1.3) (range 1-3.7) years after the highest dose increase (range 3 to 1.8, p=0.2). One patient experienced nausea, which ceased after stopping the medication.

Conclusions: These preliminary data suggest that treatment with Lamotrigine effectively reduce myotonia in selected patients with non-dystrophic myotonia. Further data analysis is ongoing.

NMJ&C06‡

Efficacy and Safety of anti FcRN treatment in Refractory MG - the UK experience of the Efgartigimod Early Access to Medicine Scheme

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Background: The landscape of treatment for generalised Myasthenia Gravis (gMG) has changed in recent years with new treatment targets becoming available. One such target is the neonatal Fc receptor (FcRN). We report our experience of patients with generalised MG (gMG) treated with Efgartigimod an FCRN antagonist, under the early access to medicine scheme (EAMS) in the UK.

Methods: Data from all UK patients treated with Efgartigimod under the EAMS June 22 -July 23 were collected retrospectively. Efgartigimod was administered as per the ADAPT protocol (consisting of a treatment cycle of 4 infusions at weekly intervals with further cycles given according to clinical need).

Results: 49 patients with AchR antibody positive gMG were treated in 12 centres. Most (76%) were female and most had a disease duration of over 10 years. The average MG-ADL score at baseline was 11.2 (SD 3.2). Most (73%) patients had undergone thymectomy in the past (mean time since thymectomy 12.5 yrs). 71.4% were taking prednisolone at baseline. All patients had utilized non-steroidal immunosuppressants treatments (NSITs), the average number of NSITS tried was 4.3 (range 1-7) and 51% had received Rituximab. 57% of patients required regular IVIg/PLEX and 38% had required rescue IVIg/PLEX in the year before starting Efgartigimod. 77% of patients had a mean reduction in the MG-ADL of ≥2 points in the first cycle and this remained stable throughout the study. The mean reduction in the MG-ADL score in the 1st, 2nd, 3rd and 4th cycles were -4.5, -6.3, -6.9 and -7.8 respectively. Side effects were generally mild though one patient stopped treatment due to severe hypokalemia. No rescue treatments were required. At the end of the study period, 96% of patients remained on Efgartigimod.

Conclusion: Efgartigimod is a safe and effective treatment for patients with refractory, treatment resistant gMG.

NMJ&C07

Real world experience of Efgartigimod in a single UK centre – 16 months of the Early Access Scheme

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Background: We present our experience of treating patients with generalised Myasthenia Gravis (gMG) with the neonatal Fc receptor (FcRN) antagonist Efgartigimod under the UK Early Access to Medicine Scheme (EAMs) in single centre over a 16 month period.

Methods: Data regarding all patients receiving Efgartimod in the National Hospital for Neurology and Neurosurgery were collected prospectively. Efgartigmod was given as per the ADAPT protocol (a cycle of 4 weekly infusions with further infusions given depending on symptoms). Response to Efgartigmod was measured with MG-ADL scores, change in prednisolone dose and need for rescue therapies

Results: 18 patients with gMG were treated with Efgartigimod over the 17 month period, 12 were female and the average disease duration was 14.6 years (range 1-40). 66% had required IVIG/PLEX regularly and 22% had required intermittent rescue treatment prior to starting Efgartigimod. All patients had previously received prednisolone and non-steroidal immunosuppressant therapies (NSITs). The average NSITs tried was 2.4. 50% had received Rituximab. The mean MG-ADL at baseline was 11.3. Following completion of the first treatment cycle 89% were defined as MG-ADL responders (a reduction of at least 2 points) with an average reduction of 7 points. Two patients stopped taking Efgartigimod due to lack of efficacy. 89% remained on it with a mean inter-cycle interval of 6.5 weeks. 83 % of patients reduced their steroid doses. Rescue IVIG and PLEX were required in the two patients that stopped treatment but otherwise no rescue treatments were required Efgartigimod was well tolerated with only minor side effects reported

Conclusion: Efartigimod is an effective and well tolerated treatment in patients with refractory gMG

NMJ&C08

Efficacy, safety, and factors predicting response in Rituximab therapy for generalised Myasthenia Gravis: A single centre study

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Background: Up to one third of patients with generalised Myathenia Gravis gMG) have unsatisfactory symptom control. Rituximab (RTX) is an anti CD20 monoclonal antibody approved for use in refractory gMG.

Aims: Our objective was to assess the efficacy and safety of RTX in the treatment of gMG and to assess factors that predict response.

Methods/Materials: This is a retrospective observational study from a single specialist centre. We analysed the case notes of patients with gMG treated with rituximab from 2019 to 2023. Treatment was deemed successful if, at the end of six months, patients experienced a) a significant reduction of at least 2 points in MG Activities of Daily Living (ADL) or 3 points in MG composite scale, or b) if at the end of one year – the patient did not need further treatment escalation to another agent or c) had been at least partially successful in reducing the dose of either prednisolone or concurrent Intravenous immunoglobulin.

Results: A total of 32 patients were included in the study. Six (18.8%) were male and 26 (81.2%) were female The mean age at start of treatment was 46.8 years (range 25 – 76). Eighteen patients (56.2%) were acetyl-choline receptor antibody positive (AchR) and thirteen were muscle-specific kinase (MuSK) antibody positive. One patient was double seropositive. The mean duration of disease was 13.2 (+/-10) years prior to commencing RTX (range 1 to 47). Patients had previously received an average of 4 other immunosuppressive agents (range 1-7). Twelve patients had undergone thymectomy. Overall, 20 (62.5%) patients responded to treatment. 10 (76.9%) of MuSK patients responded compared to 9 (50%) of AchR positive patients. In those that responded there was no significant correlation to age, previous thymectomy or time from diagnosis to treatment - though only two patients were treated within 12 months of disease onset. One patient who had medical comorbidities died of Covid19 two months after receiving Rituximab.

Conclusion: Rituximab is effective for the treatment of MuSK positive gMG but its effect in AchR positive gMG is variable.

Mitochondrial Disease

MD01

A comparative analysis of guidelines in the interpretation of sequence variants identified in *POLG*

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Background: Over the last two decades, much effort has been made to produce comprehensive guidance to interpret genetic variants. Two types of guidance are available. First, general non-disease/gene specific such as the American College of Medical Genetics and Genomics /Association for Molecular Pathology and the Association for Clinical Genomic Science in the United Kingdom. Second, disease/gene specific guidance. Mitochondrial disease is one area for which specific guidance was developed. However, how the current published variant guidelines compare is uncertain.

Aims: To compare the performances of the two widely used general and one gene-specific variant interpretation guidelines in a cohort of individuals identified to harbour sequence variants in *POLG*.

Methods/Materials: The three variant interpretation guidelines were used to classify all *POLG* variants identified by the NHSE Highly Specialised Rare Mitochondrial Disorders Service Oxford over a period of six months. Cases were identified through searches of in-house databases. There were no age, gender, or diagnostic exclusion criteria applied, but well-known benign and likely benign variants in *POLG* were filtered out as part of routine bioinformatic analysis and so were not included in the study.

Results: Twenty-one *POLG* variants identified in 15 individuals were included in our study. Nine (60 %) of individuals harboured a single variant. Variants comprised 17 (81 %) single nucleotide variants and four (19 %) insertion-deletion variants. The most frequent variant classification was 'variant of uncertain significance' and differences in variant classification were identified for two (10 %) variants.

Conclusion: We found a small but clinically relevant difference between the variant interpretation guidelines examined and identified an absence of data available for classification of variants. To avoid patients and families being left in diagnostic limbo, more needs to be done to facilitate variant assessment.

MD02

Mitochondrial Cristae Disruption and Cytosolic DNA Sensing in Inclusion Body Myositis

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Background: Inclusion body myositis (IBM) is an inflammatory myopathy with distinct mitochondrial abnormalities. IBM is typically a sporadic disease with a late-onset, typically manifesting in patients over 50 years old. Previous research has shown IBM is associated with mitochondrial abnormalities including mitochondrial DNA (mtDNA) deletions, disrupted cristae organisation , and COX- fibres. Additionally, polymyositis with mitochondrial pathology (PM-Mito) has been proposed a potential precursory stage to IBM, further suggesting a relationship between mitochondrial dysfunction and IBM. Although it is not fully understood how the mitochondrial changes observed in PM-Mito and IBM correlate with the disease progression, recent evidence suggests that cristae disruption is associated with mtDNA release and inflammation.

Aims: To further characterise how changes in mitochondrial cristae organisation underlie the progression of PM-Mito and IBM and lead to the inflammatory changes associated with the disease.

Methods/Materials: Muscle tissue samples from control, PM-Mito, and IBM patients were collected and assessed for changes in key mitochondrial and inflammatory proteins.

Results: Patients with PM-Mito display early changes in mitochondrial dysfunction, including deficient ATP synthase and depletion of cristae organisational protein MIC19, which are mirrored in IBM patients. Activation of the cGAS/STING and inflammatory pathways is primarily evident in IBM patients.

Conclusion: Mitochondrial changes underlying IBM likely occur early in disease progression, as seen already by in PM-Mito. However, main activation of inflammatory pathways primarily occurs in the later-stage IBM patients. This preliminary evidence supports further work into understanding the molecular differences between PM-Mito and IBM patients in effort to identify the trigger for the inflammatory changes observed in IBM.

MD03‡

In vitro 3D-model of mitochondrial myopathy human skeletal muscle

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Background: Mitochondrial myopathy is one of the most common neuromuscular disorders for which no cure is currently available. Performing mechanistic study and screening compound libraries on myoblast cultures is not time and cost effective, since myoblasts are structurally and metabolically distinct from mature muscle fibres.

Aims: The aim of this study is to develop functional *in vitro* 3D-models of mitochondrial myopathy human SKM (skeletal muscle) that could be applied for the search of a suitable compound to treat mitochondrial myopathy patients.

Methods/Materials: We used primary myoblasts obtained from mitochondrial myopathy patients carrying nuclear variants in *TWNK*, *RRM2B* and *SURF1* genes. After differentiation into myotubes, the characterization of the *in vitro* 3D-models of mitochondrial myopathy included the assessment of SKM structure by immunofluorescence and contraction and Ca^{2+} (calcium) dynamics induced by electric pulse stimulation.

Results: The generated *in vitro* 3D-models present different SKM structure. *RRM2B* myoblasts are not able to differentiate, therefore the SKM specific marker SAA (sarcomeric alpha-actinin) is not expressed in the 3D-model, oppositely to *TWNK* and *SURF1* 3D-models, which both present good SKM directionality (d=0.76 and d=0.93, respectively) and express high SAA level. Moreover, *TWNK* and *SURF1* 3D-models functionally contract when electrically pulse stimulated, although displaying different SKM Ca^{2+} dynamics compared to healthy control 3D-muscles.

Conclusion: We generated functional and contractile *in vitro* 3D-models of mitochondrial myopathy human SKM using primary cells from patients carrying nuclear variants. In the future, primary cells from patients carrying mtDNA (mitochondrial DNA) variants will be used. Overall, the application of such an *in vitro* 3D-model will accelerate the search of a suitable cure for mitochondrial myopathy patients.

MD04

Patient satisfaction following Phase I and Phase II/III primary mitochondrial myopathy trials

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Background: Primary mitochondrial myopathies (PMMs) are emerging as a major target for drug development. However, inherent challenges to trial design in this group of rare disease remain. Increasingly, patient preference concerning symptom management is used to inform trial design. Nevertheless, there is limited data for patient-reported experience during participation in PMM drug studies.

Aim: To explore patient satisfaction during Phase I and Phase II/III clinical trials in PMMs.

Methods: Data was collected from people with PMMs who had previously participated in Phase I and Phase II/III clinical trials at The National Hospital for Neurology and Neurosurgery, using a patient-administered survey with the Likert scale 0-10.

Results: Seventeen participants responded. Mean age was 55.9 years The main reason provided for joining a trial was to improve health outcomes in others The least important factor was receiving compensation for participating. The most burdensome factor was traveling to site while the least burdensome was too much contact with the study team. Seventy one per cent of participants considered questionnaires, and 65% thought assessments, were relevant to PMMs. Weekend visits were suggested to improve accessibility compared with home/remote visits. Nineteen per cent of participants received information about publications and 22% had received information on whether they had the drug or placebo post-trial.

Conclusion: Improved accessibility could potentially enable a more diverse PMM population to participate in clinical trials. There is also an opportunity for assessments and questionnaires to be more relevant to participants with PMM. Nevertheless, overall satisfaction was rated high for trial visits.

MD05

Elucidating CHCHD10 Mutant Phenotypes in Inducible Cell Models and Designing Effective Therapeutic Strategies

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Background: Mutations in the mitochondrial protein CHCHD10 cause autosomal dominant neuromuscular disorders including frontotemporal dementia (FTD)/ALS, mitochondrial myopathy, lower motor neuronopathy (SMAJ, spinal muscular atrophy Jokela type), and a familial form of ALS. These mutations have a strict genotype-phenotype relationship, with the p.G58R mutation causing a pure myopathy and the p.G66V mutation causing a pure lower motor neuronopathy. The molecular basis for this genotype-phenotype relationship is not well understood, and the current model of pathogenicity is toxic gain-of-function, as CHCHD10 knock-in mice but not CHCHD10 KO mice recapitulate the myopathy phenotype seen in patients with the p.G58R or p.S59L variants, as demonstrated by our lab and others.

Aims: To investigate the genotype-phenotype relationship among CHCHD10 mutations and develop an effective ASO therapy targeting diseased tissues (i.e. skeletal muscle and motor neurons).

Methods/Materials: In Vitro cell culture lines, including patient iPSCs (induced pluripotent stem cells) to model mitochondrial disease and to test ASOs. Western blotting and qRT-PCR for protein and RNA analyses.

Results: We are using transcription factor mediated differentiation of human wild type iPSCs into motor neurons and myocytes. After inducing mitochondrial stress, we measured the transcriptional signature of these cells. Using a CRISPR-Cas9-based approach, we've generated an allelic series of iPS cells with the homozygous pathogenic CHCHD10 mutations p.R15L, p.S59L, p.G58R, and p.G66V, as well as CHCHD10 knock-out (KO), that we will differentiate into motor neurons and myocytes to assess for phenotypic differences among mutant lines. Finally, we are designing non-allele-specific antisense oligonucleotides (ASOs) targeting *CHCHD10* mRNA. We have screened and identified several ASO candi-

dates that are effective in knocking down CHCHD10.

Conclusions: Mutant CHCHD10 leads to dominant inheritance of neuromuscular diseases, including mitochondrial myopathy and adult onset SMA, by toxic gain-of-function. The mechanism leading to a strict genotype-phenotype relationship in these CHCHD10 disorders is unknown. By differentiating human iPSCs into relevant cell types of CHCHD10 neuromuscular diseases, such as motor neurons and myocytes, we are determining these cells' unique disease phenotype and use these to develop effective ASOs to target CHCHD10.

MD06

Bi-allelic variants in *COXFA4* are associated with isolated cytochrome *c* oxidase deficiency and paediatric mitochondrial disease

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Background: Cytochrome *c* oxidase (COX; complex IV) is the terminal enzyme of the mitochondrial respiratory chain. COX comprises 14 protein subunits which originate from both the mitochondrial and nuclear genome and the modular assembly of the holocomplex relies upon an intricate process requiring a number of distinct assembly factors. COXFA4, previously NDUFA4, was recently reassigned as a structural subunit of COX following the description of a homozygous, loss-of-function *COXFA4* consensus splice variant causing human COX deficiency in a single family (**PMID: 23746447**). Here we present two further unrelated families, each with an affected proband presenting with complex IV deficiency and harbouring homozygous splice variants in the *COXFA4* gene.

Aims: To characterise the clinical and functional nature of homozygous splice variants in *COXFA4* leading to human COX deficiency and mitochondrial disease phenotypes.

Methods/Materials: Steady-state levels of COXFA4 protein and structural components of the oxidative phosphorylation (OXPHOS) complexes in patient-derived fibroblasts and muscle were compared to those of healthy age-matched controls utilising SDS-PAGE and immunoblotting. Blue-Native (BN)-PAGE and high-resolution MS-based complexome profiling were applied to patient cell lines to assess complex IV assembly.

Results and conclusions: Whilst results remain at a preliminary stage, the muscle biopsy from one patient has revealed a significant COX deficiency, a finding confirmed by direct enzyme assay of patient fibroblast mitochondria. SDS-PAGE and western blotting has demonstrated a significant reduction, likely a complete loss, in the steady-state levels of COXFA4 protein with BN-PAGE confirming a reduction in holoenzyme assembly; ongoing proteomic experiments will determine the consequences of rare COXFA4 splice variants in patient cells. Alongside providing diagnostic clarity for these two families, this work aims to generate insight into the role of COXFA4 in human COX deficiency and hence add to the literature regarding the reassignment of COXFA4 as an essential subunit of this important OXPHOS component.

MD07‡

Uplift in diagnosis after renanalysis of WES data in a 10-year-old cohort of patients with suspected mitochondrial disorders

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Background: The genetic diagnosis of mitochondrial disorders is complicated by their genetic and phenotypic complexity. Next-generation sequencing techniques have much improved the diagnostic yield for these conditions but a molecular diagnosis is still not reached in around 40% of patients. The challenges in diagnosis include the vast number of genes involved in mitochondrial function and metabolism, the phenotypic diversity of these disorders and the phenotypic overlap with other nonmitochondrial disorders. Reanalysis of existing exome data has been shown to increase diagnostic yield in cohorts of patients with rare disease.

Aims: We reanalysed the whole exome sequencing (WES) data for a 10-year-old cohort of individuals with multiple respiratory chain deficiencies previously reported by Taylor et al., 2014 (JAMA). We aimed to establish a genetic diagnosis in the undiagnosed branch of this cohort.

Methods/ Materials: Where patient consent was obtained, raw WES data was transferred to and processed by the RD-Connect Genome-Phenome Analysis Platform (GPAP). Variant prioritisation was carried out using the RD-Connect GPAP.

Results: Singleton WES data from 14 individuals was reanalysed and a possible or likely genetic diagnosis was found in 8 (57%). The variants identified included mitochondrial DNA point mutations (n=1, *MT-TN*), nuclear encoded mitochondrial genes (n=2, *PDHA1,* and *SUCL*A2) and nonmitochondrial nuclear genes (n=5, *PNPLA2, CDC40, NBAS* and *SLC7A7*) further highlighting the advantages of untargeted exome wide analysis. We increased the diagnostic yield for the original cohort by 15% without generating any further genomic data.

Conclusions: In the era of multiomics we highlight that reanalysis of existing WES data continues to be a valid tool for generating additional diagnoses, particularly when enough time has passed to allow for improved bioinformatic pipelines, new tools in variant interpretation and emergence of new genes. Importantly, five of the possible diagnosis in our cohort were in nonmitochondrial disease genes, which is an important consideration for clinicians looking after patients with genetically undiagnosed mitochondrial disorders.

MD08

The role of magnetic resonance imaging and spectroscopy biomarkers in primary mitochondrial myopathies

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Background: Primary mitochondrial myopathies (PMM) are genetic disorders with defects of the oxidative phosphorylation affecting predominantly the skeletal muscles. Currently, there are no disease modifying therapies for PMM. One major difficulty to clinical trials in PMM is the lack of reliable and reproducible biomarker that can catch the disease severity and progression.

Aims: This is the first study aiming to examine the validity of MRI quantified muscle volume, fat fraction, and 31 phosphorous magnetic resonance spectroscopy (31P-MRS) of thigh muscles before, during, and after a knee extension/flexion exercise with tailored ankle weight, as outcome measures with a direct correlation of clinical and functional measures used in PMM.

Methods/Materials: We did a prospective observational cohort study of patients with genetically confirmed PMM. Age and sex-matched healthy subjects (HS) are also recruited. Assessments are done at baseline and 12 months. Correlations between MRI outcomes and clinically relevant outcome measures are performed. The MRI session consists in quantitative MRI (volume, fat fraction, diffusion) of the thighs bilaterally, and 31P-MRS of the left thigh. Assessment includes measures of functional performance, clinician/patient reported scales of muscle involvement and fatigue, and blood tests.

Results: Only baseline results are reported (follow-up is still ongoing). Twenty adults with PMM (10 m.3243A>G and 10 single deletion of mitochondrial DNA) were recruited, 6 females and 5 males in each group, mean ages of 51.2±9.3 and 48.2±14.4 years, respectively. They were matched with 10 HS, with mean age of 47.9±14.3 years. All patients had fatigue. A direct correlation between age and fat fraction in thigh muscles in PMM patients was present. Muscle strength was reduced in those with increased fat fraction. The vastus lateralis 31P-MRS showed a more profound normalised phosphocreatine signal reduction during knee extension exercise, and a slower recovery time compared to HC. Peak torque, 12-minute-walk test, 30-second sit to stand test, and fatigue scale show a more severe impairment in the m.3243A>G group compared to the single deletion group.

Conclusion: Muscle involvement is heterogeneous within the PMM group. MRI and 31P-MRS might be valuable biomarkers in PMM and might be useful in better characterise participants for clinical trials.

MD09‡

Investigating mitochondrial alanyl-tRNA synthetase defects in neurons

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Background: Mutations in mitochondrial aminoacyl-tRNA synthetases (MT-ARS) result in a spectrum of severe tissue-specific diseases. Healthy cells rely on MT-ARS to facilitate mitochondrial protein translation by charging mitochondrial tRNAs with cognate amino acids. Although, defects in mitochondrial translation may impair oxidative phosphorylation (OXPHOS), the molecular mechanisms and the clinical heterogeneity of MT-ARS defects remain poorly understood.

Aims: We investigated the molecular mechanisms underlying the neurological phenotypes in mitochondrial diseases resulting from distinct AARS2 defects.

Methods/Materials: We generated a novel patient-derived in vitro model of neuronal cells carrying specific variants of AARS2. We applied cell reprogramming techniques to generate neuronal progenitor cells (NPC) and cortical neurons from iPSC carrying a homozygous mutation in the *AARS2* gene (c.1774C>T; p. R592W). We obtained transcriptomic data from the mutant NPCs and neurons. We characterised defects in mitochondrial respiratory chain using Western blotting and the Seahorse XFe96 Extracellular Flux Analyzer to further investigate differences in mitochondrial respiratory function. A click-chemistry approach was used to quantify the effect of MT-ARS mutations on protein synthesis.

Results: Transcriptomic analysis of our model shows that AARS2$^{R592W+/+}$ NPCs are primarily enriched with GO terms linked to cell differentiation and development, whereas neurons predominantly exhibit GO terms associated with neuronal development. Western blotting revealed a selective reduction in the steady state levels of OXPHOS proteins. Seahorse analysis showed mitochondrial respiratory impairments in the AARS2$^{R592W+/+}$ NPCs. Quantification of mitochondrial translation in AARS2$^{R592W+/+}$ neurons revealed two distinct mitochondrial populations with impaired rates of protein translation.

Conclusions: Our model offers a novel strategy for reproducing the neurological phenotypes resulting from MT-ARS defects. We suggest that different types of mitochondrial dysfunction trigger distinct stress responses which activate specific compensatory mechanisms depending on the MT-ARS defect leading to impaired neuronal differentiation. Understanding the pathological and compensatory pathways is essential to understand the pathogenic mechanism and tissue-specificity of MT-ARS defects.

MD10

A novel case of adult onset autosomal recessive progressive external ophthalmoplegia with mtDNA deletions caused by a homozygous variant in the Twinkle mtDNA helicase

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Background: Disorders of mitochondrial DNA (mtDNA) maintenance are caused by pathogenic variants in nuclear genes that function in mtDNA replication and repair. Twinkle (*TWNK*) encodes the mtDNA helicase which is required for mtDNA replication. Monoallelic pathogenic variants in *TWNK* are known to cause adult onset progressive external ophthalmoplegia (PEO) with mtDNA deletions. Biallelic pathogenic *TWNK* variants have previously been associated with childhood onset disorders including hepatocerebral mtDNA depletion syndrome, infantile-onset spinocerebellar ataxia (IOSCA) and Perrault syndrome (characterized by sensorineural hearing loss and ovarian dysfunction).

Aims: To identify the genetic diagnosis in an adult female with PEO and a suspected disorder of mtDNA maintenance.

Methods/Materials: Comprehensive genetic analysis was carried out including analysis of a large panel of nuclear encoded genes associated with mitochondrial disorders, whole mitochondrial genome sequencing and analysis for mtDNA rearrangements in muscle DNA. Extensive clinical assessment of the proband was performed, including brain MRI and muscle histology. Available first and second degree family members were also clinically examined and underwent genetic testing.

Results: A homozygous *TWNK* variant (NM_021830.5:c.904C>T p.(Arg302Trp)) was detected. Analysis of a muscle biopsy showed early ragged red fibres and a couple of rare cytox negative / cytox pale fibres. Multiple mtDNA deletions were also detected in muscle DNA. Assessment using ACMG criteria resulted in classification of the *TWNK* variant as likely pathogenic in association with autosomal recessive disease. Molecular modelling also supported a deleterious effect on protein function. Genetic analysis in other family members without PEO identified that the proband's sister, daughter, and granddaughter were all heterozygous for the *TWNK* variant.

Conclusion: We believe that this is the first description of adult onset autosomal recessive PEO caused by biallelic *TWNK* variants.

MD11‡

Defective Mitochondria Induce Quantitative Easing of Cholesterol Leading to Neurological Disease

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Background: Many brain disorders are caused by defects in cholesterol metabolism or impaired mitochondrial energy production. In 2017, we discovered that defective mitochondria in the form of ATAD3 dysfunction drastically alter cholesterol metabolism, thereby linking these two important areas of biomedicine. Given the scarcity of cholesterol in mitochondrial membranes, no one anticipated such an outcome from a mutant mitochondrial protein. However, the causes and consequences of the cholesterol changes owing to mitochondrial dysfunction were obscure.

Aims: To further our understanding of the pathogenesis of ATAD3-related disease and to determine the impact of cholesterol perturbation on cell metabolism.

Methods: As models, we used fibroblasts derived from patients with different genetic alterations in ATAD3 gene cluster and a fly carrying an orthologous pathological mutation. To study the cholesterol metabolism, we employed, among others, a fluorescently tagged cholesterol-binding domain as a reporter of membrane–bound cholesterol in vivo.

Results: Cholesterol perturbation is a conserved feature of pathological *ATAD3* variants and when mitochondria have difficulty obtaining cholesterol the entire cell increases cholesterol levels. This leads to an expanded lysosome population containing membrane whorls characteristic of lysosomal storage diseases. Additionally, using nutrient restriction and cholesterol supplementation, we show that the *Drosophila Atad3* mutant displays heightened cholesterol dependence. Together, these findings suggest a disease cascade in which elevated cholesterol enhances the cell's tolerance of pathological *ATAD3* variants, at a cost of inducing cholesterol aggregation in membranes, which lysosomal clearance only partly mitigates.

Conclusion: Our studies demonstrate the essential role of cholesterol in mitochondria, and show that mitochondria can, if necessary, reconfigure cellular cholesterol homeostasis. Thus, when the organelles have difficulty obtaining sufficient cholesterol, cholesterol levels are increased in mitigation. While the process ensures the mitochondria obtain the cholesterol they need, this comes at the expense of cholesterol aggregation in membranes, which then clog up the lysosomes- the organelle central to the cell's recycling machinery. The findings highlight the im-

portance of studying cholesterol and mitochondrial metabolism together, across the full spectrum of neurological and neurodegenerative diseases, to unveil disease pathogenesis and identify new treatments to implement or avoid.

MD12

Quantitative proteomics of patient fibroblasts reveal biomarkers and diagnostic signatures of mitochondrial disease

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Background: Mitochondrial disease encompasses a wide spectrum of disorders arising from genetic defects in both the nuclear and mitochondrial genomes that impact mitochondrial bioenergetics; manifesting diverse clinical presentations. This genetic and clinical heterogeneity has often precluded rapid diagnosis, and despite advances in next generation sequencing technologies, many patients remain genetically undiagnosed. Recent advances in mass spectrometry allow for the quantitative detection of large numbers of proteins, potentially adding an additional layer to the diagnostic pathway of mitochondrial disease. Recent reports integrating multi-omics approaches into healthcare have demonstrated the feasibility of this strategy, increasing understanding of mitochondrial biology and disease mechanisms with further applications implicated in the monitoring and validation of treatment strategies.

Aims: We aimed to use mass spectrometry-based proteomics to improve patient diagnostics, correlate changes in metabolic pathways to specific disease groups and validate variants of unknown significance (VUS).

Methods/Materials: A cohort of 61 patients harbouring nuclear-encoded variants affecting five, distinct elements of mitochondrial biology, were identified and investigated. Clinical presentations were structured according to specific human phenotype ontology (HPO) terms, and mass spectrometry-based label-free quantification (LFQ) proteomics was performed in patient-derived primary fibroblast cultures. Additionally, we tested cells from 6 patients harbouring VUSs to examine a role for proteomics in identifying additional diagnoses.

Results: We demonstrate that fibroblast proteomics can classify patients according to their biochemical and genetic characteristics, with differential expression analysis identifying several proteins - the expression levels of which - correlate with the disease cohort, and thus, represent putative biomarkers. Pathway analysis further revealed the deregulation of pathways associated with inflammatory and mitochondrial stress responses, presenting potential contributions to disease mechanism. This included the upregulation of glycosphingolipid metabolism and mitochondrial protein import, as well as the downregulation of arachidonic acid metabolism. Together, these results reveal a general deregulation of inflammatory and mitochondrial stress responses, with both pro- and anti-inflammatory factors exhibiting changes in fibroblasts from patients with mitochondrial dysfunction.

Conclusions: Our results establish quantitative mass spectrometry-based proteomics as a viable and versatile tool for the diagnosis of mitochondrial disease using patient fibroblasts, highlighting the potential of proteomics to understand mechanisms of mitochondrial disease.

Other Diseases

OD01

IFNγ promotes satellite cell senescence and myofibre atrophy via JAK/STAT1 in post-lesional myogenesis: Implications in inclusion body myositis

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Background: Idiopathic inflammatory myopathies (IIM) form a heterogeneous group of muscle-degenerating disorders that remain poorly understood. Typical histological features include focal myonecrosis, chronic leukocyte infiltrate, and abnormal MHC expression. IIMs are affected by distinct signatures of inflammatory cytokines.

Aims: To investigate the link between MHC expression, inflammation, and muscle lesions in IIM, focusing on the potential role of IFNγ in inclusion body myositis (IBM).

Methods/Materials: Muscle biopsies from IIM patients were analysed by transcriptomics and immunohistochemistry. Muscle tissue clearing was used to improve MHC imaging. *In vitro,* mouse-isolated myofibres and human satellite cells were extracted and cultured for cell treatment. *In vivo,* wild-type mice with myoinjury were grafted with an ALZET osmotic pump chronically releasing IFNγ.

Results: Anti-synthetase syndrome (ASS) and inclusion body myositis (IBM) displayed the upregulation of both IFNγ and cell senescence signalling. Notably, IFNγ expression significantly correlated with myofibre atrophy in ASS and IBM muscle biopsies. IFNγ delivery elicited myofibre atrophy and scar tissue deposition. The mechanism of action of the IFNγ-induced sarcopenia was investigated *in vitro* in cultured human myoblasts and *ex vivo* in mouse-isolated myofibres. IFNγ stimulation dramatically impaired MuSCs activation, proliferation, fusion, and promoted cell senescence. *In vitro* and *in vivo*, Ruxolitinib, an antagonist of JAK1/2 counteracted the effects of IFNγ on muscle atrophy and premature senescence.

Conclusion: Our study provides multiple lines of evidence of the causative role of IFNγ in muscle atrophy affecting ASS and IBM patients, suggesting that the JAK1/2 pathways may represent a new therapeutic target to reduce sarcopenia.

OD02

Using Phage Display to Identify Peptides for Targeted Antisense Oligonucleotide Delivery to Muscle Interstitial Fibroblasts

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Background: Collagen 6 (COL6) is a major component of skeletal muscles extracellular matrix. COL6-related congenital muscular dystrophies (COL6-CMD) are genetic disorders where mutations affect the ability of collagen 6 subunits to form tetramers. This causes gradual muscle degeneration and weakness, with early death in severe cases. Currently, no cures are available, but antisense oligonucleotide (ASO) therapies show great promise. ASOs are nucleic acid-based therapies, targeting RNA through Watson-Crick base pairing for degradation or splice switching, restoring functional protein. We have previously provided strong *in-vitro* evidence and proof-of-concept regarding ASOs as a therapeutic approach to COL6-CMDs. However, efficient uptake of ASOs in target cells (muscle interstitial fibroblast, MIFs) is crucial for clinical application. *In vivo* data indicates poor uptake in MIFs using naked ASOs. To address this, we are actively working on developing ASO targeted delivery using ASO-peptides conjugates preferentially binding and internalized in fibroblasts and not other cell types such as myoblasts, hepatocytes (liver), podocytes (kidney) etc. Here we use Phage Display to identify peptides for use in ASO conjugation, allowing specific internalisation in fibroblasts, while avoiding undesirable cell types.

Aims: To use peptide phage display to identify peptides with preferential binding and internalisation in fibroblasts, while avoiding targeting to myoblasts, hepatocytes, podocotyes etc.

Methods/Materials: Multiple rounds of biopanning with the outlined cells are performed with the Ph.D.-C7C Phage Display Library. The outputs after each round are amplified and cells challenged again. The principle is, that after multiple rounds, phage displaying peptides specific for target cells will be selected and then identified through sequencing. Peptides identified in fibroblasts will then be utilised to challenge other cell types.

Results: After 3 rounds of biopanning in fibroblasts we successfully identified sequences from 5 library inserts. However, 32/40 sequences did not contain the insert, implying low selective pressure.

Conclusion: Phage Display is a viable method of identifying peptides for targeting fibroblasts, though further optimisation is needed to increase selective pressure and number of peptides identified. Further validation will be conducted in more fibroblast cell lines, alongside other cell types, to identify peptides specific to fibroblasts and rule-out those targeting undesirable cell types.

OD03

Exploring fibroblast-homing peptides to enhance the delivery of antisense oligonucleotides in Collagen VI-related congenital muscular dystrophies

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Background: Collagen VI-related congenital muscular dystrophies (COL6-CMDs) are a group of neuromuscular diseases affecting skeletal muscle and connective tissue. They are caused by mutations in genes encoding the three major α-chains of collagen VI. There is no cure for COL6-CMDs. Antisense oligonucleotide (ASO) is a promising therapeutic approach for COL6-CMDs. We have identified ASO sequences correcting common dominant mutations in COL6-CMD patient-derived fibroblasts. However, subsequent *in vivo* studies revealed a major challenge in delivering ASOs to muscle interstitial fibroblasts (MIF), the major cell population producing collagen VI protein.
Aims: This study aims to identify MIF-targeted peptides that can be conjugated to ASOs, enhancing their uptake into MIFs.

Methods/Materials: Thirteen short peptides fragmented from the ligand of a fibroblast surface recep-

tor were synthesized and labelled with fluorescence tags. Peptide binding specificity was tested in various cell lines, including human and mouse fibroblasts, myoblasts, podocytes, endothelial cells, human kidney cells, hepatocytes, neuroblastoma cells, and myotubes. Cellular internalization of receptor mediated lead peptides was verified by siRNA knock-down of receptor mRNA and confocal imaging. The efficiency of Peptide-ASO conjugates in correcting specific mutations was tested in patient fibroblasts carrying the *COL6A1* mutation *c.930+189C> T*. Furthermore, lead peptides localization in skeletal muscle was tested in wild-type mice by intramuscular injection.

Results: We have identified two peptides able to effectively target human and mouse fibroblasts, with low or no binding affinity to other cell types. We confirmed that these peptides bind to the receptor then internalize into fibroblasts. We showed that a lead peptide localizes in the muscle extracellular matrix *in vivo,* where MIFs are located. Furthermore, we have identified a lead peptide-conjugated ASO that significantly improves mutation correction efficiency *in vitro* compared to the naked ASO.

Conclusion: We have successfully identified a lead peptide strongly enhancing ASO delivery to fibroblasts. Our next step is to test Peptide-ASO conjugates efficacy on COL6 mutations using different methods of conjugation and linkers. The lead compounds will be tested in the appropriate mouse model to investigate their biodistribution. We expect to develop a MIF-targeted peptide-ASO delivery system to address the current bottleneck of ASO therapy in COL6-CMDs.

OD04

Multiomics approach to identify a novel recessive pathogenic variant in the *TNNT3* gene in two siblings with congenital myopathy

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Background: The troponin complex consists of three subunits, troponin T, I and C and plays an important role in the process of muscle contraction. Pathogenic missense variants in the fast skeletal muscle troponin T (*TNNT3*) gene were described to cause autosomal dominant distal arthrogryposis. Two reports have associated recessive *TNNT3* variants to congenital myopathy (CM) with distal arthrogryposis with and without nemaline bodies.

Aims: To identify and characterise the underlying genetic cause of the CM in two affected siblings from the UK.

Methods/Materials: We report on two sisters who presented at birth with hypotonia, little spontaneous movements, bell shaped chest, facial dysmorphisms, bilateral dislocated hips and hyperextensible joints. Muscle weakness was not progressive and functional abilities improved over time, with both patients (currently in their early teens) able to walk independently. Early respiratory and swallowing difficulties also markedly improved with no current respiratory concerns. Muscle biopsies performed in infancy in both, showed increased fibre size variation, clusters of small atrophic fibres, internal nuclei and increased connective tissue and focal fat infiltration. Diagnostic gene panel analysis was negative. Trio whole

exome sequencing (WES) was performed through the Broad Institute in patient 2 and parents. Gene agnostic whole genome sequencing (WGS) was performed in both sisters through the SOLVE-RD project; WGS data was analysed using the GPAP platform. Muscle derived whole RNA sequencing (RNAseq) of patient 1 was performed through SOLVE-RD. RNA from the muscle of patient 2 was analysed by RT-PCR and Sanger sequencing.

Results: We identified a novel homozygous intronic variant in *TNNT3*, c.67+128G>A, in both sisters. Unaffected parents are heterozygous carriers. The variant creates a new acceptor splice site. RNASeq showed insertion of 10 nucleotides from intron 5 and very low levels of *TNNT3* transcript in patient 1.

Conclusion: We report a novel recessive variant in *TNNT3*. The N-terminal region of TNNT3 is alternatively spliced, and not all isoforms are affected by the variant. The presence of residual full-length protein might explain the improving phenotype of these patients. This work also highlights the role of multiomics and RNAseq in patients with unsolved CM.

OD05

Assessing the measurement properties of the Inclusion Body Myositis Functional Rating Scale

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Background: With ongoing development of therapeutic agents and research in Inclusion Body Myositis (IBM), it is imperative to identify and validate appropriate outcome measures for assessing disease progression. The IBM Functional Rating Scale (IBMFRS), established in 2008 as a disease-specific Clinician-Reported Outcome. Our aim was to evaluate the validity, reliability, responsiveness, and interpretability of the IBMFRS in participants of the arimoclomol trial in IBM.

Aims: To evaluate the validity, reliability, responsiveness, and meaningful change threshold of the Inclusion Body Myositis (IBM) Functional Rating Scale (FRS).

Methods: Data from a 20-month multicentre, randomised, double-blind, placebo-controlled trial in IBM were used (arimoclomol trial). Construct validity was tested using Spearman correlation with other health outcomes. Discriminant validity was assessed using standardised effect sizes (SES). Internal consistency was tested using Cronbach's alpha; equivalence in stable patients, test-retest reliability, and equivalence of face-to-face and telephone administration were tested using intraclass correlation coefficients (ICCs) and Bland-Altman plots. Responsiveness was tested using standardised response mean (SRM). A ROC curve anchor-based approach was used to determine meaningful change in IBMFRS.

Results: Among the 150 patients, mean (SD) IBMFRS total score was 27.4 (4.6). Construct validity ranged from medium to large (r_s modulus: 0.42-0.79). Discriminant validity ranged from moderate to large (SES=0.51 to 1.59). Internal consistency was adequate (overall Cronbach's alpha: 0.79). Test-retest reliability (ICCs=0.84-0.87) and reliability of telephone versus face-to-face administration (ICCs=0.93-0.95) were excellent, with Bland-Altman plots showing good agreement. Responsiveness in the worsened group defined by various external constructs was large at both 12 (SRM=-0.76 to -1.49) and 20 months (SRM=-1.12 to -1.57). In ROC curve analysis, a drop in two IBMFRS total score points was shown to represent meaningful decline.

Conclusions: The IBMFRS is a reliable, valid and responsive tool that can be used to evaluate the impact of IBM and its treatment on physical function, with a 2-point reduction representing meaningful decline

OD06‡

Retrospective deep phenotypic and genotypic analysis of UK patients with recessive early onset titinopathy

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Background: Biallelic *TTN* variants cause a congenital/childhood onset myopathy (*TTN*-CM) with paucity of phenotypic and natural history data to date.

Aims: Perform a deep phenotypic analysis of paediatric patients with *TTN*-CM.

Methods/Materials: Monocentric retrospective case notes review of UK patients with *TTN*-CM assessed in the period 2001-2023.

Results: Twenty-one children (15 males, 6 female) were reviewed. Median (range) age at last ascertainment was 13.4 (1.9-17.6) years. Two patients died of cardiomyopathy at 13 and 19 years and one from respiratory failure at 7 weeks. Six patients had prenatal onset, and median (range) age at postnatal onset was 6 (0-60) months. Of those ≥2 years (n=19), 32% (n=6) could run, 53% (n=10) could walk but not run and 16% (n=3) were non-ambulant. Weakness was commonly symmetric (95%, n=20), proximal-predominant (76%, n=16) with equal upper and lower limb distribution (62%, n=13). Limb power was sub-gravity or weaker in 52% (n=11). Neck and/or axial weakness was present in 81% (n=17), facial weakness in 48% (n=10) and dysmorphic features were evident in 33% (n=7). No patients exhibited ophthalmoplegia. Scoliosis was observed in 5 patients aged >10 years (42%), with two requiring spinal surgery. Contractures were evident in 91% (n=19). Three patients (14%) had arthrogryposis multiplex congenita (AMC), 1 of which carries biallelic metatranscript only *TTN* variants (*TTN*-mo), 1 patient is compound heterozygous with a single *TTN*-mo and 1 patient is homozygous for a *TTN* splice variant implicating the N2A and N2BA isoforms. Cardiomyopathy was present in 29% (n=6), all harbouring biallelic *TTN* variants implicating the N2BA cardiac isoform. Restrictive lung disease was present in 24% (n=5), with 4/5 patients requiring non-invasive ventilation (NIV).

Discussion: Our data is consistent with previous *TTN*-CM cohorts, with an early onset, four-limb, symmetrical, proximal-predominate myopathy. Axial weakness was prevalent and consistent with high rates (24%) of scoliosis and NIV requirement, although at lower rates compared to published cohorts. The established correlation between biallelic N2BA isoform *TTN* variants and cardiomyopathy is upheld. However, our data suggests that AMC may also occur in the absence of *TTN*-mo. These findings expand the phenotypic and genotypic knowledge of this rare condition.

Diagnostics and Cross-cutting Therapies

DCC01‡

Raman spectroscopy as a translational biomarker of muscle health

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Background: Diagnosing and monitoring neuromuscular disorders can be challenging. A translational readout of muscle health could therefore benefit clinical practice, clinical trials and preclinical studies. Raman spectroscopy is an emerging, non-destructive technique that uses light of a single wavelength to produce a biochemical fingerprint of tissue.

Aims: To assess how well Raman spectroscopy can differentiate between healthy, neurogenic and myopathic pathologies and explore the spectral features that are used to identify disease.

Methods/Materials: A fibre optic Raman needle probe was used to obtain spectra. In vivo preclinical recordings were undertaken using the mdx model of Duchenne muscular dystrophy at two ages (30 and 90 days; representing disease onset and a more regenerative phase of the model), the SOD1^{G93A} model of motor neurone disease (at 90 days, an established disease stage) and associated wild-type/non-transgenic controls. Human muscle biopsies were studied from patients with a variety of myopathies. Data was analysed using multivariate statistics (partial least squares discriminant analysis) and hierarchical modelling. Protein secondary structure profiling was performed to gain insight into biochemical features.

Results: The spectra were able to accurately identify the four preclinical conditions (acute mdx, chronic mdx, SOD1^{G93A} and healthy muscle) with an overall accuracy of 92%. Spectral analysis demonstrated a reduction in alpha helix profiles in mdx mice. A similar reduction was also noted in human myopathy muscle samples.

Conclusion: Raman spectroscopy of muscle offers the potential for a highly accurate assessment of muscle, with novel insights into protein secondary structures. The findings appear to translate from preclinical models to human specimens. We are now developing technology for first-in-human studies.

DCC02

Optimising muscle fibre type quantification using multiplexed capillary immunoelectrophoresis to enable disease and exercise physiology research

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Background: Adult skeletal muscles are generally heterogeneous, composed of different myofibre types with varying metabolic and contractile properties and different functions. The 3 principal mature types present in large mammals (including humans) are Type I – Slow twitch, Type IIa – Fast oxidative - glycolytic and Type IIx – Fast glycolytic fibres. Disease, aging and exercise regimens can all have a substantial effect on the fibre type populations. Thus, characterising the fibre type profile is often consid-

ered vital when studying muscle physiology and pathology. Historically, enzymatic histochemical stains such as adenosine triphosphatase, immunohistochemistry or gel electrophoresis were used for fibre identification, but methods are somewhat laborious or prone to error. Fibre types express specific myosin heavy chain (MyHC) isoforms that can act as potential markers for fibre typing. Capillary immunoelectrophoresis (CIE) facilitates accelerated separation of proteins based on their molecular weight and has potential for identifying MyHC isoforms of specific fibre types.

Aims: To investigate if CIE is a suitable method for accurate and expedited muscle fibre type identification and quantification in mammalian skeletal muscle extracts in comparison with histological immunofluorescence fibre typing.

Methods/Materials: CIE was performed using 3 antibodies that together recognise all 3 fibre mature types in protein extracted from equine semimembranous muscle, via 2 detection channels in a Jess Simple Western® instrument (ProteinSimple). Multiplexed Zenon® labelling was carried out in serial sections to quantitate fibre types via conventional immunohistochemistry.

Results: The antibodies can be multiplexed and reprobed via CIE to be used in tandem and continue to generate linear and consistent signals over antibody dilutions of 1:10 to 1:50 and protein concentration ranging from 0.00625 to 0.1µg/µl.

Conclusion: CIE holds promise for identification and quantification of muscle fibre types in protein extracts. Further work will examine samples from different muscle groups, different breeds and diseases to evaluate the efficacy of the method across various conditions and in comparison with conventional immunohistochemical methods. We gratefully acknowledge the funding from MARS Equestrian.

DCC03

Myoguide.org: a web-based portal supporting the analysis of MRIs for the diagnosis of neuromuscular patients

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Muscle magnetic resonance imaging (MRI) proves valuable in examining muscle structure in genetic neuromuscular disease (NMD) patients, efficiently revealing fat replacement and alterations in water content. Despite its widespread use in NMD diagnosis, the intricate analysis of MRIs and identification of distinct muscle involvement patterns pose challenges demanding a high level of specialization. Our objective was to establish a web platform facilitating NMD diagnosis through MRI. Integration of MYO-Guide, a machine learning algorithm, enhances patient diagnosis based on MRIs. The platform also features an informative catalogue encompassing typical muscle involvement patterns, accompanied by detailed reports guiding MRI analysis. The collaborative effort of 30 global centres led to the launch of www.myoguide.org. Housing the MYO-Guide algorithm, the platform accurately predicts 15 neuromuscular diseases with an 85% accuracy rate, utilizing a dataset of 2276 muscle MRIs from the pelvis and/or lower limbs. In addition to disease progression insights, the website provides valuable resources, including articles to support healthcare professionals in MRI analysis. Ongoing efforts involve the creation of an automatic segmentation tool aimed at identifying individual muscles and quanti-

fying fat content seamlessly. Myoguide.org serves as a comprehensive online platform, offering valuable resources for the analysis of muscle MRIs. It features an artificial intelligence prediction tool alongside a plethora of training resources designed to support the medical community in MRI analysis.

DCC04

Identification of MYO-SEQ patients with the newly described HMGCR-related muscular dystrophy

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Background: Limb-girdle muscular dystrophies (LGMD) are rare, typically progressive autosomal diseases that manifest with a range of symptoms including muscle weakness and elevated serum creatine kinase (CK) levels. In 2023, Yogev et al, reported for the first time the association of genetic variants in the *HMGCR* gene with the development of LGMD, which was corroborated by a publication by Morales-Rosado et al. *HMGCR* encodes 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a rate-limiting transmembrane enzyme key to cholesterol biosynthesis. Thus far, 15 individuals from six unrelated families have been identified. The common clinical symptoms were weakness affecting proximal and axial muscles and respiratory insufficiency. However, the age at onset, serum CK levels and progression showed a wide variability spectrum.

Aim: Identify patients carrying disease causative variants in *HMGCR* from the MYO-SEQ project.

Methods/Materials: We interrogated our cohort of >1000 undiagnosed neuromuscular disease patients for variants in *HMGCR*, applying standard filtering criteria. We performed family segregation studies of the likely causative variants.

Results: We found five individuals from three unrelated families carrying missense variants in *HMGCR*. Following the reported autosomal recessive inheritance pattern, two cases of consanguineous families carried homozygous changes [Case 1: c.557G>C; p.Gly186Ala and Case 2: c.2186T>C; p.Ile729Thr], and case 3 presented two heterozygous variants [c.1469G>A; p.Arg490His and c.2403G>T; p.Glu-801Asp]. All the variants were absent in the control population (gnomAD) and predicted to have a deleterious effect according to *in silico* tools. Three of these changes map in the catalytic domain of the protein, while the remaining affect the Sterol-sensing domain. Similar to the clinical picture provided by the aforementioned papers, patients presented progressive proximal muscle weakness in upper and lower limbs. Interestingly, Cases 2 and 3 had an early onset, while case 1 started with symptomatology during adulthood. An ample variability in CK levels was observed among them. Overall, the clinical presentation is consistent with LGMD.

Conclusions: Here, we report three additional families with likely causative variants in *HMGCR*, further confirming the disease-association. This also highlights the importance of capturing detailed phenotypic patient records and re-analysing unsolved exome data periodically, in order to solve hitherto undiagnosed cases of rare genetic diseases.

DCC05

Mitigating against the effect of freeze artefact in skeletal muscle biopsy samples

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Background: Frozen skeletal muscle is generally favoured over formalin-fixed paraffin-embedded muscle for histological interpretation in the research and diagnostic setting, and its collection enables molecular studies at mRNA and protein levels. However, freezing muscle can be challenging due to its high water content: improper freezing or accidental thaw followed by freeze (e.g. following freezer breakdown or inadvertent thawing) of stored samples creates significant artefact - in particular ice crystal formation that disrupts cellular architecture and structural integrity, compromising or negating histological interpretation.

Aims: To investigate the hypothesis that ice crystal artefact in frozen muscle can be reversed by a specific thaw and refreezing protocol. Furthermore, to evaluate RNA degradation following thawing and re-freezing.

Methods/Materials: Three muscles (tibialis anterior, quadriceps and triceps) from wild type (WT, n=3) and a glycogen storage myopathic mouse model (GSL30, N=3) were frozen with conventional methods and confirmed as being histologically valid; thereafter, muscles were thawed at room temperature (RT) for 30 minutes and refrozen in a -80˚C freezer, to generate severe ice crystal artefact. Samples were rethawed at RT (30 mins) or on wet ice (1 hour) prior to refreezing using liquid N2-cooled isopentane and cryosectioned, H&E-stained and quantified using ImageJ software. Additionally, changes in RNA integrity were measured using RNA TapeStation®.

Results: Refreezing severe freeze artefact-containing muscle from RT resulted in better histological outcome than from wet ice ($P<0.01$). The method resulted in almost complete resolution of ice crystals in both WT and myopathic muscle. However, RIN value revealed RNA degradation with both protocols.

Conclusion: Thawing and refreezing from RT is a simple, effective way to restore histological integrity of muscle with ice crystal artefact and offers resolution following poor freezing or freezer breakdown issues, however, RNA quality might be affected.

DCC06

An integrated transcriptomics and genomics approach to detect an X/autosome translocation in a female with Duchenne Muscular Dystrophy

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Background: Dystrophinopathies, including Duchenne and Becker muscular dystrophies, are the most common inherited neuromuscular conditions in childhood. These diseases are caused by pathogenic variants in the *DMD* gene and present an X-linked recessive inheritance pattern, thus, mainly males are affected. Most prevalent pathogenic variants are Copy Number Variants (CNVs) thus, genetic diagnosis is mostly achieved through MLPA and exome sequencing of the *DMD* gene.

Aims: Here, we aimed to identify the genetic diagnosis of a female patient presenting with muscular dystrophy, resembling a dystrophinopathy.

Methods/Materials: We performed MLPA, exome sequencing, aCGH, X-chromosome inactivation assay, and genome sequencing on DNA extracted from

patient's peripheral blood. Routine histological immunostaining and total RNA sequencing were performed from patient's muscle biopsy.

Results: Histological analysis revealed dystrophic features and a reduction in α, β, γ, δ-sarcoglycan, β-dystroglycan and dystrophin staining. MLPA, exome sequencing and aCGH were negative. RNA sequencing outlier expression analysis identified the *DMD* gene as a statistically significant downregulated gene with an 85% reduction in expression compared to all muscle samples analysed in the cohort. Trio whole genome sequencing revealed a *de novo* balanced translocation between chromosome 17 and the X chromosome (t(X;17)(p21.1;q23.2), disrupting both the *DMD* and *BCAS3* genes. X-inactivation from patient's peripheral blood showed a non-random X-chromosome inactivation pattern (73%-27%).

Conclusion: A combined analysis of RNA and whole genome sequencing played a crucial role in the detection, characterisation, and interpretation of the disease-causing variant in this patient, who had been followed up for several years. This case illustrates the diagnostic odyssey in female *DMD* patients with complex structural variants that are not detected by or panel or even exome sequencing.

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