Muscular Dystrophy UK
Fighting muscle-wasting conditions

MDUK Oxford Neuromuscular Centre

UNIVERSITY OF OXFORD

MDUK Oxford Neuromuscular Centre 3rd Annual Meeting

26TH SEPTEMBER 2023

ST ANTONY'S COLLEGE, OXFORD

Annual Meeting Programme

TABLE OF CONTENTS

Muscular Dystrophy UK
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AGENDA

MDUK Oxford Neuromuscular Centre Annual Meeting

08:30	-	09:00	***Registration, Tea, & Pastries in Investcorp Foyer***

Session 1: Welcome & Keynote speaker

09:00	-	**09:10**	**MDUK Oxford Neuromuscular Centre – Welcome from the Director** Prof Matthew Wood *Professor of Neuroscience, Department of Paediatrics, University of Oxford | Deputy Head (Innovation), Medical Sciences Division | Director of MDUK Oxford Neuromuscular Centre*
09:10	-	**10:10**	**Advanced in vitro modelling of neuromuscular diseases and therapies** Prof Francesco Saverio Tedesco *Professor of Neuromuscular Biology and Regenerative Medicine, University College London & The Francis Crick Institute*
10:10	-	10:35	***Tea & Coffee in The Buttery***

Session 2: Pre-clinical NMD research – Chair: Prof David Bennett

10:35	-	**12:00**	**A novel mouse model of muscle nAChR clustering deficiency CMS** Dr Yin Dong *MRC Career Development Fellow, Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford* **Mitochondrial dysfunction in iPSC-derived motor neurons from ALS patients** Dr Ruxandra Dafinca *Senior Postdoctoral Researcher, Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford* **Novel insights into dystrophic pathology and dystrophin restoration therapies from gene expression analysis** Dr Thomas Roberts *Research Fellow and Group leader, Department of Paediatrics, Medical Sciences Division, University of Oxford and Institute of Developmental and Regenerative Medicine* **Insights into ALS from the cerebrospinal fluid proteome** Dr Alex Thompson *Clinician Scientist, Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford | Honorary Consultant Neurologist, Oxford University Hospitals NHS Foundation Trust* **Chemogenetic silencing of sensory neuron-driven pain** Dr Jimena Perez-Sanchez *Postdoctoral Researcher, Nuffield Department of Clinical Neuroscience, Medical Sciences Division, University of Oxford*
12:00	-	13:00	***Lunch in The Buttery and poster setup***

Session 3: Bridging the divide: where bench and bedside meet – Chair: Prof Laurent Servais

13:00	**-**	**14:10**	**The Genomics England Generation Study** Dr Katrina Stone *Clinical Fellow in Genomics, Genomics England* **What academics should know if they want to see their drug used in clinic** Cliff Bechtold *President and General Manager, Biohaven Bioscience Ireland Ltd | Chief Operating Officer and Compliance Officer, Biohaven Pharmaceuticals, Inc.* **Planning for translation in Duchenne Muscular Dystrophy** Dr Amy Donner *Senior Director, Medical Communications, Wave Life Sciences* **Developing an experimental medicine approach to testing drugs in ALS** Prof Martin Turner Professor of Clinical Neurology & Neuroscience, Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford
14:10	-	14:35	***Tea & Coffee in The Buttery***

Session 4: NMD in the clinic – Chair: Assoc Prof Carlo Rinaldi

14:35	**-**	**15:30**	**The importance of natural history studies – with nemaline myopathy as an example** Dr Gemma Fisher *Paediatric Neuromuscular Clinical Research Fellow, Department of Paediatrics, Medical Sciences Division, University of Oxford* **Microdystrophin in DMD: hopes, fears, and limitations** Dr Serge Braun *Chief Scientific Officer, AFM-Telethon | President, Genosafe | Director of Neuromuscular Diseases, Genethon* **Congenital Myasthenia Syndromes- Defining outcome measures for clinical trials** Dr Sithara Ramdas Consultant Paediatric Neurologist, Department of Paediatric Neurology, John Radcliffe Hospital, Oxford

Session 5: Closing speaker & Wrap-up – Chair: Prof Kevin Talbot

15:30	**-**	**16:20**	**Expanding genetic therapies to treat all rare disorders** Prof Stephan Sanders *Professor of Paediatric Neurogenetics Department of Paediatrics, Medical Sciences Division, University of Oxford | Department of Psychiatry and Behavioral Sciences, UCSF Weill Institute for Neurosciences, University of California*
16:20	**-**	**16:30**	**Wrap-up and Closing** Prof Kevin Talbot *Professor of Motor Neuron Biology and Head of Department, Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford | Co-Director of MDUK Oxford Neuromuscular Centre*
16:30	-	18:00	***Posters & Drinks Reception in The Buttery***

VENUE – ST ANTONY'S COLLEGE

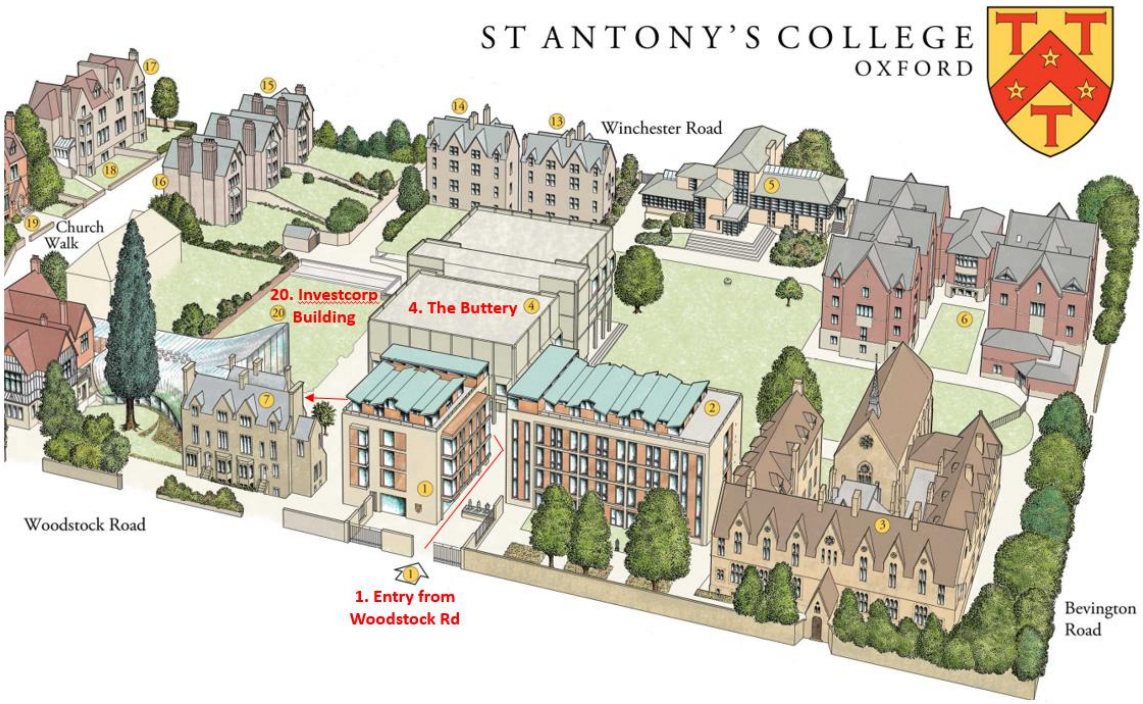
Address

62 Woodstock Rd, Oxford OX2 6JF
+44 (0)1865 284700

Venue

St Antony's College is located on the corner of Woodstock Rd and Bevington Rd. It is directly accessible by the following buses: 300, 6, S1, S2, S3, and ST2, or it is a 15-min walk from City Centre. There will not be parking available on site, but on-street parking is possible on Bevington Rd or paid parking is available a 10-min walk away at Port Meadow South Parking.

Lectures will be held in the **Investcorp Auditorium**, which can be easily accessed by turning left just past the Lodge Building at the entrance (route shown below in red). A registration desk will be set-up as you enter the Investcorp Building and meeting volunteers will be present to guide you. Tea breaks, lunch, and the Poster & Drinks Reception will take place in The Buttery.



SPEAKER BIOGRAPHIES

Matthew Wood

Professor of Neuroscience | Director of MDUK Oxford Neuromuscular Centre | Deputy Head od Medical Sciences Division (Innovation)

Department of Paediatrics, Medical Sciences Division, University of Oxford

Matthew Wood F.Med.Sci. is Professor of Neuroscience and Deputy Head of the Medical Sciences Division at the University of Oxford. He directs the Laboratory of RNA biology and Neuromuscular Disease investigating development of RNA-based medicines for neuromuscular disease focusing on the development of advanced generation antisense oligonucleotides for Duchenne muscular dystrophy and related neuromuscular conditions. He is currently Director of MDUK Oxford Neuromuscular Centre and Director of the Oxford Harrington Rare Disease Centre. He has pioneered the development of novel drug delivery systems including peptide and exosome-based technologies for the targeted delivery of macromolecular biologics, including oligonucleotides, to tissues including the brain. He is a co-founder of the biotech spin-outs Evox Therapeutics, PepGen, Orfonyx Bio and ISOgenix, and has recently led a major UK national initiative to establish a UK Nucleic Acid Therapy Accelerator (NATA). Matthew is an advisor to numerous research funding agencies including UKRI, to Genomics England and to industry. In his role as Deputy Head of the Medical Sciences Division of the University of Oxford, Matthew leads strategic coordination of all innovation related activities. He currently serves as a Non-Executive Director of the University of Oxford's technology transfer organisation, Oxford University Innovation (OUI).

Professor Francesco Saverio Tedesco

Professor of Neuromuscular Biology and Regenerative Medicine

University College London, Division of Biosciences | The Francis Crick Institute

Prof. Tedesco is a paediatric neurologist with expertise in neuromuscular disorders, muscle regeneration and advanced disease modelling. He graduated in Medicine and Surgery with honours at the Sapienza University of Rome (Italy). Before his doctorate he was a visiting scientist at the Institut Pasteur (Paris, France) studying muscle stem cell biology. He obtained his PhD investigating novel gene and cell therapies for muscular dystrophy at the San Raffaele Scientific Institute of Milan (Italy). Prof. Tedesco received the 2015 Young Investigator Award by the European Society of Gene and Cell Therapy. He was then awarded an NIHR Academic Clinical Fellowship, followed by a Clinical Lectureship, and a prestigious European Research Council (ERC) Starting Grant. He received the 2020 Simon Newell Investigator of the Year award by the Royal College of Paediatrics and Child Health and the 2021 MacKeith Prize by the British Paediatric Neurology Association.

The Tedesco laboratory studies skeletal muscle regeneration, focusing on the development of novel therapies for incurable neuromuscular disorders of childhood. They work pioneered the use of cutting-edge technologies such as human induced pluripotent stem (iPS) cells, artificial chromosomes and tissue engineering for advanced disease modelling and gene/cell therapies of muscle diseases. Current projects investigate iPS cell-derived myogenesis for complex neuromuscular disease and therapy modelling, as well as the use of small molecules to improve myogenic cell delivery. The overall goal of the Tedesco laboratory is the translation of the aforementioned regenerative strategies into novel therapies to improve outcomes for children with neuromuscular disorders.

Dr Yin Dong

MRC Career Development Fellow

Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford

Dr Yin Dong studied Biochemistry at the University of Warwick before undertaking his PhD with Prof Karen Morrison to study the molecular mechanisms of ALS at the University of Birmingham. He then investigated the structure and function of human membrane proteins with Prof Liz Carpenter at the Structural Genomics Consortium as a post-doctoral associate. He obtained his MRC career development fellowship to work with Prof David Beeson on congenital myasthenic syndromes, with a particular focus on glycosylation related CMS and congenital diseases of glycosylation. More recently, he has been working with Prof Jacqueline Palace as part of the national CMS referral service, managing their functional diagnostics team.

Dr Ruxandra Dafinca

Senior Postdoctoral Researcher

Nuffield Department of Clinical Neurosciences, Oxford MND Research group, Medical Sciences Division, University of Oxford

I obtained my Bachelor of Science degree in Biochemistry and Cell Biology at Jacobs University Bremen in Germany, followed by an MSc in Neuroscience at the University of Oxford. In 2009, I started my DPhil studies on ALS/FTD in Prof Wade-Martins' laboratory at the University of Oxford where I developed novel fluorescently tagged BAC constructs carrying the human TARDBP genomic locus with ALS-related mutations. My constructs were used to develop a new transgenic mouse model of ALS, which has subsequently shown disease-like degeneration, becoming an essential physiological tool for modelling ALS.

In 2014, I joined Professor Kevin Talbot's research group in the Oxford MND Centre and I took the lead on establishing a new programme of research on iPS models of ALS/FTD. As co-investigator on a project grant from the MND Association, I performed and optimized differentiations of human iPSCs to motor neurons and I identified significant disease-associated phenotypes in ALS/FTD iPS-derived neurons, such as ER stress, loss of mitochondrial function and Ca2+ buffering deficits. With a fellowship from the Oxford-Celgene/BMS Translational Research program in 2019, I focused my research on molecular pathways involved in axonal transport deficits and mitochondrial dysfunction in ALS, with the goal of identifying new potential therapeutic targets. During this time, I also developed expertise in using CRISPR/Cas9 technology to generate essential iPS models for disease modelling.

With a Brain Science Fellowship awarded by the University of Oxford, I am now establishing a research programme on identifying the mechanistic link between mitochondrial deficits and dysfunctional synaptic transmission in ALS/FTD, which is a promising candidate pathway for therapeutic intervention.

Dr Thomas Roberts

Research Fellow and Group leader

Department of Paediatrics, Medical Sciences Division, University of Oxford | Institute of Developmental and Regenerative Medicine, University of Oxford

Tom Roberts is a research fellow at the Institute of Developmental and Regenerative Medicine and senior research scientist at the Department of Paediatrics, University of Oxford. He leads the RNA Medicine research group, with interests including neuromuscular disorders, RNA biology, extracellular nucleic acids, biomarkers, epigenetics, and gene/oligonucleotide therapies. He studied at the University of Oxford for both his undergraduate degree in Molecular and Cellular Biochemistry and doctoral degree in Physiology, Anatomy and Genetics in the laboratory of Professor Matthew Wood. He subsequently moved to San Diego, California where he undertook postdoctoral training positions at the Scripps Research Institute, Department of Molecular and Experimental Medicine (laboratory of Professors Kevin Morris and Marc Weinberg) and Sanford Burnham Prebys Medical Discovery Institute, Development, Aging and Regeneration Program (laboratory of Professor Lorenzo Puri). In 2022 he founded a biotech start-up company, Orfonyx Bio, that aims to develop genetic medicines for rare diseases.

Dr Alexander Thompson

Clinician Scientist | Honorary Consultant Neurologist

Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford | Oxford University Hospitals NHS Foundation Trust

Alexander Thompson is an MRC Clinician Scientist and Motor Neurone Disease Association Lady Edith Wolfson fellow at the Nuffield Department of Clinical Neurosciences, University of Oxford, and honorary consultant neurologist in the Oxford MND Centre, Oxford University Hospitals NHS Foundation Trust. Alex completed my BMBCh in Clinical Medicine at the University of Oxford in 2007 and undertook clinical training in London and Oxford before completing my DPhil in Clinical Neurosciences in 2018 at the University of Oxford. My research focuses on the development and implementation of neurochemical markers for the fatal neurodegenerative disease amyotrophic lateral sclerosis (ALS) in order to accelerate therapy development and understand early biochemical alterations before the onset of symptomatic ALS. This involves methods including integrated analysis of the CSF protein network using mass spectrometry, analysis of CSF and blood extracellular vesicles and development of pathology-specific biochemical assays, as well as the analysis of large scale population datasets.

Dr Jimena Perez Sanchez

Postdoctoral Researcher

Nuffield Department of Clinical Neuroscience, Medical Sciences Division, University of Oxford

I obtained my PhD from Laval University in Canada, where I studied how inhibition shapes local synaptic plasticity in dorsal horn circuits of the spinal cord. The spinal cord is an ideal system to study plastic changes that occur after injury because it is a relatively simple neural circuit, with direct input from sensory neurons and a measurable output. Since joining the Nerve Injury Group at the Nuffield Department of Clinical Neurosciences in Oxford, my research is focused on understanding how sensory neurons within the peripheral nervous system contribute to neuropathic pain. Increased activity in these neurons, which relay information from the periphery to the central nervous system, has been identified as a common driver for many of the maladaptive changes associated with neuropathic pain. One of the strategies I have adopted to study altered activity in these circuits is chemogenetics; expressing a modified chloride channel gene in specific sensory neuron subtypes, which is activated by low doses of a non-toxic agonist, to selectively silence sensory neurons.

Dr Katrina Stone

Clinical Fellow in Genomics

Genomics England

Katrina studied Medicine at the University of Cambridge and King's College London. She is currently training in clinical genetics at St George's Hospital. She joined Genomics England in September 2022 to work on the Newborn Genomes Programme.

Clifford Bechtold

President and General Manager | Chief Operating Officer and Compliance Officer

Biohaven Bioscience Ireland Ltd | Biohaven Pharmaceuticals, Inc.

Cliff has served as the Chief Operating Officer and Chief Compliance Officer for Biohaven Pharmaceuticals taking the company from early start-up to post Pfizer acquisition. He is a 30-year veteran of the Pharmaceutical industry with a broad drug development experience and a track record of optimizing organizations and capabilities. In addition, Cliff serves as a scientific advisor to a number of start-up companies and patient focused advocacy organizations.

Prior to joining Biohaven, Cliff was the Development Lead for Genetically Defined Diseases at Bristol-Myers Squibb Company (BMS). In this role he led the teams which developed innovative strategies and executed on two novel programs in neuromuscular and neurodegenerative diseases. Cliff has in-depth experience in all stages of program advancement from discovery to launch in diverse disease areas including virology, oncology, immunology, cardiovascular and neuroscience. This includes key leadership roles for the development and launch of Reyataz®, Baraclude®, Nurtec ODT® and Sprycel®. At BMS, he also served as Head of Biologics Strategy and Operations with a key role in optimizing the company's biologics development and manufacturing capabilities.

Through his career, Cliff has held multiple positions in Discovery, Clinical Development and leadership roles in Project Planning and Management. Cliff currently serves as a consult for Project Parent Muscular Dystrophy (PPMD) and a number of small start-up organizations. Cliff holds a Master of Science degree in Medical Microbiology from Creighton University School of Medicine and a Bachelor of Science degree from South Dakota State University.

Dr Amy Donner

Senior Director, Medical Communications

Wave Life Sciences

Amy Donner, PhD is currently Senior Director, Head of Medical Communications at Wave Life Sciences, overseeing scientific and medical communications and publications. She has >15 years of experience in scientific and medical communications, having served as an editor at multiple Nature journals, a program director for a private foundation, and a communications director for a venture capital fund and a public-private research partnership before joining Wave in 2018. Dr. Donner received her PhD in Biology on protein-nucleic acid interactions from University at Buffalo, with post-doctoral training in Molecular, Cellular and Developmental Biology and Genetics at Yale University and Harvard Medical School, respectively.

Prof Martin Turner

Professor of Clinical Neurology & Neuroscience

Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford

Martin Turner is Professor of Clinical Neurology & Neuroscience within Oxford University's Nuffield Department of Clinical Neurosciences, and Honorary Consultant Neurologist to the John Radcliffe Hospital in Oxford. His research focus is the development of biomarkers (including advanced neuroimaging and biofluid analysis), to accelerate therapy discovery in ALS (MND). He has been awarded Medical Research Council Clinician Scientist and Senior Clinical Fellowships, and the Royal College of Physicians' Graham Bull Prize with the Goulstonian Lecture in 2016. He has co-authored ~300 peer-reviewed articles and several books.

Dr Gemma Fisher

Paediatric Neuromuscular Clinical Research Fellow

Department of Paediatrics, Medical Sciences Division, University of Oxford

Gemma is a paediatric neurology trainee who has paused her clinical training to gain research experience. She has spent the last year or so working with STRONG at MDUK Oxford Neuromuscular Centre and is due to begin a doctorate in nemaline myopathy.

Dr Serge Braun

Chief Scientific Officer | President | Director of Neuromuscular Diseases

AFM-Telethon| Genosafe | Genethon

Member of the French National Academy of Pharmacy

Serge Braun, PharmD, PhD, 63 y. old, is currently:

- Scientific Director (CSO) of AFM-Telethon, the French Muscular dystrophy Association acting in innovative therapies of rare diseases, Evry (France)
- President of Genosafe, a CRO company dedicated to QC of biotherapeutic products, Evry
- Director Neuromuscular diseases at Genethon, Evry.

He prior had:

- 10 years of experience in the neuromuscular diseases field in the academic sector (Univ. Strasbourg France and USC Neuromuscular Center, Los Angeles)
- 10 years in the biotechnology sector (Vice-President Research of Transgene SA, Gene therapy biotech company) in the field of gene therapy of genetic diseases and of immunotherapeutics.

He was:

- Co-founder of Neurofit, a contract research organization specialized in preclinical testings of both the central and the peripheral nervous system.
- Vice-President of Alsace BioValley, the tri-national initiative, non-profit making organization, for the development of a major biotech cluster in Europe.

He also serves as advisor for Venture Capital companies and bioclusters.

Dr Sithara Ramdas

Consultant Paediatric Neurologist

Department of Paediatric Neurology, John Radcliffe Hospital, Oxford

Sithara Ramdas is a Consultant Paediatric Neurologist at the Oxford Children's Hospital and Honorary Senior Clinical Lecturer with the University of Oxford. Her areas of specialist interest are neuromuscular disorders and neuroimmunology. She leads a multi-disciplinary regional paediatric neuromuscular service. She is the paediatric lead for the National Highly Specialised Services for Congenital Myasthenia Syndromes and for Neuromyelitis Optica. She also leads a national rare disease clinical network on Juvenile myasthenia gravis. She is the principal investigator for several past and current clinical trials and her current areas of research interest are on DMD, SMA, Congenital and Acquired Myasthenia.

Professor Stephan Sanders

Professor of Paediatric Genetics

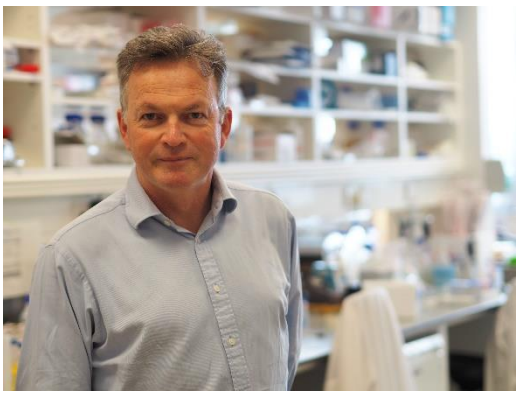
Department of Paediatrics, Medical Sciences Division, University of Oxford | Department of Psychiatry and Behavioral Sciences, UCSF Weill Institute for Neurosciences, University of California

Stephan Sanders is a Professor of Paediatric Neurogenetics in the Department of Paediatrics at the University of Oxford and a member of faculty at the University of California, San Francisco (UCSF). He trained as a paediatrician before undertaking a PhD and Postdoctoral studies in Genetics at Yale University. In 2014, he started his lab at the University of California, San Francisco (UCSF) before moving to Oxford in 2022. His group specialises in the genetics of neurodevelopmental disorders, including genomics, functional genomics, and therapeutics. Dr. Sanders is a leader of the Autism Sequencing Consortium, the BrainVar Project, and a SFARI autism sex-bias project. He was awarded the Theodore Reich Young Investigator Award by the International Society of Psychiatric Genetics (ISPG) in 2019 and a NARSAD Young Investigator Award by the Brain and Behavior Research Foundation in 2015.

Prof Kevin Talbot

Professor of Motor Neuron Biology | Head of Department, NDCN | Co-Director of MDUK Oxford Neuromuscular Centre

Nuffield Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford



Kevin Talbot is Head of the Nuffield Department of Clinical Neurosciences at the University of Oxford. He qualified in medicine from the University of London and trained in Neurology in London and Oxford. He doctoral research was on the childhood motor neuron disorder spinal muscular atrophy (SMA) in the laboratory of Professor Kay Davies. In addition to providing a clinical service for patients with motor neuron disorders, especially amyotrophic lateral sclerosis, he runs a research group developing pre-clinical models of ALS to understand the molecular basis of motor neuron degeneration. Using these cells his laboratory has identified disease-specific changes in motor neurons and this allows the development of ways of screening drugs which have the potential to slow degeneration of motor neurons.

POSTER VOTING DETAILS

Two poster awards, one for best DPhil student poster and one for best Postdoc/Fellow poster, will be awarded. Posters will be marked so that you know in which category each poster is. Each award is for £500 to be used for travel to attend a future conference.

To vote

In the poster area, there will be QR codes present to scan with your smart phone and access the voting page where you will be asked to input your name (which will be kept strictly confidential and only be used to verify that attendees vote only once) and vote for the best poster in each category.

If you are not able to vote via QR code, please contact me to make alternative arrangements.

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POSTER ABSTRACTS

Table 1: MDUK ONMC Annual Meeting 26th Sep 2023 Poster Abstracts List – coloured text indicates presenters competing for the DPhil student or Postdoc/Research Fellows travel awards

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

Setareh Alabaf[1], Richard Webster[1], Judith Cossins[1], Wei-wei Liu[1], David Beeson[1], Yin Dong[1]

[1]*Neurosciences Group, Weatherall Institute of Molecular Medicine, University of* Oxford

Congenital Myasthenic Syndromes (CMS) are genetic disorders of the neuromuscular junction (NMJ) characterised by fatigable muscle weakness. In CMS patients with mutations in the RAPSN gene, the mutations impair acetylcholine receptor (AChR) clustering at the motor endplate and cause reduced AChR surface expression. A CMS patient with clinical features of RAPSN CMS including muscle weakness and respiratory crises from birth was found to have compound heterozygous mutations in the AChR delta subunit. One of these was p.R396H in the cytoplasmic loop of the delta subunit. We have previously confirmed that AChR clustering in myotubes is impaired if AChR harbours this mutation. We further designed a δR399H C57BL/6 mouse model, provided by the MRC GEMM program and characterised the mouse model up to postnatal week 20 confirming it reflects many characteristics of congenital myasthenic syndrome. The muscle adapter protein Dok-7 is essential for amplifying and activating the receptor kinase MuSK which ultimately orchestrates the clustering and maintenance of AChR clusters. In this study we administered therapeutic dose of adeno-associated virus serotype 9 (AAV-9) vector encoding the human DOK7 gene to δR399H C57BL/6 mouse model at postnatal week 11 to mice that showed symptoms of fatigable muscle weakness tested by inverted screen test. This was compared to model mice treated with only 0.9% saline. Neuromuscular junction (NMJ) function was evaluated by weekly inverted screen tests, electromyography at age 6 and 20 weeks postnatal life, and ex-vivo electrophysiological recordings of hemidiaphragm-phrenic nerve preparations. NMJ morphology was assessed by fluorescent immunostaining followed by confocal microscopy at the end of the study. Administration of DOK7-AAV9 resulted in enlargement of NMJs, reversed the fatigable muscle weakness and improved decrement tested by repetitive nerve stimulation in approximately half of the mice. Positive ex-vivo electrophysiological features such as an increase in the amplitude of miniature endplate potentials and endplate potentials as well as increased quantal content was seen. These results suggest that DOK7-AAV9 gene therapy may be beneficial for a range of neuromuscular junction disorders that cause AChR clustering defects and the DOK7-AAV9 gene therapy may also be beneficial when administered later in life when symptoms are present.

Serum periaxin is elevated in patients with Guillain-Barré syndrome and chronic inflammatory demyelinating polyradiculoneuropathy

Roberto Bellanti[1,2], Ryan Keh[2,3], Stephen Keddie[4], Michael P Lunn[2], Simon Rinaldi[1]

[1] *Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK*

[2] *Centre for Neuromuscular Diseases, Queen Square, University College London Hospitals NHS Foundation Trust, London, UK*

[3] *Manchester Centre for Clinical Neurosciences, Salford Royal NHS Foundation Trust, Manchester, UK*

[4] *Department of Neurology, The Royal London Hospital, Barts Health NHS Trust, London, UK*

Introduction. Immune-mediated demyelination is the primary pathological process in the majority of patients with Guillain-Barré syndrome (GBS) and chronic inflammatory demyelination polyradiculoneuropathy (CIDP). However, no clinical reliable serum biomarkers of demyelination exist for the diagnosis and management of GBS and other demyelinating inflammatory neuropathies. We have previously described peripherin[1] as a useful biomarker of peripheral axonal damage. Periaxin is a structural protein exclusively expressed by myelinating Schwann cells.[2] Due to its high specificity for peripheral nerve myelin, we postulated that periaxin could serve as a biomarker of peripheral demyelination.

Methods. We developed an electrochemiluminescence (ECL) based immunoassay to measure serum periaxin in patients with inflammatory neuropathies. Using full-length recombinant periaxin purified from transiently-transfected HEK cells to optimise assay conditions, we established a lower limit of detection of 30 pg/ml. We tested serum samples from patients with the acute inflammatory demyelinating polyneuropathy (AIDP, n=11) and acute motor axonal neuropathy (AMAN, n=7) variants of GBS (n = 19 in total), CIDP (n=22), non-neurological disease controls (NNC, n=17) and healthy controls (HC, n=5). We also evaluated whether periaxin is released in myelinated co-cultures following immune-mediated demyelination and axonal damage, and compared results with control, uninjured cultures.

Results. We found overall higher concentrations in GBS (679.2 pg/ml) and CIDP (425.2 pg/ml) compared with NNC (66.23 pg/ml) and HC (22.0 pg/ml): GBS vs NNC, $p = 0.0005$; GBS vs HC, $p = 0.0051$; CIDP vs NNC, $p = 0.0151$; CIDP vs HC, $p = 0.0184$). AIDP levels (874.4 pg/ml) were higher than controls (AIDP vs NNC, $p = 0.0083$; AIDP vs HC, $p = 0.0275$). Median levels of periaxin were overall higher in AIDP compared with AMAN and CIDP, however differences did not reach statistical significance. In vitro, 48 hours after antibody- and complement-mediated demyelination, periaxin levels were higher (8119.9 pg/ml) compared with axonal damage (3171.2 pg/ml) and control conditions (217.6 pg/ml).

Conclusions. Our findings suggest that periaxin is elevated in patients with acute and chronic inflammatory neuropathies and may serve as a biomarker of peripheral nerve demyelination in GBS and CIDP. Larger cohorts of samples from patients with GBS, CIDP and other neuropathies are being tested. We will validate the assay using cell-based models of immune-mediated neuropathy, where we will correlate periaxin, peripherin and neurofilament light to establish their individual contributions to clinical assessment.

References. (1) Brain. 2023 Jul 12:awad234. (2) Neuron. 1994 Mar;12(3):497-508.

Myonuclear domain-dependent spatial restriction of the dystrophin and associated proteins in dystrophic myofibres

Katarzyna Chwalenia[1], Jacopo Oieni[1], Britt Hanson[1], Yulia Lomonosova[1], Annemieke Aartsma-Rus[2], Maaike van Putten[2], Chase P. Kelly[3], Eric T. Wang[3,4], Matthew J.A. Wood[1,5], Thomas C. Roberts[1,5]

[1] *Department of Paediatrics, University of Oxford, Institute of Developmental & Regenerative Medicine, Oxford, OX3 7DQ, UK*

[2] *Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands*

[3] *Department of Molecular Genetics & Microbiology, Centre for NeuroGenetics, Genetics Institute, University of Florida, Gainesville, FL, USA*

[4] *Myology Institute, University of Florida, Gainesville, FL, USA*

[5] *MDUK Oxford Neuromuscular Centre, South Parks Road, Oxford, OX1 3QX, UK*

Therapeutic strategies for Duchenne muscular dystrophy (DMD) that aim to restore expression of the dystrophin protein include CRISPR-Cas9 gene editing and peptide-PMO (PPMO)-mediated exon skipping. The efficacy of these approaches is typically assessed in terms of the amount of dystrophin restored, while correct sarcolemmal localisation of the protein is largely assumed. However, we previously showed that CRISPR-Cas9-mediated exon excision results in a patchy, myonuclear domain-restricted pattern of dystrophin expression, whereas PPMO-mediated exon skipping leads to uniform dystrophin distribution. To investigate the relevance of this phenomenon further, we have established a novel genetic mouse model (*mdx52-Xist*$^{\Delta hs}$) which exhibits patchy dystrophin expression from birth. Analyses in isolated single myofibres from these mice are revealing fundamental new insights into the function of dystrophin and the myonuclear domain. The microtubule network (which is crucial for macromolecular transport along the fibre and is significantly disrupted in dystrophic mice) was partially disorganised in *mdx52-Xist*$^{\Delta hs}$ mice suggesting interrupted trafficking of gene products in patchy-dystrophin muscles. Accordingly, components of dystrophin associated protein complex (i.e., α-dystrobrevin, β-dystroglycan, and utrophin) co-localised with dystrophin in a spatially-restricted manner. Surprisingly, neuronal nitric oxide synthase was uniformly distributed in these myofibres. RNA FISH analysis revealed reduced levels of dystrophin mRNA in both *mdx52-Xist*$^{\Delta hs}$ and *mdx52* models, in addition to significant nuclear enrichment of dystrophin transcripts in dystrophic *mdx52* mice. Interestingly, the distribution of dystrophin mRNA was also dependent on the regenerative state of the fibre. These findings have important implications for the development of improved therapies for DMD.

Data-independent acquisition proteomics of cerebrospinal fluid implicates endoplasmic reticulum and inflammatory mechanisms in amyotrophic lateral sclerosis

Elizabeth R Dellar[1], Iolanda Vendrell [2,4], Kevin Talbot [1.3], Benedikt M Kessler [2,4], Roman Fischer [2,4], Martin R Turner [1], Alexander G Thompson [1].

[1] *Nuffield Department of Clinical Neurosciences, University of Oxford, UK*

[2] *Target Discovery Institute, Centre for Medicines Discovery, Nuffield Department of Medicine, University of Oxford, UK*

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While unbiased proteomics of human cerebrospinal fluid (CSF) has been used successfully to identify biomarkers of amyotrophic lateral sclerosis (ALS), high abundance proteins mask the presence of lower abundance proteins that may have diagnostic and prognostic value. However, developments in mass spectrometry (MS) proteomic data acquisition methods offer improved protein depth. In this study, MS with library-free data-independent acquisition (DIA) was used to compare the CSF proteome of people with ALS (n=40), healthy (n=15) and disease (n=8) controls. Quantified protein groups were subsequently correlated with clinical variables. Univariate analysis identified 7 proteins, all significantly upregulated in ALS versus healthy controls, and 9 with altered abundance in ALS versus disease controls (FDR<0.1). Elevated chitotriosidase-1 (CHIT1) was common to both comparisons and was proportional to ALS disability progression rate (Pearson r=0.41, FDR-adjusted p=0.035) but not overall survival. Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1; upregulated in ALS versus healthy controls) was proportional to disability progression rate (Pearson r=0.53, FDR-adjusted p=0.003) and survival (Kaplan Meier log rank p = 0.013) but not independently in multivariate proportional hazards models. Weighted correlation network analysis was used to identify functionally relevant modules of proteins. One module, enriched for inflammatory functions, was associated with age at symptom onset (Pearson r=0.58, FDR-adjusted p=0.005) and survival (Hazard Ratio=1.78, FDR=0.065), and a second module, enriched for endoplasmic reticulum proteins, was negatively correlated with disability progression rate (r=-0.42, FDR-adjusted p=0.109). DIA acquisition methodology therefore strengthened the biomarker candidacy of CHIT1 and UCHL1 in ALS, whilst additionally highlighted inflammatory and endoplasmic reticulum proteins as novel sources of prognostic biomarkers.

The Friedreich's Ataxia Alliance at Oxford

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Friedreich's ataxia (FA) is an autosomal recessive cerebellar ataxia affecting around one in 50,000 globally. It is characterised by degeneration of the large sensory neurons and spinocerebellar tracts, cardiomyopathy and an increased incidence of diabetes. In >96% of cases, the cause is a homozygous GAA trinucleotide repeat expansion in intron 1 of the frataxin (FXN) gene, resulting in transcriptional silencing and very low production of the mitochondrial protein FXN. FXN plays an important role in the biogenesis of iron sulfur clusters, important components of several mitochondrial enzymes including respiratory chain complexes and aconitase. FXN deficiency in various cellular and animal models of FA causes mitochondrial dysfunction resulting in the cellular and neural dysfunction observed in FA.

The Oxford-Harrington Rare Disease Centre (OHC) initiated a program with the aim of developing novel therapies to cure or treat FA. A philanthropic gift launched the Friedreich's Ataxia Alliance at Oxford (FA Alliance), a multidisciplinary consortium of investigators combining their expertise to develop novel therapies for FA. The OHC identifies and endeavours to fund investigators committed to implementing translational research projects focusing on developing therapeutic candidates for FA. So far, six new FA-focused research projects have been developed, with funding secured for three. The OHC organises workshops and events to support FA Alliance members and works with them to facilitate their research. Additionally, it circulates a monthly FA Alliance email bulletin to inform investigators of important developments in the field and works closely with partners at the Friedreich's Ataxia Research Alliance and Ataxia UK (leading FA patient organisations) to integrate its activities into the global landscape of FA research and therapeutics development.

Optogenetic stimulation reveals activity-dependent mechanisms of neurodegeneration in C9orf72-HRE ALS motor neurons and neuromuscular co-culture

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Background: Increasing evidence suggests that abnormal activity and excitotoxicity underlie the selective vulnerability of motor neurons (MNs) in ALS. The C9orf72 hexanucleotide repeat expansion (HRE) is the leading genetic cause, linked to early hyper- and late hypo- excitability in induced pluripotent stem cell (IPSC)-MNs. However, whether these changes in excitability drive neurodegeneration is unclear. To answer this question requires control of MN activity. We utilised optogenetics to model the consequences of early disease hyperexcitability for the MN and the neuromuscular junction (NMJ) in C9orf72-HRE ALS.

Objectives: To determine whether mechanisms of neurodegeneration in C9orf72-HRE ALS are activity-dependent, using optogenetically-stimulated C9orf72-HRE IPSC-MNs alone and in neuromuscular co-culture. Given that immaturity of IPSC-MNs may hamper efforts to recapitulate key hallmarks of ALS in IPSC-derived models, we also sought to determine the effect of optogenetic stimulation on MN maturity *in vitro.*

Methods: We differentiated IPSC-MNs from 3 C9orf72-HRE ALS patients, 1 isogenic and 3 healthy control lines. We characterised spontaneous activity using multi-electrode array (MEA). IPSC-MNs expressing channelrhodopsin (ChR2) underwent chronic low- and high-level light stimulation and effects on (i) IPSC-MN maturity and (ii) mechanisms of C9orf72-ALS were determined. Optogenetic neuromuscular co-cultures were generated using ChR2-IPSC-MNs and C2C12 myotubes.

Results: MEA recordings confirmed optogenetic control of MN firing, associated with elevation of activity-dependent transcription factor cFos. Chronic stimulation of non-mutant IPSC-MNs led to increased features of maturity, including choline acetyltransferase expression, and bursting on MEA. C9orf72-HRE IPSC-MNs displayed baseline hypoactivity on MEA with reduced bursting and network activity relative to controls. High level chronic stimulation activated the apoptosis pathway in C9orf72-HRE MNs, rescued by CRISPR-Cas9 correction of the HRE. This was associated with a reduction in C9orf72 protein, and evidence of lysosomal dysfunction. Finally, we successfully generated optogenetic neuromuscular co-cultures with light-evoked contraction and characterised these at baseline and after stimulation.

Discussion: With optogenetic control of MN activity, we show intrinsic hypoactivity of C9orf72-IPSC-MNs and activity-dependent vulnerability linked to C9orf72 loss of function. Together, these findings provide support for activity as a driver of neurodegeneration in C9orf72-HRE ALS, and this novel application of optogenetics as a platform for further study of activity-dependent pathways in ALS.

Peptide-Oligonucleotide Conjugate Therapy for Cardiac Cells in Myotonic Dystrophy Type 1

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Myotonic dystrophy type 1 (DM1) is a genetic muscle degenerative disease that not only produces myotonia and endocrine abnormalities but also heart defects. This leads to a decrease in mobility and reduces the life expectancy of patients, primarily due to respiratory failure and malignant cardiac arrhythmias. An expansion in the CUG repeat, located in the 3'UTR of the DMPK gene, is responsible for the disease. Our lab is developing new cell-penetrating peptides conjugated with antisense oligonucleotides (ASO) against the CUG expansion (PPMOs), aiming to enhance the systemic distribution of the oligomer. Our primary goal is to develop a PPMO with high effectiveness and minimal toxicity, hoping for it to advance into clinical trial for DM1. Other objectives involved the development of new biomarkers that could be used in these trials. To do that we proceeded to quantify the differences in expression levels of mRNA, miRNA, and lncRNA between the nucleus and cytoplasm in both WT and DM1 myotubes. Building on these findings, we aimed to validate the differences in expression levels of a selected subset of mRNA, miRNA, and lncRNA in cardiomyocytes derived from iPSCs. Our results indicated that the vast majority of the subset of transcripts presented differences in expression between nucleus and cytoplasm, which aligned with the findings in myotubes. Considering these results, we treated cardiomyocytes with PPMOs at varying concentrations. Initial observations detect a partial or total correction paving the way to develop new biomarkers and validating the potency of PPMOs in cardiac cells.

Investigation of RIKEN genes in a mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a fatal, X-linked recessive neuromuscular disorder caused by mutations in the dystrophin gene at Xp21. Extensive research effort has been directed towards therapies that can restore dystrophin expression. The leading dystrophin restoration strategy is currently exon skipping. Preliminary work from our group shows that exon skipping with peptide-PMO (PPMO) antisense oligonucleotide conjugates is more effective in adult (14-week-old) versus aged (~80-week-old) dystrophic *mdx* mice. Specifically, dystrophin expression levels, histopathological correction, and restoration of the transcriptome were all better in adult compared with aged *mdx* mice. These findings have implications for the application of dystrophin restoration therapies in DMD patients, as they suggest that initiating treatment early may be beneficial. To investigate novel aspects of dystrophic pathology, we have investigated RIKEN genes (as identified by the FANTOM consortium). Such genes are often neglected due to their relatively poor annotation and paucity of existing literature. However, we have identified multiple interesting RIKEN genes in our RNA-seq datasets that are differentially expressed in dystrophic muscle. Interestingly, multiple of these RIKEN genes are also differentially expressed in during muscle injury and regeneration following notexin injection, and during myogenic differentiation in C2C12 mouse myoblasts, suggestive of important roles for these genes in muscle (patho)physiology. Notably, that the majority of our mouse RIKEN candidate genes have human homologues, and experiments in human myoblasts are currently underway. Similarly, RIKEN candidate gene function will be assessed through knock-down/out and overexpression experiments.

In vitro generation of disease-associated autoantibodies from patients with an aggressive, highly lethal autoimmune nodopathy

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Autoantibodies targeting both nodal/axonal neurofascin-186 (NF186) and paranodal/glial neurofascin-155 (NF155) are associated with a severe and rapidly progressive 'pan-neurofascin' (panNF) autoimmune nodo-paranodopathy. Although B-cell depletion with rituximab has shown promise in improving some patients' symptoms, revealing a central role for B-cells in disease pathogenesis, others do not improve or subsequently relapse. Thus, the underlying immunobiology of panNF patient B cells and their antibodies warrant further investigation. The first step in this process is successfully generating autoantibodies from patient B cells *in vitro*. We cultured bulk, unsorted healthy control PBMCs under various conditions to optimise total IgG production as measured by ELISA. To assess the proportion of $CD27^+CD38^+$ plasma cells present, we used flow cytometry. We then measured total, NF155, and NF186 IgG production using ELISA and a transiently-transfected, cell-based assay from supernatants of bulk, unsorted and sorted $NF155^+$ and $panNF^+$ patient cell cultures. B cells were sorted into new emigrant, mature naïve, and unswitched and switched memory subpopulations using FACS. We achieved consistent total IgG production in the μg/mL range in bulk, unsorted PBMC cultures using $2x10^5$ cells/well cultured for 14 days with Resiquimod (R848), interleukin-2 (IL-2), and soluble CD40 ligand (sCD40L) in a supplemented, RPMI-based B-cell media. These results were supported by expansion of $CD27^+CD38^+$ plasma cells at day 6 of culture. However, these conditions did not elicit antigen-specific IgG production from $NF155^+$ nor $panNF^+$ patient PBMCs. Results from healthy controls indicated the addition of IL-1β, IL-21, and TNFα augments total IgG production, confirmed in supernatants from both bulk and singly sorted panNF patient B cells in which 323/480 wells (67.3%) had detectable levels of total IgG. Furthermore, 2/480 (0.4%) wells from single switched memory B cells of a panNF patient demonstrated reactivity against NF186 and 1/480 wells (0.2%) demonstrated reactivity against NF155. Our data overall demonstrate the first instance of successful NF155 and NF186 autoantibody generation *in vitro* from panNF patient PBMCs. Ongoing work will investigate the sequences and characteristics of the receptors of single panNF switched memory B cells demonstrating NF155 and/or NF186 reactivity.

PRMT inhibitor promotes SMN2 exon 7 inclusion and synergizes with nusinersen to rescue SMA mice

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Spinal muscular atrophy (SMA) is the leading genetic cause of infant mortality. The advent of approved treatments for this devastating condition has significantly changed SMA patients' life expectancy and quality of life. Nevertheless, these are not without limitations, and research efforts are underway to develop new approaches for improved and long-lasting benefits for patients. Epigenetic regulation of gene expression, which involves covalent and sequence-specific modifications of histone and non-histone proteins, is a dynamic and reversible process that establishes normal cellular phenotypes and, when dysregulated, contributes to a wide range of human diseases, including SMA. Protein arginine methyltransferases (PRMT) are emerging as druggable epigenetic targets, with several small molecule PRMT inhibitors already in clinical trial. From a screen of epigenetic molecules, we have identified MS023, a potent and selective type I PRMT inhibitor, able to promote SMN2 exon 7 inclusion in preclinical SMA models. Treatment of SMA mice with MS023 results in amelioration of the disease phenotype, with strong synergistic amplification of the positive effect when delivered in combination with the antisense oligonucleotide nusinersen. Moreover, transcriptomic analysis revealed that MS023 treatment has minimal off-target effects and the added benefit is mainly due to targeting neuroinflammation. Our study warrants further clinical investigation of PRMT inhibition both as a stand-alone and add-on therapy for SMA.

Machine learning-guided AAV capsid bioengineering for enhanced EV loading and tissue specificity

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Gene therapy has emerged as a promising avenue for addressing the needs of the 350 million patients worldwide who are suffering from rare monogenic diseases. Currently, five FDA-approved gene therapies utilize adeno-associated virus (AAV) vectors, establishing AAVs as the gold standard delivery vehicle. Compared to other viral vectors, AAVs dominate due to their low toxicity profile, minimal integration into the genome, and capability of extrahepatic delivery. Past research has shown that AAVs are more resistant to neutralizing anti-AAV antibodies and mediate functional gene delivery more efficiently when encapsulated in extracellular vesicles (EVs). EVs are biological lipid nanoparticles that transport inter-cellular signaling molecules, and are hijacked by pathogenic viruses to boost transduction and evade the immune system. Consequently, AAVs enveloped in EVs (creating EV-AAVs) have the potential to deliver gene therapies to patients who test seropositive for anti-AAV antibodies. However, manufacturing costs, achieving required yields and the accumulation of EV-AAVs in non-target tissue are keeping the EV-AAV technology from clinical translation. Accumulation in nontarget tissue not only diminishes therapeutic efficacy but also necessitates the administration of higher doses, elevating the risk of adverse effects. To overcome the major bottlenecks keeping the EV-AAV technology from clinical translation, we are developing a machine learning (ML)-guided directed evolution approach to engineer AAV capsid proteins with enhanced EV loading properties and tissue tropism. Directed evolution has proven to be a successful strategy for similar protein engineering tasks, particularly when comprehensive understanding of the underlying biophysical processes is lacking. For engineering AAVs via directed evolution, an established strategy involves incorporating a random sequence into an exposed part of virion capsid protein 1. Given a random sequence of seven amino acids, the potential sequence combinations exceed 1 billion, and this combinatorial space expands dramatically if multiple sequence edits are required (e.g., to tune tissue tropism). Leveraging ML will allow us to screen this vast sequence space by learning patterns from previously studied protein variants to predict new protein variants with enhanced capabilities. In addition to transforming directed evolution using advanced data science methodologies, this work will contribute to a broader understanding of how ML can accelerate discovery.

Multi-platform mass spectrometry analysis suggests Immunoglobulin G-related metabolic phenotype of Guillain-Barré syndrome (GBS) patient serum samples

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Guillain-Barré syndrome (GBS) covers several variants of a disabling immune-mediated neuropathy which affects ~100,000 people every year. The relationship between infection, immune-mediated damage to peripheral nerves and the development of some GBS subtypes has been established, though the precise nature of the underlying immune process is in most cases unknown, and likely variable. Current treatments involve non-specific immunomodulation, and are only partly effective. Insight into the potential molecular pathways of metabolic dysregulation could help reveal links between specific targets and distinct variant phenotypes, thus pointing towards new therapeutic approaches. Here we report untargeted mass spectrometry metabolomics analyses of serum samples from GBS patients aiming to generate hypothesis about signalling pathways associated with GBS.

Metabolomics analysis was carried out using patient serum samples from three experimental groups: 1) MAG (Immunoglobulin M(IgM)-mediated), 2) GBS (seronegative or unknown) and 3) autoimmune nodopathies mediated by Immunoglobulin G (IgG) pan-neurofascin antibodies (AIN panNF). Principal Component Analysis revealed that all experimental groups overlap significantly. One-way ANOVA indicated differences predominantly between the MAG group and the other two groups. Supervised multivariate analysis corroborated those observations and showed that part of the GBS samples tend to divide in two subgroups, one associated with MAG and the other with AIN panNF. Binary comparisons and functional analysis further confirmed those findings. The only comparison that returned statistically significant pathways was GBS vs MAG. The top three identified pathways were: 1) Butanoate metabolism, 2) Valine, leucine and isoleucine degradation and 3) Tyrosine metabolism. Thus, statistical and functional analyses indicated that the GBS and MAG experimental groups were most dissimilar.

In conclusion, here we report untargeted metabolomics analysis of patient serum samples from GBS, MAG and AIN panNF. The results indicated that the GBS group is metabolically distinct from the IgM-mediated MAG group and most similar to the IgG-mediated AIN panNF. Statistical and functional analyses showed that it is polar analytes and butanoate and amino acid signalling pathways that drive the metabolic differences between the GBS and the AIN panNF groups. Thus, the results suggest that GBS is phenotypically similar to IgG-mediated nodopathies. However, these findings need further investigation using larger datasets.

Targeting metabolism to purge mutant mitochondrial DNA

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Background: Pathological variants of human mitochondrial DNA (mtDNA) were first reported over 30 years ago and, since then, they have emerged as a major cause of human disease; yet, treatments are still lacking for these devastating disorders. Deleterious mutations often affect some, but not all, of the many copies of the mtDNAs -a state known as heteroplasmy- and, typically, disease manifests only if the proportion of the mutant molecules exceeds a threshold. This means that it is not necessary to eradicate all the mutants to restore mitochondrial function; instead, a modest increase in the proportion of wild-type mtDNA should be sufficient to transition from a disease to a healthy state.

Results: Because mitochondrial fitness does not favour the propagation of functional mtDNAs in disease states, we sought to create conditions where it would be advantageous. Glucose and glutamine consumption are increased in mtDNA dysfunction, and so we targeted the use of both in cells carrying the pathogenic m.3243A>G variant with 2-Deoxy-D-glucose (2DG), or the related 5-thioglucose. Both compounds selected wild-type over mutant mtDNA, restoring mtDNA expression and respiration. Mechanistically, 2DG selectively inhibits the replication of mutant mtDNA; and glutamine is the key target metabolite, as its withdrawal, too, suppresses mtDNA synthesis in mutant cells. Additionally, by restricting glucose utilization, 2DG supports functional mtDNAs, as glucose-fuelled respiration is critical for mtDNA replication in control cells, when glucose and glutamine are scarce. Hence, we demonstrate that mitochondrial fitness dictates metabolite preference for mtDNA replication; consequently, interventions that restrict metabolite availability can suppress pathological mtDNAs, by coupling mitochondrial fitness and replication.

Conclusion: There is an urgent unmet clinical need for therapies to treat patients with heteroplasmic mtDNA disorders. The identification of small molecules that favour the selection of wild-type mtDNA represents an important advance. One of these compounds has been extensively tested in preclinical setting and administered to human subjects previously and so it can potentially be translated to the clinic in near future. Moreover, since the compounds rectify the genetic defect itself, by decreasing the number of copies of the mutated gene, they can potentially arrest and even reverse disease progression.

Effect of myonuclear domain structure on the efficacy of dystrophin restoration therapies

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Therapies that can restore the expression of the dystrophin protein in the muscles of Duchenne muscular dystrophy (DMD) patients are presumed to correct the disease. Factors that affect the success of such therapies are the total amount of dystrophin restored, the quality of the restored dystrophin (i.e. degree of internal deletion), and the correct localization of dystrophin at the sarcolemma. To address the latter point, we have investigated the pattern of dystrophin distribution after treatment with various dystrophin restoration therapies in DMD mouse models. Treatment of *mdx* mice with peptide-PMO conjugates designed to induce skipping of *Dmd* exon 23 resulted in a uniform pattern of dystrophin expression, even at low doses. By contrast, treatment of dKO (dystrophin/utrophin double knock-out) mice with an AAV-delivered CRISPR-Cas9-mediated exon deletion strategy resulted in a within-fiber patchy pattern of dystrophin distribution, which was insufficient to extend lifespan in these animals. Patchiness in the CRISPR-treated mice was attributed to the resulting myofibers being heterokaryons containing a mixture of both edited and non-edited myonuclei. Furthermore, long read sequencing across the cut sites revealed a plethora of non-productive editing events, including asymmetric cleavage and AAV backbone integration. We therefore propose that non-productively-repaired (i.e. corrupted) myonuclei further contribute to sarcolemmal dystrophin patchiness. These studies highlight an under-appreciated facet of dystrophin restoration therapeutic strategies.

Cellular and axonal transport phenotypes due to the C9orf72 HRE in iPSC-motor and sensory neurons

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Introduction: Induced pluripotent stem cell (iPSC)-derived motor neurons (MNs) from patients with Amyotrophic Lateral Sclerosis (ALS) who are carriers of the C9orf72 hexanucleotide repeat expansion (HRE) have multiple cellular phenotypes, but it remains unknown whether these phenotypes reflect the biology underlying the cell-specific vulnerability seen in this disease. We took advantage of the capability of iPSCs to generate different cell types, to compare phenotypes due to the C9orf72 HRE in MNs with sensory neurons (SNs), which are relatively spared in ALS.

Methods: We differentiated iPSC-MNs and SNs from five controls and three patients carrying the C9orf72 HRE and performed an in-depth phenotypic characterisation of the two cell types, including comparison with the relevant adult cells using post-mortem datasets, and an assessment of C9orf72 HRE foci and dipeptide protein. Survival, stress granule formation and TDP-43 mislocalisation were assessed with and without 0.5 mM sodium arsenite treatment for one hour. Axonal transport was studied using microfluidic devices.

Results: RNA sequencing confirmed the divergent transcriptome of iPSC-MNs and SNs and expression of their relevant cell type markers. The iPSC models were able to reproduce some, but not all, of the differential expression seen between sensory and motor neurons in healthy adult tissues. Sense and antisense RNA foci and dipeptide protein synthesis were confirmed in both MNs and SNs from C9orf72 patients. Compared to SNs, MNs had fewer arsenite-induced stress granules ($p<0.01$) and a higher relative cytoplasmic concentration of TDP-43 ($p<0.001$), but no differences in stress granule frequency or TDP-43 distribution were observed due to the C9orf72 HRE. The speed of retrograde lysosomal and bidirectional mitochondrial axonal transport was reduced in both C9orf72 MNs and SNs compared to controls ($p<0.05$).

Conclusion: These results suggests that these *in vitro* phenotypes seen in iPSC-derived MNs are not sufficient to explain the cell type selectivity of ALS in isolation. The detailed molecular and transcriptomic comparisons of MNs and SNs in this study confirm the potential of iPSC neuronal models, but also highlight marked differences to their corresponding adult cell types, which need to be considered when comparing findings from this model with human postmortem studies

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 Services 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.

Conclusions: 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.

Investigating RNA editing dysregulation in polyglutamine disorders

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Introduction: RNA editing by deamination is a ubiquitous post-transcriptional modification, catalysed by the family of Adenosine Deaminase Acting on RNA (ADAR) proteins 1 and 2, which upon binding to double-stranded RNAs, convert adenosine into inosine (A-to-I). As inosine is interpreted as guanosine by the translation machinery, editing of a single nucleotide can have dramatic effects, such as changing the amino acid within a coding region, and creating or eliminating a stop codon or a splice site. RNA editing is pivotal for survival: tight control of this mechanism of RNA modification is critical for cellular homeostasis. Recent advancements in high-throughput sequencing techniques have shown that neurons are amongst the cells with the highest levels of RNA editing, with millions of A-to-I conversion events across the transcriptome. Aberrant RNA editing has been identified in many diseases, from cancer to neurodegeneration, resulting in deleterious consequences, such as genomic instability and DNA damage, by mechanisms which are largely unexplored. Whether RNA editing is impaired in polyglutamine diseases is unknown.

Methods: The aim of my PhD is to explore the RNA-mediated mechanisms of toxicity in polyglutamine diseases, focusing my interest on RNA editing activity. By analysing transcriptomic data from iPS-derived motor neurons of patients affected by spinal and bulbar muscular atrophy (SBMA), I have observed reduced overall RNA editing, as expressed by the Alu editing index, a commonly used metric of global editing activity. Currently, I am interrogating transcriptomic data-sets to provide an accurate and comprehensive map of A-to-I changes in diseased neurons. My working hypothesis is CAG repeat mRNAs fold into hairpin structures, which sequester ADAR enzymes and affect their ability to edit the transcriptome. In my DPhil, I will employ a combination of techniques including analysis of recoding events, dsRNA pull down-and single molecule tracking microscopy to evaluate ADAR binding dynamics in neurons expressing untranslated CAG repeats.

Expected results: Successful completion of this project not only has the potential to shed light onto a previously unrecognised mechanism of pathogenesis for repeat expansion conditions, but may also allow the identification of new therapeutic approaches targeting restoration of ADAR editing activity.

How much transferrin receptor is enough: the therapeutic consequences of different levels of transferrin receptor in preclinical models

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The human transferrin receptor, TfR1, is the primary cellular importer of iron into cells. In recent years, TfR1 has become an important target for enhancing the delivery of drugs across muscle membranes and the blood-brain barrier for the treatment of a number of neuromuscular diseases, including myotonic dystrophy (DM1), Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA). Despite this exploitation of TfR1, very little is known about the receptor turnover, its expression on the cell surface of different tissue types and the potential expression differences among animal disease models and most importantly, between animal models and humans.

Here we demonstrate, using antisense oligonucleotides (ASOs) conjugated to an anti-TfR1 antibody in mouse models of DM1 and SMA, that anti-TfR1-ASOs are able to enhance ASO delivery to specific tissues but with differing ability in different mouse models, suggesting that TfR1 expression across tissues is inconsistent between models. To explore this hypothesis we have measured and compared the expression of TfR1 mRNA and total protein in 4 highly studied neuromuscular disease mouse models: MDX52, HSA-LR, LC15 and SMA, and their respective WT strains: FVBC and C57BL/6. More importantly, using the same models, we seek to quantify TfR1 protein expression on the cell surface on a panel of key tissues, including skeletal muscle, heart and brain. Our data provides valuable information on receptor availability, with implications for drug delivery systems seeking to exploit TfR1. Furthermore, a comparison of human and murine TfR1 highlights species differences that must be considered upon therapeutic translation into humans.

Long-term outcomes of offspring of mothers with fetal acetylcholine receptor antibodies (FARAD)

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Rare cases of arthrogryposis multiplex congenita (AMC) can be strongly associated with maternal antibodies targeting the fetal acetylcholine receptor (AChR); these antibodies paralyse the baby i*n utero* which leads to fixed joints and other defects. More recently children with milder myopathic presentations, originally termed fetal AChR inactivation syndrome, have been identified in rare families. This study brought together clinical and antibody data on 30 novel families from a multi-national cohort (n=46 offspring; 24 centres; 11 countries), re-named Fetal Acetylcholine Receptor-Antibody-related Disorders (FARAD).

All 30 mothers had AChR antibodies (by definition) and most of the maternal sera bound more strongly to the fetal AChR than to the adult AChR. Remarkably, 50% of the mothers had no diagnosis of MG. *In utero,* 15.2% had severe AMC and the pregnancies were terminated. Less severe outcomes were antenatal contractures (26.1%), polyhydramnios (51.1%), reduced fetal movements (26.2%) and/or intrauterine growth retardation (17.9%). Postnatally, four (8.7%) died during early life usually from respiratory failure. Thereafter, weakness, contractures, bulbar and respiratory involvement were prominent but improved gradually over time. Striking longer-term features in survivors were facial (73.5%), velopharyngeal insufficiency (75%), and feeding difficulties (44.4%). In addition, there were unexpected features particularly hearing loss (37.5%), diaphragmatic paresis (14.3%), pyloric stenosis (8.1%) and CNS involvement (17.5%). Better outcomes, including fewer offspring deaths, were achieved for offspring of the 15 mothers who were treated with combined immunotherapies during pregnancies. In addition, oral salbutamol, used empirically in 16/37 (43.2%) offspring, resulted in symptom improvement in 81.3%.

Maternal AChR antibody-associated disorders are not necessarily fatal, mimic other neuromuscular disorders, and are probably more common than previously recognised. Maternal AChR antibody testing is widely available; testing could be conducted on mothers of babies with polyhydramnios, reduced fetal movements, mild or severe arthrogryposis, or whose children present unexplained hypotonia, fixed joints or velopharyngeal insufficiency.

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Enhancing EV-AAV incorporation: Insights into natural loading and exogenous binding strategies for improved gene therapy vector delivery

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Adeno-associated viruses (AAVs) are one of the leading gene therapy vectors for clinical applications. However, AAV serotypes are limited by their inherent tropisms to specific tissues, and the presence of neutralizing antibodies (NAbs) in a patient's bloodstream can prevent efficient biodistribution of AAVs and make repeated treatment challenging. It has been shown that a small proportion of the AAVs incorporate into extracellular vesicles (EVs) to evade immune detection. These EV-AAVs have several advantages over standard AAVs including protection from NAbs, improved transduction efficiency and ability to co-package additional molecules for improved targeting or immunomodulation. Nonetheless, EV-AAV candidacy for

clinical therapy is limited by low levels of natural incorporation into vesicles. EVs are lipid nanoparticles produced by cells that can be subdivided into three groups based on origin: exosomes, microvesicles and apoptotic bodies. It is the goal of this study to characterize EVs that naturally incorporate AAVs, to inform future engineering strategies in increasing EV-AAV yield. Here, using a flow nanoanalyzer and two independent staining methods, we show that the percentage of EVs that incorporate AAVs is less than 2%, highlighting the need for the development of engineering strategies that can increase the incorporation of AAVs inside EVs. Evaluation of three different serotypes – serotypes 2, 8 and 9 – demonstrates similar (low) percentages of AAVs being secreted in association with EVs. Ultracentrifugation separation of large (20k pellet) and small (100k pellet) EVs, reveals that larger vesicles incorporate AAVs more efficiently. The biogenesis pathway of EV-AAVs was investigated using siRNA knockdown, as well as chemical inhibition of exosome and microvesicle release pathways. To explore the potential incorporation of AAVs into EVs via interactions with common EV markers, we conducted a study wherein 12 different markers were overexpressed in HEK293T producer cells. Our findings revealed that the overexpression of TSPAN2, CD63, and PTTG1IP significantly enhanced the yield of EV-AAVs. Pulldown experiments reveal that EV-AAVs are enriched in phosphatidylserine, but surprisingly devoid of tetraspanin markers. In order to overcome the challenging unknowns of endogenous loading, initially we evaluated exogenous binding of AAVs at the surface of EVs. Laminin Receptor and AAV Receptor (AAVR) were both evaluated for loading capabilities. AAVR engineered EVs were able to bind AAVs efficiently and showed protection from NAbs *in vitro*. These findings contribute valuable insights for future advancement of EV-AAV candidacy for clinical gene therapy applications.

Acute Oxidative Stress Exacerbates ALS-Related Pathology

and Impairs Translation of *UNC13A* and *PURA* in *C9orf72*-ALS Motor Neurons

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Background: Abundant evidence suggests that normal cellular response to stress is impaired in *C9orf72*-ALS, which is partly attributed to the RNA foci and dipeptide repeats (DPRs) derived from the hexanucleotide repeat expansion (HRE) mutation. In this study, we comprehensively characterized the acute stress response in human induced pluripotent stem cell-derived motor neurons (iPSC-MNs), and studied the associated transcriptomic and translatomic profiles.

Methods: We treated iPSC-MNs from 3 healthy controls (HCs) and 3 *C9orf72*-ALS patients with 0.5 mM sodium arsenite (ARS) for one hour, and characterized the ARS-induced stress response for up to 24 h after stress removal. We also obtained the parallel transcriptome and translatome of iPSC-MNs by Translating Ribosome Affinity Purification (TRAP) at baseline, immediately after stress, and after 2 h of recovery.

Results: C9-ALS iPSC-MN displayed a heavier burden of antisense $(CCCCGG)_n$ compared to sense $(GGGGCC)_n$ foci at baseline, both of which significantly increased after transient oxidative stress. Poly (GP) and poly (GA) were detected in the C9-ALS iPSC-MNs but did not change after ARS treatment. Nuclear depletion of TDP-43 was also induced by ARS and was more severe in the diseased MNs compared to controls, as indicated by the ratio of nuclear versus cytoplasmic TDP-43. However, we did not find any significant difference between the HC and C9-ALS group in C9orf72 protein level, SG dynamics or translation activity. While the transcriptomic profiles were similar between the two groups before and after stress, a discrete group of 68 Differentially Expressed Genes (DEGs) were identified in the translatome of C9-ALS iPSC-MNs after 2 h of recovery. Notable DEGs relevant to ALS pathogenesis included *UNC13A* and *PURA*, and GO term analysis of the DEGs showed enrichment in synaptic function, neuronal projection and small GTPase signalling transduction. Analysis of the subcellular distribution of UNC13A and PURA revealed deficient export of these transcripts from the nuclei in the disease MNs following exposure to ARS, as well as delayed incorporation of UNC13A mRNAs into SGs compared to the control group. Future work will explore pathomechanisms for nuclear retention of mRNAs during acute stress response and potential links to HRE toxicity in C9-ALS iPSC-MNs.

Unveiling Neural Disruptions in ALS: Insights from Magnetoencephalography (MEG)

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Background: Amyotrophic lateral sclerosis (ALS) presents a considerable obstacle to drug discovery, partly due to the absence of objective and sensitive biomarkers for assessing therapeutic benefits. Magnetoencephalography (MEG), a non-invasive neuroimaging technique that measures the brain's electrical activity, may be a valuable tool in this context. MEG offers a more direct measure of brain function than other neuroimaging techniques, such as MRI, by providing higher temporal resolution with millisecond precision. It also allows for the assessment of frequencies and large-scale networks with relatively good spatial resolution.

Methods: In an ongoing study, 71 age-matched healthy controls and 56 ALS participants underwent a standardised MEG protocol involving resting state and a gripper task. Metrics were extracted from the alpha, beta, delta, and theta frequency bands, including power, amplitude envelope correlation and coherence (connectivity), 1/f exponent (excitability) and Higuchi fractal dimension (complexity), using a permutations-based general linear model with correction for multiple comparisons.

Results: Notably, a worsening ALSFRS-R score correlated with larger areas of sensorimotor beta power decrease and gamma increase, as well as increased frontal and occipital theta connectivity and temporal gamma connectivity, compared to the healthy control group. In the premotor cortex, increased disability was associated with increased fractal dimension and a lower 1/f exponent. Increases in temporal connectivity characterised the intra-hemispheric analysis, with increases in frontal and occipital connectivity noted inter-hemispherically. Furthermore, a significant reduction in beta-band corticomuscular coherence (CMC), reflecting the functional coupling between cortical oscillations and muscle activity, was observed in ALS participants compared to controls during the bilateral gripper task. Interestingly, no significant difference in absolute grip strength was observed between the groups. Additionally, asymmetrical changes were noted in the unilateral gripper tasks, with only the left-hand task showing significant reductions in beta-band CMC in the contralateral hemisphere, irrespective of handedness, grip strength, or initial onset side/site.

Conclusion: MEG-derived measures of motor system functional connectivity can identify specific neural dynamics disruptions associated with ALS pathology. This highlights the potential of MEG as a valuable tool in ALS research and therapeutic assessment.