Abstracts of the
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29th and 30th March 2023

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Index

Invited Speakers March 29	S3
Invited Speakers March 30	S6
Posters and Platform Presentations	S9
Dystrophy Pre-Clinical	S9
Dystrophy Clinical	S21
Peripheral Neuropathy	S36
Motor Nerve Disorders	S49
Neuromuscular Junction Disorders and Channelopathies	S57
Mitochondrial Disease	S61
Other Diseases	S75
Diagnostics and cross-cutting therapies	S90
Author index	S97

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Invited Speakers

Wednesday 29th March 2023

S01

The MRC Nucleic Acid Therapy Accelerator (NATA) - its role in the translational research panorama in the UK

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The Nucleic Acid Therapy Accelerator (NATA) is a new established MRC Unit based on the Harwell Science and innovation Campus. NATA's mission is to advance the development of nucleic acid therapies and associated technologies and improve healthcare for the benefit of patients. NATA operates a collaborative research model - partners can access NATA's capability and expertise in oligonucleotide design, synthesis and scale up production for target screening, validation and preclinical studies. Currently, NATA is focused on ASO and siRNA therapeutic modalities and can provide a full range of chemical modifications. NATA has an inter-disciplinary team of chemists and biologists that work together to accelerate target validation; NATA can perform *in vitro* studies to establish specificity, efficacy and on/off target effects. In addition, NATA is co-located with the MRC Mary Lyon Centre facilitating *in vivo* tolerability and biodistribution studies as well as access to mouse models available through the National Mouse Genetics Network. NATA has also funded an international academic-industry research consortium to address issues facing the tissue-specific delivery of oligonucleotide therapeutics with a focus on delivery to CNS, skeletal muscle and heart muscle.

S02

Preclinical development of a gene therapy for calpainopathy

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Limb-girdle muscular dystrophy type R1 (LGMDR1 or LGMD2A) is a neuromuscular disorder caused by mutations in the calpain 3 gene (CAPN3). It affects predominantly the proximal skeletal muscles, leading to progressive weakness and atrophy and consequently loss of motor function. We aim at developing a therapeutic product based on gene transfer mediated by a vector derived from the adeno-associated virus (AAV). As some mutations in the calpain 3 gene have recently been observed to manifest as a dominant form of calpainopathy, we wanted to define whether these mutations corresponded to haploinsufficiency or gain of function. The study of these particular mutations demonstrated that the first mechanism was correct, indicating that these forms would also benefit from gene replacement as for the recessive forms, *de facto* extending the indications for our gene therapy in development. To ensure the highest efficacy and safety possible of the gene transfer, we engineered an AAV vector that present a high tropism for the skeletal muscle and that will express calpain 3 only in this tissue. We developed a new capsid through rational design with enhanced properties compared to the parental serotype. In particular, we demonstrated *in vivo* in mice using a GFP-luciferase transgene that it displayed a decreased tropism for the liver and an increased tropism for the skeletal muscles. In addition we showed a 10-fold increase in infection of human primary and IPs-derived differentiated myogenic cells compared to AAV9 and 8. We demonstrated the potential of transfer of the calpain3 gene using this newly devel-

oped AAV to correct the pathological signs in mouse and rat models for LGMD-R1 after intravenous injection. All the preclinical development is well advanced for this program in preparation for a clinical trial.

S03

RNA therapeutics for cardiac repair

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Cardiac disorders are common, lethal and expensive. Heart failure, which is a common consequence of cardiomyocyte (CM) damage and loss, now affects over 1-3% of the global adult population, has a 5-year mortality of 50-75% and absorbs 2-3% of national health expenditures in high-income countries. Loss of CMs is not offset by new cell generation after birth, as the regenerative capacity of the adult human myocardium is less than 1% per year over an average lifetime. No drugs exist for myocardial survival or regeneration. Even more notably, no biological therapy has yet been developed for any primary cardiac condition.

A main goal of my laboratory is to develop RNA therapeutics to treat cardiac disease, with the objectives of preventing CM loss after damage, stimulating cardiac regeneration and achieving precise gene editing for the correction of hereditary cardiac mutations. Using whole genome siRNA and miRNA libraries, over the last years we have performed a series of high throughput screenings for ncRNAs that prevent CM death, stimulate CM proliferation and promote homology directed repair and prime editing using CRISPR/Cas9 tools. We have identified a few miRNAs that are very effective at stimulating re-entry of CMs into the cell cycle. Once administered to mice or pigs after MI, one of these miRNAs promotes clinically relevant cardiac regeneration and remuscularisation of infarcted hearts. Other miRNAs stimulate precise gene editing by reawakening expression of the homologous recombination machinery in both cultured CMs and in neonatal and adult mouse hearts. We can achieve expression of these miRNAs either using AAV vectors or by delivering their synthetic mimics using lipid nanoparticles (LNPs) generated with the SNALP technology. Precise cardiac gene editing can be achieved using single LNP preparations containing three RNAs (the Cas9 mRNA, single guide CRISPR RNA and the microRNA enhancing homologous recombination) and using an AAV DNA as a template for correction.

S04

Genetic therapies for demyelinating CMT neuropathies

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Charcot-Marie-Tooth (CMT) inherited neuropathies are caused by mutations in numerous genes that are highly expressed in neurons and their axons, or in Schwann cells and the myelin sheath they form, leading to either loss-of-function or toxic gain-of-function cellular mechanisms. The most common demyelinating CMT neuropathies result from either cell-autonomous loss of function or toxic molecular mechanisms in myelinating Schwann cells. Thus, in order to develop effective treatments, we generated Schwann cell-targeted gene replacement or gene silencing approaches. Adeno-associated viral (AAV) vectors were used to deliver disease-specific therapeutics. Following lumbar intrathecal injection, we showed widespread biodistribution and transduction of Schwann cells throughout the PNS. Delivery of the *GJB1* gene encoding the gap junction protein Cx32 associated with CMT1X neuropathy, resulted in restoration of Cx32 expression in Schwann cells. Pre- and post-onset gene replacement therapy provided therapeutic benefit including improved motor function, nerve conduction velocities, and nerve pathology in the *Gjb1* knockout and in two transgenic mouse models of the disease. Likewise, replacement of the *SH3TC2* gene associated with autosomal recessive early onset CMT4C neuropathy resulted in functional and morphological improvement in the *Sh3tc2* knockout mouse model of the disease. For the treatment of CMT1A, the commonest CMT type, which is caused by *PMP22* gene duplication resulting in a toxic gene-dosage effect, we developed a

microRNA-based gene silencing approach. Delivery of microRNA by AAV9 both at early as well as late stages of the disease efficiently silenced PMP22 expression in PNS tissues, leading to functional and morphological phenotypic improvement in a CMT1A model overexpressing the human *PMP22* gene. Across the neuropathy models we also evaluated clinically relevant and treatment-responsive blood biomarkers, including neurofilament light (NF-L), neural cell adhesion molecule 1 (NCAM1) and growth differentiation factor 15 (Gdf15. Currently we perform vector dose-escalation and safety studies in these disease models to facilitate translation. Our studies provide proof of concept for the therapeutic potential of gene replacement or gene silencing therapies to treat patients suffering from demyelinating CMT neuropathies.

S05

Mitochondrial-targeted small molecule therapy for CMT2A and other neurodegenerative conditions

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Mitochondrial fragmentation and dysmotility are hallmarks of neurodegenerative diseases. Mitochondrial involvement may be direct, as in CMT2A caused by mutations of the mitochondrial fusion protein MFN2, or indirect as in ALS, Huntington's disease (HD) and others. Dependence upon mitochondria-derived ATP for neuronal signaling, repair and regeneration combined with susceptibility of neuronal mitochondria to damage from intrinsic or extrinsic factors likely underlays mitochondrial involvement in these conditions. Also, focal mitochondrial damage can provoke greater mitochondrial damage in a vicious cycle leading to widespread mitochondrial degeneration. We considered that therapeutic approaches which generally enhance resistance of mitochondria to injury might break this cycle and alleviate neurodegenerative diseases.

Mitofusins (MFN) 1 and 2 mediate the initial steps of mitochondrial fusion, which is central to mitochondrial health and repair. MFNs are also essential for mitochondrial motility through neuronal axons. Mutations of MFN2, uniquely involved in mitophagic mitochondrial quality control, cause most cases of CMT2A. Thus, mitofusins directly enhance mitochondrial fitness and transport while generally contributing to overall health of the neuronal mitochondrial collective. We posited that mitofusin activation would interrupt the cycle of mitochondrial degeneration in neurological disease. Indeed, recently developed small molecule allosteric mitofusin activators reversed mitochondrial fragmentation, depolarization and dysmotility in cultured fibroblasts or reprogrammed neurons of patients with CMT1, CMT2A, ALS, HD and FTD, but not Friedrich ataxia. *In vivo* administration of short- and long-acting mitofusin activators to mice expressing the CMT2A MFN2 mutant T105M in motor neurons corrected mitochondrial fragmentation and dysmotility, stimulating neuronal regrowth and fully reversing functional neuromuscular degeneration. Likewise, sustained (but not intermittent) mitofusin activation delayed neuromuscular degeneration and prolonged survival in mice expressing the ALS SOD1 mutant G93A; the major effects were on neuromuscular function (i.e. quality of life) rather than lifespan. By contrast, and despite correcting mitochondrial abnormalities in HD GABAergic neurons, mitofusin activation had no measurable effect on neuromuscular degeneration in R6/2 mice (120 *htt* CAG repeats).

Pharmacological mitofusin activation exhibits pre-clinical efficacy for CMT2A and some other etiologically diverse neuropathies in which primary or secondary mitochondrial dysfunction contributes to neuronal degeneration. The pharmacokinetic and pharmacodynamic properties of lead compounds, their potential applications in human disease, and a possible time-line for human trials will be discussed.

S06

The fifth Morgan-Hughes Thomas lecture

Solving the undiagnosed neurogenetic diseases

Professor Henry Houlden
UCL Queen Square Institute of Neurology

Hereditary neurological diseases represent the largest group of undefined disorders in the UK. Rational management and therapeutic development rely on accurate diagnosis, yet rare disease diagnostic rates remain disappointingly low at around 25% of patients and families. This is particularly the case for

patients from ethnically diverse backgrounds. We have developed a program to investigate undiagnosed hereditary neurological disorders, initially using cost effective exome, and more recently genome sequencing and an optimised neurology analysis pipeline. To enhance diagnostic yield, we have integrated short-read transcriptome sequencing and we have started to establish long-read sequencing and optical genome mapping to enhance the identification of camouflaged genomic region such as repeat expansions.

To identify and diagnose neurological disease in the UK we plan to build a coordinated undiagnosed neurological disease network, with support from existing genetic and rare disease infrastructure, forming a Registry with deep phenotyping, on which to work with Genomics England and the NIHR BioResource enhance diagnostic yield and gene discovery.

Thursday 30 March 2023

S07

Experimental gene therapy in mitochondrial disorders

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A number of different pharmacological and genetic approaches have been attempting to mitigate or cure mitochondrial disorders. Interesting results have been obtained for improvement of isolated or predominant mitochondrial myopathies, for instance by using the pro-autophagic anti-TORC1 rapamycin, activators of the mitochondriogenic PGC1-alpha, moderate overexpression of the cristae organizer OPA1 and, most notably, AAV-based gene replacement.

Leigh disease, a genetically heterogeneous condition characterized by defective mitochondrial bioenergetics, is the most common oxidative-phosphorylation disease in infancy. Leigh's brain lesions are mimicked by the ablation of the mouse respiratory chain complex I subunit *Ndufs4*. We previously delivered the human *NDUFS4* gene to the mouse brain using either single-stranded adeno-associated viral 9 (ssAAV9) recombinant vectors or the PHP.B adeno-associated viral vector. Both these approaches significantly prolonged the lifespan of the *Ndufs4*$^{-/-}$ mouse model but the extension of the survival was limited to a few weeks by the former approach, whereas the latter was applicable to a limited number of mouse strains, but not to primates. We then exploited the recent development of new, self-complementary AAV9 (scAAV9) vectors, in which the transcription rate of the recombinant gene is markedly increased and can be applied to all mammals, including humans. Either single intra-vascular or double intra-vascular and intra-cerebroventricular injections were performed at post-natal Day 1. The first strategy ubiquitously conveyed the human *NDUFS4* gene product in *Ndufs4*$^{-/-}$ mice, doubling the lifespan from 45 to ≈100 days after birth, when the mice developed rapidly progressive neurological failure. However, the double, contemporary intra-vascular and intra-cerebroventricular administration of self-complementary-adeno-associated viral NDUFS4 prolonged healthy lifespan up to 9 months of age. Robust expression of hNDUFS4 was detected in different cerebral areas preserving normal morphology and restoring Complex I activity and assembly. Future work is warranted to explore translatability of scAAV9-*NDUFS4* in the prodromal phase of the disease in mice and eventually humans.

S08

Generating mouse models of mitochondrial DNA disease

Jim Stewart
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We have known for 35 years that mutations in the mitochondria's own multi-copy, maternally inherited DNA (mtDNA) can lead to metabolic diseases in humans. During this time hundreds of different mitochondrial DNA mutations have been identified and linked to dozens of different disorders with variable

age of onset, clinical severity, and tissue specificity. Although each one of these diseases is rare, it is estimated that 1:7500 people are at risk of developing a disease due to mutations of their mitochondrial DNA. Unfortunately, effective treatments are still lacking.

Animal models play a crucial role in basic research and pre-clinical translation studies into how mitochondrial DNA mutations translate into cell-level defects and tissue-level pathologies. Unfortunately, animal models with deleterious mitochondrial DNA mutations have not been available for basic research or preclinical studies. This has been because, until recently, it has not possible to directly manipulate the mitochondrial DNA using standard gene editing techniques. For these reasons we employed a strategy which involved the use of a mouse strain with a proof-reading deficient mitochondrial DNA polymerase to generate and transmit mutations in their mitochondrial DNA. These mitochondrial DNA mutations were segregated into maternal lineages of mice where we could directly identify mutations causing mitochondrial dysfunction in permissive tissues.

Lineages of mice carrying pathogenic mitochondrial DNA mutations have been utilized as models for the study of how mitochondrial DNA mutations cause pathogenesis and in translational studies to evaluate possible treatments for mitochondrial DNA-based genetic disorders. Our first two models bore pathogenic mutations in the mitochondrial DNA-encoded $tRNA^{Ala}$ gene. We were very surprised to find that these two mutations affected the tRNA and the mitochondria's function, in very different ways. I will present my lab's work on defining the similarities and differences between these two mouse models, and highlight examples of the preclinical collaborations that we have undertaken with these mice.

S09

The role of mitochondrial S-Adenosylmethionine in health and disease

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Production of the methyl-group donor S-adenosylmethionine (SAM) depends on one-carbon metabolism, with vital intermediary steps being localised to mitochondria. It remains uncertain how one-carbon availability connects to mitochondrial function. By generating tissue-specific knockout mice as well as patient-specific *Drosophila melanogaster* models for the mitochondrial SAM carrier (SLC25A26), we show a differential response to declining mitochondrial SAM level. Reduced SAM (mitoSAM) import causes hierarchical defects in fly and mouse, comprising loss of metabolites and OXPHOS assembly. Complex I stability and iron-sulfur cluster biosynthesis are directly controlled by mitoSAM levels, while other protein targets are predominantly methylated outside of the organelle prior to import. Interestingly, methylation modifications on mitochondrial RNAs seem to be least affected by a diminishing mitoSAM pool. Further, our data establishes that the mitoSAM pool follows cytosolic production, forming a feedback loop and identifying mitochondria as responsive receivers of one-carbon units. Thus, we demonstrate that cellular methylation potential is connected to energy metabolism, with direct relevance for aging and disease.

S10

The fifth Victor Dubowitz Lecture

The therapeutic potential of mitochondrial genome engineering

Michal Minczuk
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Mitochondria are subject to unique genetic control by both nuclear DNA and their own genome, mitochondrial DNA (mtDNA), of which each mitochondrion contains multiple copies. In humans, mutations in mtDNA can lead to devastating, heritable, multisystem diseases that display different tissue-specific presentation at any stage of life. Despite rapid advances in nuclear genome engineering, for years, mammalian mtDNA has remained resistant to genetic manipulation, hampering our ability to understand the mechanisms that underpin mitochondrial disease. I will present recent developments in the genetic modification of mammalian mtDNA and discuss the progress towards using genome editing technologies, such as programmable nucleases and base editors, for the treatment of hereditary mitochondrial disease.

S11

When is a variant in *TTN* pathogenic?

Marco Savarese
Folkhälsan Research Center & University of Helsinki, Finland

While interpreting titin variants, clinicians and geneticists are often faced with dubious clinical implications. Titin (*TTN*) gene (OMIM #188840) contains 364 exons (363 coding exons and the first non-coding exon), a high-complexity repeated region, and isoform-specific elements. In particular, the isoform-specific elements result in muscle and developmental stage specific isoforms through extensive alternative splicing, significantly increasing the complexity of titin variant interpretation.

The wide use of sequencing technologies has resulted in the identification of an increasing number of causative variants, which has increased the number of titinopathy patients, and widened the spectrum of *TTN*-related diseases. However, technical and interpretation issues is preventing us from uncovering the full landscape of titinopathies. The pathogenicity of missense variants is difficult to prove. Moreover, truncating variants are now required to be reported as incidental findings in any sequenced patient, creating ethical and clinical implications that should be addressed in genetic counselling, pertaining both the patient and their family.

The aforementioned complexity suggests that a comprehensive 'genotype-up' approach, which combines the genetic analysis with assessment of clinical, pathology, imaging, transcript, and protein findings, is mandatory for an improved management of patients and families with *TTN* variants. The fruitful collaboration of neurologists, cardiologists, geneticists, and other professionals is essential in recognizing, diagnosing and managing patients with titin related diseases. and coordinate the cardiac and neurological consultations.

S12

Solve-RD: European Rare Disease genomic analysis and interpretation

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Solve-RD is a European Union funded research program that has the aims to solve unsolved rare diseases and diagnose undiagnosed rare disease patients. Solve-RD has been following two main approaches that are re-analysis of existing exome and genome data sets and novel multi-omics approaches.

Six European Reference Networks form the clinical and genetic basis of Solve-Rd as regards data submissions from existing cohorts and available expertise for interpretation of genetic variants.

Solve-Rd has organized its re-analysis effort in three data freezes. In data freezes 1 and 2, clinical and molecular scientists from 36 centers of expertise across Europe joined efforts to systematically re-analyze 9,874 previously negative genomic datasets for 6,449 affected, but undiagnosed individuals from 6,004 RD families.

Solve-RD established a genetic diagnosis in 506 (8.4%) families by systematic reassessment of exome and genome datasets. The majority of disease explanatory variants (84%) were single nucleotide variants or short insertions/deletions (SNVs/InDels). Novel variant types counted for the remaining 16% of disease-causing variants.

In addition to the systematic re-analysis, 250 (4.2%) families were diagnosed by ad hoc expert review leading to an overall diagnostic yield of more than 12.5%.

Three conclusions can be drawn:

(i) There remains a continued need for re-analysis of genomic data from individuals with RD even among centers of expertise for these conditions.

(ii) Effective diagnosis of rare genetic diseases (RD) depends on the ability to accurately identify and interpret variants from genomic data.

(iii) The Solve-RD approach prove the impact of sharing data and expertise across Europe for genomic analysis and interpretation and is going to be piloted as a European diagnostic care service in the Joint Action for the Integration of the European Reference Networks in the national healthcare systems.

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Posters 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

Rescue of dystrophin (DMD) in the central nervous system after antisense oligonucleotide therapy in the mdx23 mouse model of Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder caused by mutations in the *DMD* gene that produces several dystrophin proteins. DMD mutations prevent the production of functional dystrophin and lead to pro-

gressive muscle weakness and premature death. The full length *DMD* gene is mainly expressed in skeletal and cardiac muscle with a lower expression in the brain, where other shorter isoforms are also expressed. Neurobehavioral complications such as anxiety and autism spectrum disorder occur in one third of DMD patients, related to dystrophin deficiency in the brain. Mdx23 mice lacking the full-length Dp427 isoform show significantly higher anxiety compared to wild-type mice in the elevated zero maze (EZM) behavioural test. Dystrophin expression can be induced using exon-skipping, which aims to skip mutated exons and restore the reading frame. The phosphorodiamidate morpholino oligomer (PMO) which induces skipping of exon 23, can restore dystrophin expression in muscle, and its intracerebroventricular administration has been shown to partially rescue the enhanced fear response in mdx23 mice.

Aims: We investigated whether intracisternal magna injection of this PMO into mdx23 mouse brain can restore dystrophin expression in different brain regions (olfactory bulb, cerebellum, cortex, hippocampus and spinal cord) and lead to phenotypic improvement. We used intracisternal magna injections due to their minimal invasiveness and the translational potential of this modality.

Methods/Materials: Male mdx23 mice were tested for restraint-induced fear response in EZM after treatment with three intracisternal PMO injections, at 6 weeks of age at intervals of three days. DMD exon skipping, and dystrophin protein restoration were analysed in brain regions of PMO injected mdx mice using RT-qPCR and capillary western blot.

Results: Mdx23 mice treated with three intracisternal PMO injections showed a small but significant rescue of the enhanced fear response in the EZM. Detectable but low dystrophin protein restoration and DMD exon skipping were measured following PMO administration, with a higher biodistribution in cerebellum and olfactory bulb at both the RNA and protein level.

Conclusion: We demonstrate that the direct delivery of a PMO can restore limited dystrophin expression and modestly improve the mdx23 behavioural phenotype when administrated by intracisternal magna injection.

D02

Gene expression profiles and spatial localisation of dystrophin isoforms in adult and foetal human brain

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Background: Duchenne Muscular Dystrophy (DMD) is an X-linked neuromuscular disorder caused by frameshift or nonsense mutations affecting the *DMD* gene, producing multiple tissue-specific transcripts. Although being primarily a progressive muscle-wasting disorder, due to the lack of the full-length dp427m dystrophin in muscle, about 35% of affected patients display a variety of central nervous system (CNS) co-morbidities. These cognitive and/or neuropsychiatric phenotypes are due to the down-regulation or absence of brain-specific dystrophin isoforms. Currently, localisation at the cellular level of different isoforms in the human brain is missing and much needed to better correlate dystrophin mutations and phenotype.

Aims: To study the regional expression of dystrophin isoforms at both transcript and protein levels in the embryonic, foetal and adult human brain.

Methods/Materials: Protein expression studies relied on immunohistochemistry (IHC) assays employing a panel of antibodies targeting three distinct

dystrophin epitopes. *DMD* transcripts were quantified and visualised by TaqMan Real-time quantitative PCR (RT-qPCR) and *in-situ* hybridisation (ISH), respectively. Optimisations and experiments were performed on formalin-fixed, paraffin-embedded (FFPE) human brain section (5 µM thick).

Results: We generated a comprehensive human brain developmental dataset including a) dystrophin isoforms gene and protein expression profiles, b) *in-situ* localisation and semi-quantification of 5' and 3' *DMD* transcripts (RNAscope) and, c) *in-situ* localisation of six *DMD* isoforms (Basescope) across the selected region of interest (cerebellum, hippocampus, amygdala and prefrontal cortex).

Conclusion: Our preliminary findings show regional, temporal and cell-specific patterns of different dystrophin isoforms expression in brain. In addition, we identified a set of high-level dystrophin areas which role in CNS comorbidities could be further investigated. Our studies provide foundation for future efforts to restore dystrophin expression postnatally in the brain, to address the DMD related CNS comorbidities.

‡D03

Decoding the transcriptome of Duchenne muscular dystrophy to the single nuclei level reveals clinical-genetic correlations

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Background: Duchenne muscular dystrophy (DMD) is characterized by early onset progressive muscle weakness leading to irreversible severe disability. The process of muscle degeneration in DMD involves a complex interplay between muscle fibers, muscle resident cells and, circulating cells invading the muscle. Despite considerable progress in the understanding of this process, there is still a considerable lack of knowledge of what are the cellular and molecular consequences of the absence of dystrophin in humans.

Aims: To study the changes in the gene expression profile to the single nuclei level of muscle samples from patients with DMD and age/gender matched controls

Methods: We have performed single nuclei RNA sequencing analysis of 7 samples of the quadriceps muscle of DMD patients aged 2 to 4 years before steroids were started and 5 samples from age and gender matched healthy controls. Bioinformatic analysis was performed using packages developed for the analysis of single cell/nuclei RNA sequencing in R and python.

Results: A total of 30857 nuclei from controls and 25817 nuclei from DMD were analyzed. Using Seurat package we identified 19 different nuclei clusters that were reduced to 11 putative identities after differentially expressed gene signatures were investigated including slow and fast myofibers, regenerative fibers, satellite cells, endothelial cells, smooth muscle cells, fibroadipogenic progenitor cells (FAPs), adipocytes, macrophages and, lymphocytes. We observed significant differences in the proportion of cell populations in the DMD samples versus controls, characterized by a reduction in the number of nuclei from slow muscle fibers and smooth muscle cells and an increase in the nuclei from regenerative fibers, satellite cells and FAPs. Patients with a better muscle function at the time of the biopsy had a significant higher number of regenerative fibers, while those with a worse muscle function had a significant increase in FAPs. Analysis of gene expression profile revealed significant differences in genes expressed in several clusters. In the case of myonuclei we observe a increase in gene involved in myogenesis, muscle growth, axon guidance and linking of muscle fibers to cytoskeletal with a re-

duced expression of genes involved in metabolic pathways such as glycolysis and oxidative phosphorylation. FAPs from DMD patients were characterized by an increase expression of genes coding for components of the extracellular matrix and genes involved in cell dividion. A deep analysis of the FAP cluster allowed us to identify seven different population, which proportion varied from controls to DMD. Two of these clusters were exclusively present in DMD samples, and were characterized by the expression of genes involved in cell division and proliferation. The population of these later FAP subtypes was increased in patients with worse muscle function at baseline.

Conclusion: We have observed substantial differences in the population of cells present in skeletal muscle samples of DMD patients compared with controls even at earlier stages of disease progression. Moreover, we have identified a large number of genes which expression is dysregulated in DMD cell population pointing towards an enhanced regenerative activity in DMD patients associated with an increase proliferative activity of FAPS, which produce high levels of extracellular matrix components.

D04

DMD isoforms spatial localization and topography in human adult brain areas

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Background. Duchenne muscular dystrophy (DMD) is an X-linked neuromuscular disease due to pathogenic variants in the *DMD* gene causing the absence of dystrophin protein.

Dystrophin function is not only limited to muscle but is also relevant in several brain circuits and during brain development. Pathogenic variations occurring in the 3' of the gene and thus disrupting both full-length (Dp427) and short 3' isoforms as Dp140 and Dp71 are known to impact on the neurobehavioral phenotypes.

Aim. We intended to assess the topography of *DMD* isoforms in relationship with the known brain comorbidities in human adult brain areas.

Methods. All dystrophin isoforms were firstly analysed in 24 adult human brain areas using TaqMan Real-time PCR on TissueScan cDNA array. The areas showing highest DMD isoforms' levels were prioritized for Basescope analysis with probes designed to detect each selected *DMD* isoforms. This latter technique was used in samples from 8 adult human male donors for a total of 34 brain areas, obtained from Edinburgh Brain-Tissue Bank. So far, we have completed the analysis of FFPE tissues from cerebellum, vermis, paracentral gyrus, corpus callosum, cingulate gyrus, and hippocampus.

Results. The more abundant isoforms in studied brain areas were Dp427c, Dp427m, Dp427p2, Dp140, Dp71 and Dp40, based on a preliminary Real-time PCR screening.

BaseScope showed that these isoforms were transcribed in all brain areas with a different abundance among donors.

Three main *DMD* isoforms clusters were identified, including i) low represented, as Dp427m and Dp427p2, ii) medium represented, as Dp140 and Dp40, which are mainly localized in the cerebellum, and iii) highly represented, as Dp427c and Dp71, which are enriched in hippocampus and paracentral gyrus. Vermis, corpus callosum and cingulate gyrus were the areas with lowest isoforms' representation. We could also recognize isoforms with cell- layer-specific localization in cerebellum and hippocampus.

Conclusion. We defined spatial and topographic representation of *DMD* isoforms in human adult brain. The enrichment in Dp140 isoform in cerebellum and in Dp427c and Dp71 in hippocampus are well aligned with the brain phenotypes observed in individuals affected by DMD.

D05

Horizon scan of datasets reporting asymptomatic and mildly symptomatic males carrying DMD deletions shows phenotype-related mutation clusters

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Background: Males carrying DMD deletions with absent, mild, or cardiac phenotypes have been sparsely described.

Aims: We performed a horizon scan on public datasets to enroll males with above phenotypes and carrying DMD deletions to delineate genotype-phenotype relationships.

Methods/Materials: We inventoried 88 males and proposed the following clinical categorization: (A)- fully asymptomatic males aged >43 years; (CK)- isolated hyperCKemia; (W)- mild weakness (any age); (CKW)- high CK plus W. We considered isolated dilated cardiomyopathy (XLDC) and dilated cardiomyopathy with CK and/or W (XLDCW). We counted: A (N=18 patients), CK (N=28), W (N=6), CKW (N=11), XLDC (N=7), XLDCW (N=18). We could not consider cognitive involvement since not consistently reported.

Results: In all cases, deleted intervals were exons 1 to 55, no downstream exons were never involved. Single exon deletions were exon 1, 2, and 48 only. Deletions in category A (N=18) were exon 2 (1), 45-51 (1), 45-55 (6), 48 (1), 48-51 (3), 48-53(1), 49-51 (1), 50-51 (2), and 51-52 (2). Deletions in categories "plus" (CK, W, and CKW) vary, deletion 45-55 represent the 38% of cases. All deletions were in-frame, apart from the known exception of exon 2 and 3-7. In patients with XLDC -XLDCW phenotypes, 19/25 have deletions of exons 45-55, exon M1, and exon 48, all together representing 76% of cardiac mutations. Apart from exon M1, all cardiac deletions were in frame.

Conclusion: In asymptomatic males, deleted exons involved R17-22 repeats, and Hinge 3 domain only; other repeats, Hinge1, and Hinge2 were never involved. Notably, deletions of exons 50-51 and 51-52 occur exclusively in asymptomatic males, and conversely deletions of exons 45-55 occur in all phenotypes. Obviously, domains downstream exon 55 are intact in all considered phenotypes and preserve Dp116 and Dp71 isoforms, H4, EFH1, EFH2, WW, ZZ domains, and the C-terminus translation. To be noted that this region is the ancient sea urchin DMD gene, which function is purely annelid striated muscle related. Deletion clusters definition may contribute to understanding the diverse pathogenic mechanisms underlining these mild and cardiac phenotypes and may also help to address the "critical" exon content needed to preserve a semi-functional DMD gene.

D06

Analysing the effect of human fibro-adipogenic progenitor cells from DMD on myogenic differentiation *in vitro*

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Background: Fibroadipogenic precursor cells (FAPs) are involved in the expansion of fibro-fatty tissue in muscles of patients with muscular dystrophies. Growing studies have shown that FAP regulation in muscular dystrophies is altered, contributing to muscle degeneration, defective muscle regeneration and also reducing the effective delivery of potential drugs into the muscle tissue. Although some

of the pathways governing FAPs have been studied, the precise mechanisms underlying FAP differentiation in muscular dystrophies remains not fully understood. Although it seems that FAPs have a different behavior in DMD patients than in healthy muscle, the different pathways and pathomechanisms regulating the function of these cells are unknown.

Aims: Our principal aim is to analyse the effect of FAPs isolated from healthy control and DMD patients muscle biopsies in the myogenic process, in order to understand if FAPs can influence this process in any way and identify if there are differences depending on the source of the cells.

Methods: FAPs and myoblasts will be isolated from muscle biopsies from healthy young males and DMD patients. These cells will be co-cultured both directly and indirectly with healthy myoblasts to analyse what effects FAPs have on myogenesis and whether these effects are due to cell-to-cell contact or whether they are communicating through the cell secretome. Proteomic analysis obtained from the secretome will be further analysed.

Results: We have observed that FAPs from healthy and DMD patients are different. DMD FAPs promotes a reduced myogenesis *in vitro* both in direct and indirect co-culture. Results from the secretome shows a different pattern of upregulated proteins in DMD compared to healthy controls.

Conclusion: The present study shows that FAPs from DMD patients are different compared to FAPs from healthy controls. The results of this work will shed some light on the complex process FAPs in muscle degeneration, in order to understand the different interactions between cells that occur in muscular dystrophies.

D07

Using *in situ* hybridization to identify and locate collagen VI-expressing cells in skeletal muscles of wild-type and COL6-related dystrophies mice

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Background: Collagen VI (COLVI) is a critical myomatrix protein for skeletal muscle health and maintenance. Pathogenic variants in any of the 3 major COL6 genes (*COL6A1-COL6A3*) cause COL6-related dystrophies (COL6-RDs) with early-onset muscle weakness and loss of ambulation. To develop therapies for COL6-RDs, it is critical to further our understanding of COLVI's physiological roles through identifying and locating all cell types that express COL6 genes in skeletal muscles. Although fibro-adipogenic progenitors (FAPs) are thought to be the major COLVI-synthesizing cells, there has not been any comprehensive characterization of COL6 genes' expression patterns in skeletal muscles. Here, using RNAscope, we aim to identify, quantify, and locate all cell types that express COL6 genes in the diaphragm and limb skeletal muscles of wild-type and COL6-RDs male mice at 10-day-old, and 6 and 20-month-old.

Methods/Materials: RNAscope staining of fresh-frozen quadriceps of two 6-month-old male mice (2 10 µm sections/mouse, 3 fields of view/section). QuPath was used for RNAscope quantification. *Pdgfra*, *Dpt*, and *Clec3b* were used as FAPs mRNA markers.

Results: The preliminary results show that FAPs only make up 42%, 46%, and 59% of the cells that express *Col6a1*, *Col6a2*, and *Col6a3*, respectively. Additionally, the preliminary data indicate that the 3 major genes are not always co-expressed in the same cell. Only 44% and 50% of the cells that express *Col6a1* or *Col6a2* co-expressed all 3 genes, respectively. However, 89% of *Col6a3*-expressing cells also co-expressed the 2 other genes.

Conclusions: Our preliminary data suggest that FAPs are not the only COL6-expressing cells in skeletal muscles. Furthermore, the 3 major COL6 genes are not always co-expressed in the same cell to form the predominant monomer structure of COL6 (α1(VI)α2(VI)α3(VI)). We are currently validating mRNA markers to identify the non-FAPs cells that express COL6. The findings of this project will provide additional insights into the roles of COLVI-producing cells in the pathogenesis of COL6-RDs and help direct therapeutic approaches.

D08

Characterising dystrophy-associated changes in muscle fibre type in the DE50-MD dog model of Duchenne muscular dystrophy

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Background: The fatal muscle wasting disease Duchenne muscular dystrophy (DMD) is associated with altered fibre type composition of skeletal muscle: muscle repair elicits expression of the regenerative myosin heavy chain genes, *MYH3* and *MYH8*, but dystrophic damage has also been reported to preferentially affect fast (type II) fibres, with disease progression consequently driving a fast-to-slow transition. Such changes have functional consequences and might also serve as biomarkers for evaluating disease progression or therapeutic efficacy. Dog models of DMD are valuable pre-clinical tools, with disease that closely mirrors young human patients, and moreover permit detailed body-wide assessment of fibre-type changes that would be impractical in human patients.

Aims: To investigate fibre type changes in the skeletal muscle of DE50-MD dogs with age and across different muscle groups, using gene expression and immunofluorescence analysis.

Methods/Materials: For longitudinal analysis, muscle samples (*vastus lateralis*) were biopsied from healthy (WT) and dystrophic (DE50) dogs at 3-monthly intervals from 3-18 months. For body-wide analysis, multiple muscles were collected post-mortem. Multiplex immunolabelling and in-house digital analyses were used to assess fibre type composition and distribution, complemented by qPCR measurement of myosin heavy chain (MHC) expression.

Results: All DE50-MD muscles expressed elevated regenerative myosins (*MYH3*, *8*), but behaviour of other MHCs was more variable. Within the vastus, despite high numbers of hybrid fibres (both regenerating and mature) overall fast/slow MHC expression remained essentially unchanged. Within the diaphragm, a marked fast-to-slow transition was observed (alongside profound fibre splitting), while other muscles instead displayed slow-to-fast changes, including increased expression of *MYH1* (IIX).

Conclusion: Dystrophic changes in skeletal muscle fibre composition and expression in this canine model are more complex than a simple fast-to-slow transition: different skeletal muscles exhibit distinctive alterations that might reflect muscle loading, frequency of use, severity of dystrophic pathology or other mechanisms. Similar varied changes might be present in different muscles of DMD patients of different ages.

D09

Developing advanced human myo-fibrogenic 3D models for disease modelling and therapy development

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Background: Extracellular matrix (ECM) represents the three-dimensional (3D) non-cellular network that exists within all tissues. In skeletal muscle tissues, ECM exerts pivotal roles in providing structural stability, mediating contractile force transmission whilst also regulating muscle development, homeostasis and regeneration. Abnormal muscle ECM caused by mutations in the genes encoding crucial ECM constituents such as collagen type VI (COL6) lead to the development of a spectrum of muscle disorders collectively known as COL6-related dystrophies (COL6-RDs). These disorders, including the most severe Ullrich congenital muscular dystrophy (UCMD), are still incurable and the exact pathogenesis mechanisms remain unresolved. Thus, a robust human model that can recapitulate key disease hallmarks *in vitro* is required to further decipher the disease mechanisms whilst also to provide novel tools for precision diagnosis and therapeutic development.

Aims:

1. Generate human myo-fibrogenic 3D cultures that further enhance physiological resemblance of the *in vivo* muscle tissues by recapitulating muscle-matrix interactions *in vitro*.
2. Identify faithful phenotypic readouts of the derived myo-fibrogenic 3D cultures that can depict key disease hallmarks and reflect patient-/mutation-specific phenotypes.

Methods/Materials: Based on our previously developed 3D artificial skeletal muscle platform, we further advanced the fibrin-based hydrogels to encapsulate both ECM components deposited by fibrogenic cells and myotubes derived from myogenic cells.

Results: The constructed myo-fibrogenic model can serve as a quasi-vivo 3D culture system which demonstrates close spatial interactions between aligned, straited myotubes derived from myogenic cells and COL6 proteins deposited by fibroblasts. This model can further recapitulate clinically relevant UCMD phenotypic readouts (i.e., muscle/joint contractures) which would have been otherwise not detected in standard 2D monolayer models.

Conclusions: The 3D culture platform developed in this study can reveal novel insights into the pathomechanisms of COL6-RDs and elaborate the genotype-phenotype correlations as well as identifying early pathogenic phenotypes of COL6-RDs *in vitro*. This platform will open new avenues for downstream etiological and prognostic studies along with facilitating future therapeutic development for COL6-RDs.

D10

Enhancement of muscle function in healthy and dystrophic muscles by downregulation of ribosome specific Ribosomal Protein L3-Like

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Background: Ribosomal protein L3-like (RPL3L) is one of the ribosomal proteins constituting the eukaryotic ribosome, responsible of protein translation from mRNA templates. RPL3L is poorly characterized and its function has never been fully clarified. While RPL3L is exclusively expressed in cardiac and skeletal muscle, its paralogue RPL3 is ubiquitously expressed except in these tissues, suggesting a crucial role for RPL3L in muscle. We recently showed that AAV-shRNA mediated RPL3L downregulation in skeletal muscles of normal mice induces significant increase in muscle strength. Since several transcriptomic studies have found RPL3L to be downregulated in Duchenne muscular dystrophy (DMD), a fatal muscle wasting disorder affecting 1 in 5000 boys, we also downregulated RPL3L in muscles of a mouse model of DMD and we detected a significant increase in muscle strength.

Aims: The aim of this study is to generate new evidences supporting the beneficial effects of RPL3L downregulation in skeletal muscle using a knock-out (KO) mouse model of RLP3L (Rpl3L -/-).

Methods/Materials: AAV vectors designed to downregulate RPL3L were injected in normal or

dystrophic muscles. A knock-out (KO) mouse model of RLP3L (Rpl3L -/-) was generated and back-crossed with the dystrophic mdx strain to make a double dystrophin and RPL3L KO mouse (RDKO). We performed histological, molecular and functional characterization of these muscles where RPL3L was downregulated to assess its role in muscle physiology.

Results: We found that in both Rpl3l -/- and RDKO mice, RPL3L loss triggers strong upregulation of its paralogue RPL3 while expression of neither utrophin or dystrophin was changed. Maximal specific force generated by tibialis anterior muscles depleted of RPL3L was also significantly enhanced compared to normal muscles.

Conclusions: Here we show that in both normal and dystrophic muscles where RPL3L is absent, muscle strength is enhanced. Our findings suggest that RPL3L downregulation could potentially alleviate some of the muscle force deficit observed in DMD.

D11

Human iPSC-derived muscle models for development of a CRISPR-based exon skipping therapy for *LMNA*-related muscular dystrophies

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Background: Skeletal muscle laminopathies are a diverse group of severe and incurable muscular dystrophies caused by mutations in the *LMNA* gene which encodes the nuclear lamins A and C. With lamins B1 and B2, these form a filamentous meshwork structure called the nuclear lamina which lies beneath the inner nuclear membrane and has important roles in maintaining nuclear structure and integrity, chromatin organisation and transcriptomic regulation. Lack of effective disease models that recapitulate human genetics and the skeletal muscle tissue environment currently hamper efforts to research potential disease mechanisms and therapeutic strategies. Our previous work has shown that *in vitro* patient-derived induced pluripotent stem cell (iPSC) skeletal muscle models recapitulate major disease phenotypes such as nuclear dysmorphism.

Aims:
1. Model hallmark laminopathy-associated skeletal muscle phenotypes such as nuclear dysmorphism *in vitro* using a protocol for differentiation which mimics *in vivo* muscle development and repair.
2. Apply CRISPR-based exon skipping strategies to *LMNA* mutant iPSCs to skip targeted disease-causing mutations.
3. Assess the potential of these therapies to ameliorate disease-associated phenotypes.

Methods/Materials: Three laminopathy patient-derived iPSCs carrying different *LMNA* mutations were differentiated into skeletal muscle and disease phenotypes assessed. CRISPR-based exon skipping strategies were applied to muscle cells and iPSCs respectively of one LMNA mutant line with an amenable mutation and phenotypic amelioration of nuclear dysmorphisms was assessed following treatment.

Results: *LMNA* mutant lines differentiate to skeletal muscle and recapitulate dysmorphic nuclear phenotypes observed *in* vivo. Moreover, development of an exon skipping strategy for amenable mutations efficiently excises the affected exon and produced shortened mRNA and protein corresponding to skipped exon lamins A and C. Assessment of amelioration of disease-associated phenotypes is currently ongoing.

Conclusion: Our iPSC-based platforms can be used to model hallmark laminopathy phenotypes such as nuclear dysmorphism and to develop potential mutation-specific therapeutic strategies.

D12

Dysferlinopathies – Flow cytometry, Molecular genetics, and mitochondrial proteome study

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Background: Dysferlinopathy, an autosomal recessive limb girdle muscular dystrophy includes two major phenotypes: Miyoshi muscular dystrophy(MMD) presenting with weakness and wasting of distal muscle and limb-girdle muscular dystrophy type R2 (LGMDR2) with proximal muscle weakness. The high clinical variability of this disease, as well as the secondary effect of some other structural muscle gene mutations makes the diagnosis really complex.

Objectives: The study was undertaken:

(a) To develop flow cytometry based, non-invasive and rapid diagnostic method
(b) To genetically characterize dysferlinopathies
(c) To analyze alterations in the total muscle mitochondrial proteome and to validate.

Methodology with Results: The study included 20 cases, clinically suspected and /or immunohistochemically confirmed Dysferlinopathies. FACS analysis in all 20 cases showed low cell count and no shift/ lesser shift of FITC pick towards double positive quadrant as compared to controls suggesting low expression of Dysferlin which corroborated with immunohistochemical diagnosis.Sanger Sequencing done revealed eight variation in 10/20 cases. Of which, five are reported as pathogenic while three had novel variations[Exon26 c.2771G>A;Exon42 c.4522C>T and Exon51 c.5713c>T]. Proteomic analysis revealed 446 mitochondrial proteins with down-regulation of subunits of electron transport chain, assembly factors and tricarboxylic acid cycle enzymes. While four upregulated mitochondrial proteins (L-lactate dehydrogenase A chain isoform 1, Threonine--trna ligase, mitochondrial isoform c, Mitochondrial calcium uniporter regulator 1, Calcium uniporter protein, mitochondrial isoform 3) were specific to dysferlinopathy.

Conclusion: Dysferlinopathy constituted 26% of AR- LGMDs seen at our centre (hospital based data). Flow cytometry based dysferlin assay helps in rapid and economical diagnostic technique. Diverse upstream events correlated with altered mitochondrial proteome. Four upregulated mitochondrial proteins were specific to dysferlinopathies. Genetics study revealed eight variation (five previously reported pathogenic and three novel variations) in 10/20 cases.

D13

Longitudinal assessment of tibiotarsal extensor and flexor torque dynamics and resistance to flexor eccentric stretch in the DE50-MD dog model of Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy, a fatal muscle wasting disease, is caused by mutations in the dystrophin gene and absence of dystrophin protein. Patients have progressive reduced skeletal muscle strength and lower resistance to eccentric muscle stretch. Dystrophic DE50-MD dogs are used as final translational models for evaluation of promising treatments.

Aims: To compare force dynamics of the tibiotarsal (ankle) joint extensors and flexors in DE50-MD and WT dogs and resistance of dystrophic muscle to eccentric stretch as dogs age.

Methods/Materials: Male dogs (DE50-MD: N=13; WT: N=12) were analysed every 3 months until 18 months of age, under general anaesthesia. The tibial and fibular nerves, supplying the tibiotarsal extensors and flexors respectively, were supramaximally stimulated sequentially via percutaneous needle electrodes, with a single pulse (twitch) followed by a tetanic pulse run. The tibiotarsal flexors then underwent 30 eccentric contractions in 3 sets of 10, with a 4-minute rest between sets. The cranial tibial muscle (the main tibiotarsal flexor) was fibre typed by immunohistochemistry and by RT-qPCR of myosin heavy chain isoforms.

Results: Maximum absolute and relative force of the tibiotarsal flexors was significantly lower for DE50-MD compared to WT dogs for both twitch and tetanic isometric contractions. Although there was a trend towards lower force of DE50-MD tibiotarsal extensors the difference was only significant for absolute twitch force. Time to reach maximum force was shorter in DE50-MD compared to WT dogs following both tibial and fibular twitch, while time for muscle relaxation was prolonged in DE50-MD dogs for tibial and fibular twitch, and fibular tetanus. There was an age-associated decline in force generation following eccentric stretch in DE50-MD tibiotarsal flexors compared to WT. The cranial tibial muscles of 18-month old DE50-MD dogs had significantly fewer type I fibres and more hybrid and regenerating fibres than those of WT animals.

Conclusion: DE50-MD tibiotarsal flexors produced lower force, were quicker to reach maximal contraction force and slower to relax, had reduced resistance to eccentric contraction and fewer type I fibres compared to age-matched WT dogs. These parameters could be used as objective outcome measures for pre-clinical testing in DE50-MD dogs.

D14

Effects of golodirsen on *DMD* transcript imbalance and nuclear trafficking in muscle biopsies from patients who completed the clinical study 4053-101

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Background: Antisense oligonucleotides (AONs) are one of the most promising genetic therapies for the treatment of Duchenne muscular dystrophy (DMD), an X-linked rare disease characterized by progressive muscle weakness and wasting. AONs aim to therapeutically skip *DMD* exons to reframe the dystrophin transcript that is altered by deletions occurring in patients. Golodirsen is a phosphorodiamidate morpholino oligomer (PMO) for the treatment of DMD in patients with confirmed *DMD* gene mutations amenable to exon 53 skipping. mRNA is the biological target of antisense therapies and the DMD's mRNA shows the unique peculiarity of the transcript imbalance, consisting of being expressed differently throughout its length, with a significant decrease towards the 3'. Moreover, recent works showed a possible mRNA trafficking impairment between nuclei and cytoplasm in myogenic cells of DMD patients and in myofibres of the mdx mouse model of DMD, compared to healthy controls. Our previous work, conducted on MyoD-lentivirally induced fibroblasts from 25 patients enrolled in the clinical study 4053-101, revealed that golodirsen is able to significantly restore the DMD transcript imbalance. Moreover, our preliminary evidence suggested that therapeutically skipped DMD transcript is preferentially located in the cellular cytoplasm, in contrast to the non-skipped transcript, which tends to be retained in the cellular nuclei.

Aims: In order to confirm the restorative role of golodirsen on transcript imbalance and to better delineate if it might have a functional role in repristinating the mRNA intracellular trafficking affected by DMD mutations, we repeated our previous in vitro experiments on muscular biopsies of the same 25 DMD patients.

Methods: We used FluiDMD cards to evaluate the transcript 5'-3' imbalance and the in-situ RNA hybridization Basescope approach to investigate the transcript subcellular localization; both investigations were conducted on muscle biopsies taken at baseline and after 48 weeks of the clinical study.

Conclusion: Our findings will allow us to better understand the mode of action of golodirsen, the variability in patient response to AON treatments and will be invaluable in predicting outcomes of new AONs developed for DMD.

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D15

Advanced *in vitro* human striated muscle platforms to model multi-tissue involvement in Duchenne muscular dystrophy

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Background: Muscular dystrophies are a heterogenous group of genetic disorders characterised by skeletal muscle wasting and weakness. Importantly, several muscular dystrophies are also characterised by cardiac muscle involvement, such as Duchenne muscular dystrophy (DMD): the most common paediatric muscular dystrophy. However, this cardiac phenotype is limitedly studied in patients and poorly recapitulated in most animal models, which primarily focus on skeletal muscle abnormalities. Although there are multiple *in vitro* DMD modelling platforms, none of them has been designed to recapitulate phenotypic readouts in both striated muscles together.

Aims:
1) Use DMD patient-specific human induced pluripotent stem cells (hiPSCs) to generate cardiac and skeletal muscle cells to study disease pathogenesis.
2) Recapitulate the complex multicellular tissue architecture of cardiac and skeletal muscles using engineered 3D tissue systems to improve maturation *in vitro*.
3) Establish robust DMD disease hallmarks in our 3D DMD models and validate them by testing therapeutic strategies.

Methods/Materials: DMD and healthy control hiPSCs were differentiated into cardiomyogenic cells (CMC) using a transgene-free protocol and thereafter assembled with biomaterials into 3D engineered heart tissue (EHT). Molecular analyses were performed to assess tissue maturation. Effects of dystrophin mutations on the electrophysiological behaviour of cells were explored via Ca^{+2} imaging. Construction of 3D artificial skeletal muscles is currently in progress for concurrent analysis with DMD EHTs.

Results: Gene expression analysis indicated enhanced tissue maturation in EHTs vs. conventional cardiomyogenic monolayer cultures. Electrophysiological analysis of the DMD CMC demonstrated contraction abnormalities resembling *in vivo* disease phenotype. Fewer CMC were detected in dystrophic EHTs than in healthy controls, meanwhile, dystrophic gels had higher non-cardiomyogenic fibroblast-like cells.

Conclusion: We could successfully establish DMD EHTs with enhanced cardiac maturation which recapitulated disease-associated functional phenotypes *in vitro*. These EHTs will now be used in conjunction with 3D engineered skeletal muscles to test therapeutics on both tissues in mutation-specific and mutation-independent strategies.

Dystrophy Clinical

DC01

The International Clinical Outcome Study for Dysferlinopathy- COSII baseline cohort characteristics

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Background: Mutations in the gene *DYSF* cause a variety of phenotypes collectively known as dysferlinopathies, including Limb Girdle Muscular Dystrophy 2B and Miyoshi Myopathy. Although there are currently no disease modifying treatments for dysferlinopathies there was a need to develop clinically validated outcome measures in dysferlinopathy in preparation for future clinical trials. The International Clinical Outcomes Study for Dysferlinopathy (COS), a three year multicentre study of 193 participants with dysferlinopathy, funded by The Jain Foundation and sponsored by Newcastle upon Tyne NHS Foundation Trust has enabled the development of appropriate outcome measures including the newly developed North Star Assessment for limb girdle type muscular dystrophies (Jacobs and James, 2022). We now need to validate these in a new cohort of participants through the COSII.

Aims:
COSII objectives include
1. To validate the outcome measures defined in part 1 of this study and confirm potential exclusion criteria as valid.
2. To gain a greater understanding of the transition from ambulant to non-ambulant in this population.
3. To expand the ethnic diversity of studied cohorts

Methods: All COSI participants were eligible to continue. We aimed for a validation cohort of 70 participants. Data will be collected at four visits over

two years, including physiotherapy and medical assessments, qualitative and quantitive MRI, biomarkers and patient report outcome measures.

Results: Four of the original 15 sites opted not to continue with their participation in COS2 due to disease progression of their site cohorts or staff turnover. Four new sites were initially added, in Denmark, Chile and the USA, with a further site in South Korea added at a later date to manage the risk to recruitment caused by the COVID-19 pandemic. 16 sites in total recruited 202 participants by June 2022. 118 new participants were recruited, and 84 continuing participants were retained. Baseline demographic data will be presented. In COSI the ratio of ambulant to non-ambulant participants was reported as 3:1. For COSII, self-reported ambulation status for the validation cohort was comparable.

Conclusion: Completion of this study and subsequent analyses will enhance our understanding of the natural history this variable condition.

DC02

The DMD Hub Central Recruitment Pilot Project

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Background: Over the past 10 years, there has been an increased number of natural history studies and clinical trials in Duchenne Muscular Dystrophy (DMD) conducted to better understand the natural course of the disease, explore innovative biomarkers, and evaluate the effect of new interventions and treatments. Due to the complexity of the disease and of setting up trial sites, they are often only run in a limited number of centres, limiting opportunities for participation for people with DMD and creating recruitment challenges to clinicians.

Aims: The pilot project is establishing a centrally coordinated national recruitment contact database for people with DMD in the UK, who are interested in participating in research studies. The online database will be active for an initial 12 months and contains information that will support clinical sites in identifying potentially eligible candidates for research studies in DMD.

Methods: Information on a participant's condition, including genetic diagnosis and motor abilities, and preferences relating to participation in research studies are being collected via an online questionnaire completed by the participant. The participants are asked to consent for sharing the information provided directly with sites looking for potential candidates for a specific research study.

Results: The Central Recruitment Database launched on the 31st March 2022 and has 158 registered participants with a confirmed genetic diagnosis of DMD. To date 36 individual referrals have been made to 7 research studies at 5 sites. These referrals have led to the successful recruitment to a gene therapy and an exon skipping clinical trial, as well as a physiotherapy study comparing AFO's and CCD's. 6 months after launch a participant and a clinical trial site survey was conducted to measure their satisfaction with the Central Recruitment Database.

Conclusion: The project has demonstrated that it is delivering fairer and more equitable access for patients to research studies in the UK, regardless of their location. It is also actively assisting clinical sites in the UK with recruitment to research studies in DMD. The data collected may be used to inform decision making on recruitment into UK clinical trials for people with DMD and their families, trial sites and study sponsors in the future.

‡DC03

Genotypic and phenotypic spectrum of *ANO5*-associated muscle disorders

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Background: The spectrum of *ANO5*-associated muscle disorders includes asymptomatic carriers, limb girdle muscle dystrophy R12 (LGMDR12), Myoshi myopathy distal subtype 3 (MMD3), isolated elevation in serum creatine kinase (CK), myalgias and recurrent rhabdomyolysis..

The most common reported genetic mutation is c.191dupA, with no clear genotype-phenotype correlation described to date.

Aims: To describe the genetic and clinical spectrum of disease in patients diagnosed with recessive *ANO5* variants at the Highly Specialised Service for Limb Girdle Muscular Dystrophy in Newcastle-upon-Tyne, United Kingdom.

Methods: Retrospective file review of patients identified through the local genetic database.

Continuous data were summarized using medians and ranges; proportions were used for categorical data. The genetics, clinical presentation and progression of disease by different phenotypes were described and compared.

Results: We identified 44 patients from 39 non-consanguineous families: 41/44 (93.2%) had c.191dupA mutations.

The median age at disease onset was 34 (range 9-62) years; two patients presented in childhood. Thirty-five (79.5%) were male. The median duration of follow-up was 20 (range 2-40) years.

The most common phenotype was LGMD (27/44 - 61.3%), followed by MMD3 (9/44-20.5%), myalgia (4/44 - 9.1%) and isolated high CK levels in one patient (2.2%).

Proximal lower limb (LL) weakness was present in 35/44 (79.5%), distal LL weakness in 20/44 (45.5%) and upper limb weakness in 20/44 (45.5%). Muscle atrophy was observed in 18/44 (40.1%), scapular winging in 12/38 (32%) and myalgia in 20/39 (51.3%).

Weakness was asymmetric in 63.1% and slowly progressive in all patients. At time of last evaluation, 68.2% retained ambulation.

Cardiac disease was present in 4/44 (9.1%), respiratory involvement in 5/44 (11.4%) and mild dysphagia in three (6.8%). Two deaths occurred in patients over 70 years of age from unknown causes.

Non-LGMD patients had an earlier presentation compared to LGMD patients (29.4 vs. 37.5 years, p=0.04), but no significant differences were observed in demographics, genetics, clinical severity, CK levels and lung function between phenotypic groups.

Conclusions: Recessive ANO5-related muscle disease had variable age of onset, but slow progression in all patients. No genotype-phenotype correlations were observed.

DC04

DMD Hub: A UK network enabling trials in Duchenne muscular dystrophy

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Background: The DMD Hub was established in 2015 as an innovative collaboration between Duchenne UK and two leading UK neuromuscular centres of excellence, the JWMDRC in Newcastle and GOSH in London, with the initial aim of increasing capacity and sharing expertise in Duchenne muscular dystrophy (DMD) trials. With investment exceeding £3.5 million the initial aims were successfully achieved, 34 posts have been funded at 11 DMD Hub sites and 450+ boys have been recruited to interventional and non-interventional DMD clinical trials.

Aims: With the number of clinical trials in DMD continuing to increase and the arrival of adeno associated virus (AAV) based gene therapy trials it is important that the DMD Hub ensures sites and staff continue to be trial ready.

Methods: The DMD Hub is focused on supporting the network sites, facilitating the exchange of information via online resources, peer-to-peer support networks and providing training and education opportunities.

Results: The DMD Hub has developed a website (dmdhub.org) as a key resource for sites as well as industry and patients. Specifically, it hosts the:

- Online training platform, sharing training resources and exchanging trial specific information.
- Expanded toolkit, a repository of information and tools for sites.
- Clinical Trial Finder, a comprehensive list of all DMD clinical trials and appropriate recruitment status at each site.

The DMD Hub has also set up peer-to-peer support networks, facilitated secondments and exchange visits and delivered workshops and meetings to enable sharing of resources and exchanging of knowledge.

- The PI network enables early engagement with sponsors to consult with potential UK investigators to understand the logistics and capacity issues that may be present.
- The nurse and clinical trial coordinator networks enable sharing of trial specific information to facilitate efficient set up and delivery.

Conclusion: The DMD Hub has helped implement significant changes to the UK clinical trial environment for patients, sites, and pharma. The collaborative work continues to increase the DMD community's knowledge and is ensuring we are in a strong position to deliver trials effectively and efficiently. Outputs are being shared so that other countries and other diseases may benefit and replicate the model.

DC05

Filamin C myopathy masquerading as mitochondrial disease

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Background: Filamin C myopathies are autosomal dominant conditions caused by pathogenic variants in *FLNC*. They typically cause adult onset muscle weakness, which can be distal, proximal or axial. Respiratory muscle involvement and cardiomyopathy are often prominent features. Muscle biopsy can show variable myopathic changes. A myofibrillar pattern (associated with variants in the rod domain) may be observed.

Aims: We report the case of a 59-year-old man with a filamin C myopathy clinical phenotype masquerading as a mitochondrial disorder on muscle biopsy.

Methods: Clinical presentation compatible with a myopathy, which led to muscle biopsy, neurophysiological examination, and genetic analysis.

Results: A 59-year-old man was seen in the muscle clinic. He had a background of type 2 respiratory failure treated with NIV, and cardiac failure. He gave a 3 year history of progressive limb weakness, loss of muscle bulk, and paraesthesia in his fingers. He had a strong family history of cardiomyopathy and sudden cardiac death. Examination revealed weakness of ankle dorsiflexion bilaterally and lower limb sensory impairment.

He had a creatine kinase of 556 IU/L. Neurophysiology showed evidence of a mild axonal neuropathy with additional scattered myopathic changes on EMG. He was unable to have an MRI. Muscle biopsy showed fibre size variability, scattered atrophic fibres and a strong type 2 fibre predominance. The remaining type 1 fibres had uneven staining suggestive of myofibrillar disarray. There were striking signs of mitochondrial dysfunction including 3% COX negative and 5% ragged red fibres. However, the pattern was not classical for a mitochondrial disorder.

Mitochondrial genetic analysis including the whole mitochondrial genome and extended nuclear gene panel did not detect any pathogenic variants, nor were there any mitochondrial rearrangements detected in muscle. The cardiomyopathy gene panel detected a heterozygous variant in *FLNC*, c.6031G>A p.(Gly2011Arg), reported as likely pathogenic.

Conclusion: We describe a pathogenic variant in *FLNC* causing typical filamin C myopathy. His muscle biopsy was strongly suggestive of a mitochondrial disorder, leading to extensive mitochondrial genetic testing which was negative. We conclude that the pathological findings are a very unusual consequence of filamin C myopathy, not previously reported in the literature.

‡DC06

Molecular Diagnosis of Facioscapulohumeral Muscular Dystrophy in low and middle-income countries in the ICGNMD consortium

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is the second most common muscular dystrophy in adults. The typical phenotype involves progressive, often asymmetric involvement of facial, scapular and humeral muscles. FSHD is caused by chromatin relaxation of the D4Z4 macrosatellite repeat, mostly by a repeat contraction (FSHD1), resulting in toxic expression of DUX4 in skeletal muscle. In some cases, FSHD is caused by pathogenic variants in D4Z4 chromatin modifiers (FSHD2). Genetically FSHD is mainly studied in patients with a European or Northeast Asian (Japanese, Chinese and Korean cohorts) background which suggested a difference in disease susceptibility. Southern blot-based analysis is the gold standard for FSHD diagnostics but comparable results can be obtained using optical genome mapping, molecular combing or D4Z4 methylation analysis.

Aims: Access to FSHD genetic diagnosis of populations in low and middle-income countries (LMIC) such as India, Brazil and South Africa is limited. We aimed to investigate FSHD in patients from these 3 countries using pulsed-field gel electrophoresis (PFGE)-based Southern blotting and methylation analysis.

Methods/materials: Analysis of blood DNA from almost 200 individuals (patients and relatives). For D4Z4 repeat sizing, high molecular weight DNA was digested with restriction enzymes followed by PFGE, Southern blotting and sequential hybridization with radioactive labeled probes. DNA methylation analysis was performed to identify D4Z4 hypomethylation associated with FSHD2.

Results: Genetic testing revealed a 1-10 unit (U) repeat on a 4qA allele (Europe-based FSHD1 threshold) in 31 of 57 Indian families, 5 of 11 Brazilian families and 12 of 15 South African families. More detailed analysis of the Indian cohort revealed a de novo FSHD contraction in 9/31 probands. Interestingly, for 26/31 Indian probands we identified a 1-7U FSHD-allele. In the remaining 5 cases, two

were FSHD2 and for one we found a different genetic explanation for the phenotype. The remaining two cases are under investigation with more extensive genetic analysis. In both FSHD2 patients we identified a missense variant in SMCHD1, the most common FSHD2 gene.

Conclusion: Based on our initial genetic data, the size distribution of the FSHD1 allele in India resembles the distribution previously found in the Northeast Asian population (1-7U). Further analyses are ongoing to better define the spectrum of the genotype-phenotype correlation in India, Brazil and South Africa.

DC07

Data highlights and insights from the Global Registry for COL6-related Dystrophies

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Background: The Global Registry for COL6 Related Dystrophies is an international patient registry for individuals with Bethlem Myopathy, Ullrich Congenital Muscular Dystrophy, and intermediate diseases. The registry is funded by MDUK and coordinated at Newcastle University.

Aims:
- Further understanding of the natural history of COL6-related dystrophies
- Facilitate clinical research
- Provide a valuable dataset to stimulate research into COL6-related dystrophies
- Disseminate information to the disease community
- Support identification of patients for clinical trials as they become available

Methods/Materials:
The registry collects longitudinal, self-reported data through an online portal (http://www.col6.org) from both patients and their clinicians, including:
- Age of onset
- Presenting symptoms
- Family history
- Motor function
- Muscle strength
- Respiratory and cardiac function
- Qualify of Life and Pain

Data from the registry can be made available to interested parties upon approval from the steering committee.

Results: The registry has 184 pariticpants representing 34 countries. 47% report a diagnosis of Bethlem Myopathy, 31% Ullrich muscular dystrophy, 15% Ullrich-Bethlem intermediate and 7% not specified. 42% have a mutation in the *COL6A1* gene, 26% *COL6A2,* 25% *COL6A3* and 7% not specified.

A review of the registry dataset is underway to harmonise with the TREAT-NMD LGMD Core Dataset. This also involves a review of the differential data to be collected from patients with Bethlem Myopathy and Ullrich Congenital Muscular Dystrophy, as the latter is not an LGMD.

We are working to further align with the FAIR Data Principles. This is being achieved by making the dataset's metadata available in collaborative efforts such as the RDCA-DAP and (ERDRI). Work is also underway to align UK registry participants with biobank samples that they may have provided.

Conclusion: The registry has the potential to be a key resource to support continued research into the COL6-related dystrophies. By involving as many participants as possible, this value continues to increase. Continued efforts are being made to raise awareness of the registry worldwide.

DC08

A qualitative study assessing the acceptability of risks associated with gene therapy for Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is a progressive, life limiting muscle wasting condition resulting in a reduced life expectancy (median 28.1 years). Gene therapies (GT) for DMD are currently under clinical trial, with initial results showing GT to be disease modifying, but not curative. There are significant risks, including the risk of death.

An initial study was completed in 2017 to assess the maximum acceptable risk (MAR) of death in U.S.-based adults with DMD and caregivers.

Aims: Given the emerging GT trial results and the increasing knowledge in the DMD community since 2017, the present study aims to assess the current MAR in the USA and UK, and explore experiences and attitudes of clinicians.

Methods/Materials: Three focus groups with adult patients and caregivers (n=16) and clinician interviews (UK n=8, US n=8) were conducted by experienced researchers using semi-structured guides. Data were coded using a matrix based approach.

Results: Clinician responses were highly variable with regards to benefit (modest to curative) and duration (one year to lifelong). This variation is likely due to their differing levels of experience with gene therapy and clinical trials. Clinicians indicated risk to the liver and side effects associated with the use of viral vectors. Clinicians emphasised the need to dose with GT early for maximum benefit, with greater risks for older patients.

Adults and caregivers anticipated benefits including stopping/slowing progression of muscle, cardiac and pulmonary decline, which would be highly meaningful. They were uncertain about the origin of the risks and side effects, the degree of risk, and the ability to mitigate risk. Adults and caregivers did not describe gene therapy as curative, however clinicians perceived that families may have unrealistic expectations and reported challenges in managing expectations.

Conclusion: The results from this study were used to update a quantitative study designed to understand the wider DMD community's priorities, risk tolerances and therapeutic optimism towards gene therapy, which is ongoing, with results expected to be published in 2023.

DC09

Global FKRP Registry - the research database for limb girdle muscular dystrophy R9 (2i)

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Background: The Global FKRP Registry is an international registry for individuals with conditions caused by mutation of the *Fukutin-Related Protein* gene (*FKRP*): limb girdle muscular dystrophy R9 (LGMDR9, formerly LGMD2i) and the congenital muscular dystrophies MDC1C, Muscle-Eye-Brain Disease and Walker-Warburg Syndrome.

Aims: The registry seeks to further understanding of the natural history and prevalence of FKRP-related muscular dystrophies and to aid the rapid identification of eligible patients for clinical studies. It disseminates FKRP-relevant information; provides a source of information to academics, industry and healthcare professionals; and supports the FKRP community.

Methods: Registration is patient-initiated through a secure online portal (http://www.fkrp-registry.org/). Participants give their consent and are invited to complete a questionnaire about their condition. Data is reported by both patients and their healthcare professionals and includes: gene mutation, age of onset, presenting symptoms, family history, motor function and muscle strength, respiratory and cardiac function, and medication. In addition, participants are invited to complete validated questionnaires on quality of life (INQoL) and pain (McGill).

Results: The registry has 909 participants (772 adult:137 paediatric (<18years)), 48% of whom have shared confirmation of their genetic diagnosis. Participant age range is 1-82 years (mean and median ages of 38 years). Registrations are from 50 countries, with greatest numbers from the USA (27%), Germany (21%) and the UK (10%). Diagnoses are reported as LGMDR9 (90%), MDC1C (2%), other FKRP-related MD (2%), unspecified (6%).

In recent years, the registry has assisted recruitment to natural history studies and clinical trials in LGMDR9. It has facilitated research by responding to data enquiries and circulating surveys, demonstrating its effectiveness as a repository of patient data, a tool for data collection and assembly of a trial-ready patient cohort. The registry participated in the working group to create the first TREAT-NMD LGMD Dataset, was used as a pilot registry to collect patient feedback and will shortly implement the newly developed dataset.

Conclusion: As knowledge of rare neuromuscular conditions increases and advances in the development of potential therapies are made, the registry is centrally placed to help support the accumulation of natural history and post-marketing surveillance data and facilitate recruitment to clinical trials.

DC10

Autosomal Dominant Distal Myopathy in two South African families with heterozygous nebulin deletions

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Background: Distal myopathies form a group of muscular dystrophies with over twenty causative genes including nebulin (*NEB*) with autosomal recessive inheritance. An autosomal dominant (AD) Finnish family was reported with a large heterozygous deletion in *NEB* (exon 14-89). We report two unrelated South African (SA) families with both a similar phenotype and *NEB* deletions.

Methods: Three symptomatic and two asymptomatic members of each family were evaluated. Next-generation sequencing (Invitae) and analysis with deletion/duplication testing of a myopathy gene panel were performed on the probands and subsequently a focused *NEB* analysis on the remaining family members.

Results: Family 1 has SA mixed-genetic ancestry. The proband is a 40-year-old female with early childhood onset of distal lower limb anterior compartment weakness, and subsequent proximal in-

volvement. In addition, she had mild bifacial and neck flexor weakness and subtle glove-and-stocking pinprick impairment. Muscle biopsy revealed mild atrophy of both fibre types. The two other symptomatic family members had a similar phenotype; however, symptom onset was in adulthood (40 and 56 years). In family 2, of Caucasian ancestry, the proband was a 23-year-old female with early childhood onset. She had mild bifacial weakness, nasal speech, neck flexion weakness, mild scapular winging, hand and distal lower limb weakness (predominantly anterior compartment), as well as glove-and-stocking pinprick impairment. Her affected mother and brother (age at onset 14 and 13 years respectively) had a similar phenotype. Muscle biopsy was not performed. Creatinine kinase levels and sensory nerve conduction studies were normal in both probands. A large in-frame heterozygous deletion of exons 14-77 in *NEB* was found in the three affected and one unaffected family member (age 35) of family 1, as well as in all three affected members of family 2.

Conclusion: We report two unrelated SA families with AD distal myopathy (predominantly anterior compartment) showing similar heterogenous phenotypic expression to a reported Finnish family and a large heterozygous *NEB* deletion. The heterozygous shortening of nebulin could suggest alternative pathogenetic mechanism(s) of *NEB*-associated myopathies such as a dominant negative effect. However, a digenic pathogenesis should also be considered.

DC11

Neurodevelopmental Disorders and Mortality in a Cohort of Adults with Duchenne Muscular Dystrophy

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Background: Duchenne Muscular Dystrophy (DMD) is the most common muscular dystrophy worldwide. With increasing survival there is now a greater awareness of neurodevelopmental co-morbidities in DMD, including autism spectrum disorder and learning difficulties & disability. Cardiorespiratory failure remains the most common cause of death, but there is currently a limited understanding of the effects of neurodevelopmental disorders on mortality.

Aims: The aim of this study was to establish the prevalence of neurodevelopmental disorders (NDs) in a cohort of deaths and assess for relevant differences between this sub-group and the remainder of the cohort.

Methods/Materials: Our service registered 37 deaths between 2011-2022. The notes of these patients were reviewed retrospectively for cause of death, neurodevelopmental status, compliance with therapy and physiological parameters. A neurodevelopmental disorder was defined as a documented diagnosis of Autistic Spectrum Disorder (ASD), Attention Deficit Hyperactivity Disorder (ADHD) or learning difficulty (LD). The data were then analysed to assess for differences based on neurodevelopmental status.

Results: Within our population of deceased patients, 43.2% had a diagnosis of a ND (ASD: 21.62%, ADHD: 0%, LD: 40.54%, including those with a combination of more than one ND). Those with NDs had a lower mean age of death compared to the remainder of the cohort, although this was not statistically significant (22.5 *vs* 24.9, p=0.10). A lower proportion were on steroids at death (12.5% *vs* 33.3%) and compliance with non-invasive ventilation was also lower (58.3% *vs* 88.33%).

Conclusion: Our data show a high prevalence of NDs in this cohort of deceased patients, exceeding prevalence figures noted in living populations elsewhere. This may suggest that co-morbid NDs convey greater risk of early death in patients with DMD. Indeed, mean age at death was lower in those with NDs, although this was not statistically significant. Given the centrality of steroids and ventilatory support in the management of DMD, the poorer compliance observed with these therapies may underly any such risk. More comprehensive data collection is required to assess this question further.

DC12

POGLUT1 related muscular dystrophy

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Background: WES analysis may show various variants in many genes. Sometimes this can cause confusion and difficulty in diagnosis. Deep phenotyping and muscle biopsy and imaging findings can be supportive in the definitive diagnosis.

Aim: We aimed to present our case who was diagnosed with muscle MRI findings and WES analysis.

Case: A seventeen year old male was admitted to our pediatric neurology clinic with progressive difficulty in walking and standing. He was born to consanguineous parents at term. Frequent falls started at the age of 12. Progressive proximal lower limb weakness was noted on examination. Serum CK levels were elevated. Electromyography revealed myopathic motor unit potentials. Muscle biopsy showed myopathic changes without dystrophy. Other investigations were not helpful in diagnosis. The patient was included in the ICGNMD project. WES analysis showed some TTN variants and variants in the *POGLUT1* gene. Muscle MRI was performed and it showed an ”inside to outside” involvement pattern of lower limbs. The patient was diagnosed as Limb-girdle muscular dystrophy 21 (*POGLUT1*-related muscular dystrophy) with the help of MRI findings and WES analysis results.

Conclusion: Muscle satellite cells take a role in skeletal muscle regeneration which is activated by muscle damage and regulated by Notch signaling. Protein O-glucosyltransferase 1 (POGLUT1) is the main protein that modulates the glycosylation of the extracellular domain of Notch receptors. Thus, disruption of POGLUT1 leads to defective muscle regeneration and secondary dystroglycanopathies.

Acknowledgement: We would like to thank our patients who agreed to participate in the project and the ICGNMD project team for their contributions

DC13

Prevalence of scoliosis in a large cohort of non-ambulant paediatric patients with Duchenne muscular dystrophy

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Background: Glucocorticoid (GC) therapy improves strength and reduces need for scoliosis surgery in patients with Duchenne muscular dystrophy (DMD).

Aims: To review rate of occurrence of scoliosis in a large cohort of non-ambulant paediatric patients with DMD from the UK.

Methods/Materials: Review of case notes and radiological reports was completed in a cohort of DMD patients attending a dedicated non-ambulant clinic at the Dubowitz Neuromuscular centre between 2019-2022. Patients were considered affected

by scoliosis when Cobb angle was greater than 20° (moderate/severe scoliosis).

Results: Records of 111 patients with DMD who attended at any time point the dedicated clinic were retrospectively reviewed. Age of patients ranged between 5.7-18.6 years at last appointment. Average age at loss of ambulation (LoA) was 10.6 years, with a median of 10 years. Fifty-seven patients had scoliosis at any time point (median age at last review 16 years, median age at LoA 9.3 years). Cobb angles range was 20-108° (average 47.6°, median 40°). Twenty-four patients with scoliosis (24/57; 42%) were on intermittent prednisolone treatment, 18/57 (31%) were not on GC, with remaining on either prednisolone daily (3/57; 5%), deflazacort intermittent (8/57; 14%) or deflazacort daily (3/57; 5%). Nine patients underwent spinal surgery (9/57; 16%), 5 of which being on prednisolone intermittent regime. 54 patients had no or mild scoliosis (median age 15 years; median age at LoA 11 years). The most common GC therapy in this group was prednisolone intermittent (14/54; 26%), followed by deflazacort daily (13/54; 24%) and prednisolone daily (12/54; 22%).

Conclusion: This review indicates a relatively high occurrence of scoliosis in this cohort of non-ambulant DMD patients (51%). Scoliosis was more common in GC naïve patients and those on intermittent prednisolone regime, with 63% of patients on this GC regime developing moderate or severe scoliosis at any time point. Our data also supports the lower rate of scoliosis in patients on daily GC regime, with only 19% developing scoliosis at any time point. While statistical analysis is key to better interpret this data, this first review may help in counselling and assist long term management of patients.

‡DC14

Quantifying Variability in Duchenne Muscular Dystrophy: Centiles by Age for the Rise from Floor Velocity and 10m Walk Run Velocity in Glucocorticoid-steroid Treated Boys

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Background: There is substantial clinical heterogeneity in the pattern of change of motor function in Duchenne Muscular Dystrophy (DMD), and this represents a significant hurdle to clinical monitoring. The 10-metre walk/run and rise from floor are two of the motor function outcomes most commonly used in clinical trials and in routine clinical assessments.

Aims: We describe for the first time centiles of the 10m walk/run velocity (10MWRV) and rise from floor velocity (RFFV) in Glucocorticoid-steroid (GC) treated boys with DMD, between 5 and 16 years. This includes RFFV-for-age and 10MWRV-for-age lines and tables to calculate the centile and Z-score for a given age and total score.

Methods: Participants were included from the NorthStar registry if they had a confirmed diagnosis of DMD, were on GC (primarily deflazacorts or prednisolone, intermittent or daily) and were not enrolled in a trial. In addition, where the assessment was not performed but the corresponding NorthStar Ambulatory Assessment item was scored as a 0, the corresponding velocity was imputed as a 0 to avoid informative drop-out. Both centiles were fitted using a GAMLSS model with the 0-1 inflated logit Normal family, and a cubic spline of age with one knot was selected for the mean models.

Results: We analysed 4143 observations in 833 DMD boys, of which 1905 of the 10MWRV (46%) and 1983 of the RFFV (48%) were imputed as 0. We present the 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} centiles to visualise progression on each. The centiles show loss of rise from floor (RFFV of 0) at 8.5, 10.0 and 11.8 years for the 25^{th}, 50^{th} and 75^{th} centiles, respectively. The centiles show a 10MWRV of 0 at 8.6, 10.4 and 12.3 years for the 25^{th}, 50^{th} and 75^{th} centiles, respectively. The 10MWRV displays a much flatter

centile over time, whilst the RFFV is much steeper over time.

Conclusions: The 10MWRV and RFFV centiles could provide insights for clinical monitoring of DMD boys, particularly to identify conventional and unusual change in motor function. Future work will look to validate the 10MWRV and RFFV centiles in trial placebo arms and natural history cohorts.

DC15

Ten years of collecting natural history data in the International Clinical Outcome Study for Dysferlinopathy

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Background: Patients with dysferlinopathies have highly variable clinical presentations and a lack of appropriate outcome measures to define progression. This presents significant challenges for clinical trial readiness. The International Clinical Outcome Study for Dysferlinopathy (COS) was the first international, multi-centre longitudinal natural history study in dysferlinopathy.

Aims: COS aimed to characterise phenotypes and determine appropriate outcome measures (OMs) across function, MRI, biomarkers and quality of life.

Methods/Materials: 193 participants from 15 sites in eight countries attended for three years, with some sites collecting data for up to six years. The extension study, COSII, recruited 203 participants, from 16 sites globally in nine countries. Inclusion criteria was two predicted pathogenic mutations in DYSF, or one predicted pathogenic mutation plus either absent dysferlin expression on immunoblot or < 20% dysferlin monocyte expression (NCT01676077). In the absence of any dysferlinopathy specific OMs, participants completed a variety of standardised assessments of strength and function, patient reported questionnaires, qualitative and quantitative MRI and biomarker studies.

Results: The North Star Assessment for limb girdle type muscular dystrophies (NSAD), was developed, validated and allowed accurate capture of dysferlinopathy presentation and progression. Utilising NSAD and MRI, COS has confirmed that Miyoshi and LGMD2B/R2 are not two distinct phenotypes, but both dysferlinopathies, a critical finding for clinical management, clinical trial population definition and access to disease modifying treatments. Participants experienced the steepest functional decline in the first ten years after symptom onset. Qualitative MRI defined a characteristic pattern of muscle involvement. Quantitative MRI methods including MRS and fat fraction captured change over three years. Standards of care for dysferlinopathy are under development.

Conclusion: COS, conducted with patient advocacy partners the Jain Foundation, has determined appropriate OMs that define the phenotype and progression of dysferlinopathy, driving the community much closer to clinical trial readiness.

DC16

The first standards of care guidelines for a limb girdle muscular dystrophy

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Background: The International Clinical Outcome Study (COS) for Dysferlinopathy, a large international natural history study, was initiated in 2012 (NCT01676077).

Aims: COS was developed to characterize common and rarer phenotypic features, and to describe clinical, functional and muscle MRI outcome measures in dysferlin-deficient patients longitudinally.

Methods/Materials: This study includes 15 sites from 8 different countries, recruiting >200 patients, who were evaluated initially six times over a three-year period. As the largest natural history study in LGMD, the results from the first phase of the study add significant knowledge to the course of the disease. A second phase is underway to validate the findings from phase 1, expand the cohort and initiate a care discussion with patients, as it was realised that access to therapy and care was highly variable across trial sites.

Results: Based on the results of the study and the development of validated outcomes measures, the field can now begin to translate some of this knowledge into international care guidelines (Standards of Care, SoC). With a number of potential therapies for dysferlinopathy entering human clinical trials (NCT025792, NCT01863004, NCT02710500), the development and implementation of internationally agreed SoC is becoming more urgent.

Conclusion: SoC are important for patients in order to proactively maximise their function and quality of life over their lifetime and for the robustness of clinical trials by ensuring patients are receiving a similar care standard across all trial sites and international boundaries. As pharmacological agents are still in development or early testing stages, the dysferlinopathy patient community requires informed and expert led SoC to be delivered via their routine clinical care. Empowering patients as the experts in their condition and ensuring that whilst other treatments are in the pipeline, they receive internationally recognised SoC in diagnosis, clinical assessments and follow up via access to the most appropriate multidisciplinary management.

DC17

Improving and harmonising care standards for Duchenne muscular dystrophy in the UK through collaboration and consensus building – the DMD Care UK Project

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Background: DMD Care UK was established in 2020 as a collaboration between Newcastle University and Duchenne UK, embedded in the North Star network. The project is reaching the end of phase 1 and is planning activities and sustainability for the next 3 years – phase 2.

Aims: DMD Care UK aims to improve and harmonise care provision across the country for Duchenne muscular dystrophy (DMD) by reaching expert-consensus, facilitating implementation and providing education for UK-context standards of care (SoC) based on international guidelines. We also aim to identify nd address barriers to SoC implementation.

Methods: Through a series of sub-specialist working groups comprising clinical experts in their fields, neuromuscular specialists and patient representatives, the project consults throughout the North Star network to reach consensus based on latest evidence, patient and clinician experience, feasibility and expert opinion. Engagement with relevant professional bodies helps support implementation of guidelines within clinical care. Patient information resources are produced to explain the SoC so that patients become empowered in making decisions about their own care and can better advocate for themselves/their children. Impact of the recommendations will be measured to make a strong business case for their implementation.

Results: DMD Care UK has produced and is publishing a series of UK-specific guidelines with endorsement from the neuromuscular community and relevant professional bodies.

Progress so far includes SoC development in bone

and endocrine, respiratory, cardiac, physiotherapy, emergency and psychosocial care. We also summarise next steps as we enter phase 2 with a focus on implementation, impact, education and research questions to inform and support recommendations.

Conclusions: DMD Care UK is an example of how the clinician and patient community can work together to improve standards of care for all those living with DMD. One of our successes has been the reach beyond the neuromuscular expert community to raise awareness of the multi-systemic nature and the needs of people living with DMD. The project has taken international guidelines and turned them into actions for implementation within the UK healthcare system. This is a model that can be adopted in other national healthcare settings and in other disease areas.

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DC18

Identifying barriers and increasing patient enrolment - UK Myotonic Dystrophy Registry.

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Background: Patient registries collect information from individuals affected by specific conditions, such as myotonic dystrophy. They can contribute to natural history, support the creation of care standards, and aid development of new treatments or therapies by identifying participants for clinical trials. Registries play an important role in providing a link between community and research.

Aims: The JWMDRC developed an online, anonymised survey, with the aims of identifying patient perceived barriers to joining the UK DM patient registry, and to understand what additional support could be provided.

Methods/Materials: Participants were invited to complete a survey about their experiences with the UK DM Patient Registry, with the opportunity to provide comments and feedback. The survey was disseminated to the existing registry participants via email, and to the wider DM community via social media through UK DM charities. The registry engagement survey launched 23rd August 2022 and remains open to continue to collect ongoing data.

Results: To date the survey has received 189 responses, of which 168 have a diagnosis of myotonic dystrophy and 17 are unaffected caregivers. 78% currently participate in the registry, but only 11 individuals reported logging in annually to update data. 10 respondents stated they are not enrolled with reasons including most not knowing of the existence of the registry, not understanding what the registry is, or 'not seeing the point'.

54 responders provided comments on how the registry could be improved, and how to better support sign up for new patients. Of the registration support options suggested, the most popular was clinicians passing on contact details. 45 people stated they would like the registry curator to contact them for further help.

Conclusion: Difficulties within the patient community in understanding or accessing the registry were identified. This information and feedback will be used by the registry curator and steering committee to support requests to fund additional registry work, identify new strategies and create better resources for the patient community. Registry enrolment and longitudinal participation could be increased by facilitating direct contact between the patient and registry curator, increasing the quality and quantity of data available to support DM research.

DC19

Clinical Features of the UK FSHD Patient Registry

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Background: The UK Facioscapulohumeral Muscular Dystrophy (FSHD) Patient Registry is a patient self-enrolling online database collecting clinical and genetic information about FSHD type 1 (FSHD1) and type 2 (FSHD2). The registry was established in May 2013 with support from Muscular Dystrophy UK and is coordinated by Newcastle University.
Aims: The registry aims to facilitate academic and clinical research, better characterise and understand FSHD, and disseminate information relating to upcoming studies and research advancements.

Methods/Materials: The registry captures longitudinal, self-reported data through an online portal available to patients and clinicians. Where specialised clinical or genetic information is required, the neuromuscular specialist involved in the patient's care can be invited to provide some additional information and the patient can select them from a pre-populated list at the registration stage. The registry is a Core Member of the TREAT-NMD Global Registries Network for FSHD.

Results: As of January 2022, there were 952 active, UK based patient registrations. For those reporting a clinical diagnosis, 96% have FSHD or FSHD1, and 4% have FSHD2. Overall, 52% of patients have had genetic confirmation of their condition. In addition to collecting specific genetic data inputted by clinicians, the registry is now able to receive digital copies of patient's genetic reports directly via a secure upload portal. The registry has supported almost 30 registry enquiries to date, recent examples including three COVID-19 surveys, and various surveys capturing information on dysphagia, pregnancy, sleep and the patient/caregiver experience.

Conclusion: The registry is currently one of the largest national FSHD patient registries and is an example of a versatile, cost-effective research tool, helping facilitate and advance a wide range of FSHD research. Additional work continues to be done to improve reporting of genetic information on the registry and there are future data linkage plans between the registry and the Newcastle Research Biobank for Rare and Neuromuscular Diseases.

DC20

Patient Registries at the JWMDRC: where we are now and where we might go next

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Background: Patient Registries are more than just databases; they can facilitate translational research by collecting and providing genetic, clinical, and quality of life data on neuromuscular diseases. The JWMDRC has developed and managed UK neuromuscular patient registries since 2008, for NMDs including SMA, FSHD, DM, and international regis-

tries for MTM and CNM, FKRP-related conditions and Col6 related dystrophies.

Aims: The purpose of the registries include aiding the rapid identification of eligible patients for clinical studies, collection of longitudinal natural history data, and as a communications interface between patients, healthcare professionals, and the research community.

Methods: For all JWMDRC registries, registration is patient-initiated through a secure online portal. Participants give their informed consent and are invited to complete a questionnaire about their condition, including: genetic confirmation, clinical diagnosis, current and best motor function, and ventilation status. Most include questions on patient perception and experiences, captured using PROM questionnaires: EQ-5D-5L; and/or Patient Global Impression of Change, and a free-text box, and all contain additional condition-specific questions.

Results: The JWMDRC registries currently contain over 4,000 participants. The poster will provide an overview of the individual registries, describe some recent registry use cases, and discuss how the registries can respond to changing needs from the neuromuscular community.

Conclusion: The JWMDRC Patient Registries represent several cohorts of individuals, across a number of neuromuscular conditions, and are valuable tools for the collection of patient data which informs academics, healthcare professionals and industry. UK registry datasets are being updated and expanded when possible in order to collect data for evaluation by UK regulatory authorities considering potential approval of new therapies.

Peripheral Neuropathy

PN01

An induced pluripotent stem cell-based model to study neurodegeneration in *RFC1* disease

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Background: Cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) is a late-onset, slowly progressive ataxia associated with bi-allelic AAGGG expansions in RFC1.The phenotypic spectrum of RFC1 disease is broad but sensory neurons are constantly involved. The disease mechanisms of this disorder remain elusive and previous studies on patients' cell lines (i.e., fibroblasts, lymphoblasts) or post-mortem brain did not show a reduction of RFC1 RNA or protein, nor an overt dysfunction of DNA replication and repair. Recently, induced pluripotent stem cells (iPSCs) have been proposed as a powerful experimental model for several diseases, as they allow to generate patient-specific cell lines from different sources (e.g., fibroblasts, peripheral blood).

Aims: We generated IPSC-derived sensory neurons from CANVAS patients and controls to investigate the disease mechanisms of RFC1 disease.

Methods: We used Chamber's modified protocol for the differentiation of sensory neurons starting from IPSC lines derived from patients' and controls' fibroblasts. We confirmed the correct maturation and purity of colonies by staining for the sensory

neurons-specific marker BRN3A. We assessed morphological parameters such as neurite outgrowth and number of branching points. We then compared the transcriptome profile of CANVAS and control lines by RNAseq (Illumina Next Seq 500). Finally, given the role of RFC1 in DNA damage, we quantified DNA damage response in basal conditions and after pharmacological stress, as well as axonal damage markers (neurofilament light chain).

Results: We successfully generated mature and pure colonies of post-mitotic sensory neurons derived from IPSCs lines (n=3 CANVAS; n=3 controls). No significant difference in neurite outgrowth and branching points was observed between patients and controls. The transcriptomic analysis on three CANVAS vs three control lines revealed unchanged RFC1 transcription and splicing. Quantification of RFC1 protein, DNA and axonal damage markers is still ongoing.

Conclusion: The study confirmed no overt reduction of RFC1 transcript or abnormal splicing in a disease relevant model as IPSCs-sensory neurons. Future studies on long-term cultures of IPSCs-derived neurons will provide a better insight into the mechanisms and pathways underlying neurodegeneration in this disorder.

PN02

Mycophenolate maintenance in CIDP facilitates in IVIG dose and improvement in outcome measures

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Background: Intravenous immunoglobulin (IVIG) maintenance therapy in CIDP is costly treatment and limited resource. There is limited evidence to support mycophenolate (MMF) as a potential replacement therapy.

Aims: To assess the effect of the introduction of mycophenolate on IVIg dose, IVIg cost and clinical outcome measures in a cohort of CIDP patients who were maintained on ultra-high dose of IVIG

Methods/Materials: Retrospective review of electronic records of patients treated with MMF after being established on IVIG therapy for CIDP. Statistical analysis was performed in Microsoft Excel. For each patient 6 months prior to and after MMF therapy, we analysed the total amount of IVIG needed, number of days of therapy required, frequency of IVIG and outcome measures of RODS score and MRC strength score.

Results: Eleven patients with CIDP (1/11 NF155 antibody positive, 1/11 motor variant) were treated with IVIG in the NHNN inflammatory neuropathy cohort. 8/11 male, mean age 54.5 (s.d 15.5) years, mean duration of disease was 5 (s.d. 3.4) years. Mean duration of mycophenolate treatment was 17.2 (s.d. 20.5) months, with a mean dose of 2.8 (s.d. 0.4) g. MMF therapy was well tolerated by all patients. At latest follow up there was a mean IVIg dose reduction of 0.7g/kg/month, mean day care reduction of 1.5 days/month. Median I-RODS improved from 24/48 to 39/48, with an improvement in median MRCSS from 58/70 to 69/70. Overall, this has a total IVIg saving of 4137 grams over 6 months within our cohort of patients. The estimated savings for the IVIG were £165,514 in over 6 months. There was also a reduction of day care unit use by 80 days over the 6 months.

Conclusion: Mycophenolate is a well-tolerated alternative and or IvIg "sparing agent" maintenance therapy in CIDP with positive impact on IVIg requirement and cost as well as patient clinical outcome measures. MMF can help in reducing ultra-high dose IVIG required in treatment resistant CIDP.

PN03

Patient Reported Data for Charcot-Marie-Tooth Disease

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Background: Charcot-Marie-Tooth disease (CMT) is a hereditary motor and sensory neuropathy affecting 1 in 2500, approximately 3 million people worldwide. The disease is highly heterogeneous and more than 100 different CMT-causative genes have been identified. There are currently no curative treatments for CMT.

Aims: A quality improvement initiative was launched in 2018 with the goal of advancing CMT research through the collection of patient reported data. The initiative aims to engage the patient community in research, the development of treatments, and ultimately a cure, for CMT. This is the first publication of this patient reported data.

Methods/Materials: Data was collected via secure online questionnaire and anonymized prior to analysis.

Results: By October 2022, 6091 individuals had created a patient reported data profile. Analysis of the anonymized data shows average (mean) age is 47.7 years (SD 22.3, range 8 months to 105 years, n=1961). 5911 individuals (97%) shared data on CMT type, distributed as, Type 1 35.3% (n=2085), Type 2 16.6% (n=980), Type X 4.3% (n=252), Type 4 2.8% (n=167), HNPP 0.9% (n=53), GAN 0.03% (n=2) and 40.1% (n=2369) do not know their type. 4034 individuals (66%) shared data on CMT subtype, 47 different sub-types are represented. The largest subtype groups are CMT1A 39.4% (n=1590), CMT2A 7.4% (n=297), CMT1X 5.1% (n=204), CMT1B 4.2% (n=170), CMT4C 1.6% (n=63) and 32.0% (n=1291) reported undiagnosed/unknown. Average (mean) age at diagnosis is 31.9 years (SD 20.9, range 0 to 81 years, n=1494). Location data, age at symptom onset and rate of accessing genetic testing was also analyzed.

Conclusion: This initiative represents one of the largest available collections of CMT patient reported data, it is a valuable tool for patient engagement, research and clinical trial recruitment. We will present a summary of the anonymized data, real-world examples of how the initiative has supported CMT research and highlight opportunities for global research collaboration.

PN04

Clinical and neurophysiological characteristics of a Brazilian cohort of patients with Charcot-Marie-Tooth type 4C (CMT4C)

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Background: Charcot-Marie-Tooth neuropathy type 4C (CMT4C) is an autosomal recessive demyelinating neuropathy caused by mutations in the SH3TC2 (SH3 domain tetracotripeptide repeats 2) gene. Patients classically present in the first decade of life with distal weakness and sensory impairment, areflexia, foot and spine deformities and sometimes cranial nerve involvement or respiratory distress.
Aim: To describe a Brazilian cohort of patients with a confirmed diagnosis of CMT4C, highlighting electrophysiological studies.

Methods/materials: Eight individuals from 6 families were analyzed. Clinical and electrophysiological data were retrospectively collected from clinical records. Sex, age of onset, presenting symptoms and electrophysiological data were collected for further analysis.

Results: Six out of 8 patients (75%) were female. Frequent falls and difficulty walking were the most common initial features in up to half of the patients. In 6 patients (75%), symptoms started in the first decade of life. Interestingly, nerve conduction studies

revealed a non-uniform reduction of conduction velocity and temporal dispersion in all of the patients.

Conclusion: The presence of temporal dispersion in all studied patients may suggest that SH3TC2 plays an important role in peripheral nerve conduction. Indeed, recently, nerve pathology data from SH3TC2-knockout animal models revealed loss of internodal architecture that could ultimately explain this feature. Temporal dispersion might be a frequent finding in CMT4C patients which may aid in differentiation from other CMT subtypes. Clinicians must be aware of this to avoid extensive investigation and misdiagnosis with acquired neuropathies.

PN05

Novel Dynamin 2 mutations causing Charcot-Marie-Tooth Neuropathy

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Background: Charcot-Marie-Tooth disease (CMT) and related disorders are the commonest group of inherited neuromuscular diseases. They represent a heterogeneous group of disorders. There are now over 100 genes that are known to be responsible for several forms of CMT due to increasing use of next-generation sequencing technologies, including Dynamin-2 (DNM2), with a constant evolving phenotypic spectrum as new variants are identified. DNM2 a type of guanosine triphosphatase that is involved in multiple cellular functions including endocytosis, membrane trafficking, actin assembly and centromere cohesion. DNM2 mutations are associated with intermediate CMT and CMT2, centronuclear myopathy, congenital contracture syndrome type 5 and hereditary spastic paraplegia indicating phenotypic variability.

Aim: We report 4 patients with novel DNM2 related neuropathy causing CMT.

Method: All genetically confirmed cases with novel DNM2 mutations in our cohort were retrospectively included. Clinical features, variant type and neurophysiological data were extracted and analysed.

Results: From our cohort we identified 4 patients from 4 different families. 3/4 were females. 2 patients had a family history of CMT and 2 were apparently sporadic. Symptoms appeared in the first 2 decades of life. Clinical and neurophysiological examination showed neuropathy in all patients. Charcot-Marie-Tooth Examination score varied between patients (Mean 8.75, Range 4-12). Novel variants were as follows; c.1736T>C p.(Phe579Ser), c.1733G>A p.(Gly578Asp) and c.1660A>G p.(Lys554Glu). Segregation was confirmed for the latter 2. The c.1660A>G p.(Lys554Glu) was also identified in another patient, but this was apparently sporadic.

Conclusion: We report novel DNM2 mutations leading to CMT. DNM2-related diseases are phenotypically heterogeneous and may manifest differently, emphasising the importance of a comprehensive clinical examination, neurophysiological findings and pursuing causative gene variants.

PN06

Clinical and Electrodiagnostic Characterization of Primary Hereditary Neuropathies in a cohort of patients with genetic neuromuscular disorders in Zambia

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Background: Little is known about the clinical phenotypes, electrodiagnostic (EDX) or molecular characteristics of primary inherited neuropathies in sub-Saharan Africa (SSA). As gene-targeted therapies are on the horizon for the most common Charcot-Marie-Tooth (CMT) gene mutations found in populations from the global north, there is urgent need for knowledge about these disorders in SSA populations to enable clinical trial readiness and participation.

Objective: Describe inheritance patterns, clinical and electrophysiologic phenotypes of primary inherited neuropathies in a cohort of patients with genetic neuromuscular disorders (GNMDs) in Zambia.

Methods: We identified probands with primary inherited neuropathies among participants enrolled in a prospective GNMD study at the University Teaching Hospital in Lusaka, Zambia. Molecular studies were pending at the time of this analysis. HIV positive persons were excluded.

Results. Twelve (16%) cases of primary inherited neuropathies were identified among 74 probands. Eight (67%) were male with median age 13 years (absolute range: 6-29 years). The majority (n=8; 67%) reported symptom onset in the first decade of life, while the remaining developed symptoms in the second (n=3; 25%) and third decades (n=1; 8%). Inheritance patterns included 5 (42%) sporadic/de novo, 4 (33%) autosomal dominant, and 3 (25%) autosomal recessive. All cases had weakness in the distal lower limbs and most (n=9; 75%) had weakness in the distal upper limbs. Median modified MRC sum score was 62 (absolute range 46-68) out of 70. Associated clinical characteristics included tongue fasciculations (n=1;8%), hyperkinetic movements and childhood-onset epilepsy (n=1; 8%), and hearing loss and hoarse voice (n=1; 8%). EDX findings included sensorimotor axonal polyneuropathy in 10 (83%) cases, one (8%) pure motor axonal polyneuropathy, and 3 (75%) cases with indeterminate findings.

Conclusion: Primary inherited neuropathies were common in a GNMD cohort in Zambia. The clinical and EDX profile of the majority of cases appears most consistent with CMT2A, compared to other populations where CMT1A is most common. We suspect that referral bias may exist towards more severe presentations and other health system factors may also be contributing. Improved community engagement and referral systems for rare diseases are needed to improve identification and enable clinical trial readiness.

PN07

The role of riboflavin in Strachan's syndrome in the UK Caribbean population

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Background: Riboflavin-5 -phosphate (FMN) and flavin adenine dinucleotide (FAD) are generated from riboflavin (vitamin B2) and serve as essential cofactors for over 90 flavoproteins collectively named the 'flavoproteome' which play a crucial role in various processes including beta fatty acid oxidation and the electron transfer chain. Riboflavin has recently emerged as a treatment of certain hereditary neurological diseases, such as Multiple Acyl Coenzyme-A Dehydrogenase Deficiency (MADD) and Brown-Vialetto-Van Laere syndrome (BVVLS). The phenotype of the latter closely resembles Strachan's syndrome, a presumed nutritional neuropathy, comprised of a triad of painful sensory neuropathy, and optic and auditory neuropathy.

Strachan's disease is seen in black African and Caribbean individuals following dietary restriction or acute illness. Treatment with B-vitamin supplementation has led to clinical improvement or stabilisation in some patients. We propose genetic variants in flavoproteome genes may be responsible for this phenomenon.

Aims: The purpose of this study was to define the clinical, metabolic and genetic features of Strachan's syndrome.

Methods/Materials: The clinical, metabolic and pathological features of a cohort of UK patients with Strachan's syndrome were collected from five neuroscience centres in the UK. Whole genome sequencing was performed in 13 affected subjects.

Results: Thirty Black Caribbean patients, including two sets of unrelated siblings, were identified with a clinical diagnosis of Strachan's syndrome. Plasma acylcarnitine and urine organic acid profiles measured acutely revealed elevated short, medium and long chain acyl carnitines. Screening for known mitochondrial DNA and nuclear mutations was negative. Muscle biopsies revealed a reduction in mitochondrial DNA copy number and ragged red fibres in some subjects. Sural nerve biopsies showed perivascular inflammatory cell infiltrate. Genetic analysis in one patient revealed two missense variants in the *FDXR* gene.

Conclusion: Diseases such as MADD and BVVLS are caused by genetic mutations in the flavoproteome, are responsive to riboflavin and are known to give phenotypes similar to that seen in Strachan's syndrome. The striking similarities suggest that there may be a genetic susceptibility to Strachan's syndrome, which is linked to the riboflavin pathway, that can be aggravated at times of metabolic stress. Identifying such patients and commencing riboflavin supplementation may reduce disability.

PN08

In vivo axonal transport of mitochondria is impaired in mice modelling Charcot-Marie-Tooth disease type 2D

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Background: Dominant, toxic gain-of-function mutations in *GARS1* cause Charcot-Marie-Tooth disease type 2D (CMT2D). We recently identified impairments in *in vivo* axonal transport of signalling endosomes in both $Gars^{C201R/+}$ and $Gars^{\Delta ETAQ/+}$ mouse models of the neuropathy, correlating with disease progression. However, it remains unknown whether this trafficking disruption is organelle-specific or if additional cargoes are affected.

Aim: To evaluate mitochondrial dynamics in live axons of two different mouse models of CMT2D.

Methods/Materials: To visualise mitochondria in the nervous system, $Gars^{C201R/+}$ and $Gars^{\Delta ETAQ/+}$ mice were crossed with Thy1-CFP-MitoS mice, which selectively express cyan fluorescent protein (CFP) in neuronal mitochondria. *In vivo* imaging was performed in surgically exposed sciatic nerves of anaesthetised mice to capture mitochondrial axonal transport dynamics at one and three months of age – representing early and later symptomatic timepoints. Manual tracking of mitochondria in Image J was used to evaluate average transport speeds, pausing, frame-to-frame speed distributions and directionality of transport. In addition, wholemount muscles will be dissected and immunohistochemically assessed to analyse mitochondrial density in distal motor neurons and neuromuscular junctions (NMJs) using anti-CFP and α-bungarotoxin.

Results: $Gars^{C201R/+}$ mice present with an increase in average mitochondrial speed at 1 month, which progresses to a decrease at 3 months. By 1 month, the more severe $Gars^{\Delta ETAQ/+}$ mice already display a decrease in average mitochondrial speed, which is also present at 3 months. Both mutant *Gars* alleles also show a directionality phenotype, with a greater percentage of mitochondria being trafficked anterogradely compared to wild-type controls. The density of mitochondria both in sciatic nerves and at the NMJ is currently being investigated.

Conclusion: These findings reveal that in addition to a disruption in axonal transport of signalling endosomes, the trafficking of axonal mitochondria is also perturbed in CMT2D mice, changing with disease progression. These data indicate that a general disruption of axonal transport contributes to *GARS1* neuropathy and could be a therapeutic target for treatment of the disease.

‡PN09

Mutant allele-specific silencing of SPTLC1 by antisense oligonucleotides to treat Hereditary Sensory Neuropathy Type 1A

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Background: Hereditary sensory neuropathy type IA (HSN1A) is a severe and debilitating condition caused by missense 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. Mutant *SPTLC1* causes accumulation of neurotoxic metabolites within the body, leading to predominantly axonal loss of the sensory nerves. There is no cure for HSN1A.

Aims: We aim to develop a novel therapy for HSN1A by selectively suppressing the mutant transcripts of the *SPTLC1* gene, using antisense oligonucleotides (AONs), to reduce the amount of the toxic metabolites and alleviate the neurological symptoms.

Methods/Materials: Patients fibroblasts were cultured to screen AONs designed to target the UK founder mutation C133W. Silencing efficiency was determined by allele-specific quantitative RT-PCR for the differential expression of the mutant or wild-type transcripts. Neuro2A cells over-expressing the V5-tagged C133W or WT SPTLC1 proteins were used to evaluate the efficiency of the lead human AONs at protein level. As a proof of concept study, a Sptlc1 ^{S331F} mouse model was used to evaluate the *in vivo* efficacy of allele-specific silencing AON approach, with mouse AONs designed to target the S331F mutation.

Results: We have identified two lead human AONs which can selectively silence the C133W mutant transcripts in patients' fibroblasts. In the reporter cell lines, the lead human AONs can also reduce the production of the mutant protein, with no effects on wild-type protein expression. In Sptlc1^{S331F} mouse model, after a single systemic injection, the lead AON targeting the murine gene achieved robust silencing effect on the S331F mutant transcripts, with approximately 95% silencing in liver and approximately 65% silencing in dorsal root ganglia, respectively.

Conclusion: our study suggests that the AON mediated allele-specific silencing approach is feasible and promising in treating gain-of-function genetic disorders such as HSN1A. We have identified two promising lead AONs targeting human C133W mutation, which can be further developed as potential RNA drugs to treat HSN1A. Future studies will focus on the functional assessment of the lead human AONs, followed by validation *in vivo* in a novel humanized SPTLC1^{hC133W} mouse model.

PN10

Progressive changes in mitochondrial bioenergetics in skeletal muscle and peripheral nerve during postnatal development are responsible for the delay in Wallerian degeneration observed in neonatal mice

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Background: Following traumatic or hypoxic injury, axons and synaptic terminals in adult mice breakdown rapidly via in a process known as Wallerian Degeneration (WD). In mice aged 2 weeks or less, the equivalent injury results in a markedly attenuated rate of synaptic breakdown. Between 2 and 2 weeks of age, there is an acceleration in the rate in degeneration following injury, until the adult phenotype is established. In order to gain insight into the mechanisms which underlie the delay in synaptic degeneration observed in young mice, we performed proteomics during this critical time window in peripheral nerve and skeletal muscle.

Aims: Here we aim to perform a proteomic analysis of skeletal muscle and peripheral nerve in mice aged between 2 and 4 weeks old, and identify the proteins which could be responsible for the delay in synaptic degeneration observed.

Methods/Materials: Tandem mass tagging proteomics was performed on lumbrical muscles and sciatic nerves at 5 time points between 12 and 24 days of age inclusive. The identified proteomics changes were validated using western blot and ELISA. The role of complex I of the respiratory chain in the rate of axon degeneration was validated using cellular and ex-vivo models of nerve injury.

Results: We identified a consistent and progressive increase in proteins pertaining to oxidative phosphorylation between P12 and P24 in peripheral nerve and skeletal muscle in mice. We observed a specific enrichment for proteins involved in complex I of the mitochondrial respiratory chain and validated this in nerve and muscle using western blot and ELISA. We demonstrate that inhibition of complex I prevents the axotomy-induced rise in reactive oxygen species and protects axons following injury. Furthermore, we reveal that pharmacological activation of oxidative phosphorylation significantly accelerates degeneration at the neuromuscular junction in neonatal mice.

Conclusion: In summary, we reveal dramatic changes in the neuromuscular proteome during post-natal maturation of the neuromuscular system, and demonstrate that endogenous dynamics in mitochondrial bioenergetics during this time window have a functional impact upon regulating the stability of the neuromuscular system.

PN11

Motor Unit Recovery Following Smn Restoration in Mouse Models of Spinal Muscular Atrophy

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Background: Although Smn dependant therapeutics have recently been approved for the treatment of SMA, there is a clear therapeutic time window, after which the delay in treatment results in a dramatic reduction in therapeutic efficacy. Hallmarks of motor unit pathology in SMA includes a loss of motor neurons and degeneration of neuromuscular junction (NMJs). Following an increase in Smn levels, it is

unclear how much damage can be repaired, whether normal patterns of innervation are re-established, or whether some defects remain.

Aims: Here we perform a detailed analysis of motor unit pathology before and after restoration of Smn levels.

Methods/Materials: Smn levels were restored in mouse models of SMA using either an Smn inducible mouse model or admnsitration of an ASO targeting SMN2. We then quantified the recovery of neuromuscular junctions using high resolution imaging and quantification.

Results: Using an Smn inducible mouse model of SMA, we show that genetic restoration of Smn at P4 results in a dramatic reduction in NMJ pathology, with restoration of innervation patterns, preservation of axon and endplate number and normalised expression of P53 associated transcripts. Notably, endplate atrophy and pre-synaptic swelling remain, and transcript levels of PMAIP remained elevated. We next analysed the effect of either early or delayed treated of an ASO targeting SMN2 on a range of differentially vulnerable muscles. Following either early or delayed treatment with ASO, the percentage of occupied endplates increased, with the majority appearing fully occupied. However, there was an underlying significant loss of axons and endplates, which was more prevalent following a delay in in treatment. The loss of axons in the absence of significant levels of denervation resulted in an increase in average motor unit size following both early and delayed treatment.

Conclusion: Together this work demonstrates the remarkably regenerative capacity of the motor neuron following Smn restoration, but highlights that recovery is incomplete. This work suggests that there is an opportunity to enhance the efficacy and extent of neuromuscular junction recovery following administration of Smn enhancing therapeutics.

PN12

Dysautonomia in Guillain-Barré syndrome: A multicentre retrospective cohort study of screening in clinical practice

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Introduction: Guillain–Barré syndrome (GBS) is an acute, inflammatory neuropathy which affects the autonomic nervous system in up to two-thirds of cases. Dysautonomia manifests as blood pressure and temperature dysregulation, urinary retention, paralytic ileus and cardiac arrhythmia. Dysautonomia, particularly cardiovascular dysfunction, is linked to increased intensive care admissions and increased mortality (1). However, there is little evidence to guide screening for GBS dysautonomia in clinical practice.

Aims: To assess the frequency of, and adequacy of screening for, dysautonomia in GBS cases in the first two weeks of hospital admission.

Methods: We performed a retrospective cohort study of GBS cases presenting to four UK regional neurosciences centres between December 2019 and October 2022. Cases were identified from intravenous immunoglobulin (IVIg) prescription databases

and local diagnostic coding according to published criteria (2). Data were collected by review of each patient's medical notes and clinical observation chart using a consensus, piloted proforma. Data are expressed as mean (standard deviation, SD) or median (interquartile range, IQR).

Results: 47 patients were included across 4 sites. 30/47 were female (58%) and the mean age was 54 (SD=15.9) years. Median modified Erasmus GBS outcomes score (mEGOS) on day 7 of admission was 4 (IQR=5), median length of hospital stay was 26 (IQR=29) days and 16/47 (34%) required intensive care. 45/47 patients (96%) developed at least one feature of dysautonomia within the first two weeks of admission. In this cohort, the most frequent types of dysfunction were constipation (83%), labile blood pressure (68%) and tachy/bradyarrhythmias (55%). Urinary retention (26%), paralytic ileus (9%), and gastroparesis (2%) were less common. Screening for dysautonomia was variable. There were relatively high levels of screening for gastrointestinal dysfunction (96%), pupillary abnormalities (72%) and urinary dysfunction (68%). In contrast, only 32% had postural blood pressure assessments, 38% had baseline electrocardiography, and 47% underwent continuous cardiac monitoring.

Conclusion : We have demonstrated that dysautonomia is common in GBS, but that its screening is inadequate, particularly with cardiovascular dysfunction. We recommend that improved screening for dysautonomia in line with routine screening for motor, sensory and respiratory complications could reduce morbidity and mortality in GBS.

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PN13

Skin biopsy as a diagnostic tool for hereditary ATTR amyloid neuropathy in the UK

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Background: The introduction of gene silencing therapy for hereditary transthyretin (ATTRv) amyloidosis has markedly improved treatment and the diagnosis of neuropathy is critical for access to this new therapy. In minimally symptomatic early neuropathic disease where neurophysiology is normal, skin amyloid deposits have been demonstrated to be a marker of ATTRv amyloid neuropathy with intraepidermal nerve fibre density (IENFD), a marker of disease progression.

Aims: To study the value of skin biopsy in the diagnosis of UK ATTRv patients and to assess the influence of this on accessing gene silencing treatment.

Methods: 53 patients had skin biopsies between July 2021 and October 2022. These were stained for amyloid and if positive, were typed by immunohistochemistry and were also analysed for IENFD.

Results: A total of 59 skin biopsies were performed from July 2021 to October 2022, including 6 patients who had a repeat biopsy. The highest number of biopsies was undertaken in individuals carrying the T60A variant (27%) closely followed by the V30M and V122I variants. Normal neurophysiology was demonstrated in 62% of cases. From results that were available, 48% of cases had abnormal IENFD, 37% had amyloid identified and 24% had both. This allowed 27% of these patients to start gene silencing therapy.

Conclusion: Skin biopsy is a useful, minimally invasive method for detecting amyloid deposits and

small nerve fibre loss. Of particular importance, it allowed early identification of amyloid neuropathy and commencement of gene silencing therapy in patients who might otherwise not have been eligible. As T60A and V122I are the commonest pathogenic TTR variants in the UK and patients often present with cardiomyopathy, early diagnosis of amyloid neuropathy is critical for treatment decisions.

PN14

Validating MRI biomarkers for clinical trials in Charcot Marie Tooth Disease type 1A using automated segmentation

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Background: Using The Queen Square Neuromuscular MRI protocol, our group have previously shown that lower limb muscle MRI fat fraction is sufficiently sensitive to detect disease progression in patients with CMT1A over 12 months. In order for muscle MRI to gain approval by regulatory authorities, validation of it will be required against other biomarkers and clinical outcome measures.

Aims: To determine whether changes in lower limb muscle MRI in adults with CMT1A correlate with clinical measures (CMT examination score) and plasma neurofilament (NfL) levels and to assess the responsiveness of fat fraction MRI using automated segmentation over 12 months.

Methods: 20 patients were recruited with CMT1A and 6 controls (with further controls recruited as part of a larger MRI study in CMT). Lower limb muscle MRI, CMTES and plasma NfL were acquired at baseline and at 12 months. The 3-point-Dixon fat water separation technique was used to determine thigh and calf muscle fat fraction at a single slice using regions of interest with Musclesense, a trained artificial neural network for lower limb segmentation.

Results: Baseline lower limb MRI, CMTES and NfL level were performed in 20 CMT1A patients. Baseline mean calf fat fraction was significantly increased in CMT1A patients versus controls (22.07±26.51% vs 1.79±0.67%, p 0.003) and also increased significantly over 12 months versus controls (1.15±1.77% vs 0.07±0.19%, paired p 0.02) while there was no significant change in CMTES or NfL levels over the same period. Standardised response mean (SRM) was 0.65 overall and 1.63 in subgroup whose baseline fat fraction was 10-70%. There was a significant correlation with mean calf fat fraction change and CMTES (r 0.54, p 0.03), which was not observed for NfL when correlated either with CMTES or mean calf fat fraction.

Conclusion: With automated segmentation, we have shown that calf fat fraction remains a responsive biomarker and is superior to clinical outcome measures like CMTES and also blood biomarkers like NfL. It also continues to demonstrate validity by correlation with clinical measures. Lower limb muscle MRI should be considered as a biomarker in future clinical trials with CMT patients.

‡PN15

Classification of *GJB1* variants

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Background: Variants in gap junction protein beta-1 (*GJB1*) are responsible for X-Linked Charcot-Marie-Tooth disease (CMTX1); a length-dependent sensory and motor neuropathy where males are more severely affected than females. Despite a characteristic phenotype, variants often cannot be classified as pathogenic and remain a 'variant of uncertain significance' (VUS). This important difference in classification may have ramifications for eligibility for clinical trials and family planning.

Aims: We hypothesise that an adapted set of classification criteria for CMTX1 will result in an increased proportion of pathogenic variants.

Methods/Materials: We developed adapted American College of Medical Genetics (ACMG)/Association for Clinical Genomic Science (ACGS) criteria. Patients with CMTX1 were recruited from 21 international sites from 2009 to 2021 and their *GJB1* variants classified.

Results: We reviewed case data from 421 patients in 324 families with CMTX1. Variant data was available for 388 patients from 296 families. After excluding one 15-year-old male who presented with a central nervous system complaint, but had no neuropathy, and considering 'combined variants' for patients in two families harbouring two GJB1 variants each, we analysed 154 *GJB1* variants. In our cohort, 109 (70.8%) variants were classified as pathogenic/likely pathogenic (P/LP) from 244 (82.7%) families, 41 (26.6%) VUS from 47 (15.9%) families and 4 (2.6%) benign from 4 (1.4%) families. Contrastingly, ClinVar classifications were: 58.1% P/LP, 41.9% VUS and zero benign.

Conclusion: In this study we show that adapted criteria for variant classification dramatically increase the number of pathogenic variants when compared with ClinVar, and correspondingly decrease the proportion of VUS. This increase is primarily due to the use of specific phenotype characteristics and inclusion of segregation data for families with private variants. This confirms that clinical assessment of patients, and communicating this with diagnostic genetic laboratories, is key to variant interpretation.

PN16

Understanding the genetic basis of tropical ataxic neuropathy

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Background: Tropical ataxic neuropathy (TAN) is a disorder peculiar to the tropics. Nutritional deficiencies and dietary toxins have been inconsistently implicated in the etiology. Since the clinical features such as ataxic neuropathy, optic atrophy and deafness are reminiscent of mitochondrial disorders, it may be hypothesized that genetic abnormalities underlie the pathophysiology of TAN.

Aims: To identify genetic variants that might have a causal role in TAN.

Methods: Patients with progressive ataxic neuropathy with any combination of optic atrophy and/or sensorineural deafness, were recruited from a single neurology unit after obtaining consent. Clinical, electrophysiological, histopathological and imaging data were used for deep phenotyping. Clinical exome sequencing (CES) was performed on Illumina platform (80-100X coverage). Identified variants were interpreted based on the recommendations of American College of Medical Genetics and Genomics.

Results: The cohort comprised of 43 patients (M:F = 18:25). Mean age at the time of evaluation was

26.06±11.05 years. Family history suggestive of TAN was noted in 13 (neuropathic illness: 7, deafness: 5, visual impairment: 1). Other salient clinical features included impaired hearing (n=26), impaired vision (n=17). Nerve conduction studies revealed length-dependent sensory predominant axonal neuropathy. Majority of the patients had impaired evoked potentials (BAER=41, VEP=31). Spine MRI (n=33) revealed abnormalities in cervical and/or thoracic regions: cord atrophy(n=20) and hyperintensities (n=7). Nerve biopsy (n=19) showed chronic axonal pathology in all with varying degree of fiber loss, being uniform in 14. The patterns of fiber loss included small fiber loss (n=6), large fiber loss (n=1), and both (n=12). CES revealed 56 variants in 51 genes in 35 patients. These included eight variants that were reported previously and 48 variants of uncertain significance. Variants identified involved genes associated with: neuropathy=28 (*MFN2*, *MORC2*, *WNK1*, etc.,), deafness=8 (*ABCC1*, *DMXL2*, *TNC*, etc.,), ataxia= 6(*SACS*, *RNF170*, *TRPC3*, etc.,) and mitochondrial phenotypes=4 (*COX15*, *TK2*, *FLVCR1*, etc.,)

Conclusion: This study provides comprehensive data on the spectrum of genetic abnormalities in 81.4% cases with the TAN phenotype. However, the pathogenicity of variants of uncertain significance needs to be established.

PN17

Radiographic Predictors for Common Peroneal Nerve Injury after Total Knee Arthroplasty

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Background: Common peroneal nerve injury (CPNI) following total knee arthroplasty (TKA) is rare but can lead to substantial disability. The goal of this study was to identify radiographic predictors of CPNI adjusted for non-radiographic factors.

Methods: Patients with CPNI following primary and revision TKA with adequate radiographic imaging were retrospectively identified. Each CPNI case was matched with 2 control primary or revision TKAs with no diagnosis of CPNI. Various patient, surgical and radiographic characteristics were also recorded.

Results: Of the 23,551 procedures performed during this period, there were 23 cases and 41 matched controls. Pre-operative valgus angle (POVA) was -0.1 ± 7.4° (valgus) in controls and 8.1 ± 9.1° (valgus) in patients with CPNI (p = 0.002). Valgus angle correction (VAC) was 0.0 ± 9.5° (varus to valgus) in controls and -5.2 ± 10.0° (valgus to varus) in cases (p = 0.002). POVA of ≥ 5° and ≥ 10° presented odds ratios of 6.42 and 12.03, respectively, and VAC of < -5° and < -10° (valgus to varus) presented odds ratios of 7.48 and 7.12, respectively, for CPNI. Using a multivariable conditional logistic regression model adjusting for sex and tourniquet time, the odds of CPNI significantly increased by 11% for each 1° increase in POVA (OR = 1.11, 95% CI 1.01 – 1.21, p = 0.035)

Conclusions: Pre-operative valgus deformity may be the most important risk factor for CPNI. Even correction of small degrees of pre-operative valgus may be clinically important and lead to CPNI.

PN18

The human neuropathy-causing *GARS1*$^{\Delta ETAQ}$ mutation causes motor and sensory dysfunction in mice

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Background: Dominant, toxic gain-of-function mutations in *GARS1* cause Charcot-Marie-Tooth disease type 2D (CMT2D). The *Gars*$^{C201R/+}$ and *Gars*$^{NMf249/+}$ mouse models for CMT2D display clear motor and sensory phenotypes, replicating the human condition, but do not model patient mutations.

Recently, CRISPR/Cas9 technology was used to create the *Gars*$^{\Delta ETAQ/+}$ mouse, which possesses a four amino acid deletion mutation identified in a patient with severe peripheral neuropathy.

Aims: To evaluate motor and sensory phenotypes of the *Gars*$^{\Delta ETAQ/+}$ mouse model for CMT2D.

Methods/Materials: Tissues were dissected from wild-type and *Gars*$^{\Delta ETAQ/+}$ mice at three months of age and processed as wholemounts or embedded and cryo-sectioned onto slides. Immunohostochemical assessment of sensory neuron subtypes in dorsal root ganglia (DRG) was completed using anti-NF200 and anti-peripherin antibodies. Analysis of muscle spindles, spinal cord motor neurons, and neuromuscular junctions (NMJs) was also performed using immunohistochemistry. Intramuscular injections of fluorescently-labelled, non-toxic fragments of tetanus neurotoxin were performed to evaluate the axonal transport of neurotrophin-containing signalling endosomes in intact sciatic nerve axons of live, anaesthetised mice. *In vivo* transport experiments were coupled with western blotting of sciatic nerves to measure levels of key endosome adaptor proteins. Finally, grip strength testing was performed to assess motor function.

Results: *Gars*$^{\Delta ETAQ/+}$ mice display a distortion in the proportions of sensory neuron subtypes found in lumbar DRG, previously identified at birth in other CMT2D alleles. There is no motor neuron loss in *Gars*$^{\Delta ETAQ/+}$ spinal cord, but cell bodies are smaller and distally there is muscle-specific NMJ degeneration, consistent with a peripheral neuropathy phenotype. Moreover, *Gars*$^{\Delta ETAQ/+}$ mice possess fewer muscle spindles combined with spindle degeneration in the soleus. *In vivo* imaging of signalling endosomes revealed impaired axonal transport in motor axons innervating the tibialis anterior, but not gastrocnemius, which was linked with reduced expression of endosome-specific adaptor proteins in CMT2D sciatic nerves. The identified phenotypes are likely to contribute to the considerably reduced weight and grip strength of *Gars*$^{\Delta ETAQ/+}$ mice.

Conclusion: These findings confirm that *Gars*$^{\Delta ETAQ/+}$ mice replicate many features of human neuropathy, including selectivity of peripheral neurodegeneration, and suggest that the mutant allele will be advantageous for identifying CMT2D pathomechanisms and testing potential therapeutics.

Motor Nerve Disorders

MND01

Effects of constitutively low NMNAT2 levels on SARM1 activation and NAD metabolism in neurons

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Background: The length, branching and high metabolic demand of axons makes them vulnerable to many stresses. In many diseases axons die long before cell bodies, with consequences including pain, sensory and motor loss. Wallerian degeneration (or programmed axon death) is a well-characterised signaling pathway regulating axon survival and contributing to genetic, toxic and metabolic disease in animals and human disorders including ALS and other motor nerve disorders. NAD-related metabolism plays a central role. The pathway is triggered when NMNAT2, an NAD-synthesising enzyme crucial for axon survival, is depleted. This activates SARM1, an NAD(P)-consuming enzyme that kills axons. Interestingly, acute or chronic NMNAT2 loss have distinct effects. In primary mouse neurons acute loss of a single *Nmnat2* allele kills axons whereas chronic depletion of one allele is consistent with long-term axon survival. *In vivo* mice survive and remain healthy with an expression down to 30% of wild-type NMNAT2.

Aims: The aim of our study is to understand whether chronic depletion of NMNAT2 can activate SARM1 in seemingly intact axons.

Methods: We have used superior cervical ganglion (SCG) neurons from mice expressing 30% of normal NMNAT2 to study the effects on neurite outgrowth and NAD and NADP.

Results: Neurons expressing low levels of NMNAT2 have neurite outgrowth defects and significantly less NAD and NADP than wild-type neurons. This loss of NAD and NADP appears to be completely SARM1-dependent, suggesting that chronic activation of SARM1 leads to a constitutive depletion of these metabolites without causing axon degeneration. We also show that application of the NAD precursor Nicotinamide Riboside (NR) does not boost NAD levels in SCG neurons from low-NMNAT2 expressing mice due to chronic SARM1 activation.

Conclusion: A deficiency of NMNAT2 partially activates SARM1 even in axons that appear intact. It will be important to extend this to studies of other neuron types, including motor neurons, to establish what compensatory mechanisms are employed to allow axons with chronically active SARM1 to survive, and whether these indicate potential new therapeutic strategies for neurological disorders involving SARM1.

MND02

Morphological characterisation of a highly prevalent, length-dependent motor axonopathy of horses

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Background: Length-dependent axonopathies in humans are somewhat rare and modelling them is sometimes challenging in rodents because (comparatively) long axons are absent. Here, we studied a common neuropathy of horses – affecting the recurrent laryngeal nerves (the longest motor nerves in this species). Horses with recurrent laryngeal neuropathy (RLN) develop variable severities of left-sided intrinsic laryngeal muscle paresis, and poor athletic performance. It is unclear if RLN is a generalised neuropathy, or if it is restricted to the recurrent laryngeal nerves (RLns). Improved understanding of the pathophysiology might help in the search for the disease's enigmatic aetiology and reveal parallels with related human disorders.

Aims: Characterise and quantify histopathological markers of distal nerve and neuromuscular junction (NMJ) pathology in selected nerves from age-matched horses with varied severities of RLN.

Methods/Materials: RLns sampled bilaterally from 17 horses, at distal and mid-cervical sites, alongside distal phrenic, radial, palmar, deep peroneal and lateral plantar nerves were examined after resin embedding and by immunohistochemistry for ChAt and tyrosine hydroxylase to identify motor and sympathetic axons respectively. Samples of right and left lateral cricoarytenoid and short digital extensor muscle underwent fibre immunotyping, teasing and NMJ immunolabelling. Phenotype was graded based on ante-mortem laryngoscopy and post-mortem RLn axon count ratios.

Results: Median myelinated fibre density, overall neuron and axon diameters were significantly smaller, and there were fewer ChAt but not TH positive axons in left RLn compared with the right RLn. Changes were evident in horses considered clinically normal and were most prominent at the distal site but were evident proximally in more severely affected horses. Largest diameter axons were preferentially lost. There was no evidence of involvement of other long nerves. Our pilot morphological characterisation of NMJs has further revealed degeneration, partial and full denervation, and commonly, multi-innervation of single laryngeal muscle fibres, generally by a single axon.

Conclusion: RLN is a length-dependent motor axonopathy with preferential loss of the widest diameter axons. Subclinical RLN is common. Various features of equine RLN reflect those seen in similar diseases of humans. Given the very high prevalence, we suggest that RLN offers translational potential for related human diseases.

MND03

Adult SMA REACH: The challenges encountered using standard of care data collection to support approval of Managed Access treatments

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Background: Adult SMA REACH is an observational study, collecting clinical data and outcome measures from adult Spinal Muscular Atrophy (SMA) patients. SMA treatments, Nusinersen and Risdiplam, are available through Managed Access Agreements (MAA) and Adult SMA REACH is responsible for capturing and reporting data to UK regulatory authorities to support their review of drug efficacy.

Aims: Although data collection for Adult SMA Reach is in its initial stages, we have identified and overcome many challenges associated with real world data collection.

Methods/Materials: The clinical care of adults with SMA was not structured or coordinated, patients were often not seen regularly at specialised sites and SoCs were not implemented equally geographically.

Real-world data was supposed to be obtained from the routine clinic visit with fixed cut-off points and time windows used in clinical trials and scheduled follow-up visits, this changed due to the Covid-19 pandemic.

Adaptation of the scope of data collection due to changes in the criteria for the administration of the treatments.

Additional resources are required up front for data entry at sites.

The research project and standard of care services had to be set up or expanded to manage capacity changes in tandem.

Expedited set up of the research project was required at sites which were sometimes research naïve.

Difficulties to access NIHR portfolio funded staff. Local research departments required additional assistance to approve the study due to the mandatory nature of data collection for MAA which is juxtaposed against the voluntary nature of research.

Results: We established the Adult SMA REACH network, which includes all sites caring for adult SMA patients with the aim of sharing knowledge and coordinating data collection.

The coordinating centre has worked together with R&D departments to allocate local support resources.

We provided additional resources to sites for data collection purposes, however the implementation of this within an NHS setting has been difficult.

Conclusion: The collection of real-world data that supports the approval of treatments using research project structures requires streamlining and simplification of project approval. The centres that collect this data require structural help that allows them to carry out this task in addition to clinical activity.

MND04

Structural investigation of SARM1, a protein that causes axon loss

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Background: SARM1 is a pro-degenerative NADase that executes the programmed axon degeneration pathway, after nerve injury and in diseases including polyneuropathies and ALS. Attenuating SARM1 NADase activity delays axon degeneration, so SARM1 has become an important drug target.

Aims: The ARM domain of SARM1 regulates its NADase activity. The first aim was to characterise activation at the ARM domain allosteric site, where NMN and NAD bind in competition, respectively activating SARM1 and blocking its activation. The

second aim was to test whether a rare, natural mutant in a patient with a complex disorder with motor and retinal symptoms, also lying within the ARM domain (SARM1^{W253C}), confers a gain-of-function consistent it having a causative role.

Methods/Materials: Site-directed mutagenesis was used to modify the ARM domain allosteric site with artificial variants, or to introduce the W253C natural mutation. Variants were expressed in HEK cells to determine their influence on NAD levels and mutant proteins isolated using immunoprecipitation for NADase assays of basal and NMN-induced activity.

Results: Several artificial mutants in the SARM1 ARM domain influence NAD levels in transfected HEK cells and alter basal and/or induced SARM1 NADase activity. Interesting patterns are emerging that will help understand how SARM1 becomes activated and potentially how to block activation therapeutically. Further characterisation of these residues is ongoing to understand more fully how they influence activation. The SARM1^{W253C} variant from the motor and retinal disorder case, was shown to decrease NAD levels in HEK cells to similarly low levels as one artificial variant. Purified SARM1^{W253C} NADase assays are ongoing to determine whether it too is a constitutively active mutant, and how its activity compares to those reported previously in ALS.

Conclusion: SARM1 ARM domain allosteric site residues regulate NADase activity, helping to understand how this site could be targeted to block activation. Initial data are consistent with W253C conferring gain-of-function, a wider heterogeneity of motor and other phenotypes associated with SARM1, but recombinant protein assays are needed to confirm this.

MND05

Evaluating the therapeutic effects of antisense oligonucleotide and small molecule on muscle satellite cells in SMN deficient mice

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Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease caused by mutations in the survival of motor neuron 1 (*SMN1)* gene. Muscle satellite cells are skeletal muscle stem cells, which are responsible for postnatal muscle growth and the regeneration and maintenance of adult muscles. Antisense oligonucleotides (AONs) and small molecules are efficacious in enhancing SMN protein expression and improving SMA-related phenotypes, particularly when administered at a pre-symptomatic or early post-symptomatic stage. Several lines of research are investigating if targeting skeletal muscles could add additional benefits to patients. However, the effects of SMN-enhancing treatments on satellite cells are currently unknown.

Aims: To investigate the effect of therapeutic AON and small molecule on satellite cell function in a mouse model of severe SMA.

Methods/Materials: The Taiwanese SMA mouse-model carrying either two (severe) or four (mild) copies of human *SMN2* transgene was used in this study. Quantitative real-time-PCR, immunofluorescence staining and western blotting were used to determine the myo-lineage markers expression, number of satellite cells (expressing Pax7) and the level of Pax7 protein in tibialis anterior muscles of the 7-day old mice. These markers were firstly compared among control, mild and severe SMA mice to evaluate the extent of the SC defects, and then investigated in severe SMA mice that have received either a 25-mer morpholino oligomer (PMO25), or a small molecule compound (RO7021707), two well-defined SMN-enhancing drugs, to evaluate the effects of these drugs on muscle satellite cells.

Results: Myogenic factor 5 (Myf5) and myogenin transcripts were significantly decreased in both mild and severe SMA mice. Significant reduction of SMN and Pax7 protein was observed in severe SMA mice.

Seven days after treatment of the severe SMA mice, both PMO25 and RO7021707 rescued the muscle-fibre size and increased the SMN protein expression. Interestingly, only RO7021707 was able to increase the transcripts of myo-lineage markers, the number of Pax7+ satellite cells and the level of Pax7 protein in treated mice.

Conclusion: Our results demonstrate that there are more pronounced satellite cells defects in severe than mild SMA mice. These defects can be restored by RO7021707 but not antisense treatment.

MND06

PROMs collection and the UK Spinal Muscular Atrophy Patient Registry

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Background: The 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 SMA REACH clinical data, anonymised, analysed and submitted to regulatory authorities for consideration as part of the treatment MAAs.

Methods: Registration in the UK SMA Patient Registry is patient-initiated through a secure online portal. Patients are invited to complete questionnaires about their condition and PROMs: EQ-5D-5L; Patient Global Impression of Change; SMA Independence Scale (SMAIS-ULM); 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 613 participants: 406 adult (16+years); 207 paediatric (<16years). PROMs have been completed by 101 adults and by the caregivers of 48 paediatric patients. The fraction of PROMs able to be aligned with SMA REACH clinical data is growing and will be presented.

Conclusion: The UK SMA Patient Registry represents a trial-ready cohort of individuals and is a valuable tool for the collection of SMA natural history data from treated and treatment-naïve patients. Expansion of the registry to collect PROMs supports UK SMA data collection and supplements SMA REACH clinical data, assisting in therapy evaluation by regulatory authorities.

MND07

Dissecting the role of oxidative stress in spinal muscular atrophy

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Background: Spinal Muscular Atrophy (SMA) is the most prevalent paediatric cause of lower motor neuron disease and the most common monogenic cause of death in infancy, triggered by the depletion of the ubiquitously expressed Survival Motor Neuron protein (SMN). It still remains unknown the mechanism for the selective vulnerability of alpha-motor neurons in this neuromuscular disorder. A role of oxidative damage in SMA has been proposed with various SMA models depicting an increase in ROS production and mitochondrial dysfunction. Despite this growing body of evidence, a comprehensive overview of how oxidative stress signalling is perturbed in SMA is still missing.

Aims: The aim of the project is to delineate whether oxidative stress contributes to SMA pathogenesis s with the ultimate goal to identify the cellular and molecular pathways needed to spearhead further therapeutic avenues for SMA treatment options.

Methods/Materials: Here we utilise the powerful genetic tools of *Caenorhabditis elegans* in a previously established *C. elegans* SMA model and a range of functional assays, pharmacological challenges, and genetic analysis to delineate the mechanism(s) by which oxidative stress perturbations control SMN function.

Results: Exposure of the *C. elegans* SMA model to compounds that induce oxidative stress through increasing intracellular superoxide levels significantly reduced the survival of the animals, indicating an increased sensitivity to oxidative stress reminiscent to mammalian studies. Furthermore, we combine pharmacological and behavioural assays to study the impact that the reduction of ROS have in the SMA neuromuscular phenotype. Our ongoing studies focus on genetic interactions of **smn-1** mutant animals with known players of the oxidative stress pathway and on assays that will further define **smn-1** oxidative stress defects.

Conclusion: The ultimate goal of this research is to gain a deeper understanding of the key cellular events that lead to SMA pathology. Understanding the root cause of SMA is critical for developing effective SMA therapies. Altogether, we highlight the strengths of *C. elegans* as an exceptional tool to understand the molecular mechanisms underlying a devastating motor neuron disease.

‡MND08

Exploring the therapeutic role of miRNA-X on RNA splicing in Spinal Muscular Atrophy

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Background: Spinal Muscular Atrophy (SMA) is a neuromuscular condition caused by mutations in the *survival motor neuron 1* (*SMN1*) gene. The complex molecular mechanisms underlying motor neuron degeneration in SMA are not fully understood, as global cellular dysfunctions mask the identification and characterisation of disease-specific pathways. This in turn complicates the identification of novel therapeutic targets. Recent studies have linked microRNAs (miRNAs) as latent contributors to the pathological mechanisms in SMA. Through miRNA-mRNA predicted consequential pairing, our group recently characterised miRNA-X as a candidate target involved in the *SMN2* exon-7 splicing.

Aims: We sought to validate the pathway through which miRNA-X alters RNA splicing in *SMN2*. Using this information, we seek to explore the therapeutic benefits of targeting miRNA-X in combination with an existing treatment.

Methods/Materials: We performed Rinella Luciferase assay to validate the interaction between miRNA-X and the 3' UTR of the *Tra2B*, a key splicing factor involved in *SMN2* exon-7 splicing. We designed and tested the effects of different doses of antisense oligonucleotides (AON) with a 2' *O*-methoxyethyl (MOE) chemistry on the expression of *SMN2-FL* (full length) transcript in patient fibroblasts (with three *SMN2* copies). We also tested the effects of commercially available miRNA-X mimic using patient and control fibroblasts.

Results: We confirmed a binding site for miRNA-X on the 3' UTR of the *Tra2B* mRNA (*p<0.01*). A significant increase in *Tra2B* and *SMN2-FL* transcripts was observed with 100nM, 200nM and 400nM AON treatment respectively. Interestingly, no changes in *Tra2B* but an increase in *SMN2-FL* and total *SMN* transcripts was observed with 25nM and 100nM miRNA-X mimic treatments.

Conclusion: AONs targeting miRNA-X seem to exert effects through Tra2B. In contrast, miRNA-X mimic appears to target a negative regulator of the *SMN* exon-7 splicing with a role in epigenetic regulations of the *SMN* gene. Whilst further studies are required to validate this, we believe it would help elucidate how mimics and AONs could converge to drive similar outcomes *in vitro*. Using SMA mice and iPSC derived motor neurones, we hope to demonstrate the clinical relevance of miRNA-X as potential co-therapy.

MND09

Validation of Optical Genome Mapping for the Molecular Diagnosis of C9orf72 and the detection of large repeat expansions

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Background: Repeat expansions are an important cause of neurological disease. The size of repeat expansions can affect disease severity, with larger expansions often associated with earlier onset of disease and more severe symptoms. 'GGGGCC' hexanucleotide repeat in *C9orf72* is the most common cause of genetic amyotrophic lateral sclerosis (ALS) and familial frontotemporal dementia (FTD). Sizing of the repeat expansion is done with Southern Blot (SB). SB is cumbersome, low-throughput, and time consuming. Furthermore, somatic mosaicism as well as the unclear cut-off between normal alleles and expanded pathogenic alleles can make the diagnosis difficult.

Aims: Using this non-sequencing-based technique, we aimed to identify and size the repeats expansion involving the *C9orf72* gene in patients diagnosed with ALS.

Methods/Materials: High molecular DNA was isolated from bloods from 15 historical ALS patients with a diagnosis of C9orf72 in their records. Optical genome mapping (OGM) was performed on the Saphyr Genome Imaging Instrument recently established in the Neurogenetics lab at UCL Queen Square Institute of Neurology.

Results: We identified *C9orf72* - hexanucleotide repeat expansion in 14 of 15 ALS patients. Repeat sizes ranged from 6 to 25 Kbp. The negative case was repeated with RP-PCR and confirmed to be a C9orf72 negative case. OGM also provided further detail on somatic instability for each sample analysed.

Conclusions: Our preliminary data showed that OGM can detect accurately hexanucleotide repeat expansion in C9orf72 and provide further detailed information on somatic instability.

MND10

The role of autophagy in spinal muscular atrophy: lessons from the nematode *Caenorhabditis elegans*

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Background: Spinal Muscular Atrophy (SMA) is a devastating autosomal recessive neuromuscular disorder characterised by the degeneration of -motor neurons in the anterior horn of the spinal cord, resulting in progressive muscle loss and ultimately death. SMA is caused by reduced levels of the ubiquitously expressed survival motor neuron (SMN) protein. Autophagy, a highly conserved lysosomal degradation pathway associated with a multitude of neurodegenerative disorders, is dysregulated when SMN levels are depleted, suggesting a potential role in SMA pathogenesis.

Aims: To characterise the perturbations of the autophagic pathway in the well-established *Caenorhabditis elegans* (*C. elegans*) SMA model and highlight putative genetic modifiers.

Methods/Materials: We challenged our *C. elegans* model pharmacologically, exposing the nematode to a variety of autophagic regulators.

Results: Our pharmacological screen identified autophagy modulating compounds targeting various stages of the pathway as modulators of SMN defects, capable of ameliorating neuromuscular and growth defects associated with depletion of SMN.

Conclusion: Our results suggest that modulation of varying steps of the autophagic pathway in the *C. elegans* SMA model has resulted in the amelioration or exacerbation of SMA neuromuscular and growth defects. Thus, these findings highlight the autophagic pathway as a viable target for novel therapeutic strategies. However, future work is required to pinpoint the exact steps at which autophagy is implicated in SMA pathogenesis.

MND11

Profiling neuroinflammatory markers in CSF in response to nusinersen treatment in pediatric SMA patients

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Background: Spinal muscular atrophy (SMA) is a progressive motor neuron disease caused by mutations in the *SMN1* gene. Intrathecal administration of the antisense oligonucleotide drug nusinersen (Spinraza) is the most commonly used treatment for SMA. It is still unknown if there is any potential neuroinflammation in the central nervous system (CNS) in SMA, or whether the repeated intrathecal injections of nusinersen may trigger any potential neuroinflammatory reaction in CNS.

Aims: We aim to investigate the response of a series of neuroinflammatory markers in Cerebrospinal fluid (CSF) from SMA patients receiving repeated intrathecal injections of nusinersen, and to correlate findings to the clinical improvement in motor function.

Methods/Materials: CSF samples were collected from 16 type 1 SMA and 4 type 2 SMA patients,

with an age range between 0.18-2.33 years old. CSF samples were collected at baseline (day 0), 15, 30, 60, and 180 days after the initiation of nusinersen treatment. Motor function scores were assessed using the Children's Hospital of Philadelphia Infants with Neuromuscular Disorders Test (CHOP-INTEND) or the Revised Hammersmith Scale, at baseline and 180 days after nusinersen . The levels of cytokines, chemokines, proinflammatory and vascular injury markers were measured by the V-PLEX neuroinflammation panel kits using the Luminex system. In total, 29 markers were measured in CSF from 20 patients at 5 time points along the treatment course.

Results: Three neuroinflammatory markers at baseline are significantly higher in SMA1 than SMA2. Twelve markers showed statistically significant dynamic changes at different time points in the first 6 months of treatment, compared to baseline levels. Eight markers were markedly increased at 60 days and then steadily reduced to baseline levels. Two markers were significantly increased at 180 days, compared to baseline levels, while two other markers decreased. Two markers also showed a significant negative-correlation between their level changes from baseline and the improved motor scores after 6 months of treatment.

Conclusion: Our study provides a detailed profile on neuroinflammatory markers in CSF in patients treated with nusinersen. Our results suggest the presence of a subtle inflammatory CNS response to nusinersen during the loading phase, which then returned to baseline levels during the maintenance phase.

Neuromuscular Junction Disorders and Channelopathies

NMJ&C01

Characterisation of a novel mouse model for Congenital Myasthenic Syndrome caused by a mutation in *CHRND*

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Background*: Congenital Myasthenic Syndromes (CMS) are genetic disorders of the neuromuscular junction (NMJ) characterised by fatigable muscle weakness. Mutations in RAPSN impair acetylcholine receptor (AChR) clustering at the motor endplate and reduce surface AChR. A CMS patient with a RAPSN-like CMS phenotype was found to have compound heterozygous mutations in the AChR delta subunit. One of these is the missense mutation p.R396H in the delta subunit cytoplasmic loop. To learn more about AChR clustering and test potential novel treatments, we designed a mouse model harbouring this mutation (δR399H in mouse), which was created through the MRC GEMM program.*

Aims: Phenotypic characterisation of δR399H C57BL/6 mouse model.

Methods/Materials: Mice were genotyped and phenotypically characterised up to postnatal week 20. Muscle weakness and coordination were measured using inverted screen, grip strength and rotarod tests. Response to oral Pyridostigmine at a dose of 14mg/kg/day was assessed. Neuromuscular junction function was evaluated by electromyography of the gastrocnemius muscle, and ex-vivo electrophysiological recordings of hemidiaphragm-phrenic nerve preparations. NMJs were visualised by fluorescent immunostaining of synapses by labelling the AChRs with α-bungarotoxin followed by confocal microscopy. Wildtype (WT), heterozygous and homozygous mice were compared separately for males and females.

Results: In model mice, up to 50% compound muscle action potential decrement was seen on repetitive nerve stimulation (RNS) at postnatal week 6. Muscle fatigability became apparent later, at 12 weeks of age. Mild weakness in grip strength and coordination was also noted. Despite worsening muscle

weakness with age, decrement did not increase at postnatal weeks 12 or 20. Only model mice showed electrophysiological evidence of a NMJ defect. Ex-vivo electrophysiological recordings in hemidiaphragm-phrenic nerve preparations gave a reduction in the amplitude of both miniature endplate potentials and endplate potentials in models. No difference was observed in quantal content. Fluorescent immunostaining of synapses showed smaller NMJs, reduced α-bungarotoxin fluorescent intensity and increased endplate fragmentation in heterozygous and model mice. Reduced endplate AChR was confirmed by labelling with radioactive α-bungarotoxin. Pyridostigmine treatment improved muscle strength and reduced decrement on RNS.

Conclusions: The δR399H C57BL/6 mouse model reflects many characteristics of a congenital myasthenic syndrome.

‡NMJ&C02

Treatment of congenital myasthenia using a novel AAV-DOK7 gene therapy

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Background: Congenital myasthenic syndromes (CMS) are a group of inherited disorders characterised by defective neuromuscular transmission and fatigable muscle weakness. Approximately 15% of CMS patients have mutations in DOK7, a cytoplasmic-adaptor protein responsible for the formation and stabilisation of the neuromuscular junction (NMJ). Overexpression of DOK7 protein in muscles generate enlarged and functional NMJs and can rescue the phenotype in several neuromuscular disease mouse models including a DOK7-CMS model.

Aims: We are conducting a pharmacology pre-clinical study in a mouse model to test the efficacy of a novel, clinically optimised AAV gene replacement therapy for DOK7-CMS. Clinical parameters measured include weight gain, muscle strength, electromyography measurements and neuromuscular junction (NMJ) morphology.

Methods/Materials: A DOK7-KI mouse model homozygous for the common CMS mutation c1124-1127dupTGCC, was used. Four day old model pups were injected with $2x10^{13}$, $6x10^{13}$ or $1x10^{14}$ vg/kg of the AAV-DOK7 vector AMP-101 in which human DOK7 is under control of a muscle-specific promoter. This directs high levels of expression specifically in skeletal and cardiac muscle. The user was blinded to the dose. Control WT littermates were injected with saline. Weight was monitored, and strength was measured using a hang test, grip test and rotarod. At 3 months of age EMG was performed and mice were culled. Pre- and post-synaptic morphology of diaphragm NMJs were analysed using confocal microscopy.

Results: Untreated DOK7-KI model is lethal, with severe muscle weakness, small stature, underweight and do not survive longer than 10 days. Two of the three doses tested rescue this phenotype in approximately 45% of treated mice who lived longer than 10 days. 90% of these mice survived the duration of the 3 month study and were as strong or significantly stronger than controls in all the strength tests. Despite the phenotypic correction, treated model mice presented residual neuromuscular transmission decrement in compound muscle action potential on repetitive stimulation at 20Hz (females 17%; males 27%). Enlarged NMJs were formed on the diaphragm, and in some mice the post-synaptic area was up to 10 times larger than those of WT controls. **Conclusion:** AMP-101 is an effective gene therapy for DOK7-CMS, and may have therapeutic value for other neuromuscular disorders.

NMJ&C03

The emerging spectrum due to *in utero* transfer of maternal antibodies against the fetal Acetylcholine Receptor (fAChR) isoform – an important and potentially preventable differential diagnosis of genetic neuromuscular disorders

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Background: *In utero* exposure to maternal antibodies targeting the fetal acetylcholine receptor isoform (fAChR) can impair fetal movement, leading to Arthrogryposis Multiplex Congenita (AMC) and, apparently rarer, myopathic presentations termed Fetal Acetylcholine Receptor Inactivation Syndrome (FARIS).

Aims: To report the full clinical spectrum associated with *in utero* exposure to maternal fAChR antibodies and to analyze the relationship between maternal treatments and offspring outcome.

Methods: Retrospective case note review; immunological analysis (RIA, CBA) of maternal and infant AChR antibody profiles; literature review.
Results: We identified 46 cases associated with exposure to maternal fAChR antibodies, 29 novel and 17 previously reported with novel longitudinal follow-up data. Remarkably, in more than half of all cases there was no previously established diagnosis of maternal MG. Offspring death occurred in 11/46 (23.9%) cases. Weakness, contractures, bulbar and respiratory involvement were prominent early in life, but improved gradually over time. Facial weakness (25/34; 73.5%), variable peripheral weakness (14/32; 43.8%), velopharyngeal insufficiency (VPI) (18/24; 75%) and feeding difficulties (16/36; 44.4%) were the most common sequelae in long-term survivors. Other features included hearing loss (12/32; 37.5%), diaphragmatic paresis (5/35; 14.3%), CNS involvement (7/40; 17.5%) and pyloric stenosis (3/37; 8.1%). Oral salbutamol used empirically in 16/35 (47.5%) offspring resulted in symptom improvement in 13/16 (81.3%). Combining our series with all previously published literature, we identified a total of 21/85 mothers treated with variable combinations of immunotherapies (corticosteroids, IVIG, PLEX) during or immediately before pregnancy either for maternal MG symptom control (12/21 cases) or for fetal protection (9/21 cases). Compared to untreated mothers (64/85), maternal treatment resulted in a significant reduction in offspring deaths ($P<0.05$) and other complications, with treatment approaches involving IVIG/PLEX administered early in pregnancy most effective.
Conclusion: Presentations due to *in utero* exposure to maternal fAChR antibodies are probably more common than currently recognized and may mimic a wide range of neuromuscular disorders. Particularly where mothers are asymptomatic, these potentially preventable presentations may lead to the erroneous suspicion of a genetic neuromuscular disorder but are vitally important to diagnose correctly to improve outcomes in future pregnancies. Oral salbutamol is a potentially effective treatment option in affected offspring.

‡NMJ&C04

Congenital Myasthenic syndrome: a Brazilian cohort study

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Background: Congenital myasthenic syndrome (CMS) encompass a group of hereditary neuromuscular junction disorders. Fatigability is the hallmark clinical feature affecting ocular, facial, bulbar, and even appendicular muscles. There are more than 30 causative genes, and with the next generation sequencing (NGS) techniques, several potentially causative genes have been recognized, expanding genotypic variability and knowledge about this syndrome.

Aims: To genetic evaluate a Brazilian cohort of patients with clinically suspected congenital myasthenic syndrome.

Methods/Materials: Patients with fatigable weakness were clinically and electrophysiologically evaluated. Pseudonymised data was uploaded to the ICGNMD Study RedCap database. WES was performed on probands according to ICGNMD study protocols, a CMS virtual panel applied, and variant data evaluated using American College of Medical Genetics (ACMG criteria). Additional family members were tested by Sanger sequencing if necessary.

Results: 41 patients from 33 different families were included, and 28 cases underwent genetic testing. A definite diagnosis (Class 4 or 5) was established in 14 probands (50%). Among the solved cases, disease onset was in the first decade of life for 11 (78.6%) patients, with seven presenting during their first year. The genes implicated were: CHRNE (8), COLQ (2), DOK7 (2), GFPT1 (1) and MUSK (1). Another two affected participants from two different families with CHRNE were diagnosed through Sanger sequencing.

Conclusion: Pathogenic variants within CHRNE are the most frequent cause of CMS in our cohort, highlighting the genetic distribution of CMS in Brazil.

NMJ&C05

In silico predictive tools versus in vivo functional characterisation of *CLCN1* genetic variants in muscle channelopathies

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Background: Variants of uncertain significance (VUS) remain a huge challenge in genomic medicine. Accurate classification of missense variants is key to clinical practice. In silico predictive tools are routinely used to score the pathogenicity of such variants. However, their statistical value is often unclear as they have usually not been validated against robust functional assays.

Aims: To validate in silico predictive tools by comparing to a robust functionally characterised data set for *CLCN1* missense variants.

Methods: We compare nine routinely utilised in silico predictive tools with detailed cell-based electrophysiology for 126 *CLCN1* variants we discovered in patients with the skeletal muscle channelopathy myotonia congenita.

Results: Most in silico predictive tools had poor accuracy - the better performing were Mutation Taster (84.58%) and REVEL (ROC 0.89) but had poor specificity. EVE performed better on specificity while maintaining good AUC and sensitivity. Combined methods based on concordance, improved performance but still lacked specificity. We also showed that the statistics for tools varied between genes. Hence, validation to determine the optimal in silico tools for individual genes should be considered.

Conclusions: Overall, tools with better specificity are urgently required to tackle the ongoing major challenge that VUS pose to the effective clinical implementation of genetic data.

Mitochondrial Disease

‡MD01

Defining the nuclear genetic architecture of a maternally-inherited mitochondrial disorder

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Background: Mitochondrial function is under bi-genomic control; pathogenic variants in the nuclear and mitochondrial genomes (mtDNA) can result in clinical mitochondrial disease. The most common cause of multi-system adult mitochondrial disease (mtDNA variant m.3243A>G; NC_012920.1) is associated with extensive unexplained clinical heterogeneity. Variant m.3243A>G allele level, age and sex explain only a small proportion of this variability (pseudo-R^2 range = 0.05-0.26), whereas high to moderate estimates of heritability for some m.3243A>G-related phenotypes provide evidence for the influence of unidentified nuclear factors.

Aims: This work aims to identify nuclear genetic factors that influence the development of m.3243A>G-related disease.

Methods: Using Haseman-Elston regression-based genetic linkage analysis in a cohort of 208 individuals from 83 pedigrees, we explored the nuclear genetic architecture of eleven phenotypes related to the m.3243A>G variant. For three phenotypes (migraine, cardiovascular involvement, and gastro-intestinal disturbance), no regions of interest were identified; simulation results suggest that any nuclear genetic contribution is highly polygenic in origin.

Results: For seven phenotypes (cerebellar ataxia, chronic progressive external ophthalmoplegia, diabetes, myopathy, psychiatric disturbance, ptosis, and stroke-like episodes), at least one region of interest (LOD > 1.8) was identified. Seven regions of interest were identified for encephalopathy, including two (LOD > 3.3) on chromosomes 7 and 11, suggesting that a small number of nuclear factors play a key role in the development of this severe neurological phenotype.

Conclusion: This work describes the genetic architecture of the nuclear factors that influence m.3243A>G-related disease, revealing that different phenotypes are influenced by nuclear variation in different ways. Using the results of this work to inform future studies will enable the further elucidation of the underlying genetic architecture of this complex disease via genome-wide association studies, polygenic scores, and whole-genome sequencing. This work will build a better understanding of disease development and progression, and will have a tangible impact on patients and patient care.

MD02

A case diagnosed with mitochondrial disease caused by SURF1 mutation by candidate gene method

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Background: Mitochondrial diseases are disorders that result from a deficiency of oxidative phosphorylation. It is caused by pathogenic variants in nuclear (nDNA) or mitochondrial DNA (mtDNA). More than 350 genes from both mtDNA and nDNA cause mitochondrial diseases. Mutations in SURF1 can cause damage to the basal ganglia, thalamus, brainstem, cerebellum, and peripheral nerves, resulting in Leigh syndrome (LS), a subacute neurodegenerative encephalopathy.

Aim: To show that a different method can be followed in the diagnosis of mitochondrial disease.

Methods/Materials: We present a 9-year-old female patient whose parents were consanguineous and who was followed for many years in the pediatric neurology outpatient clinic for developmental delay, myopatia, and seizures. Cranial MRI showed bilateral symmetric basal ganglia involvement.

Results: No pathogenic changes were detected in the chromosomal microarray analysis (CMA). However, when the CMA data were examined together with the SNP data, we found that there was a 22 Mb loss of heterozygosity (LOH) region at 9q34. There were 63 genes in this region and SURF1 from these genes was evaluated as the candidate gene explaining the patient's phenotype. Sanger sequencing revealed a homozygous mutation NM_003172.4:c.870dup(p.Lys291Ter) in SURF1 gene.

Conclusion: Whole exome sequencing plays a very important role in the diagnosis of mitochondrial diseases. However, when clinical findings are carefully examined, disease-causing variants can be found by candidate gene approach, with the help of SNP-based CMA and Sanger sequencing.

Key words: Mitochondrial disease, Leigh syndrome, SURF1, homozygous mapping.

‡MD03

Pathological variants in TOP3A cause distinct disorders of mitochondrial and nuclear genome stability

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Background: Topoisomerase 3-alpha (TOP3A) is an enzyme that removes torsional strain and interlinks between DNA molecules. TOP3A localises to

both the nucleus and mitochondria, with the two isoforms playing specialised roles in DNA recombination and the decatenation of replication products, respectively. Pathogenic variants in TOP3A have previously been reported to cause a disorder similar to Bloom syndrome, which results from segregating pathogenic variants in BLM, a nuclear binding partner of TOP3A.

In support of a crucial role for mitochondrially-targeted TOP3A in mitochondrial DNA (mtDNA) replication and separation, we previously characterised a mitochondrial disease phenotype, associated with muscle-restricted mtDNA rearrangements and a chronic progressive external ophthalmoplegia (CPEO) plus syndrome, in a patient with bi-allelic *TOP3A* variants.

Aims: Here, we aimed to further expand our understanding of the phenotypic spectrum of TOP3A variants and characterize the molecular defects associated with these variants.

Methods/Materials: Using WES or WGS, TOP3A variants were identified in ten individuals from eight families, including four families identified through targeted re-analysis of Genomics England 100,000 genomes data, with adult-onset mitochondrial disease. Using recombinant TOP3A variants with in vitro model substrates, we characterise the molecular defects associated with mutated TOP3A, including variants found in patients with mitochondrial disease and Bloom syndrome.

Results: Affected individuals share a consistent phenotype comprising bilateral ptosis, CPEO, myopathy and axonal sensory motor neuropathy; sensorineural hearing impairment and ataxia were prominent findings. To delineate the biochemical mechanisms underlying the clinical heterogeneity of TOP3A-related pathology, we used recombinant TOP3A variants with model substrates to characterise the molecular defects associated with mutated TOP3A, including variants found in patients with mitochondrial disease and Bloom syndrome.

Conclusion: Our data provide insight into the different clinical presentations that result from defects in the nuclear and mitochondrial functions of a dual-targeted enzyme, expanding knowledge of the links between dysregulated mtDNA maintenance and mitochondrial disease.

MD04

A splice-site mutation in *NDUFS6* causes peripheral neuropathy and optic atrophy

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Background: Variants in NADH ubiquinone oxidoreductase subunit S6 (NDUFS6) disrupt the function of the complex I subunit resulting in mitochondrial complex I deficiency. All reported patients with NDUFS6 variants had a severe mitochondrial disease. Here, we describe a mutation in NDUFS6 in a patient who presented with axonal peripheral neuropathy and optic atrophy.

Aims: To prove the pathogenicity of the variant and expand the phenotype-genotype correlations for NDUFS6 to include peripheral neuropathic and optic atrophy.

Methods/Materials: We performed next-generation sequencing on the patient, a healthy brother, and the consanguineous parents. To confirm pathogenicity of detected variants, we conduct functional studies,

including Western blot analyses of respiratory chain complex I-IV and proteomic profiling of white blood cells.

Results: The patient, a 10-year-old boy, presented with a childhood-onset slowly progressive peripheral neuropathy, starting with frequent falls and abnormal gait at age 7. His symptoms progressed and he developed distal muscle weakness with reduced reflexes without sensory abnormalities and bilateral optic neuropathy. Neurophysiological studies showed results consistent with axonal neuropathy. Next-generation sequencing identified the splice site mutation c.309+5G>A in the NDUFS6 gene. Proteomic analysis in whole protein extract derived from white blood cells of the patient detected a total of 36 significantly dysregulated proteins out of a total 3538, including mitochondrial complex I-related proteins, further supporting the pathophysiological effect of the NDUFS6 variant. Immunoblotting for mitochondrial respiratory chain subunits is currently ongoing.

Conclusion: These findings represent a novel phenotype of NDUFS6 variants and suggest that NDUFS6 may be considered a disease gene in patients with peripheral neuropathy and optic atrophy, without typical signs of mitochondrial disease.

MD05

Using whole exome sequencing (WES) to determine mitochondrial genetic insights and diagnostic yield in ICGNMD cohort of neuromuscular disease patients

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Background: Mitochondrial DNA (mtDNA) mutations are an important cause of neuromuscular disease, which can be called from whole exome sequencing data. MtDNA analysis has been shown to improve the diagnostic yield in patients with neurological disorders. mtDNA variations varies in different populations. Access to the next generation sequencing data in low and middle-income country is still difficult.

Aims: To determine the diagnostic yield and mitochondrial genetic architecture of whole exome sequencing (WES) in neuromuscular disease patients from low and middle-income countries.

Methods/Materials: MToolBox pipeline was used to call mitochondrial DNA mutation on whole exome sequencing data of ICGNMD cohort including probands from Brazil, India, South Africa, Turkey and Zambia. MitoMap and gnomAD were used for annotated mtDNA variants. Only variants QC is over 30, sequencing depth more than 10 and SDP is not 0 were selected for next filtering step. MitoMap reported (AF<0.05) and confirmed variants were reported as known pathogenic. Novel rare mtDNA variants (non-synonymous in protein coding genes) were searched in patients which are clinical diagnoses of mitochondrial disorders.

Results: 651 probands (Brazil 221, India 130, South Africa191, Turkey 99 and Zambia 10) were analysed on the MToolBox pipeline. Total 2254 variants were found in the entire cohort. After variant calling and filtering steps, 31 cases contained MitoMap reported or confirmed mtDNA variants. Known pathogenic variants were found in 8 cases and located in 5 genes (MT-TN, MT-CYB, MT-TS1, MT-ATP6 and MT-ND6). Diagnosis yield in different centres varied between 0-10%.

Conclusion: WES is helpful in diagnosis of neuromuscular disease. The diagnosis yield of mtDNA mutation in South Africa and Zambia neuromuscular disease are comparably high (2% and 10% separately). The most common variant m.3243A>G in UK population was absent from this cohort. Clinical in-

terpretation of the variants is still going on. Segregation in different tissue types and maternal family members will be performed for selected variants. These findings are helping in understanding mtDNA genetic basis in different populations.

MD06

The mitochondrial aminoacyl-tRNA synthetases and their clinical and genomic landscape of Turkey: A preliminary study

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Background: The aminoacyl-tRNA synthetases (AaRSs) encoded by the nuclear genome are one of the major components of the mitochondrial translation machinery. Although they also target other systems, mutations in these enzymes mostly impact the central nervous system.

Aim: To review a cohort with the mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) mutations to improve current knowledge about causative links between mutations in human mt-aaRSs and diseases of the nervous system.

Methods: We retrospectively reviewed the demographical and clinical features, brain and/or spinal MRI abnormalities, and gene variants of the mt-aaRSs genes in a Turkish cohort.

Results: There were nine patients (median age at onset, 2 years; mean age at genetic diagnosis, 9.2 years), 5 of them female, with mt-aaRSs gene variants. Eleven variants, 5 of which were detected as homozygous in consanguinous Turkish families were identified in 7 different genes by next-generation-sequencing/whole-exome-sequencing; *KARS* (P1; homozygous for c.1469T>C(p.Ile490Thr) and P2; homozygous for c.1063A>G(p.Ile355Val)), *EARS2* (2 siblings, compound heterozygous for c.319C>T(p.Arg107Cys)/ c.1005_1006del (p.Leu336Aspfs)), *RARS2* (compound heterozygous for c.426delT(p.Gly143Aspfs*8)/ c.1366C>T(p.Arg456Cys)), *NARS2* (homozygous for c.662G>C (p.Gly221Ala)), *FARS2* (homozygous for c.1082C>T(p.P361L)), *AARS2* (compound heterozygous for c.806G>A(p.G269D)/ c.595C>T(p.R199C)) and CARS2 (homozygous for c.655G>A (p.Ala219Thr)). Seven individuals (77.8%) exhibited refractory epilepsy and global development delay in the infantile or childhood period. Leukoencephalopathy (n = 6), infantile hypotonia (n = 4), ataxia (n = 2), dysarthria (n = 1), and microcephaly (n = 1) were additional signs of central nervous system involvement that were commonly seen. Other system involvements such as obesity (n:1), sensorineural hearing loss (n:1), acute metabolic crisis, and abnormal liver tests (n:1) were also observed in this cohort.

Conclusion: The mt-aaRSs mutations are rarely seen and associated with highly heterogeneous phenotypes and genotypes as described in the prestudy cohort. The genotype-phenotye correlation studies could shed light on the functions of the genes that are in the same pathway but effect different systems.

MD07

Pre-test counselling for primary mitochondrial disease the era of first line whole genome sequencing

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Background: Whole genome sequencing is being adopted as a first line test for primary mitochondrial disorders (PMDs). Its ability to simultaneously capture nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) with very deep coverage confers significant efficiency over previous technologies. However, with the increasing availability of WGS, PMD genes are now included in virtual panels for many diseases. PMD diagnoses are therefore more likely to be made in patients where these disorders were not actively considered *a priori,* and may come as a surprise to the patient and clinician alike.

Aims: To describe the ethical issues inherent in PMD diagnoses through WGS, to inform pre-test discussions.

Methods/Materials: We reviewed the gene content of virtual gene panels employed in WGS and how PMD diagnoses might now be reached in novel, but perhaps unanticipated ways. We used case exemplars from the clinic to identify issues.

Results: We identified PMD gene content in several gene panels not overtly associated with PMD, including monogenic diabetes, optic neuropathy, hearing loss, neuropathy, inherited tumour predisposition panels. We describe several considerations for genetic counselling when PMD genes are included in such panels: 1)Identification of variants that are both diagnostic and predictive (e.g., diagnostic of monogenic diabetes and predictive of stroke like episodes, cardiomyopathy); 2) *De facto* diagnoses of [certain] family members where mtDNA variants are identified; 3)Risk of late-onset dominant PMD in carriers of autosomal recessive PMD; 4)Inadvertent identification of tumor predisposition in carriers of recessive PMD; 5) Difficulty in delineating what counts as a medical result- i.e., distinguishing normal variation from disease risk e.g., low level heteroplasmic variants, low levels of mtDNA deletions.

Conclusion: PMD diagnoses are complex and often have an attendant uncertainty in the magnitude and temporal elements of the risks. We recommend clear and honest communication regarding uncertainties and suggest expert input and support for PMD diagnoses reached through clinical WGS testing.

MD08

Specialist multidisciplinary input maximises rare disease diagnoses from whole genome sequencing

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Background: Diagnostic whole genome sequencing (WGS) is increasingly used in rare diseases. However, standard, semi-automated WGS analysis may overlook diagnoses in complex disorders.

Aims: We aimed to develop an integrated clinical solution for patients with no primary findings (NPF) following WGS analysis within English national healthcare genetic services.

Methods/Materials: We undertook WGS in 102 adults (100,000 Genomes Project) with diagnostically challenging primary mitochondrial disease phenotypes. NPF cases were reviewed by a genomic medicine team, thus enabling bespoke informatic approaches, co-ordinated phenotypic validation, and functional work.

Results: Here, we show that specialist multidisciplinary analysis of WGS, following an initial 'no primary findings' (NPF) report, improves diagnostic rates and alters management. We enhanced the diagnostic rate from 16.7% to 31.4%, with management implications for all new diagnoses, and detected strong candidate disease-causing variants in a further 3.9% of patients.

Conclusions: This approach presents a standardised model of care that supports mainstream clinicians and enhances diagnostic equity for complex disorders, thereby facilitating access to the potential benefits of genomic healthcare. This research was made possible through access to the data and findings generated by the 100,000 Genomes Project

MD09

Rethinking mitochondrial diabetes: a multifaceted disease entity

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Background: Diabetes mellitus is the most common endocrinopathy reported in primary mitochondrial diseases (PMDs). An impediment to solving the mitochondrial diabetes (mDM) conundrum is its complexity: it has an insidious presentation, is often misdiagnosed as type I/II diabetes, and a clear definition does not exist.

Aims: Our aim is to investigate the clinical features of mDM to facilitate recognition among specialists, identify unique features, and reveal opportunity for interventions.

Methods/Materials: This is a cross-sectional, monocentre, registry-based study. Patients with a confirmed diagnosis of PMD and mDM were included. Extensive blood tests were performed in a subgroup of patients.

Results: From 01/01/2021 to 31/12/2022, 67 patients (38 females, 56.7%; mean age 51.8 ± 13.2 years) were included. As expected, the vast majority (51, 76.1%) had the m.3243A>G mutation. The age of onset of mDM was 38.2 ± 13.6 years. The diagnosis of mDM preceded the diagnosis of PMD in 48 cases (71.6%). Surprisingly, the name given to their diabetes was type I in 20 (29.9%) cases, type II in 40 (59.7%), and "mitochondrial diabetes" in 7 cases only (10.4%). Regarding treatment, 45 people (67.2%) were on insulin, which was required 3.1 ± 3.8 years after mDM onset. The mean HbA1c level was 59.7 ± 12.2 mmol/mol. Cardiovascular risk factors were present in a proportion of subjects: obesity (11, 16.4%), hypertension (31, 46.3%), dyslipidaemia (34, 50.7%), smoking (14, 20.9%). Type I diabetes autoantibodies were present in 5 out of 34 people (4 anti-GAD 4 and 1 anti-IA2). C-peptide levels (n=34), an indicator of endogenous insulin production, were low in 19 (55.9%), showed residual insulin production in 9 (26.5%), insulin resistance in 1 (2.9%), and appropriate values in 5 (14.7%).

Conclusion: mDM is a rare but distinct form of DM that arises as a consequence of OXPHOS dysfunction. mDM has peculiar features, including the age of onset, the need for insulin, and the different prevalence of risk factors. We have highlighted the coexistence of different subgroups characterised by insulin resistance and autoantibodies. These factors need to be considered when advising people with mDM and when including mDM as an outcome of clinical trials.

MD10

Normal outcome with prenatal intervention for riboflavin transporter defect

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Introduction*:* Riboflavin transporter deficiency is a rare but severe neurometabolic disorder. We report two siblings with pathogenic variants in *SLC52A3* gene, resulting in riboflavin transporter 3 deficiency.

Case Summaries: The first sibling was diagnosed at 11 months of age with severe respiratory compromise and regression of developmental milestones. His symptoms significantly improved with riboflavin supplementation therapy. The younger sibling was diagnosed by antenatal genetic analysis; riboflavin supplementation was initiated in utero and continued from birth. Now 2 years of age, he remains clinically asymptomatic despite genetic confirmation of riboflavin transporter deficiency.

Discussion*:* Antenatal riboflavin supplementation is a safe and effective treatment for the prevention of symptomatic manifestations of riboflavin transporter deficiency. These participants have now been recruited as genetically confirmed cases to the International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) to increase opportunities for participant access to future trials and research.

MD11

Long-term cardiovascular outcomes in adult mitochondrial diseases

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Background: Patients with mitochondrial diseases are susceptible to cardiovascular events. Studies have shown an association between mitochondrial DNA mutations, especially m.3243A>G in *MT-TL1*, with major adverse cardiac events (MACE), sudden death and high mortality. However, there is a lack of comprehensive assessment of long-term cardiovascular outcomes in these patients.
Aims: This study aims to describe the natural history and long-term cardiovascular outcomes of patients with genetically confirmed mitochondrial diseases and also identify risk factors associated with MACE.

Methods: Patients with genetically confirmed mitochondrial diseases were included in the study. Analysis of cardiovascular risk factors and MACE (cardiac death, all-cause mortality, device implantation, hospitalisation for heart failure, ischaemic stroke) was performed and risk factors for MACE were identified by multivariate analysis.

Results: One hundred and thirty-five patients (median age = 48 years [18-91], 42% male) were followed up for a median time of 5 years. Diabetes and systemic hypertension were present in 31% and 30% of patients respectively. Cardiac symptoms were frequently experienced, especially palpitations (20%) and dyspnoea (17%). Cardiac treatment was also frequently administered, in particular angiotensin converting enzyme inhibitors (18%) and beta-blockers (17%). Electrocardiogram abnormalities were present at baseline (42%) and mainly consisted of abnormalities in the conduction system (sinus node and AV node dysfunction). Left ventricular hypertrophy was observed in 24 patients (18%) and mostly in m.3243A>G carriers. Twenty-four major adverse cardiac events occurred over the follow-up period. Multivariate analysis identified left ventricular hypertrophy (HR 3.521; CI 1.365-9.085, p=0.009) and conduction disease (HR 3.168; CI 1.290-7.782, p=0.012) as risk factors for MACE. Seven of the 18 deaths that occurred had cardiac involvement and m.3243A>G carriers had a higher mortality rate in comparison to the other mutation groups (p=0.021).

Conclusion: Cardiac involvement in patients is a frequent and important manifestation of mitochondrial disease. Left ventricular hypertrophy and conduction defects are common, particularly in those harbouring the m.3243A>G mutation. Patients with mitochondrial diseases are at risk of MACE, which are independently associated with left ventricular hypertrophy and conduction disease.

MD12

National mitochondrial disease registry in England: linking genetics with routinely collected healthcare data

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Background: Understanding the natural history of mitochondrial disorders is important for giving patients accurate prognostic information, monitoring for complications and understanding disease mechanisms. Currently, most information comes from patient cohorts seen at specialist centres.

Aims: To use a population-based approach by linking genetic results to routinely collected healthcare data.

Methods/Materials: We are expanding the National Rare Disease Register to include patients with genetically confirmed primary mitochondrial disorders. Data is collected under legal permissions granted to NHS Digital under Section 254(1) and Section 254(6) of the 2012 Health and Social Care Act. We have requested data from ten laboratories. Personal details are confirmed using the Personal Demographics Service and data linkage is done for people resident in England. Asymptomatic individuals who had predictive testing are excluded. We have created an Advisory Board with stakeholders including the Lily Foundation and run two patient focus groups.

Results: The focus groups found the use of data acceptable, supported the idea of a register and were interested to find out the causes of hospital admissions. We have received lists of patients from seven regional genetic laboratories and from Genomics England. Most patients have single nucleotide variants in the mitochondrial DNA, some have large scale mitochondrial DNA rearrangements and ~25% have nuclear gene defects including *SPG7*, *OPA1* and *POLG*. The pattern of diagnoses approximately reflects the known epidemiology.

Using Hospital Episode Statistics for the cohort, we can describe the number of admissions, lengths of stay, reasons for admission and recorded co-morbidities (coded in ICD10). We are analysing linked Office for National Statistics mortality data using free text from death certificates. Our preliminary analysis shows that the ICD10 codes recorded capture expected features of the different genotypes. The causes of death differed between children and adults. The main causes of death in adults included mitochondrial disorder, cardiac, pneumonia and cancer, whereas nearly all childhood deaths were due to the underlying mitochondrial disorder.

Conclusion: We hope to reveal under-recognised features of mitochondrial disease, describe hospital healthcare usage, causes of death and median survival, and provide useful natural history data for counselling patients and families.

MD13

Two novel *MT-ATP6* variants cause mitochondrial disorder

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Background: Mutations in mitochondrial *ATP6* synthase *(MT-ATP6)* exhibit variable clinical phenotypes which includes maternally inherited Leigh syndrome (MILS), neuropathy, ataxia, retinitis pigmentosa (NARP), Charcot-Marie-Tooth disease (CMT), spinocerebellar ataxia, spastic paraplegia, motor neuron disease, neuropathy, white matter abnormalities and episodic weakness depending on the heteroplasmic load. Most of the *MT-ATP6* mutations are missense, few truncating mutations are also reported.

Aim: To describe the clinical, histomorphological, biochemical, genetic and functional characterization of patients harbouring novel *MT-ATP6* missense mutations.

Methods: Whole mitochondrial genome sequencing was carried out by Sanger's method. Trans-mitochondrial cybrid lines generated using patient-derived fibroblasts for two unrelated probands harbouring novel *MT-ATP6* missense mutation were used to investigate mitochondrial respiration, ATP6 steady state levels, mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) production to determine the pathogenicity of novel variant.

Results: Patient 1 presented with spastic ataxia with elevated lactate. Muscle biopsy showed mild variation in fibre size and type I fibre grouping, while ultrastructural analysis showed evidence of mitochondrial abnormalities. Patient 2 had multiaxial neurological involvement and showed mild variation in fibre size on muscle biopsy. Brain MRI showed mild cerebellar atrophy in both the patients. Whole mitochondrial genome analysis revealed the novel missense *MT-ATP6* variations; m.8576T>C (p.L17P) with 87.87% mutant load and m.8773A>G (p.N83D) with 98.8% mutant load, in muscle respectively. The heteroplasmy levels were varied across different tissues in these probands. Segregation analysis in blood samples of maternal family members showed the presence of variation with low levels of mutant. Western blot analysis on muscle extracts revealed marked reduction of ATP6 protein levels in proband 1 (m.8576T>C), whereas proband 2 (m.8773A>G) showed mild reduction. Microscale oxygraphy showed reduced basal respiration, ATP synthesis and maximal respiratory capacity, while ROS was increased and MMP was altered in cybrids carrying m.8576T>C (99.96% mutant load) and m.8773A>G (98.8% mutant load) compared to 143B wildtype cell line.

Conclusion: The study does expands the genetic spectrum of *MT-ATP6* related mitochondrial disorders. Impaired mitochondrial function noted in cybrid cell lines confirms the pathogenicity of the variant identified.

MD14

Evaluation of mtDNA copy number assessment in patients with suspected mitochondrial disease

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Background: Mitochondrial DNA (mtDNA) depletion syndromes are characterised by a reduced mtDNA copy number and are caused by genetic defects in nuclear encoded genes associated with disorders of mtDNA maintenance. Assessment of mtDNA copy number is offered as part of the diagnostic service provided by the NHS Highly Specialised Service for Rare Mitochondrial Diseases.

Aims: To review the results of mtDNA copy number assessment against the outcomes of other genetic analyses in patients with suspected mitochondrial disease.

Methods/Materials: MtDNA copy number was assessed in 187 muscle samples referred for investigation of possible mitochondrial disease over a 5 year period from 2016 to 2020. Analysis was performed by real-time PCR to assess mtDNA copy number and nuclear copy number, with results compared to the mean normal mtDNA:nuclear DNA ratio. Results were compared to outcomes of other genetic analyses and subsequent diagnoses.

Results: Of the samples tested, 15 (8%) had a mtDNA copy number consistent with a diagnosis of mtDNA depletion syndrome (mtDNA <30% of the mean normal level). Sequencing of nuclear encoded genes associated with disorders of mtDNA maintenance confirmed a genetic diagnosis of mtDNA depletion syndrome in only 6 (40%) of these cases; however, pathogenic or likely pathogenic variants consistent with a diagnosis of mitochondrial disease were identified in all cases with a mtDNA copy number <20% of the mean normal level. A further 78 samples had an intermediate mtDNA copy number (mtDNA 30-59% of the mean normal level) and in 2 of these, pathogenic or likely pathogenic variants were detected in genes reported to be associated with mtDNA depletion syndrome. A further 5 of the equivocal cases had a confirmed genetic diagnosis in a nuclear encoded gene associated with mitochondrial disease but not typically thought to cause mtDNA depletion.

Conclusion: Analysis of mtDNA copy number can help identify the genetic diagnosis in patients with suspected mitochondrial disease; however, interpretation of results is complicated by overlap in mtDNA copy number between mtDNA depletion syndromes, other mitochondrial diseases, and other non-mitochondrial disorders. In addition, a significant proportion of cases with reduced mtDNA copy number remain without an identified genetic diagnosis.

‡MD15

Mitochondrial DNA loss and mitochondrial dysfunction in liver are reversed by deoxynucleotide administration in mice

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Background: Mitochondrial disorders are incurable conditions that cause severe disability and high mortality. One class of these disorders results from nuclear gene mutations that compromise the integrity of the mitochondrial DNA (mtDNA). These lead to loss (depletion) or damage (deletions) of mtDNA molecules, with a typical minimum threshold for disease expression of 50% of mtDNAs affected or lost. Around this threshold small increases in the number or quality of mtDNAs can markedly enhance mitochondrial function, which could improve the clinical outcome in affected individuals.

A shortage of nucleotides underlies several forms of depletion and deletion syndromes (MDDSs). We and others have demonstrated that the supplementation of the nucleotide precursors (deoxyribonucleosides, dNs) restores the mtDNA levels in cell culture models of MDDS; yet the beneficial effect of dNs *in vivo* is established only for MDDS caused by thymidine kinase deficiency.

Aims: To develop a deoxnucleoside-based treatment for MDDS caused by mutations in *MPV17*, which, usually, presents as early-onset fatal liver disease, or, less frequently, as an adult-onset neuromuscular condition.

Methods: To evaluate the dNs efficacy *in vivo*, we used an *Mpv17*$^{-/-}$ mouse model. Like affected patients, *Mpv17*$^{-/-}$ mouse display severe hepatic mtDNA depletion and respiratory chain dysfunction from an early age. After investigating the effect of mitochondrial dysfunction on cellular and organ pathology, we treated the mouse with oral dNs and assessed the effects of the treatment on mtDNA loss, and mitochondrial and general metabolism.

Results: MtDNA dysfunction significantly alters cellular metabolism in the liver of *Mpv17*$^{-/-}$ mice as early as 1-month-of-age; indicating that the model can be useful to identify the key pathways that mediate the mtDNA-driven liver pathology. Importantly, dNs administration greatly increased mtDNA levels and respiration and restored several metabolic pathways.

Conclusion: There is currently no cure or effective treatment for MPV17 deficient patients, who often die in infancy owing to liver failure. Our study has identified effective pharmacological regimes in the *Mpv17*$^{-/-}$ mouse. This new knowledge provides a platform for instigating a clinical trial of dNs for the treatment of MPV17 disease and expands the range of disorders where deoxynucleoside-based treatment can be employed.

MD16

The use of Droplet Digital PCR to determine mitochondrial DNA copy number and mitochondrial DNA deletion heteroplasmy

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Introduction: Mitochondrial disorders result from dysfunction of the mitochondrial respiratory chain. Two mechanisms of mitochondrial disease are depleted mitochondrial DNA (mtDNA) copy number and heteroplasmic large segmental mtDNA deletions.

Background and Rationale: Droplet Digital PCR (ddPCR) has emerged as a robust, sensitive and specific assay for absolute quantification of nucleic acids. Quantitative real-time PCR (qPCR) is currently used in Oxford Genetics Laboratories (OGL) for mtDNA copy number determination but is prone to well-to-well variation and requires use of standards which are difficult to obtain. ddPCR is expected to overcome these problems. In addition, OGL are now required to develop an assay for mtDNA deletion heteroplasmy quantification as part of the NHS England Genomic Medicine Service test repertoire for mitochondrial disorders.

Aims: We aimed to develop and validate a ddPCR assay that reflects the new requirements of the service.

Assay Development: We developed a robust 'double duplex' ddPCR assay that quantifies heteroplasmic deletions and mtDNA depletion in two reactions (wells) per sample. We optimised the PCR protocol

to protect the generated droplets and performed a cost analysis between platforms.

Validation: Twelve mtDNA deletion samples where heteroplasmy had been quantified in another laboratory and fifty muscle samples where mtDNA copy number had been determined by qPCR were used to validate the assay. We obtained reproducible data which correlated well with previous results.

Conclusion: ddPCR has been determined to be a robust and efficient methodology for these two assays and will be incorporated into OGL's genomic testing pathways for mitochondrial disease.

MD17

Epidemiology of the West of Scotland Primary Mitochondrial Disease Cohort

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Background: Primary mitochondrial diseases are genetic disorders associated with oxidative phosphorylation dysfunction. Inheritance can be complex due to the involvement of both mitochondrial and nuclear genomes and, additionally, sporadic deletions may also occur. These diseases can present in a highly heterogeneous fashion over a patient's lifespan, with dysfunction in the visual, gastro-intestinal, cardiac, endocrine and nervous systems, most typical. Recent work by the Wellcome Centre for Mitochondrial Research revealed that mitochondrial diseases are some of the most prevalent inherited neurometabolic disorders with 1 in 4300 adults affected in the United Kingdom. The specialist West of Scotland neuromuscular clinic serves the largest population in Scotland (approximately 2.5 million) and, therefore, assessment of primary mitochondrial disease in this population may best reflect the Scottish population as a whole.

Aims: We aimed to genetically characterise patients with molecularly confirmed primary mitochondrial disease in the West of Scotland. Furthermore, we aimed to look at the phenotype associated with the commonest mitochondrial mt. 3243 A>G mutation.

Methods: We looked retrospectively at the patients attending the West of Scotland specialist neuromuscular clinic with molecularly confirmed primary mitochondrial disease. Electronic patient records were used to define the genotype and clinical phenotype.

Results: We have identified 95 adult patients with molecularly confirmed primary mitochondrial disease. As expected, mt. 3243 A>G mutation was the most prevalent mutation with 49 patients affected. The majority of these patients displayed the maternally inherited diabetes and deafness (MIDD) phenotype; 3 patients exhibited mitochondrial encephalopathy with lactic acidosis and stroke-like symptoms (MELAS). 15 patients carried the mt. 8344 mutation with myoclonic epilepsy with red ragged fibres (MERRF). Sporadic large-scale deletions were found in 13, POLG mutations in 3 and YARS2 mutations in 2 patients. Furthermore, there was a mixed group with a variety of other mutations (7 mitochondrial and 7 nuclear mutations).

Conclusion: Primary mitochondrial diseases are a heterogeneous group of progressive disorders associated with significant morbidity and mortality. Accurate ascertainment of this patient cohort will allow for adequate multi-specialty patient care and appropriate provision of resources. Assessment of the entire Scottish mitochondrial population would further help serve patient care on a national level.

MD18

Modelling loss of the mitochondrial release factor in rescue (MTRFR) in human iPSC-derived i^{3}Neurons

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Background: The mitochondrial release factor in rescue (MTRFR), encoded by the nuclear gene *C12orf65*, is a component of the mitoribosome quality control pathway. MTRFR responds to interruptions in mtDNA translation by releasing nascent polypeptide chains from stalled ribosomes. Most reported autosomal recessive mutations in the *C12orf65* gene generate a premature stop-codon, likely resulting in loss of function. While disease severity varies, patients present with optic atrophy, spastic paraparesis, and peripheral neuropathy. To date, no in vitro models of the disease have been developed, with the current literature being limited to clinical descriptions and the investigation of patient's fibroblasts and skeletal muscle biopsies.

Aims: To develop a reliable disease model to investigate the pathogenic phenotypes that arise from mutations in *C12orf65*. We will generate a *C12orf65* knockdown iPSC line and differentiate it into cortical and lower motor neurons to investigate the neuronal deficits that may be driving the neuromuscular phenotypes observed in patients with this mutation.

Methods/Materials: To generate the knockdown iPSC line we use a CRISPRi lentiviral vector to guide dCas9-KRAB transcriptional repression of the C12orf65 gene. Then, we perform transcription factor-mediated differentiation of the iPSCs into neurons. In short, the iPSCs stably express doxycycline-inducible neurogenin-2 (NGN2) at a safe-harbor loci. These cells can be differentiated into neurons by the induced overexpression of NGN2. Finally, we perform mitochondria functional assays in the neuronal cultures, including oxygen consumption and activity of the respiratory chain complexes, to investigate possible mitochondria dysfunction.

Results: This work is still in progress. We are currently generating the knockdown iPSC lines. We expect this model will recapitulate the defect of mitochondrial translation that have been previously reported on patient's fibroblasts and muscle biopsies, and will provide further insights into neuronal-specific disease-related phenotypes.
Conclusion: Very little is known about the neuronal deficits that may underlie the neuromuscular phenotypes observed in patients with mutations in MRFR. Having a neuronal in vitro model will allow to both delineate C12orf65-related phenotypes as well as to examine possible therapies for this ultra-rare mitochondrial disease.

Other Diseases

OD01

Anti-signal recognition particle antibody associated necrotizing myopathy: Importance of early diagnosis and treatment

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Background: Anti-signal recognition particle (anti-SRP) myopathy is a rare cause of immune-mediated necrotizing myopathy in childhood. It has been reported in only a few pediatric cases to date and is usually refractory to treatment with a poor prognosis.

Case presentation: A three-year-old girl presented with easy fatigue, frequent falls, and difficulty in walking for the last 2 months. She was the second child of non-consanguineous parents and her developmental milestones were compatible with peers. She had a history of Covid-19 infection two weeks prior to admission. Neurological examination revealed axial and symmetrical proximal muscle weakness, absent deep tendon reflexes, and nasal speech. Serum creatine kinase (CK) levels were elevated to 6506-13552 U/L. Anti-SRP antibody posi-

tivity was detected by myositis autoantibody panel. Skeletal muscle biopsy showed active necrotic and regenerating processes, with mild inflammatory changes. Based on the above findings, the patient was diagnosed with anti-SRP-associated myopathy. She was treated with pulse methylprednisolone and intravenous immunoglobulin, followed by multiple immunosuppressive therapies, including methotrexate, rituximab, cyclophosphamide, and azathioprine, maintained by oral prednisolone. The muscle weakness gradually improved and serum CK levels returned to normal. Six months later she achieved to walk independently. At 15 months follow-up, she remains clinically stable with 4/5 muscle strength.

Conclusion: Anti-SRP myopathy should be considered as a differential diagnosis in children who clinically appear with rapid progression of weakness. Early immunomodulatory therapy is crucial for obtaining a better prognosis in patients with anti-SRP myopathy.

OD02

Usefulness of Genetic Testing in a Cohort of Patients Presenting with Acute Rhabdomyolysis Secondary to Exertion

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Background: Rhabdomyolysis is characterized by severe acute muscle injury resulting in muscle pain, weakness, and/or swelling. There are many different causes of acute rhabdomyolysis both acquired and genetic. The clinical history and examination, laboratory studies, muscle biopsy, and genetic testing are useful tools for diagnosis. These diagnostic tests are usually quite expensive and sometimes invasive.

Aims: This study aimed to identify the clinical phenotype of patients presenting with acquired and genetic causes of acute rhabdomyolysis and to inform the need for genetic testing for acute rhabdomyolysis.

Methods/Materials: The subjects were patients presented with acute rhabdomyolysis and attended McArdle Disease and Related Disorders Service between January 2020 and December 2022. We retrospectively reviewed the case notes.

Results: 51 patients have attended to our clinic during the two-year period, of which 19 cases (37%) were rhabdomyolysis due to unaccustomed exercise, 10 cases (20%) were McArdle disease, 10 cases (20%) were CPT-2 deficiency and 12 cases (23%) were RYR-1-related rhabdomyolysis. 100% of the patients had genetic testing to confirm the diagnosis. The mean age was 37 (range: 19-75); 84% were males. Median CK during acute rhabdomyolysis was 55000 (range: 8000-300000). Of 30 patients (59%) had hospital admission during the rhabdomyolysis attack; 7 patients (14%) admitted to ITU and 6 patients (12%) had renal dialysis. Common triggers were exercise, heat, missing a meal, illness and coffee. Neck muscle involvement was more common in CPT-2 disease (p=0.05). Muscle pain due to rhabdomyolysis occurred in the first 2 minutes of exercise in 100% of the McArdle patients. Patients with CPT-2 & RYR-1 mutation had muscle pain 2-36 hours after the exercise whereas it was more than 36 hours later for patients who had rhabdomyolysis due to unaccustomed exercise. There was a statistically significant difference in the timing of muscle pain between different disease groups (p<0,001).

Conclusion: The differences in the clinical phenotype of patients we detected could help the differential diagnosis and decrease the need for genetic testing.

‡OD03

The Prevalence and Mortality Analysis of McArdle Disease and other rare muscle glycogenoses in the UK

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Background: McArdle disease (GSDV) is a metabolic myopathy caused by a congenital absence of the enzyme muscle glycogen phosphorylase (PYGM), the condition is inherited as an autosomal

recessive trait. The prevalence of McArdle disease in England is unknown and has been estimated to be between 1:100,000 in Dallas USA to 1:170,000 in Spain. Data on the epidemiology of muscle glycogenoses are limited, with few published epidemiological studies.

Aims: The aim of the study is to develop national disease registration for rare muscle glycogenoses with the primary aim of understanding the natural history and epidemiology of McArdle Disease in England.

Methods/Materials: People of all ages were identified from the National Congenital Anomaly and Rare Disease Registration Dataset which was a collaboration of NCARDS and Inherited Metabolic Disorder Specialised Services all over the UK.

Results: We identified 184 patients with McArdle Disease in the England (prevalence of ~1/300.000 people), 194 patients with Pompe Disease (prevalence of ~1/285.000 people), followed by 93 GSDIII patients (prevalence of ~1/600.000 people), 9 GSDVII patients and 2 patients with GSD due to muscle phosphorylase kinase deficiency. We also identified causes that contributed to deaths attributed to McArdle disease. McArdle disease was recorded as the underlying cause of death on 11 certificates; of these, 11 (100%) included at least 1 additional cause. The mean age at these deaths was 66.6 years. The most frequently mentioned cause of death overall was vascular disease (n = 6), followed by cancer (n = 4) and chronic respiratory disease (n = 4). 2 patients died died due to COVID-19.

Conclusion: This is the largest series of patients' epidemiologic data that is available to date on McArdle disease and rare muscle glycogenoses in the UK and as such can provide corroborative or novel insights on these disorders.

OD04

Exploring fibroblast-homing peptides to enhance the delivery of antisense oligonucleotides in Collagen 6-related congenital muscular dystrophies

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Background: Collagen VI-related congenital muscular dystrophies (COL6-CMDs) are a group of neuromuscular disorders that affect skeletal muscle and connective tissues. They are caused by mutations in genes encoding the three major α-chains of collagen type VI protein (*COL6A1*, *COL6A2*, and *COL6A3)*. Currently, no cure is available for COL6-CMD.

Previously we have provided promising evidence and proof-of-concept for the potential of antisense oligonucleotides (ASO) as a therapeutic approach for COL6-CMD. We have identified ASO sequences able to correct the common dominant mutations in fibroblasts cultured from COL6-CMD patients. However, subsequent *in vivo* studies suggested that a major challenge was the ASO delivery in the muscle interstitial fibroblasts (MIF), the major cell population that produces collagen VI protein. This is a major bottleneck for ASO therapy development in COL6-CMD.

Aims: This experiment aims to identify MIF-targeting peptides which can be used to conjugate ASOs to enhance their uptake in MIFs.

Methods/Materials: Thirteen short peptides fragmented from a candidate ligand of a fibroblast surface receptor were synthesized and labelled with FAM fluorescence tag. The binding specificity and cellular internalization of the peptides were tested in different cell lines, including human and mouse fibroblasts, myoblasts, hepatocytes, podocytes and human endothelial cells.

Results: We have identified eight peptides that can efficiently bind to and be internalized in fibroblasts. Two peptides can be preferentially taken up by fibroblasts, but not by myoblasts, endothelial cells, hepa-

tocytes or renal cells. Moreover, we have identified a lead peptide that can efficiently and specifically bind and be internalized in cultured fibroblasts in less than 10 minutes.

Conclusion: We have successfully identified a lead peptide that can strongly target fibroblasts. Our next step is to conjugate the lead peptide with ASOs and test the efficacy of the conjugates in correcting COL6A mutations and restoring the function of collagen VI protein in cultured fibroblasts from patients. We expect the development of a MIF-targeted peptide-ASO delivery will address the current bottleneck of ASO therapy in COL6-CMD.

OD05

NEO1/NEO-EXT studies: Muscle MRI results in patients with Pompe disease after long-term avalglucosidase alfa treatment

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Background: Magnetic resonance imaging (MRI) is progressively gaining widespread use to study patients with muscle diseases, including Pompe disease.

Aims: To use qualitative and quantitative MRI to measure the disease burden of participants with late-onset Pompe disease (LOPD) at enrollment into the NEO1 study (NCT01898364) and to evaluate the effects of avalglucosidase alfa on muscle structure in participants in the NEO-EXT study (NCT02032524), an ongoing NEO1 study extension.

Methods: The impact of avalglucosidase alfa on different components of muscle structure was assessed at NEO1 completion (Week 27) and every 2 years thereafter in NEO-EXT

NEO-EXT study patients from the Newcastle site will be discussed by the presenting author.

Results: NEO1 participants were either treatment-naïve (n=10) or had received alglucosidase alfa for ≥9 months (n=14). Twenty-one participants completed NEO1; 19 entered NEO-EXT.

At NEO1 enrolment, the degree of disease burden; muscle glycogen content, Mercuri grading, and 3-point Dixon fat fraction, was consistent with previous natural history data.

During NEO-EXT, quadriceps and hamstring 3-point Dixon fat fraction, water T2 (with/without B1 heterogeneity correction) as well as muscle mass index were generally stable for up to 4.5 years of avalglucosidase alfa in most participants (change/ year in % fat fraction from baseline: quadriceps: treatment-naïve 0.3, treatment-experienced –0.02; hamstring: treatment-naïve 1.2, treatment-experienced –1.8). Few muscle biopsies were performed in NEO-EXT; being only required if glycogen content was ≥5% or a participant showed significant clinical decline.

The results are consistent with stabilization of disease by avalglucosidase alfa in this population of previously treatment-naïve and treatment-experienced adults with LOPD. This suggests that avalglucosidase alfa may contribute to muscle preservation, contrasting the worsening fatty replacement seen in untreated LOPD patients and in some recipients of alglucosidase alfa.

Conclusions: These data further demonstrate a link between quantitative MRI indications of disease activity and disease burden as assessed by muscle glycogen content and clinical endpoints of motor function. Fat fraction along with muscle function tests can be considered good measures for stratification and longitudinal follow-up in clinical trials in participants with LOPD. **Funding:** Sanofi

OD06

The John Walton Muscular Dystrophy Research Centre Biobank

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Background: The John Walton Muscular Dystrophy Research Centre (JWMDRC) biobank was established in 2008 and is part of Newcastle University's Translational & Clinical Research Institute and supported by the NIHR Newcastle Biomedical Research Centre (BRC). We are a domestic and international banking hub and a proud EuroBioBank network member. A subset of the biobank's catalogue can be found via RD-Connect (https://samples.rd-connect.eu/).

Aims: The main aim of the biobank is to generate a large collection of samples from patients with genetic neuromuscular diseases (NMDs), alongside healthy control donors, to facilitate translational research. The JWMDRC biobank wishes to increase visibility and access to rare and NMD samples. In addition to access, researchers can request relevant clinical data pertaining to the samples. We also actively look to support clinical trials/ natural history studies and have supported 19 domestic/ international studies including: COS1 & COS2, MYO-SEQ, Seq-NMD & PhenoDM1.

Methods/ Materials: The JWMDRC biobank stores over 17,000 samples from ~9000 donors diagnosed with 123 different conditions (e.g. Duchenne muscular dystrophy, Becker muscular dystrophy, various limb-girdle muscular dystrophies among others). The types of biomaterials stored in the bank include, but are not limited to: plasma, serum, DNA, RNA, cell lines, urine, CSF and muscle tissue. Informed donor consent is a prerequisite for acceptance of samples into the laboratory.

Results: The bank has collaborated with >250 researchers in over 20 countries, including 21 different Biotech companies. In total, the bank has shipped 13,000+ vials aiding research into 77 different disorders. We ask that the biobank is acknowledged in any relevant publications, and, at the time of writing, JWMDRC biobank samples have contributed to 144 publications in 60 different journals. Sample application and submission forms for academia and industry are available upon request.

Conclusion: Future goals of the biobank are to roll-out a new database and coding system in 2023. Using patient records, update currently undiagnosed samples with a genetic diagnosis where applicable. Integration of the ACTIVLIM patient questionnaire into the collection process. As well as translation of patient information sheets and consent forms into Spanish for use in South America in the new LATIN-SEQ project.

‡OD07

Clinical, electrophysiological and radiologic profile of Hirayama disease patients from a tertiary care institute in India

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Background: Cervical flexion induced myelopathy (CFIM) or Hirayama disease (HD) is a lower motor neuron (LMN) disorder manifested as asymmetric weakness, atrophy and fasciculations of one or both distal upper limbs. There is a lack of knowledge of this entity among clinicians due to its rarity. This study aims to improve awareness to enable early diagnosis and treatment.

Aim: To review the clinical, electrophysiological and radiologic characteristics of HD patients.

Methods: All patients with clinically suspected HD between January 2018 and December 2022 were reviewed. Search criteria included insidious onset progressive pure motor LMN weakness of unilateral or bilateral distal upper limbs, electromyography (EMG) showing chronic denervation in C7-T1 myotomes, and dynamic cervical magnetic resonance imaging (MRI) findings suggesting HD like anterior dural displacement, asymmetric cord flattening, cord atrophy/ hyperintensity, epidural flow voids and enhancement of epidural crescent. Demographic details, history and examination findings from case records, and electrophysiology database were analysed using SPSS software version 26.0.

Results: Ninety-six patients met the diagnostic criteria for HD. 99% were males. Mean age of the patients was 21.3 years. Average age of symptom onset was 18 years. Median duration of symptoms prior to presentation was 3 (2-4) years. All had progressive distal upper limb weakness and wasting. Fourty-six (48%) had unilateral involvement, and the rest (52%) had asymmetric bilateral involvement. Family history was confirmed in one patient. Cold paralysis, oblique atrophy and polyminimyoclonus were seen in 70 (73%), 95 (99%) and 55 (57%) patients respectively. EMG findings of anterior horn cell involvement were noted in affected limb in all patients and unaffected limb in 17 (37%) patients. Dynamic MRI revealed anterior dural displacement in 91 (95%) patients, cord atrophy in 74 (77%), asymmetric flattening of cord in 84 (87.5%), intramedullary cord hyperintensity in 42 (44%) and epidural flow voids in 68 (71%) patients. Contrast MRI was performed in 33 (34.3%) patients of which 31 (94%) had epidural crescent enhancement. One underwent surgery.

Conclusion: HD is quite uncommon and self-limited, and it needs to be differentiated from its mimics to establish correct treatment and limit functional impairment. Dynamic MRI clinches the diagnosis.

OD08

Evolution of the research landscape for neuromuscular diseases based on activity at the John Walton Muscular Dystrophy Research Centre and lessons learned so far

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Background: The John Walton Muscular Dystrophy Research Centre (JWMDRC) is a highly specialised, multi-disciplinary neuromuscular centre focused on the development and application of genomic and translational medicine to improve the diagnosis, care and therapy opportunities for children and adults with neuromuscular diseases.

Over the past 10 years, the JWMDRC has participated in 80 clinical research studies (30 natural history studies, 50 interventional studies) and has led or is actively contributing to national and international commercial and academic clinical research projects including Jain COS 2, VISION DMD, BIND, Adult SMA REACH and conect4children.

Aims & Methods: While historically the focus of the research pipeline at the JWMDRC has been on clinical trials for Duchenne Muscular Dystrophy (DMD) and Spinal Muscular Atrophy (SMA), in recent years, the team has developed a wider portfolio of clinical trials in several neuromuscular diseases including Limb Girdle Muscular Dystrophies (LGMDs), Pompe Disease, Facioscapulohumeral Muscular Dystrophy (FSHD) and Myotonic Dystrophy (DM).

Moreover, the centre has developed expertise in setting up and delivering advanced therapy clinical trials in children and adults with a broad spectrum of neuromuscular diseases. Since the opening of the first gene therapy trial in SMA in 2018, the centre is currently participating in 5 gene therapy trials across LGMD, DMD, SMA and Pompe disease, with 4 additional trials already in the pipeline.

Results & Conclusion: As a result of expansion of the clinical research portfolio, the knowledge and experience of the team has increased and this can be shared with others. The JWMDRC team has established a paediatric and adult gene therapy Safety Taskforce to support and advise on the delivery of gene therapy trials and management of potential adverse events. We are also active members of the DMD Hub and NA-ATTC, developing national guidance on costing and set-up of gene therapy trials in the UK.

We are committed to sharing this expertise nationally in order to increase trial readiness and the ability of patients to access these cutting-edge therapies, in clinical trials as well as the clinical setting.

OD09

IBM and PM-Mito revisited: are we moving towards IBM-spectrum disease (IBM-SD)?

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Background: Polymyositis with mitochondrial pathology (PM-Mito) has been described as a distinct form of idiopathic inflammatory myopathy (IIM) with marked mitochondrial pathology. Unlike in Inclusion body myositis (IBM), rimmed vacuoles are not observed in PM-Mito. Nevertheless, studies have described possible links between these two diseases. Up to 50% of patients initially diagnosed as PM-Mito may progress to IBM. Clinically, some response to immunosuppressive therapy has been described in PM-Mito as opposed to therapy-refractoriness in sIBM. However, it remains unclear to date if PM-Mito and IBM should be considered as pathophysiologically related diseases.

Methods: In this study, skeletal muscle biopsy samples as well as clinical and laboratory data from PM-Mito and IBM patients were analyzed and compared to non-diseased controls (NDC). Biopsy samples were studied by histopathology, immunohistochemistry, and quantitative PCR. Primary outcomes included cell counts for immunohistochemistry, and gene expression (fold-change values compared to NDCs) for quantitative PCR.

Results: Twenty-five skeletal muscle biopsy samples of patients with PM-Mito and IBM were included and compared to five biopsy samples from non-diseased controls. PM-Mito and IBM qualitatively harbored a similar molecular signature and shared important histopathological features. The expression of interferon-induced *GBP6* and T-cell function-related *KLRG1* distinguished IBM from

PM-Mito biopsies with IBM patients showing significantly higher expression of *GBP6* and *KLRG1*. Skeletal muscle biopsies from IBM patients showed significantly more GBP6$^+$ cells and KLRG1$^+$ lymphocytes in comparison to biopsies from PM-Mito patients. Clinically, PM-Mito patients presented with a spectrum of muscle-related symptoms including myalgia, proximal paraparesis, proximal tetraparesis and incomplete IBM-like patterns. 13 out of 14 (93%) PM-Mito patients for whom clinical follow-up was available later developed clinically defined IBM. Notably, two follow-up biopsies obtained 5 and 7 years after the first ones were available in this cohort, both showing histopathological progress to net IBM including *GBP6* and *KLRG1* upregulation.

Conclusion: Based on these findings, we propose to include PM-Mito in the spectrum of IBM (IBM-spectrum disease, IBM-SD) as a possible early form of this disease. The identification of an early and perhaps treatable form of IBM could have a significant impact on the management of IBM patients in the future.

‡OD10

MTM1 overexpression prevents and reverts BIN1-related centronuclear myopathy

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Background: Centronuclear and myotubular myopathies (CNM) are rare and severe genetic diseases associated with muscle weakness and atrophy as well as intracellular disorganization of myofibers. The main mutated proteins control lipid and membrane dynamics and are the lipid phosphatase myotubularin (MTM1), the membrane remodeling proteins amphiphysin 2 (BIN1), and dynamin 2 (DNM2). There is no available therapy.

Aims: Here, we aimed to test a novel therapeutic strategy for BIN1- and DNM2-CNM.

Methods/Materials: We evaluated adeno-associated virus (AAV)-mediated MTM1 overexpression in faithful BIN1- and DNM2-CNM mouse models.

Results: Early systemic MTM1 overexpression prevented the development of the CNM pathology in Bin1mck-/- mice, while late intramuscular MTM1 expression partially reverted the established phenotypes after only 4 weeks of treatment. However, AAV-MTM1 injection did not change the DNM2-CNM mouse phenotypes. We investigated the mechanism of the rescue of the myopathy in BIN1-CNM and found that the lipid phosphatase activity of MTM1 was essential for the rescue of muscle atrophy and myofiber hypotrophy but dispensable for the rescue of myofiber disorganization including organelle mis-position and T-tubule defects. Furthermore, the improvement of T-tubule organization correlated with normalization of key regulators of T-tubule morphogenesis, dysferlin and caveolin.

Conclusion: Overall, these data support the inclusion of BIN1-CNM patients in an AAV-MTM1 clinical trial.

OD11

Overexpression of muscle glycogen synthase 1 *(GYS1)* leads to a polyglucosan myopathy with decreased muscle strength and variable force decline following eccentric contraction in a transgenic mouse model

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Background: Cellular polyglucosan accumulation is a feature of several glycogen storage diseases of humans and other mammals. The GSL30 mouse expresses a mutated rabbit glycogen synthase 1 (GS) transgene leading to constitutively activated GS enzyme and accumulation of excess glycogen and polyglucosan in skeletal muscle, similar to that seen naturally in a highly prevalent equine myopathy with intermittent rhabdomyolysis.

Aims: Investigate the effect of excessive glycogen/polyglucosan accumulation on the histological phenotype of GSL30 mice and on muscle function including force generation and resistance to eccentric contraction-induced damage.

Methods/Materials: Gastrocnemius muscles from GSL30 and WT mice were studied from 6 weeks to 18 months of age. Cryosections were stained with haematoxylin & eosin, (amylase-) periodic acid Schiff and IgG and scored for the presence of internalised nuclei, amylase-resistant polyglucosan and glycogen accumulation. An *in situ* muscle function and eccentric contraction protocol was performed in anaesthetised 6-month-old GSL30 and WT mice by stimulating the sciatic nerve to induce tibialis anterior (TA) contraction. Cryosections from the unstimulated and stimulated TA were stained with H&E and amylase-PAS.

Results: The percentage of myofibres containing amylase-resistant polyglucosan was higher in GSL30 compared to WT mice from 6 weeks to 18 months of age ($p<0.05$). GSL30 mice at 8-18 months of age had greater numbers of myofibres containing internalised nuclei vs WT controls ($p<0.0001$). IgG-positive myofibres were detected in GSL30 mice from 6 to 18 months and muscle inflammatory cellular infiltratation was observed in 12-18 month old GSL30 mice. GSL30 mice had lower specific muscle force than WT controls ($p<0.05$). With eccentric contraction, 3/6 GSL30 mice experienced a total loss of force, 1/6 GSL30 showed a 19.4% reduction and 2/6 GSL30 mice showed no loss of force. However, there was no histological evidence of rhabdomyolysis following eccentric contraction and differences in polyglucosan accumulation between the GSL30 mice with varied force decline responses were not identified.

Conclusion: Over-expression of glycogen synthase in the GSL30 transgenic mouse leads to a skeletal myopathy with evidence of low level muscle damage and regeneration. Mice have reduced muscle specific force production and a variable deleterious response to eccentric contraction via an as yet, unknown mechanism.

OD12

Novel pathogenic, recessive *UNC45B* variants in a UK patient with congenital myopathy with central nuclei and large cores

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Background: Bi-allelic variants in *UNC45B,* encoding for uncoordinated mutant number-45 myosin chaperone B, were recently identified in 12 patients with congenital myopathy (CM) and eccentric cores.

Aims: we describe two novel *UNC45B* gene variants in a young UK patient with CM.

Methods/Materials: Gene agnostic whole genome sequencing (WGS) was performed as a trio through the SOLVE-RD research project. WGS data was analysed using the GPAP platform.

Results: The patient is a now 18-year-old male born to non-consanguineous parents. Birth history and early development was uneventful. At age 3 years, he developed difficulties in walking long distance and running, fatigue and leg pain. Over the years, he became unable to get up from floor independently and had increasing falls. He experienced dislocations of shoulders, thumbs and knees. At age 13 years he had mild facial weakness, normal eye movements, subgravity axial strength and symmetric proximal upper and lower limb weakness (MRC power grade 3-4/5). He had mild ankles tightness, but hypermobility of other joints. Cardiac and respiratory function remains normal to date. CK is normal, MRI at age 11 years evidenced of mild fatty involvement in particular of semitendinosus, sartorious, tibialis anterior and peroneal muscles. Central nuclei, large, ill-defined cores and focal myofibrillar disorganization were noted on muscle pathology. WGS revealed two novel, compound heterozygous

biallelic missense variants in the *UNC45B* gene, c.85T>G p.(Tyr29Asp) and c.2261G>T,p.(Arg754Leu). Variants affect highly conserved residues and are predicted to damage protein function.

Conclusion: We report the first *UNC45B* variants in a UK patient with a slowly progressive CM, central nuclei and cores. Arg754 is the same residue involved in a recurrent variant described in 5 independent families and predicted to affect splicing which we aim to confirm following cDNA analysis. The MRI and pathological features of our patient strongly resemble those observed in previously reported patients with UNC45B-CM and further supports the clinical and pathological features of this novel CM. Diagnostic testing for this patient done in 2018 did not include *UNC45B* gene, and this finding highlights the importance of updating diagnostic panels and investigating newly identified disease genes in genetically undiagnosed patients.

OD13

MTM & CNM International Patient Registry: A tool to accelerate the pace of research

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Background: The MTM & CNM International Patient Registry data is patient-entered, and at a later stage, clinician reported for patients and female carriers with Myotubular Myopathy (MTM) and Centronuclear Myopathy (CNM).

Aims: To showcase the data for these rare genetic neuromuscular disorders for which limited real-world data exist. We aim to describe their demographic and clinical characteristics.

Methods/Materials: We performed a data extraction and a cross-sectional analysis from the Myotubular and Centronuclear Myopathy Patient Registry for all the patients and female carriers with MTM and CNM. Data extraction was carried out on 5th of January 2023.

Results: As of the data extraction date, the Registry contained data on 441 participants (276 male & 165 female). Out of which 56 were reported as deceased (53 male and 3 were female) and 385 as living (223 male & 162 female). In our dataset, we have 54 countries represented, with three of our largest cohorts being from the United States (121), UK & Ireland (109) and Germany (37). Out of all participants, 202 patients were genetically confirmed to have the disease and 120 of those patients have a diagnosis of X-Linked Myotubular Myopathy (XLMTM). In addition, to participants with mutations in the MTM1 gene, the Registry also collects data on those with mutations in DNM2, RYR1, BIN1 and TTN. We also capture outcome measures on motor and respiratory function and use of assistive devices to build on our knowledge natural history and disease burden.

Conclusion: The Registry provides a unique opportunity to examine real-world data from patients living with MTM & CNM. This International Registry will make the recruitment of MTM and CNM patients for clinical trials and studies easier by acting as a centralised source of data to researchers, including details of each patient's particular genetic mutation and other relevant information about their condition. Without a patient registry to gather details from all patients worldwide in one place, finding enough patients for a meaningful trial can take years, delaying the testing of potential therapies.

OD14

A Hereditary Spastic Paraplegia Type 2 Case

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Background: There can be many causes of gait disturbance in childhood. Genetic analyses can guide diagnosis as clinical findings overlap.

Aim: We aimed to present our case who was diagnosed with gene panels.

Case: An 11 year old male was admitted to our pediatric neurology clinic with gait disturbance starting after the age of 3 years. He was born to non-consanguineous parents at term. No complications were seen during prenatal and postnatal period. He gained motor milestones on time. Proximal lower limb weakness with muscle atrophy and spasticity was noted on examination. Serum CK levels were within normal limits. Nerve conduction studies and electromyography, craniospinal MRI were normal. Local gene panel revealed *PLP1* c.636G>A p.Trp212Ter, likely pathogenic variant.

Conclusion: The hereditary spastic paraplegias are a group of clinically/genetically diverse disorders. They are characterized by progressive and severe, lower extremity spasticity. *PLP1* encodes proteolipid protein 1 (PLP) expressed in oligodendrocytes and accounts for 17% of the total myelin protein. *PLP1* mutations lead to the arrest of myelination. The *PLP1* mutations cause X-linked hereditary spastic paraplegia type 2.

Acknowledgement: We would like to thank our patients who agreed to participate in the project and the ICGNMD project team for their contributions

‡OD15

King Denborough Syndrome also links to the autosomal recessive *STAC3* c.851G>C pathogenic variant in a South African paediatric neuromuscular disease cohort: is it still an entity?

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Background: King Denborough syndrome (KDS) is reported as a rare autosomal dominant disorder caused by *RYR1* pathogenic variant. The classical phenotype consists of a congenital myopathy, various skeletal abnormalities, dysmorphic features with characteristic facial appearance and a susceptibility for malignant hyperthermia. Here we describe 28 cases with the KDS phenotype. An autosomal recessive pattern with the known *STAC3* pathogenic variant, c.851G>C, was identified. The enzyme STAC3 is involved in skeletal muscle excitation-contraction coupling. Furthermore, it interacts with RYR1 and DHPRα1, and is involved with surface expression of $Ca_V1.1$. Low levels of STAC3 result in reservation of intracellular Ca^{2+}. A severe phenotype is associated with *STAC3* pathogenic variants and has been clearly described in different cohorts but has not been previously labelled as KDS.

Aims: To assess the phenotypical and genotypical profile of KDS in a cohort of South African paediatric patients with congenital myopathies.

Methods/Materials: Recruitment of neuromuscular disease patients with congenital myopathy (n=58) took place at the Steve Biko Academic Hospital in PTA, South Africa. Thorough clinical evaluations were performed on the cohort, followed by whole exome sequencing. Sanger sequencing was performed on all the probands and family members for validation and segregation analysis.

Results: Through clinical evaluations, 28 of the 58 patients were identified with the KDS phenotype.

The most prominent features were myopathy (n=28), dysmorphic features (n=17), talipes equinovarus (n=19), and cleft palate (n=19). Whole exome sequencing revealed a *STAC3* homozygous c.851G>C pathogenic variant in 25 patients and a compound heterozygous pathogenic variant with a novel heterozygous deletion, c.834_836del in three patients. Sanger sequencing confirmed an autosomal recessive pattern of inheritance in the probands and carrier status of the relatives. None of them had pathogenic variants in the *RYR1* gene.

Conclusion: This is, from available knowledge, the largest reported KDS cohort. Furthermore, these cases did not present with a *RYR1* pathogenic variant associated with KDS, but with an autosomal recessive *STAC3* pathogenic variant. These results clearly broaden the genotype of KDS, and illustrates that a syndromic diagnosis is not the finite step in the era of precise genetic diagnosis.

OD16

The clinical, genetic, and biochemical spectrum of MADD in a Southern African cohort: an ICGNMD sub-study

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Background: Multiple acyl-CoA dehydrogenase deficiency (MADD) is an autosomal recessive disorder of the riboflavin/FADH metabolism caused by >436 mutations in eight genes, most of which occur in *ETFDH*. Recently, it has been proposed that this treatable disease may have a uniquely high prevalence in the European ancestry population of Southern Africa (SA), thereby initiating this study in all SA populations groups.

Aims: As part of the ICGNMD study, we recruited a cohort of patients diagnosed with MADD from three academic medical centres in SA over a three-year period. The aim was to extensively profile the clinical, biochemical/metabolic, and genomic characteristics of MADD to improve early diagnosis and treatment.

Methods/Materials: Following the recruitment of 12 unrelated families (ten of European- and two of mixed ancestry), a thorough clinical evaluation and WES was performed on each proband, followed by Sanger validation and segregation analysis. Extensive metabolic profiling was conducted before and after treatment where possible and the allele frequencies of the identified mutations were determined in the four largest population groups of SA.

Results: Clinically heterogeneous presentations were observed in the probands, with the involvement of four *ETFDH* mutations in the cohort. Of these presentations, the most severe and fatal was associated with the homozygous c.1067G>A genotype (Type I). This, together with three milder compound heterozygous genotypes (c.1067G>A;c.1448C>T,

c.740G>T; c.1448C>T, and c.287dupA(novel); c.1448C>T; Type III), presented before the age of 5 years. By contrast, the homozygous c.1448C>T genotype (Type III) presented later in life with severe liver failure in one untreated case. Each affected individual further displayed urinary and plasma metabolic markers characteristic of MADD which, apart from the homozygous c.1067G>A genotype, normalised following treatment with riboflavin and L-carnitine. Of the identified mutations, all displayed allele frequencies of <0.01, with c.1067G>A and c.1448C>T being the most frequent.

Conclusion: This study provides the aetiology, and the first extensive clinical and metabolic profile, of MADD in the diverse and understudied SA population. Although we recognise a potential referral bias within population groups, the data is in line with the suspicion that MADD is most prevalent in the European ancestry population. Altogether, this study provides data to better implement early screening, genetic counselling, and patient-specific treatment of MADD in SA patients.

OD17

Unusual systemic features in a cohort of Myotonic Dystrophy

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Background: Myotonic dystrophy is a common muscular dystrophy caused by CTG repeat expansions in the 3' untranslated region (UTR) of the DMPK gene on Chromosome 19. Apart from muscle, the disease can involve the eye, heart, brain and endocrine system.

Aim: We describe a Myotonic dystrophy Type 1 (DM1) cohort in a tertiary care hospital in North India.

Methods: All clinically suspected participants of Myotonic dystrophy were recruited prospectively to the MRC-funded International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) cohort. Inclusion criteria included presence of myotonia and/or muscle weakness. All participants gave informed consent and underwent deep phenotyping, nerve conduction studies (NCS), electromyography (EMG) and blood creatinine kinase measurement (CK). Short PCR and triplet primed PCR (TP-PCR) tests were used to assess 3'UTR CTG repeat size.

Results: We screened 24 patients with myotonia and clinically diagnosed DM1 in 19 probands and 7 affected relatives in the ICGNMD cohort. Genetic testing confirmed diagnosis in 13 probands and 4 affected relatives. Results are pending in 6 probands and 3 affected relatives. Male: Female ratio was 11:2 Median age of onset was 21.5 years and median age of diagnosis was 30 years. Family history was present in 4 probands and consanguinity was absent in all. Clinical features included grip myotonia (73%), tongue myotonia (15.38%), percussion myotonia (57.38%), eyelid myotonia (15.38%), facial weakness (76.9%), neck weakness (19.23%), frontal baldness (42.3%), dysarthria (19.23%), distal UL weakness (65.38%) and distal LL weakness (57.69%). Ophthalmology evaluation was done in 5 patients with 4 having cataracts. Eight were evaluated for cardiac function, 3 patients had cardiac conduction abnormality. Two patients had cognitive impairment. Hyperparathyroidism was seen in two patients. Two patients had thyroid nodules, one had papillary carcinoma of thyroid. Gynecomastia, ureteric stones and hypomobile bowel were seen in single cases. All patients were ambulant. Mildly elevated CK (median 243 IU/L) and myopathic changes in EMG were present in all. Muscle biopsy was done in 2 probands.

Conclusion: We describe a cohort of DM1 from India with unusual systemic features like gynecomastia, hyperparathyroidism and thyroid malignancy.

We are currently performing further genetic and clinical characterization of this cohort to understand fully the individual variation observed.

OD18

GNE Myopathy: A case series from India

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Background: GNE Myopathy (myopathy of bifunctional UDP-N-acetylglucosamine 2-epimerase/ N-acetylmannosamine kinase) is a rare genetic neuromuscular disorder caused by mutation in GNE gene on chromosome 9. It usually presents with distal lower limb weakness (foot drop). Relative sparing of quadriceps muscle is a characteristic feature. Respiratory and cardiac involvement are rare. Previous reports of GNE myopathy from India has been from Southern and Western India.

Aim: We aim to describe a GNE myopathy cohort in a tertiary care hospital in North India.

Methods: All clinically suspected participants of GNE myopathy were recruited upon presentation at clinic to the MRC-funded International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD). Clinical criteria included distal predominant myopathy with relative sparing of quadriceps. All participants gave informed consent. Participants underwent deep phenotyping, nerve conduction studies (NCS), electromyography (EMG) and blood creatinine kinase measurement (CK). Genetic testing involved whole exome sequencing of probands and sanger confirmation of affected relatives.

Results: We suspected GNE myopathy in 39 Probands and 7 affected relatives in the ICGNMD cohort. Genetic testing confirmed diagnosis in 13 probands, with results pending in 26 probands. Male: Female ratio was 12:11. Median age of onset was 23 years and median age of diagnosis was 26.5 years. Family history was present in 10 probands and consanguinity was present in 4 probands. Clinical features included distal lower limb (LL) weakness (89.1%), distal upper limb (UL) weakness (63%), proximal LL weakness (97.8 %) and proximal UL weakness (54.3%). First presenting symptom was distal LL weakness in 41.3% and proximal LL weakness in 54.3%. Most participants were ambulant but five were wheelchair dependent. Mildly elevated CK (median 429U/L) and myopathic changes upon EMG were present in all participants. Muscle biopsy was done in 18 patients and rimmed vacuoles were seen in 6 patients.

Conclusion: We describe a series of GNE myopathy patients in the ICGNMD cohort of inherited neuromuscular disorders.

OD19

Spectrum of Limb girdle muscular dystrophies in a cohort of inherited myopathies with limb girdle weakness

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Background: Limb Girdle Muscular Dystrophy (LGMD) is a group of inherited muscular dystrophies which predominantly affects proximal muscles and achieved independent walking at some point with elevated creatine kinase, dystrophic changes in muscle histology and degenerative changes in muscle imaging. Dystrophinopathies like Becker muscular dystrophy (BMD) and manifesting carriers of the same are common differential diagnosis.

Aims: To identify the spectrum of LGMD differentiated using single gene tests and whole exome sequencing (WES) in a genetically uncharacterised cohort with clinical diagnosis of LGMD.

Methods/materials: All participants with clinically-suspected LGMD were recruited to the MRC-funded International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) cohort. All participants gave informed consent. All patients underwent deep phenotyping, nerve conduction studies (NCS), electromyography (EMG), Creatine Kinase (CK) measurement. Genetic testing included MLPA for dystrophin gene deletions and duplications, singleton whole exome sequencing of probands and sanger confirmation of affected relatives. Virtual diagnostic panels were applied to exome data and ACMG criteria was applied to classify identified variants.

Results: 249 probands and 33 affected relatives were included in the analysis. DMD-MLPA was performed in 78 probands and solved as BMD in 38 probands (49%). DMD-MLPA is awaited in 3 probands. Three probands and one relative had an overlapping presentation and tested positive for SMA. Undiagnosed LGMD cases (205 probands) were planned for singleton WES. Results of WES are available for 84 probands, of which 37 probands (44%) and 7 affected relatives are solved. 47 probands and 8 affected relatives were unsolved with no tiered pathogenic or likely pathogenic variants. WES results are awaited in 121 probands. Among the solved cases, 15 probands had LGMD-R2(*DYSF*), 10 probands and 2 affected relatives had LGMD-R1 *(CAPN3),* 8 probands and 3 affected relatives had Sarcoglycanopathy (LGMD R5 (*SGCG)*-4 probands, LGMD R3 (*SGCA)*--2 probands, LGMD R6(*SGCD)*--1, LGMD R4 (*SGCB)*--1), 1 proband and 2 affected relatives had Bethlem myopathy *(COL6A2).* Other genes among solved proband cases were *TRIM32(1), ANO5(1), and GMPPB (1).*

Conclusion: We describe the genetic characterisation of a clinically suspected cohort of LGMD in India.

OD20

Clinical and Demographic Profile of Patients with Distal Myopathy Presenting to Tertiary Care Hospital

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Background: The distal myopathies are characterized by disproportionate or selective weakness and degeneration of distal limb muscles.They commonly manifest as weakness that is limited to foot and toe muscles even in advanced stages of the disease, with mild proximal leg, distal arm involvement. However clinical presentation can be quite variable.

Aims: To study the spectrum of clinical manifestations of patients with distal myopathy

Methods/Materials: Consecutive patients attending the OP/IP services of dept neurology from NIMS were studied. Any patients with a diagnosis of distal myopathy were included. The demographics and clinical details including family history and pedigree of these patients were studied. Investigative workup including Electrophysiology, genetic evaluation and muscle biopsy were done and were analysed.

Results: Sample included 11 patients with clinical diagnosis of distal myopathy. The age range was 20 to 36 years. Duration of disease ranged from 3-5 years. Out of 11, 2 patients had history of consan-

guineous parentage. Presenting complaints were distal lower limb onset weakness in most patients with foot drop, progressive difficulty in getting up from squatting, lifting weights in ADL. 5 patients had significant proximal weakness in upper limbs. Muscle atrophy was noted in lower limbs in all 11 patients and in upper limbs among 2 patients. Deep tendon reflexes were diminished in 6 patients. Ankle Contractures were seen in 1 patient. None of patients were wheel chair dependant**.** Mean *MRC sum score* was 45. CPK levels range from 200 to 742 U/L. ENMG - suggestive of primary muscle disease in 9 patients. Muscle Biopsy was done in 4 patients and suggestive of myopathy with rimmed vacuoles. Genetic tests –showed GNE mutation (Nonaka myopathy) in 2 patients and DYSF mutation(miyoshi myopathy) in 1 patient. All patients were conservatively managed.

Conclusion: This study provides additional insight into clinical spectrum of adult onset distal myopathy patients.

Diagnostics and Cross-cutting Therapies

DCC01

Optical Genome Mapping for the Molecular Diagnosis of Facioscapulohumeral Muscular Dystrophy: advancement and challenges

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is the second most common muscular dystrophy in adults. FSHD is associated with a contraction of the D4Z4 microsatellite repeat on chromosome 4. In most diagnostic centers, FSHD1 diagnosis is Southern blot (SB)-based, however SB protocol in FSHD is technically challenging, is time consuming and requires of staff trained in results interpretation. Relevant to this, currently only a few laboratories worldwide offer a full FSHD genotyping work-up and access to FSHD genetic diagnosis of populations in low and middle-income countries (LMIC) is limited. Optical genome mapping (OGM) is a new promising technology to assess structural variants in the genome.

Aims: We aimed to investigate the use of OGM as diagnostic tool in testing FSHD cases from UK and India. We also aimed to compare the results with traditional techniques such as linear gel (LGE) and Pulsed-field gel electrophoresis (PFGE) Southern blotting.

Methods/materials: Samples were processed with the Saphyr Genome Imaging Instrument (1-color) established at the UCL Queen Square Institute of Neurology following manufacturer's guidelines and data were analysed using the custom EnFocus FSHD analysis. 31 probands with suspected or confirmed diagnosis of FSHD were analysed.

Results: OGM was able to confirm the diagnosis of FSHD1 in 22 out of 31 cases and D4Z4 sizing highly correlates with SB ($p<0.001$). Two cases were iden-

tified as mosaic on the permissive 4qA chromosome by OGM and one case was found with an homozygous 10U D4Z4 contraction. The latter was not identified by standard LGE. Eight cases were found to be negative with both OGM and SB. In one of these cases, OGM showed the presence of an 18U fragment and was then diagnosed as FSHD2.

Conclusion: OGM is a promising new technology able to unravel structural variants in the genome and seems a valid tool to diagnose FSHD1. Further data are ongoing to assess OGM efficacy in assessing complex rearrangements.

DCC02

Virtual Panel versus Exome-first analysis in Neuromuscular diseases: Outcome of a Single Centre within ICGNMD Consortium

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Background: Whole exome sequencing has proved to be an effective genetic diagnostic method for rare diseases. On average, approximately 20,000 rare variants can be called from a singleton WES data. Analysis of such large list of variants can be time consuming and it is still difficult to identify the pathogenicity and relevance of each variant. Applying a virtual panel analysis of WES results, driven by patient phenotypic data, could simplify the analysis and save a lot of time and effort to identify the causative variants.

Aims: In this study, we aim to assess the diagnostic yield of phenotype-driven virtual panel analysis compared to that of undirected analysis of WES data of a clinically well-characterized cohort of patients with neuromuscular phenotypes.

Methods/Materials: WES was performed in 90 patients with various phenotypes of neuromuscular disorders recruited from a single centre; Steve Biko Academic Hospital in PTA, South Africa. Patients were categorized according to their clinical phenotype. A pre-analysis MDT meetings were conducted to review the clinical data and suggest virtual panels to be applied using the mainstream ICGNMD bioinformatic pipelines. Variant list files were also analysed without prior application of virtual panels. High quality variants were filtered based on allele frequency and predicted impact on protein. Variant pathogenicity was classified using ACMG and UK ACGS guidelines.

Results: Potential causative variants were identified in 61/90 patients (68%); 55 patients were considered as solved with pathological variants found in 17 genes, including 25 patients with *STAC3* variants and 9 patients with *DMD* variants, and 6 patients were possibly solved. Variants in 59 patients were identified in concordance using both virtual panel and exome-first analysis methods. Variants in *PLOD1* gene that were not prioritized in exome-first analysis, were identified using virtual panel analysis. On the other side, only one patient's diagnosis, with variants in the *ADPRS* gene, was identified by an exome-first approach alone. Moreover, in 2 patients, variants in novel candidate genes were prioritized by exome-first with no other variants identified using panels.

Conclusion: Virtual panel analysis is an effective method in analysing WES data of neuromuscular pa-

tients prior to a wider, whole exome analysis being applied as a later step in unsolved cases.

DCC03

The mutational profile and yield of next generation sequencing in a South African cohort with inherited neuromuscular diseases

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Background: Genetic diagnostics for inherited neuromuscular diseases have been neglected in South Africa. The only tests available for genetic neuropathies (GN) include *PMP-22* copy number screen, and for spastic ataxias the spinocerebellar ataxia panel and Frataxin expansion screen, and none for hereditary spastic paraplegia (HSP).

Aim: We report the preliminary results of GN, HSP and spastic ataxias in our self-categorized subpopulations who remain without a genetic diagnosis.

Method: 27 probands underwent whole exome sequencing (WES) as part of the ICGNMD study. 30 probands underwent whole genome sequencing (WGS) as part of the CReATe Consortium Phenotype-Genotype-Biomarker study (n=5) or by the University of Cape Town Neurology Research Group. Appraisal of coding/splice region variants using virtual gene panels was guided by the Clinical Genome Resource's Sequence Variant Interpretation guidelines. Frequency filtering was aided by population allele frequencies derived from an internal dataset of African-ancestry control genomes. Structural variant analysis in WGS data has commenced using a combination of computational prediction by ClinSV and manual visualization.

Results: Of the 29 with GN, mainly Charcot-Marie-Tooth-2, 59% had African-genetic ancestry. Among GNs, **38% were solved** with genes harbouring pathogenic (P) or likely pathogenic (LP) variants including heterozygous *MFN2* (n=3), *MPZ, ATP1A1, MORC2*, and *WFS1* (optic neuropathy), and compound heterozygous P/LP variants in *GAN*, and homozygous variants in *SH3TC2, ADPRHL2* (motor neuropathy), and *SLC12A6* (complex phenotype).

Of the 20 with HSP, 83% had African-genetic ancestry. Among HSPs, **61% were solved** harbouring P/LP variants including *SPG11* (n=3; 2 homozygous and compound heterozygous) and compound heterozygous in *CYP7B1*. Heterozygous variants were found in *ALDH18A1* (n=2), *SPG3A/ATL1, KIF1A*, *ATP1A1,* and *PSEN1* (progressive aphasia and HSP). Strong VUS variants were found in *PCYT2* and *OPTN*. Preliminary ClinSV analysis of WGS data did not reveal any structural variants in HSP genes.

Of 7 ataxia cases, all but one had African-genetic ancestry and **42% were solved** with homozygous P/LP variants in *SETX*, and *ATM*. One Indian-ancestry case was solved with *RFT1* (consanguineous parents).

Conclusions: In this small cohort using virtual panels on WES/WGS data, we resolved more HSP than GNs. The mutational spectrum appears to differ from European cohorts.

‡DCC04

Interrogation of 5`UTR and splicing variants in ICGNMD Neuromuscular patients

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Background: Pinpointing disease-causing genomic variation informs diagnosis, treatment, and management for a wide range of rare disorders. Whole-exome sequencing (WES) is an effective tool to diagnose rare neuromuscular disorders. However, the diagnostic yield of WES is around 35%–60% and a substantial proportion of diagnostic cases remains unsolved.

Aims: To assess the application of multiple in-silico tools for identification of potentially deleterious non-coding variants including: 5 untranslated regions (5 UTR) and splice altering variants in a cohort of rare neuromuscular disorder patients recruited through ICGNMD centres.

Methods/materials: WES data from 630 ICGNMD probands were analysed in the current pipeline. For prioritisation of variants, rare variants with good quality reads (DP > 10, GQ > 20 & MAF < 0.01 according to gnomAD) were selected.

For identification of variants in the 5′UTR that create or disrupt upstream open reading frames (ORFs), we applied UTR annotator tool as a plugin to Ensembl Variant Effect Predictor (VEP_ v.103). We kept only variants that form new overlapping ORFs with start sites that are Strong or Moderate matches to the Kozak consensus sequence, or that are upstream ORFs with documented evidence of translation.

Potential splice altering variants were identified using 2 in-silico tools; SpliceAI and SQUIRLS. For splice altering prediction, we applied highly stringent criteria. We prioritised variants with high splice prediction scores by both tools (Splice AI Maximum DS > 0.5 and SQUIRLS classification as pathogenic) that does not affect canonical splice sites.

For both types of analysis, only variants in known disease-causing genes and lies in Neuromuscular panel according to Genomics England Panelapp were retained for further interpretation.

Results: The preliminary analysis identified 824 potentially deleterious 5`UTR variants. Of which 249 variants were frameshift variants in upstream ORFs. Thirty-seven 5`UTR variants were detected in known Neuromuscular disorders causing genes. None of the identified variants were reported pathogenic in Clinvar. Additionally, by applying our pipeline, 2979 potential splice altering variants were detected. These variants displayed an array of predicted molecular consequences and were present in genes across disease inheritance types. Only 8 variants were previously reported pathogenic in Clinvar. Novel candidate 5`UTR and splice altering variants in known neuromuscular genes are currently under interpretation.

Conclusion: Application of additional in-silico tools to WES data for prioritization of non-coding variants is recommended to try to improve the diagnostic yield in rare disorders. However, prioritization of variants and the validation of their pathogenicity can be challenging. Further analyses are ongoing to prioritise 5`UTR and splice altering variants in our cohort.

DCC05

Copy Number Variation Detection Pipeline for the ICGMND cohort

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Background: A genetics first approach for rare disease diagnostics can utilise whole-exome-sequencing (WES) for SNP/ Indel calling as a first step. The diagnostic yield is rarely above 60%, so the addition of Copy Number Variation (CNV) detection can improve diagnostic yields and identify novel disease variants. However, we know from many large-scale genomic projects that the majority of CNVs are benign, and that the detection of CNVs in WES samples is more error prone when compared to WGS samples. Given these challenges, we have developed a novel pipeline which uses multiple detection algorithms and annotation steps to provide high-quality data for our clinical fellows to scrutinize the detected CNVs.

Aims: The ICGNMD aims to provide genetic diagnoses for individuals recruited to their WES cohort. To improve the diagnostic yield, we have been developing a novel CNV detection pipeline. We aim to identify any disease causing CNVs through a combined coherent bioinformatic and clinical phenotype analysis.

Methods/ Materials: As recommended by systematic reviews of WES based CNV detection tools, we used three tools, each based on read-depth based algorithms, which have been demonstrated to perform best. To decrease false positives, we have annotated our detected CNVs using the 2019 ACMG CNV guidelines, millions of CNVs observed in healthy populations and applied VEP annotations to our pipeline. Genes inside CNVs have been annotated by clinVar, OMIM, mitocarta and patient specific panels selected by clinical fellows. To further increase our confidence in detected CNVs, we have implemented several data science techniques to visualise the candidate CNVs. The pipeline is written in a combination of *bash* and *R*.

Results: Over 350 ICGNMD samples have been processed by this pipeline, and over 57,000 CNVs were detected, of which 1420 CNVs coincided with coding regions of genes within disease panels which were decided by clinical fellows. High-quality results files are ready for clinical interpretation by fellows.

Conclusions: Large scale CNV pipelines are uncommonly used in rare disease diagnostics and we have developed a robust CNV detection workflow with multiple annotation steps to provide more confidence to our clinical fellows.

‡DCC06

Diagnostic yield and genetic insights from whole exome sequencing in a cohort of congenital myopathy/muscular dystrophy patients from the International Centre for Genomic Medicine in Neuromuscular diseases (ICGNMD)

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Background: Access to next generation sequencing (NGS) and genetic characterisation of populations in low and middle-income countries (LMIC) are limited.

Aims: To assess the diagnostic yield of whole exome sequencing (WES) in a cohort of genetically uncharacterised patients with clinical diagnoses of congenital myopathies (CM) and congenital muscular dystrophies (CMD) from LMIC.

Methods/Materials: Probands with genetically uncharacterised CM/CMD from South Africa, Turkey, Brazil and India underwent singleton WES with corresponding virtual diagnostic panels applied (122 CM genes, 59 CMD genes). ACMG criteria was applied to classify identified variants. Cases were considered solved where (likely) pathogenic variant(s) consistent with the phenotype were identified, likely solved where suitable variant(s) of uncertain significance were identified and unsolved where no suitable variants were found. Segregation analysis was not performed.

Results: 107 probands were analysed (72 South African, 22 Turkish, 10 Brazilian, 3 Indian). After analysis, 57 (53%) patients were solved (16 genes), 19 (18%) possibly solved (18 genes), and 31 (29%) unsolved. Diagnostic yield (solved cases) in different populations varied between 0-60%. *STAC3* (28), *RYR1* (8) and *COL6A2/3* (5) were the most common genes in solved patients. We identified novel variants in ultra-rare genes including *PIEZO2*, *MSTO1* and *CHCHD10*. Three additional likely solved patients carry VUS in *RYR1* (2 patients) and *COL6A2* (1 patient).

Conclusion: The diagnostic yield from singleton WES of CM and CMD patients in LMIC is high (50%) and segregation analysis in likely solved patients may increase this further. These results can improve our understanding of the genetic architecture of traditionally understudied populations and help to further expand genotypic and phenotypic knowledge of these rare conditions. The lack of solved patients with *TTN* or *COL6A1* gene related CM/CMD, two of the most common conditions in other countries, including the UK, is noteworthy.

Journal of Neuromuscular Diseases 10 (2023) S97–S104
IOS Press

Author Index

Abati E. PN01, S36
Abegaonkar G. OD12, S83
Abicht A. MD04, S64
Acar B.C. OD01, S75
Adcock K. MND06, S53
Agarwal A. OD07, S80
Aghaeipour A. D01, S9
Aguti S. OD04, S77
Ahl A. PN10, S42
Ahmad T. DCC01, S90 OD17, S87 OD18, S88 OD19, S88 OD07, S80
Aiestaran-Zelaia I. MD15, S72
Aimes C. DC07, S26
Aksoy A. MD06, S66
Aktas S. NMJ&C03, S59
Álvarez-Velasco R. NMJ&C03, S59
Alabaf S. NMJ&C01, S57
Alfano L. DC09, S27
Alisha Reyaz V.V.Y. DCC06, S94
Alkharji R. D02, S10
Allamand V. DC07, S26
Allen N.M. NMJ&C03, S58
Almuhana A. MND02, S50
Alonso-Perez J. D03, S11
Alston C.L. MD01, S61
Alungulese A. MD14, S72
Alvarez R. DC07, S26
Anikster Y. MD03, S63
Antoniou C. MND01, S49
Ardicli D. OD01, S75
Arikan E.D. OD02, S76 OD03, S76
Ashley E.-J. DC18, S34
Aston J. MD12, S70
Avalos G. NMJ&C03, S59

Banks J. OD08, S80
Baranello G. MND05, S52 MND11, S56 DC14, S31 MND08, S54
Bardien S. DCC06, S94
Barel O. MD03, S63
Barohn R.J. OD05, S78
Bearden D.L. PN06, S39
Beeson D. NMJ&C01, S57 NMJ&C02, S58
Benatar M. DCC03, S92
Bennett D.L. PN01, S36 PN09, S42
Bettolo C.M. MND03, S51 OD08, S80
Bhatia R. DCC01, S90 OD17, S87 OD18, S88 OD19, S88 DCC06, S94 OD07, S80
Bindu P.S. PN16, S47
Birbeck G.L. PN06, S39
Bisschoff M. DCC02, S91 OD16, S86 OD15, S85
Blake J. PN05, S39
Blakely E.L. MD01, S61
Blewitt C. MND03, S51
Boddy H. DC07, S26
Boggan R.M. MD01, S61
Bogner E. PN17, S48
Bolduc V. D07, S14
Bonne G. D11, S17
Borch L. NMJ&C03, S59
Borges Neves A. MD09, S68
Boukhloufi I. MND11, S56
Boyle G. DC01, S21, OD08, S80
Brady S. DC05, S24, MD14, S72
Brazzo K. DC09, S27
Brureau A. S02, S3
Brusa C. MND11, S56
Bryden G. PN12, S44
Bugiardini E. DCC01, S90 MD08, S67 DC06, S25 MD01, S61 OD13, S84
Bullivant J. DC20, S35
Burnett N. DC13, S30
Burns E. D06, S13
Byrne B. OD05, S78
Bythell M. MD12, S70 OD03, S76
Byun S.Y. NMJ&C03, S58
Bönnemann C.G. D07, S14
Büchner B. MD01, S61

Çavdarlı B. DC12, S30 MD06, S66 DCC06, S94
Cahalan S.D. MND02, S50
Cammish P. DC02, S22 DC04, S23
Canzonetta C. NMJ&C02, S58
Carganillo R. PN13, S45
Carlier P.G. OD05, S78
Carr A.S. PN07, S40 PN12, S44
Carrasco-Rozas A. D06, S13
Catapano F. D01, S9 D02, S10 D04, S12 D14, S19 MND08, S54
Cavalcante E. MND06, S53

Celikel E. OD01, S75
Ceylan A.C. MD02, S62 MD06, S66
Ceylan A. DC12, S30 DCC06, S94
Chambers D. D01, S9 D02, S10 D14, S19
Chan L. PN12, S44
Chan W.-K. NMJ&C03, S59
Cheng M.-H. (Mark) NMJ&C03, S59
Cheng S. OD04, S77
Childs A.-M. DC02, S22 DC04, S23 DC17, S33
Chinnery P.F. MD05, S65 MD12, S70 DCC06, S94
Chouchane M. NMJ&C03, S58
Citak Kurt A.N. OD01, S75
Claeys K. NMJ&C03, S59
Clark A.J. PN01, S36 PN09, S42
Clark J. D03, S11
Colclough K. MD01, S61
Coleman M. MND01, S49 MND04, S51
Cole T. DC14, S31
Colin Y.L. PN17, S48
Comley L.H. PN11, S43
Compton S.M.L. PN02, S37
Consortium G.E.R. MD08, S67 MD03, S63
Cope H. DC08, S27
Cordell H.J. MD01, S61
Cortese A. DCC01, S90 PN01, S36
Cossins J. NMJ&C01, S57 NMJ&C02, S58
Costa O. DC03, S22
Court F.A. PN10, S42
Cox D. D06, S13 OD06, S79
Craig K. MD03, S63
Crook V. DC13, S30
Cuisset J.-M. D11, S17
Currò R. PN01, S36

Dalal A. OD17, S87 OD19, S88
Damme OD05, S78
Dastidar S. D15, S20
Davidson S. MD15, S72
Davison J. MD08, S67
Day J.W. DC01, S21
Debnath M. PN16, S47
Deconinck N. DC07, S26
Deepha S. D12, S18 MD13, S71
Demetriou C. PN09, S42
de Moura Aschoff C.A. MD03, S63
Dercksen M. OD16, S86
Desai U. DC01, S21
Desikan M. DC11, S29
DeVile C. MD08, S67
Devine H. PN12, S44
De Vivo D. NMJ&C03, S59
Diaz J. DC03, S22
Dickson G. D10, S16
Dimachkie M.M. OD05, S78
Dimitriadi, M. MND10, S56 MND07, S54
Dissanayake K.N. PN10, S42
Dixon I. DCC03, S92
Dominguez N. S02, S3
Dominik N. DCC01, S90 MND09, S55 DC06, S25 DCC06, S94
Dong Y. NMJ&C01, S57 NMJ&C02, S58
Dorn II G.W. S05, S5
Dressman H.G. DC01, S21
Drummond E. OD08, S80
Dziewczapolski G. DC07, S26
Díaz-Manera J. D06, S13 OD05, S78 OD06, S79

Eaton S. MD15, S72
Efthymiou S. DCC01, S90 MND09, S55 DC06, S25 DCC06, S94
Eggleton K. MD08, S67
Eliyahu A. MD03, S63
Elks N. MD10, S69
Ellmers R. MD08, S67 NMJ&C05, S61
Elsaddig A. PN02, S37
Elseed M. OD05, S78 DC03, S22
Elwell T. MND03, S51 MND06, S53
Emilie Nguyen C.-T. NMJ&C03, S58
Erdinc D. MD03, S63

Evans M. PN12, S44
Eymard B. NMJ&C03, S58

Facchini S. DCC01, S90 MND09, S55 PN01, S36
Faivre M. S02, S3
Falabella M. MD08, S67
Falkenberg M. MD03, S63
Falkous G. MD03, S63
Falzarano M.S. D04, S12
Farrugia M.E. MD17, S74 MND06, S53
Fassad M.R. DCC02, S91 DCC05, S93 DCC06, S94 MD03, S63 OD16, S86 OD15, S85
Feeney C. MD01, S61
Fenwick E. MND03, S51 MND06, S53
Fergus C. D01, S9
Ferlini A. D04, S12 D05, S13
Fernandez M. NMJ&C03, S59
Fernández-Simón E. D06, S13 D03, S11
Ferreira da Rosa Sobreira C. PN04, S38 DC06, S25
Ferreira T. MD04, S64
Ferretti P. D01, S9 D02, S10
Ferré X. DC07, S26
Fisher R. DC08, S27
Fitzsimmons S. MND03, S51 OD08, S80
Footitt E. MD08, S67
Forsey K. PN03, S37
Fortunato F. D04, S12 D05, S13
Foster M.C. OD05, S78
Franklin I.G. MD01, S61

Fratter C. DC05, S24 MD12, S70 MD14, S72 MD16, S73
Frezatti R.S.S. PN04, S38 NMJ&C04, S60
Frezatti R. DCC06, S94

Gaeta A. DC04, S23
Galloway A. OD06, S79
Gangfuß A. MD04, S64
Gao F. MD05, S65 DCC06, S94
Garcia-Moreno H. MD03, S63
Garg A. OD07, S80
Gayathri N. D12, S18 MD13, S71
Georgiou E. S04, S4
Gerald W. S05, S5
Gergely D. OD08, S80
Ghimenton E. DC03, S22
Giacca M. S03, S4
Gilbertson J.A. PN13, S45
Gileadi T.E. D01, S9
Gilley J. MND01, S49 MND04, S51
Gillmore J.D. PN13, S45
Giraud Q. OD10, S82
Giunti P. MD03, S63
Goel V. OD07, S80
Goker-Alpan O. OD05, S78
Gokul-Nath R. D03, S11
Gomathy S.B. OD07, S80
Goodwin D. MND02, S50
Gorman G. MD17, S74 MD01, S61 MD03, S63
Gosal D. PN07, S40
Govindaraj P. MD13, S71
Graessner H. S12, S8
Graham A. DC19, S35
Gray A. PN03, S37
Greenbaum L. MD03, S63
Gualandi F. D05, S13
Guglieri M. D03, S11 DC02, S22 DC04, S23 DC17, S33 OD06, S79 DC03, S22
Guirguis F. D07, S14
Gustafsson C.M. MD03, S63
Güleç Ceylan G. DCC06, S94
Gündüz C.N.S. MD06, S66

Haack K.A. OD05, S78
Hacohen Y. NMJ&C03, S59
Haddad S. PN05, S39
Hadden R.D.M. PN07, S40
Hahn A. NMJ&C03, S58
Hahn K. OD09, S81
Hanna M.G. DCC01, S90 MD07, S66 MD08, S67 MD09, S68 MD12, S70 NMJ&C05, S61 OD18, S88 PN04, S38 DC06, S25 DCC06, S94 MD01, S61 MD15, S72 NMJ&C04, S60 OD07, S80
Hanna M. OD19, S88
Hardy S.A. MD12, S70
Harms M. DC01, S21
Harris E. DC03, S22
Harron R.C.M. D13, S18
Harron R. D08, S15
Hathazi D. MD18, S74
Hattersley A.T. MD01, S61
Hawkins A. NMJ&C02, S58
Heales S. MD15, S72
Heckmann J. DC10, S28 DCC03, S92 DC06, S25 DCC06, S94
Henning F. DC10, S28 OD16, S86 DC06, S25 DCC06, S94
Hentschel A. MD04, S64
Herrmann D. PN15, S46
Heslop E. DC02, S22 DC04, S23 DC08, S27
Hildyard J.C.W. D13, S18
Hildyard J. D08, S15
Hill C. DC08, S27
Hilsden H. DC15, S32 DC16, S32 OD06, S79 OD08, S80
Hodsdon P. MD14, S72
Hofer M. DC05, S24
Holt I.J. MD15, S72
Hong Y. MND11, S56
Hopkins E.L. MND04, S51
Hopton S. MD03, S63
Horton R.H. MD07, S66
Horvath R. DC12, S30 MD04, S64 MD05, S65 MD12, S70 MD18, S74 OD09, S81 OD14, S84 DCC06, S94
Houden H. PN04, S38 DCC01, S90 MD08, S67 NMJ&C05, S61 PN01, S36 S06, S5 DC06, S25 DCC04, S93 DCC06, S94 NMJ&C04, S60
Howdle D. D08, S15
Human R. DCC02, S91 OD15, S85
Hurtado M.L. PN10, S42
Hız S. OD14, S84

Irvin A. DC04, S23
Irwin J. MND06, S53

Jabeen S.A. OD20, S89
Jacobson L.W. NMJ&C03, S59
Jalal S. D11, S17
James M. DC01, S21 DC15, S32 DC16, S32 OD08, S80

Japzon N. PN02, S37
Jassen A. OD16, S86

Jayaseelan D. NMJ&C05, S61
Jiang Y. D09, S15
Johnson A. DC02, S22 DC04, S23 DC08, S27 DC17, S33
Johnson N. DC09, S27
Jou C. D03, S11
Jungbluth H. NMJ&C03, S59
Júnior W.M. PN04, S38 NMJ&C04, S60

Kagiava A. S04, S4
Kao B. D10, S16
Kapapa M.M. PN06, S39
Kasapkara Ç.S. MD02, S62
Kearns G. NMJ&C03, S58
Keh R. PN12, S44
Kelly V.P. D01, S9
Kent L. DC05, S24 MD14, S72
Khedr M. D15, S20
Kim D.-S. NMJ&C03, S58
Kirby A.J. MD03, S63
Kishnani P.S. OD05, S78
Kleefeld F. OD09, S81
Kleniuk J. MD18, S74
Kleopa K.A. S04, S4
Kline R.A. PN10, S42 PN11, S43
Klopstock T. MD01, S61
Koebel P. OD10, S82
Koken O.Y. DCC06, S94
Kornblum C. NMJ&C03, S59 MD01, S61
Kozma I. NMJ&C02, S58
Kramarz C. PN07, S40
Kretkiewicz M. DC06, S25
Kuntzer T. NMJ&C03, S59
Köken Ö.Y. MD06, S66
Kölbel H. MD04, S64 NMJ&C03, S58
Köylü O.K. MD02, S62

Labrum R. MD08, S67
Ladha S. OD05, S78
Laforêt P. OD05, S78
Laidler Z. D03, S11
Lamont D.J. PN10, S42
Landeros E.V. PN11, S43
Lang Q. PN08, S41 PN18, S48
Laporte J. OD10, S82
Laura M. PN05, S39 PN07, S40 PN13, S45 PN14, S46 PN15, S46
Laurent J.-P. DC09, S27 NMJ&C03, S59
Lavin T. PN07, S40 PN12, S44
Leite I. NMJ&C03, S59
Lemmers R.J.L.F DC06, S25
Lench N. S01, S3
Lilleker J. MND06, S53
Lin J.P. NMJ&C03, S59
Lionello V. D11, S17
Liu W.-W. NMJ&C01, S57
Lloyd T. PN15, S46
Lofra R.M. MND03, S51 OD08, S80
Lopes L. MD11, S69
Loreto A. MND01, S49
Lorson C.L. PN11, S43
Lubbe E. DCC02, S91 OD15, S85
Luca MND09, S55
Lucassen A.M. MD07, S66
Lunn M. PN05, S39
Lyman S. PN17, S48

Macken W.L. MD07, S66 MD08, S67 OD17, S87 OD18, S88 OD19, S88 DCC06, S94 OD07, S80 DC06, S25
Mackenzie S. MD03, S63
Madeleine Wilmshurst J. DC06, S25
Mahadevan A. PN16, S47
Mahmoud R. DCC05, S93
Mahungu A. DCC03, S92
Main M. DC13, S30
Malerba A. D10, S16
Malhotra J. D14, S19
Malinova M. DC03, S22 MD01, S61
Manera J.D. OD08, S80 D03, S11
Manguoğlu E. MD06, S66
Manjunatha M.V. D12, S18
Manzur A. DC13, S30 DC17, S33 DC14, S31
Margari N. MD09, S68
Marina A.D. MD04, S64 NMJ&C03, S58
Marini-Bettolo C. DC07, S26 DC18, S34 DC19, S35 DC20, S35 MND06, S53 OD06, S79 OD13, S84 D03, S11 DC03, S22
Marques Jr W. DCC06, S94 DC06, S25
Martínez N.W. PN10, S42
Ma S. PN09, S42
Massey C. OD11, S82
Masud A. NMJ&C03, S59
Mathews K. DC09, S27
Matthews E. DC19, S35
Matthews I. D06, S13
Mayhew A. DC15, S32 DC16, S32
McAlister B. DC07, S26
McCallum M. OD08, S80
McCauley J. OD12, S83
McClarty N. OD08, S80
McConville J. MND06, S53
McDonald S. DC07, S26 DC20, S35
McDowell A. PN14, S46
McFarland B. DCC05, S93
McFarland R. DCC02, S91 MD17, S74 OD16, S86 DCC06, S94 MD01, S61 MD03, S63 MD15, S72 OD15, S85
McKittrick C. MD08, S67
McNiff M. DC08, S27
Mehra P. D03, S11
Mejat A. DC07, S26

Mendell J. DC01, S21
Mengel K.E. OD05, S78
Menger K.E. MD03, S63
Meng J. MND05, S52 PN09, S42
Merrison A. MND06, S53
Messaddeq N. OD10, S82
Michael Hanna T.K, OD17, S87
Michell-Sodhi J. MND03, S51
Mietto M. D04, S12
Milev E. DC14, S31
Minczuk, M. S10, S7
Miossec P. OD05, S78
Mishra R. DCC01, S90 OD17, S87 OD18, S88 OD19, S88 DC06, S25 OD07, S80
Mitsogiannis M. D01, S9
Moat D. MND03, S51 OD08, S80 DC03, S22
Mole A.J. PN10, S42
Montanaro F. D01, S9
Moore D. D11, S17
Morceau A. D03, S11
Morgan J. D02, S10 D04, S12 D14, S19 D01, S9 MND05, S52
Morrow J.M. PN14, S46
Morsy H. OD16, S86 DC06, S25 DCC04, S93 DCC06, S94
Mozzafar T. DC01, S21
Mueller A. NMJ&C03, S59
Mueller J. D02, S10 OD12, S83
Muni-Lofra R. DC19, S35 MND06, S53 DC03, S22
Muntoni F. D01, S9 D02, S10 D04, S12 D07, S14 D09, S15 D14, S19 DC13, S30 DC17, S33 MND05, S52 MND11, S56 OD04, S77 OD12, S83 DC14, S31 MND08, S54 PN09, S42
Murphy L. DC09, S27 DC20, S35 MND03, S51
Murray L.M. PN10, S42 PN11, S43
Männikkö R. NMJ&C05, S61

Nagappa M. MD13, S71 PN16, S47
Naidu K. DC10, S28 DCC03, S92 OD16, S86 DC06, S25 DCC06, S94
Nalini A. D12, S18
Nandeesh B.N. D12, S18
Nart L. DC11, S29
Nascimento A. D03, S11
Natera-de Benito D. NMJ&C03, S59
Natera D. D03, S11
Naveena M. OD20, S89
Navya Gopal N.V. OD20, S89
Nel M. DCC03, S92 DCC06, S94
Neri M. D05, S13
Nesbitt V. MD14, S72
Neuhan T. MD04, S64
Ng Y. MD17, S74
Nguyen M.P.H. PN17, S48
Nguyen N.L. D10, S16
Nicholls T.J. MD03, S63
Nitzani D. MD09, S68
Nonyane M. DCC02, S91 OD15, S85
Norwood F. DC19, S35 NMJ&C03, S59
Novacic D. MD03, S63
Nur Semerci C. DCC06, S94

Oh E.J. PN17, S48
Oktay Y. DC12, S30 OD14, S84 DCC06, S94
Olive M. DC01, S21
Orrell R. DC19, S35
Ortez C. D03, S11
Osborne P. DC04, S23
Oskoui M. NMJ&C03, S59
Osman E.Y. PN11, S43
O'Brien D. PN12, S44
O'Donnell L. PN13, S45 PN14, S46
O'Hara V. MND02, S50
O'Rahelly M. NMJ&C03, S58
O'Reilly E. DC14, S31
Özyılmaz B. OD14, S84
Ørstavik K. DC09, S27

Pacheco Torres P. MND07, S54

Padma Srivastava M.V. DCC01, S90 OD17, S87 OD18, S88 OD19, S88 DCC06, S94 OD07, S80 DC06, S25
Page J. OD13, S84
Palace J. NMJ&C03, S59
Palmowski P. D06, S13
Pangou I. DC20, S35 OD13, S84
Paradas C. DC01, S21
Pareyson D. PN15, S46
Parton M. MND06, S53
Patel K.A. MD01, S61
Patel K. DCC02, S91 DCC05, S93 OD16, S86 DCC06, S94 OD15, S85
Patel P. MND11, S56 MND08, S54
Patel Y. MD08, S67
Peay H. DC08, S27
Pegg K. DC03, S22
Pegoraro E. DC01, S21
Pena L.D.M. OD05, S78
Perez Rodriguez D. MD15, S72
Periyasamy G. PN16, S47
Perkins J. MND02, S50
Perrone B. DCC01, S90 MND09, S55 DC06, S25
Perry L.D. DCC06, S94

Perry L. OD12, S83
Pestronk A. DC01, S21
Phadke R. D01, S9 D02, S10 D04, S12 MD08, S67 OD12, S83
Pickett S.J. MD01, S61 D08, S15 D13, S18 MND02, S50
Piercy R. OD11, S82
Pietrusz A. DC11, S29
Pinder E. MD16, S73
Pini V. OD12, S83
Pinol-Jurado P. D03, S11
Pinton L. D11, S17
Pipis M. PN14, S46
Pitceathly R. OD17, S87
Pitceathly R.D.S. MD07, S66 MD08, S67 MD09, S68 MD12, S70 OD18, S88 PN04, S38 DCC06, S94 MD01, S61 MD15, S72 NMJ&C04, S60 OD07, S80
Pitceathly R. MD11, S69 OD19, S88
Pizzamiglio C. MD08, S67 MD09, S68 MD01, S61
Piñol-Jurado P. D06, S13
Poh R. PN15, S46
Polat İ. DC12, S30 OD14, S84 DCC06, S94
Polke J.M. PN15, S46 MND09, S55
Popplewell L. D10, S16
Porter A. D06, S13
Poulton J. MD14, S72 MD03, S63 S02, S3
Poupiot J. S02, S3
Prabha Turaga S. OD20, S89
Preusse C. OD09, S81
Priyanka Y. OD07, S80
Prokisch H. MD01, S61

Quinlivan R. DC11, S29 DC17, S33 MD08, S67 OD02, S76 OD03, S76

Raga S. DCC03, S92 MD10, S69 DC06, S25 DCC06, S94
Rahman M. MD11, S69
Rahman S. MD08, S67 NMJ&C03, S59
Ramdharry G. DCC06, S94
Ramond F. MND04, S51
Rashid S.A. MND10, S56
Rating P. MD04, S64
Record C.J. DCC06, S94 NMJ&C04, S60
Record C. PN04, S38 PN14, S46
Reid E. MD18, S74
Reilly M.M. MD08, S67 PN01, S36 PN04, S38 PN05, S39 PN07, S40 PN13, S45 PN14, S46 DCC06, S94 NMJ&C04, S60 PN09, S42 PN15, S46
Reimann J. NMJ&C03, S59
Reuben E. DC04, S23
Reyaz A. DCC01, S90 OD17, S87 OD18, S88 OD19, S88 OD07, S80
Rhymes E.R. PN18, S48
Ribchester R.R. PN10, S42
Richard I. S02, S3
Richard J.L.F.L. DCC01, S90
Richardson M. OD08, S80
Riddell D. D08, S15
Riddell D.O. D13, S18
Ridout D. DC13, S30 DC14, S31
Rivas J.B. DC01, S21
Robb S. DC13, S30
Roberts M. DC19, S35
Robertson A. OD08, S80
Robert W. MD01, S61
Robinson E. OD08, S80
Rodney S. DC17, S33
Rodríguez-Luis A. MD03, S63
Roefke K. NMJ&C03, S59
Roos A. MD04, S64 OD09, S81
Rossi R. D14, S19
Rossor A. PN05, S39 PN07, S40 PN13, S45 PN14, S46 PN15, S46
Roudaut C. S02, S3
Rowczenio D. PN13, S45
Roy D. D12, S18
Ruiz-Cabello J. MD15, S72
Ryburn L. MND06, S53

Sacconi S. OD05, S78
Sadjadi R. PN15, S46
Sankaran B.P. MD13, S71
Sargiannidou I. S04, S4
Sarkozy A. DC07, S26 DC13, S30 DC17, S33 MD08, S67 OD12, S83 DCC06, S94
Sasaki M. PN17, S48
Saute J.A.M. MD03, S63
Savarese M. S11, S8
Savvatis K. MD11, S69
Scadeng A. OD08, S80
Schaefer A. MD17, S74
Schaefer A.M. MD01, S61 MD03, S63
Schara-Schmidt U. MD04, S64 NMJ&C03, S58
Scherer S.S. PN15, S46
Schiava M. D03, S11
Schiavo G. PN08, S41 PN18, S48
Schneider-Gold C. NMJ&C03, S59
Schon K. DC12, S30 MD05, S65 OD14, S84
Schon K.R. MD12, S70 OD16, S86 DCC06, S94
Schoonen M. DCC02, S91 OD16, S86 OD15, S85
Schoser B. OD05, S78

Schäfer J. MD01, S61
Schänzer A. MD04, S64
Scoto M. MND06, S53 MND11, S56 DC02, S22
Segovia S. MND03, S51 OD08, S80
Selvatici R. D04, S12 D05, S13
Senbil N. OD01, S75
Sepulveda P. NMJ&C02, S58
Sergeant K. DC05, S24 MD14, S72 MD16, S73 MD03, S63
Seshagiri D.V. PN16, S47
Sharma S. D12, S18 PN16, S47
Shepherd M.H. MD01, S61
Shetty T. PN17, S48
Shiau Ng Y. MD01, S61
Shin J.-H. DC01, S21
Shy M. PN15, S46
Sickmann A. MD04, S64
Siddle M. D01, S9
Simkin R.L. PN18, S48
Singh S. D01, S9 D02, S10 D14, S19
Skorupinska I. MD09, S68
Skorupinska M. PN05, S39 PN13, S45 PN14, S46 PN15, S46
Sleigh J.N. PN08, S41 PN18, S48
Smith C. MD14, S72
Smuts I. DCC02, S91 OD16, S86 DCC06, S94 OD15, S85
Soares Frezatti R.S. DC06, S25
Sobreira C.F.R. NMJ&C04, S60 DCC06, S94
Sodhi J. OD08, S80 DC03, S22
Sokolowska E. D01, S9
Somwe W.S. PN06, S39
Sparks K. DCC03, S92
Spiegelhalter C. OD10, S82
Spinazzola A. MD15, S72
Srinivas Bharath M.M. D12, S18
Srirangalingam U. MD09, S68
Straub V. D03, S11
Stavrou M. S04, S4
Steele-Stallard H. D11, S17
Stenzel W. OD09, S81
Stewart J. S08, S6
Stilwell P. MD12, S70
Stimpson G. DC14, S31
Stojkovic T. DC01, S21
Straub V. DC01, S21 DC09, S27 DC10, S28 DC15, S32 DC16, S32 DC20, S35 OD05, S78 OD06, S79 OD08, S80 OD19, S88 DC03, S22 DCC06, S94
Su E.P. PN17, S48
Suel L. S02, S3
Sunitha B. D12, S18
Surana S. PN18, S48
Suárez-Calvet X. D06, S13 D03, S11
Symon-Allen A. MD16, S73
Talim B. OD01, S75
Taly A.B. PN16, S47
Tasca G. D03, S11
Taylor-Brown F. D08, S15 D13, S18
Taylor R. MD17, S74 OD16, S86
Taylor R.W. DCC05, S93 DCC02, S91 DCC06, S94 MD03, S63 MD15, S72 OD15, S85
Taylor S. D08, S15 MD01, S61
Tedesco F.S. D09, S15 D11, S17 D15, S20
Tekin H. DC12, S30
Thaele A. MD01, S61
Thakrur S. DC02, S22
Thangaraj K. OD18, S88 OD19, S88
Thiele S. DC09, S27
Thieme A. NMJ&C03, S59
Thomas M.A. NMJ&C03, S59
Thomas R. MD01, S61
Thomson A.K. PN11, S43
Thornton J.S. PN14, S46
Tomaselli P.J. PN04, S38 NMJ&C04, S60 DCC06, S94 DC06, S25
Tonelli L. D05, S13
Topaloglu H.A. DCC06, S94
Topaloğlu H. MD06, S66
Topf A. DC10, S28 OD19, S88 DCC06, S94
Torelli S. D14, S19
Toro C. MD03, S63
Torron R.F. DC01, S21
Torun T. D15, S20
Trainor C. DC03, S22 DCC06, S94
Trivedi J. OD05, S78
Tunçer G.Ö. MD06, S66
Turnbull D.M. MD01, S61 MD17, S74
Turner C. DC17, S33
Töpf A. OD06, S79

Ul Huda S.S. PN12, S44

Valenzuela S. MD03, S63
Vale T. MD14, S72
Van den Bergh P. NMJ&C03, S59
van der Maarel S.M. DCC01, S90 DC06, S25
van der Ploeg A.T. OD05, S78
van der Vliet P.J. DCC01, S90 DC06, S25
van der Watt G. OD16, S86
van der Westhuizen F.H. OD16, S86 OD15, S85
van der Westhuizen F. DC06, S25 DCC02, S91 DCC06, S94
Vandrovcova H.M.J. OD15, S85
Vandrovcova J. DCC02, S91 DCC03, S92 MD05, S65 MD08, S67 OD16, S86 DCC04, S93 DCC06, S94
Verdu-Díaz J. D03, S11
Verma M. OD08, S80
Villalobos E. D03, S11

Villarroel-Campos D. PN18, S48
Vincent A. NMJ&C03, S59
Viscomi C. S07, S6
Vishnu V.Y. DCC01, S90 OD17, S87 OD07, S80 DC06, S25 OD18, S88 OD19, S88
Vissing J. DC01, S21 DC09, S27 OD05, S78
Vivekanandam V. NMJ&C05, S61
Voit T. DCC06, S94
Volker S. DC07, S26
Vorster A. DCC02, S91 OD15, S85
Vorster B.C. OD16, S86

Walker H. DC18, S34 DC19, S35 DC20, S35
Walter M.C. DC09, S27
Wanders R.J.A. OD16, S86
Wan H.-S. NMJ&C03, S59
Wastling S. PN14, S46
Watson-Fargie T. MD17, S74
Watt S. DC19, S35
Webster R. NMJ&C01, S57
Wells D. D08, S15 D13, S18 OD11, S82
Willis T. DC19, S35
Wilmshurst J. DCC03, S92
Wilmshurst J.M. MD10, S69 DCC06, S94
Wilson L.A. MD05, S65 DCC06, S94
Wilson L. DCC01, S90 DC06, S25 DCC04, S93 OD15, S85
Wishart T.M. PN10, S42
Wolfe A. DC14, S31
Wolfe L. MD03, S63
Wong J. DC17, S33
Wong K. OD08, S80
Woods L. DC09, S27
Woodward C.E. MD08, S67 MD01, S61
Woodward C. MD11, S69
Woon J.T. PN17, S48
Woschitz V. PN11, S43
Wredenberg A. S09, S7
Wu G. DCC03, S92

Xie X. MD03, S63

Yareeda S. OD20, S89
Yau J. MND03, S51 MND06, S53
Yiş U. DCC06, S94 DC12, S30 OD14, S84
Yoshimura M. DC01, S21
Young P. OD05, S78

Zafeiropoulos N. PN14, S46
Zaharieva I. OD12, S83
Zammit P. D11, S17
Zarate M. MD18, S74
Zhang Q. MND05, S52 MND11, S56 PN09, S42
Zhou H. D07, S14 MND05, S52 MND11, S56 OD04, S77 MND08, S54 PN09, S42
Zhou T. OD05, S78